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Title: Cross-sectional and longitudinal neuroanatomical profiles of distinct clinical (adaptive) outcomes in autism

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One Sentence Summary: In autism, different clinical (adaptive behaviour) outcomes are linked to different cross-sectional and longitudinal neuroanatomical profiles.

Abstract: Individuals with autism spectrum disorder (ASD) display significant variation in clinical outcome. For instance, across age, some individuals' adaptive skills naturally improve or remain stable, while others' decrease. To pave the way for 'precision-medicine' approaches, it is crucial to identify the cross-sectional and, given the developmental nature of ASD, longitudinal neurobiological (including neuroanatomical and linked genetic) correlates of this variation. We conducted a longitudinal follow-up study of 333 individuals (161 with ASD and 172 neurotypicals, aged 6-30 years), with two assessment time points separated by ~12-24 months. We collected behavioural (Vineland Adaptive Behavior Scale-II, VABS-II) and neuroanatomical (structural magnetic resonance imaging) data. ASD participants were grouped into clinically meaningful "Increasers", "No-changers", and "Decreasers" in adaptive behaviour (based on VABS-II scores). We compared each clinical subgroup's neuroanatomy (surface area and cortical thickness at T1, ΔT (intra-individual change) and T2) to that of the neurotypicals. Next, we explored the neuroanatomical differences' potential genomic associates using the Allen Human Brain Atlas. Clinical subgroups had distinct neuroanatomical profiles in surface area and cortical thickness at baseline, neuroanatomical development, and follow-up. These profiles were enriched for genes previously associated with ASD and for genes previously linked to neurobiological pathways implicated in ASD (e.g., excitation-inhibition systems). Our findings suggest that distinct clinical outcomes (i.e., intra-individual change in clinical profiles) linked to ASD core symptoms are associated with atypical cross-sectional and longitudinal, i.e., developmental, neurobiological profiles. If validated, our findings may advance the development of interventions, e.g., targeting mechanisms linked to relatively poorer outcomes.

INTRODUCTION

Autism spectrum disorder (ASD), estimated to occur in approximately 1 out of 54 individuals (1), is one of the most common neurodevelopmental conditions. ASD is characterized by social communication difficulties and restricted and repetitive patterns of interests and behaviours (2). These symptoms can converge to disrupt adaptive behaviour, i.e., "the development and application of the abilities required for the attainment of personal independence and social sufficiency" (3). Accordingly, difficulties in adaptive behaviour are thought to represent a distinctive feature of ASD, compared to other neurodevelopmental conditions (4); play a crucial role in ASD diagnosis (e.g., measures of adaptive behaviour improve diagnostic accuracy beyond that provided by gold-standard instruments (5)) and intervention planning (4, 6); have been recommended as an outcome measure by both the food and drug administration [FDA] and stakeholders) in both children and adults (7, 8); and so have been used as the primary target in numerous clinical trials across the age-span.

Combined, ASD core and associated symptoms (including disrupted adaptive behaviour) can significantly affect individuals and society. For instance, only 12% of autistic adults are in full-time paid work (9). Also, a recent study estimated the cost of supporting autistic individuals with (or without) intellectual disability over their lifespan at $2.4 million ($1.4 million) in the United States and £1.5 million (£0.92 million) in the United Kingdom (10). Hence there is an urgent need for effective interventions and support strategies in ASD.

However, clinical trials addressing core symptoms in ASD have largely failed (11). A key reason for this is the substantial clinical and biological heterogeneity within ASD. For instance, across

the lifespan, some individuals' adaptive behaviour skills naturally improve or remain stable, while others' decrease (12). This natural variation in clinical outcome (i.e., intra-individual change in clinical profiles over time) may distort the results of clinical trials. Also, it highlights the need to develop 'precision medicine' approaches by gaining a better understanding of the mechanisms that contribute to differences in adaptive clinical outcomes. In the future, this knowledge may help to e.g., tailor treatments more effectively to those individuals with a relatively poor prognosis.

Previous research investigated how (change in) adaptive behaviour is linked to variation in cognitive ability, brain functional connectivity and neuroanatomy. For example, studies reported that relatively poor adaptive behaviour and outcome may be underpinned by reduced overall cognitive ability (i.e., the intelligence quotient (IQ); (13, 14)) and/or particular resting state functional connectivity patterns (15). Also, we recently demonstrated that ASD subgroups with distinct future adaptive outcomes differed in baseline neuroanatomy (including cortical thickness, surface area, and cortical volume) in multiple brain regions relevant to ASD and enriched for genes relevant to ASD (16). Moreover, in these regions, greater deviation from the neurotypical neuroanatomical profile predicted poorer adaptive outcome at the individual level. Together, these studies represent important first steps, but they had several limitations. For instance, the relationship between IQ and adaptive outcome may be complex and vary across individuals, e.g., based on sex, age, or cognitive ability (17, 18). Hence, some individuals with high IQ also have poor adaptive outcomes (19). Also, resting state functional connectivity patterns were not always specific to individuals with particular adaptive outcomes (maximum specificity 67%; (15)). Further, in our previous work (16), we only examined neuroanatomy cross-sectionally (at baseline); and compared neuroanatomy between different ASD subgroups. However, ASD is a

developmental condition where not only clinical, but also associated neuroanatomical, development may vary – both within ASD and in ASD compared to neurotypicals (e.g., reviewed in (20, 21)).

Hence, if we want to better understand the neuroanatomical correlates of variation in adaptive outcome, we need to examine them not only cross-sectionally, but also longitudinally (i.e., across time and age); and in ASD subgroups compared to neurotypicals.

Therefore, here we extend our previous work (16) by investigating if differences in adaptive outcome in ASD are paralleled by differences (compared to neurotypicals) in neuroanatomical developmental trajectories. We leveraged one of the largest deep-phenotyped longitudinal ASD datasets worldwide (EU-AIMS Longitudinal European Autism Project (22)) and our final sample included 333 individuals (161 ASD, 172 neurotypicals, age 6-30 years). We collected longitudinal adaptive behavioural (Vineland Behavior Scale-II, VABS-II) and neuroanatomical (structural magnetic resonance imaging) data at two assessment time points (T1 and T2) separated by ~ 12-24 months. Following recently published criteria (23), we grouped ASD individuals into three clinically meaningful outcome groups – "Increasers", "No-changers", and "Decreasers" in adaptive behaviour (based on VABS-II scores, as in (16)). Note that we chose to group individuals based on the VABS-II, because, for the VABS-II (unlike for other metrics, such as the gold standard Autism Diagnostic Observation Schedule [ADOS] and the Autism Diagnostic Interview-Revised [ADI-R]), there exists an empirical measure of the Minimal Clinically Important Difference (MCID). This MCID quantifies the amount of change required to be clinically (rather than statistically) meaningful; is approved by the FDA (7); and has previously been used to

quantify clinical outcome in ASD (16). First, to identify the clinical outcome groups' cross-sectional and longitudinal neuroanatomical profiles, we compared each group's neuroanatomy (surface area and cortical thickness at T1, ΔT (intra-individual neuroanatomical change), and T2) to that of the neurotypicals. Next, we explored the neuroanatomical profiles' potential genomic (genetic and transcriptomic) associates. Specifically, we leveraged the Allen Human Brain Atlas (24) to identify genes whose spatial expression maps resembled our patterns of neuroanatomical differences between ASD subgroups and neurotypicals. We then examined the enrichment of those genes for genes broadly associated with ASD; and for genes linked to various biological pathways implicated in the aetiology of ASD. We hypothesized that, compared to the neurotypicals, each outcome group would present with distinct cross-sectional and longitudinal neuroanatomical profiles. We further expected that these neuroanatomical profiles would be enriched for genes previously found to be associated with atypical (adaptive behaviour-related) neuroanatomy in ASD.

MATERIALS AND METHODS

Study design

Our data was part of the Longitudinal European Autism Project (LEAP) described in (22). We included participants if they or their parents/guardians were able to provide informed written or verbal consent/assent to their participation in this study. Our study was approved by national and local ethics review boards at all study sites and carried out to Good Clinical Practice (ICH GCP) standards. See the supplement for a full description of clinical assessments, inclusion and exclusion criteria, and ethics review boards.

Measures of adaptive functioning using the VABS-II

The autistic participants' adaptive behaviour was assessed by trained and reliable interviewers using the VABS-II (25), which assesses a person's current level of everyday functioning across three domains (communication, daily living skills, and socialization). We calculated age-normed standard scores (mean=100, standard deviation=15) for each domain and generated composite scores (i.e., total degree of impairment across all three domains) at T1 and T2. We then quantified the change between T1 and T2 (Δ=T2-T1) and used recently published estimates of what constitutes an MCID (23), to classify individuals with ASD into three adaptive clinical outcome groups: those whose scores could be said to meaningfully improve ("Increasers"; $\Delta V \geq 4$), showed no meaningful change/stasis ("No-changers"; $-4 < \Delta V < 4$), and those whose scores declined ("Decreasers"; $-4 \geq \Delta V$). Note that the MCID quantifies the amount of change required to be clinically, rather than statistically, meaningful. Accordingly, the MCID has been supported as a means to evaluate (treatment) outcomes, including by the Food and Drug Administration (FDA) (7). Note that VABS-II scores are age-normed and should therefore be interpreted considering the

expected ('normative') value at a given age. For instance, an individual's adaptive behaviour skills may increase between age at T1 and age at T2; however, if such an increase is to be expected during this period, the individual will be classified as a "No-changer" (i.e., not changing in relation to the age-normed value), and their (age-normed) VABS-II scores at T1 and T2 may be the same. For more detail, refer to the supplement.

MRI data acquisition

We used standard 3T magnetic resonance imaging (MRI) scanners to obtain high-resolution T1-weighted volumetric structural images with full head coverage (field of view=27 cm, slice thickness=1.2 mm, in-plane resolution=1.1*1.1 mm^2, for more detail see (16)).

Cortical reconstruction using FreeSurfer

Images were (pre)processed using well-validated, automated procedures (see supplement). Of the initial 709 scans at baseline, we retained 639 scans. Of the initial 459 scans at follow-up, we retained 428 images. After excluding all participants who did not have both T1 and T2 structural data, and those autistic individuals who did not have both T1 and T2 adaptive behavioural data, our final sample consisted of 333 individuals (161 ASD, 172 TD) (Table 1). We computed vertex-wise (site-corrected) cross-sectional and longitudinal measures of surface area and cortical thickness (for more information, see supplement).

Statistical analyses

First, we examined differences in neuroanatomy at T1 (baseline) between the neurotypicals and each outcome group. We included group and sex as factors; and linear (surface area/cortical thickness) and quadratic (cortical thickness) age at T1 (as in e.g., (16)), IQ, and total brain measures (total surface area, mean cortical thickness) as continuous covariates. Second, we examined differences in intra-individual change in neuroanatomy between T1 and T2 between the neurotypicals and each outcome group. We used separate models for each cortical feature that included the terms above and also corrected for the interaction between age at T1 and the follow-up duration (ΔT). Third, we investigated differences in neuroanatomy at T2 (follow-up) between the neurotypicals and each outcome group. We performed separate models as specified above, while correcting for age at T2. We corrected for multiple comparisons across the whole brain using random-field theory (RFT)-based cluster-correction for non-isotropic images (cluster-forming and cluster-p value threshold both $<.01$, two-tailed) (26). As surface area and cortical thickness are thought to have distinct neurobiological underpinning mechanisms (e.g., (27)), we treated them as separate analyses and did not correct for multiple comparisons across these two features. Also, we did not correct for multiple comparisons across the three subgroups, as we treated them as clinically separate (for more information, see supplement and (16, 28)). To establish the robustness of our results in view of additional potential confounders, we repeated our analyses i) while correcting for medication; ii) while not controlling for total brain measures; and iii) while excluding individuals with intellectual disability. To explore the generalizability of our results to other cognitive-behavioural features associated with adaptive behaviour, we repeated our analyses using different approaches to stratify ASD individuals into clinical outcome subgroups. In particular, we grouped individuals into “Increasers”, “No-changers” and “Decreasers” based on

change in i) each of the VABS-II domains, i.e., communication, daily living, and social skills; ii) the ADOS social domain; and iii) the ADOS restricted and repetitive behaviour domain. We acknowledge that analyzing change in these measures in conjunction with a cut-off is not a widely used approach to assess clinical development longitudinally. Therefore, we highlight that these analytical steps were taken only as a secondary and exploratory means to investigate the relationship between our primary results (computed using the VABS-II) and those results obtained using alternative (and ASD core symptom-related) measures. To evaluate the association between adaptive outcome and neuroanatomy using a dimensional (rather than categorical) approach, we assessed the effect of change in adaptive behaviour on neuroanatomy across ASD subgroups. Finally, to further explore the impact of age, we repeated our analyses while stratifying our sample into age-groups (children, adolescents, and adults). (For more information, see supplement).

Next, we aimed to link our neuroanatomical results to putative genomic (genetic and transcriptomic) mechanisms. First, we identified genes expressed in spatial patterns similar to the neuroanatomical differences between ASD subgroups and neurotypicals using the Allen Human Brain Atlas (AHBA) (24). Second, we tested the enrichment of these identified genes. We restricted our enrichment analyses a priori to a set of genes that were selected because of their previous implication in ASD and adaptive behaviour. We opted for this hypothesis-driven approach because it allowed us to investigate a broad set of genes (genetically and transcriptomically) linked to ASD etiology, and because it increased our statistical power. However, the trade-off of our approach was that we were limited in discovering enrichment beyond our chosen gene sets; and we encourage future work that extends our analyses to additional gene sets. In particular, we evaluated how the identified genes overlapped with genes that have

previously been associated with ASD at the genetic and transcriptomic level (29, 30, 31, 32) and that we have previously linked to cross-sectional neuroanatomical variation in ASD (16). We corrected our analyses for multiple comparisons across all subgroup contrasts and gene sets (p_{FDR}<.05). For more detailed information, see (16, 33) and the supplement. To examine the robustness of our findings, we repeated our analyses using a more restrictive background list of genes specifically estimated to be expressed in cortical tissue (34). Also, we extended our analyses to test the association between the observed neuroanatomical differences and specific (developmentally relevant) cell-types and neurobiological processes linked to both ASD and adaptive behaviour. Specifically, we examined enrichment for three gene sets of interest: i) genes expressed prenatally in specific cell types; ii) genes linked to excitatory-inhibitory pathways; and iii) microglial immune genes.

RESULTS

Demographics

Note that, to increase the generalizability of our results, we aimed to recruit a broad and representative number of participants. For instance, in both groups we included individuals with and without intellectual disability and participants across age (i.e., from childhood to adulthood), Also, the ASD group comprised individuals with a wide range of symptom severity. ASD subgroups and neurotypicals did not differ significantly in age, sex, total surface area, mean cortical thickness, and the time between visits. However, as expected, FSIQ was significantly higher in neurotypicals. Table1.

Within ASD, subgroups did not differ significantly in Autism Diagnostic Interview-Revised (ADI-R) (35) social and communication measures, Autism Diagnostic Observation Schedule 2 (ADOS-2) (36) Calibrated Severity Scores (CSS), T1 VABS (daily living and social domain) scores, mean cortical thickness, and time between visits. Nonetheless, in addition to VABS change scores (which is how ASD subgroups were derived), groups differed in ADI restricted and repetitive behaviour scores (Increasers<Decreasers<No-changers), FSIQ (Decreasers<Increasers<No-changers), sex, T1 VABS (communication domain and total) scores (Increasers<No-changers<Decreasers), T2 VABS scores (Decreasers<No-changers<Increasers), and total surface area (Decreasers<Increasers<No-changers) (see Table 1; information on medication: table S4).

Neuroanatomical differences

Primary analyses

Briefly, ASD subgroups and neurotypicals displayed neuroanatomical differences at T1, ΔT, and T2 in frontal, temporal, parietal, and occipital regions that are associated with adaptive behaviour and implicated in ASD. Increasers (compared to neurotypicals) had largely 'typical' neuroanatomical profiles. Specifically, the group showed no differences in cross-sectional and longitudinal surface area, or in longitudinal cortical thickness. However, the group had lower frontal cortical thickness at both T1 and T2 (Fig. 1). No-changers (compared to neurotypicals) showed both cross-sectional and longitudinal atypicality. Specifically, the group had greater temporal surface area at T1; both greater and lower Δsurface area in distinct frontal regions; and greater Δsurface area in parietal regions. At T2, No-changers no longer differed in surface area. No-changers displayed no differences in cortical thickness at T1 or T2; but greater Δcortical thickness in frontal and posterior cingulate regions, and lower Δcortical thickness in parietal and occipital regions (Fig. 2). Decreasers (compared to neurotypicals) also showed both cross-sectional and longitudinal differences. In particular, Decreasers had greater temporal and lower anterior cingulate surface area at T1; reduced parietal, occipital, and temporal Δsurface area; but no differences in surface area at T2. Further, the group showed greater frontal cortical thickness and lower temporal cortical thickness at T1; no differences in Δcortical thickness; and reduced frontal cortical thickness at T2 (Fig. 3). Results are also summarised in more detail in the supplement in table S1-3 (uncorrected T-values: fig. S1-3; effect sizes: fig. S4-6).

Secondary analyses

Secondary analyses established that our results remained robust in view of additional potential confounders, including correcting for medication effects (fig. S7-9); not covarying for total brain measures (fig. S7-9); and when excluding individuals with intellectual disability (fig. S10-12). This suggests that our results were not confounded by these measures. Further, our secondary analyses demonstrated that neuroanatomical differences between neurotypicals and ASD subgroups were also present when employing alternative strategies to identify clinical subgroups. Specifically, we obtained results similar to our main findings when comparing neuroanatomy between neurotypicals and clinical subgroups ("Increasers", "No-changers", and "Decreasers") based on change in i) each of the VABS-II domains, ii) the ADOS social domain, and iii) the ADOS restricted and repetitive behaviour domain (fig. S13-21). Also, we identified neuroanatomical regions associated with adaptive outcome across ASD subgroups (fig. S22); as well as neuroanatomical between-group differences within age-groups, i.e., children, adolescents, and adults (fig. S23-28).

Genomic associates

Primary analyses

Neuroanatomical differences between ASD subgroups and neurotypicals were associated with genomic mechanisms implicated in ASD and previously linked to cross-sectional neuroanatomical variation within ASD (16). Specifically, differences between Increasers and neurotypicals in cortical thickness at T1, and differences between Decreasers and neurotypicals in surface area at T1 corresponded to spatial expression patterns of gene sets previously reported to be

downregulated in ASD (cortical thickness: OR=2.51, p_{FDR}=.006; surface area: OR=3.81, p_{FDR}=.018) (30). All other imaging contrasts showed no significant enrichments. Fig. 4.

Secondary analyses

Our results remained largely unchanged when we repeated our analyses using a more restrictive background of those genes specifically estimated to be expressed in cortical tissue (34) (fig. S29). Also, secondary analyses demonstrated that our neuroanatomical results were associated with a range of genes linked to specific (developmentally relevant) cell-types and neurobiological processes implicated in both ASD and adaptive behaviour. First, differences between Increasers and neurotypicals in cortical thickness at T1 were enriched for gene expression associated prenatally with excitatory deep layer II cells (OR=2.37, p_{FDR}=.020) and maturing excitatory cells enriched in upper layers (OR=4.01, p_{FDR}=.012) (37). Also, neuroanatomical differences between No-changers and neurotypicals in Δcortical thickness corresponded with spatial expression patterns of genes linked prenatally to migrating excitatory cells (OR=15.82, p_{FDR}=.019) (37) (fig. S30). Second, neuroanatomical differences between Increasers and neurotypicals in cortical thickness at T2 were associated with spatial expression patterns of genes implicated in GABAergic pathways (OR=8.73, p_{FDR}<.001) (fig. S31). Third, neuroanatomical differences between No-changers and neurotypicals in Δsurface area corresponded with expression patterns of microglial immune genes (OR=6.63, p_{FDR}=.013) (38) (fig. S32). We observed no significant enrichments for other gene sets or between-group contrasts.

DISCUSSION

Here, we examined the cross-sectional and longitudinal neuroanatomical correlates of adaptive outcome (i.e., intra-individual change in adaptive behaviour across time) over a period of ~1-2 years in ASD, as well as their putative associated genomic mechanisms. This study extends our previous research into the cross-sectional neuroanatomical associates of variation in adaptive outcome within ASD (16). Specifically, it demonstrates that ASD subgroups with different adaptive outcomes have distinct neuroanatomical atypicality profiles (compared to neurotypicals) concerning measures of surface area and cortical thickness i) at baseline, ii) in their neuroanatomical development, and iii) at follow-up. These neuroanatomical profiles were enriched for genes previously reported to be associated with ASD itself and for genes linked to specific neurobiological pathways implicated in ASD (e.g., excitation-inhibition systems). Taken together, our findings suggest that distinct clinical outcomes related to ASD core symptoms are associated with atypical cross-sectional *and* longitudinal (i.e., developmental) neurobiological profiles.

As noted earlier, previous studies in ASD have linked adaptive outcome to brain function and structure. For example, we recently reported that adaptive outcome was associated with, and predicted by, neuroanatomical variation within ASD (at both the group- and individual level) (16). However, this previous work was limited to examining cross-sectional predictors of adaptive outcome; whereas ASD is a neurodevelopmental condition associated with atypical (compared to neurotypicals) clinical *and* neuroanatomical development (e.g., see (20, 28, 39, 40)). Therefore, to better understand the neurobiological correlates of adaptive behaviour and outcome, here we examined them both cross-sectionally and longitudinally, i.e., across time and age, and in relation

to neurotypicals. Our results suggest that a change in adaptive behaviour is paralleled by not only cross-sectional but also longitudinal neuroanatomical variation. Specifically, ASD subgroups (compared to neurotypicals) displayed distinct neuroanatomical profiles at T1, ΔT, and T2; and these profiles were robust when considering several potential confounders, including age, total brain measures, medication, and intellectual disability (information concerning other types of interventions, education, employment, and living arrangements was not available; and future studies are required to examine how these factors relate to our results).

The observed neuroanatomical profiles were characterized to varying degrees by atypicality in *both* surface area and cortical thickness. However, the atypicality patterns of these features displayed little or no spatial overlap. This is in line with previous evidence that surface area and cortical thickness represent distinct aspects of cortical architecture – with separate developmental origins and roles in brain development (41). Combined, this suggests that different neurodevelopmental mechanisms underpin variation in discrete aspects of cortical anatomy and that to better understand outcome-related neuroanatomy in ASD, it is essential to examine multiple different cortical features across time.

Further, the neuroanatomical differences we observed between ASD subgroups and neurotypicals occurred in regions that have previously been implicated both in ASD and in adaptive behaviour. For example, we identified neuroanatomical differences in frontal lobe regions, such as the superior/middle/inferior frontal gyrus, precentral gyrus, premotor cortex and supplementary motor area, and caudal/dorsal anterior cingulate cortex. These regions have previously been noted to be involved in ASD and linked to (interpersonal) emotion regulation, facial emotion recognition, and

399 adaptive behaviour in ASD and neurotypicals (42, 43, 44, 45, 46, 47, 48, 49, 50, 51). We also
400 identified temporal lobe regions, including the superior temporal gyrus, temporal pole, and
401 parahippocampal gyrus. These regions have been reported to be neuroanatomically different in
402 ASD and have been associated with social-emotional cognition (e.g., language and empathy
403 processing) and behavioural adaptation in both ASD and neurotypical populations (42, 46, 52, 53,
404 54). Parietal regions highlighted in our study included the superior/inferior parietal cortex,
405 postcentral gyrus, and posterior cingulate cortex, which are also frequently reported structures in
406 previous neuroimaging studies: among other functions, they have been linked to social cognition,
407 emotional representation, behavioural evaluation, and decision making in both autistic individuals
408 and neurotypicals (44, 55, 56, 57, 58). Occipital regions included the cuneus and lateral occipital
409 cortex. Both have been neuroanatomically implicated in ASD, and linked to the processing of
410 empathy, social inclusion/exclusion, and sensitivity to social and emotional cues in ASD and
411 neurotypicals (42, 46, 59, 60, 61). Several regions were implicated in more than one between-
412 group contrast. For instance, both No-changers and Decreasers displayed atypicality in parietal
413 and occipital cortex. Nonetheless, groups differed in how these regions were implicated (i.e., at
414 which timepoint or in which feature). Hence, despite the regional overlap, groups displayed largely
415 distinct neuroanatomical profiles. Taken together, these studies add biological plausibility to our
416 findings by linking the regions where we observed outcome-relevant neuroanatomical variation to
417 adaptive (and related) behaviour and to ASD. Specifically, they reinforce the notion that these
418 regions are both structurally and functionally implicated in (the development of) adaptive
419 behaviour in ASD. (Note that, as the regions we identified were relatively large and associated
420 with a broad set of functions, it is inherently difficult to relate them to the specific neural
421 mechanisms underlying adaptive behaviour. We further address this difficulty below, when

discussing the i) genomic correlates of our results, and the ii) specificity of our neurobiological findings to adaptive behaviour).

Additional research is required to discern if the observed reductions and enlargements in specific neuroanatomical features are primary or secondary, and detrimental or beneficial to (better) adaptive outcome. This is because the mechanistic relationship between neuroanatomical and clinical outcome remains unclear. Previous studies suggest that neuroanatomy may influence adaptive outcome, e.g., by limiting or enhancing the neural substrate available to adaptive behaviour. However, adaptive behaviour may also affect neuroanatomy, e.g., through activity-dependent alterations of synaptic and dendritic spine density (62). We previously reported that neuroanatomical differences at baseline (i.e., prior to subsequent clinical change) were predictive of adaptive outcome (16) – suggesting that (atypical) neuroanatomical variation may give rise to (atypical) behavioural development. However, these neuroanatomical differences may themselves have been influenced by/resulted from clinical change prior to our study etc. Moreover, clinical and neuroanatomical atypicalities may accumulate and compound each other across the lifespan. Taken together, this suggests that associations between neuroanatomical and clinical outcome need to be understood in the context of life-long developmental trajectories.

The neuroanatomical differences we observed in the ASD subgroups are likely modulated by a variety of genetic and other (e.g., environmental) factors. For instance, previous studies have associated variability in cortical thickness in ASD with variation in genes involved in synaptic transmission pathways (63). Also, we have previously linked adaptive outcome-related cross-sectional neuroanatomical variation between ASD subgroups to gene sets broadly associated with

ASD (16). These sets comprised genes involved in key pathological pathways in ASD, such as neurogenesis, cell proliferation, neuronal development, and synaptic processes (30). Here, we report that spatial patterns of cross-sectional differences between Increasers/Decreasers and neurotypicals were associated with these same gene sets. This suggests that (atypical) clinically meaningful change in behaviour related to ASD core symptoms is – through neuroanatomical variation – associated with key aetiological (genetic) mechanisms in ASD. Moreover, we found that both cross-sectional and longitudinal outcome-related neuroanatomical variation was associated with genes linked to specific (developmental) neurobiological processes implicated in ASD. For example, group differences in cortical thickness were enriched for genes preferentially expressed during prenatal periods in migrating excitatory cells, maturing excitatory cells enriched in upper layers, excitatory deep layer II cells (37); GABAergic pathways (64); and differences in surface area were enriched for microglial-expressed genes involved in immune functions (38). However, we observed these enrichments only in adaptive Increasers and No-changers, and not in Decreasers. This is in line with results from previous studies in toddlers with ASD, that examined early development in language ability (which may be linked to adaptive behaviour) (65, 66). Specifically, these studies reported that better outcome was linked to variation in cortical thickness genetically enriched for prenatal excitatory cell types; and to variation in surface area genetically enriched for prenatal glial (including microglial) cells (65, 66). Combined, our and these previous results suggest that the observed enrichments may indicate normative/compensatory mechanisms that help prevent or 'rescue' regression in adaptive behaviour.

Given that we compared neurotypicals to three (adaptive behaviour-based) ASD subgroups, we may have expected to consistently observe ASD-related differences, possibly

468 overshadowing/camouflaging any subgroups-specific atypicalities. Instead, we observed no
469 overlap in the between-group differences, i.e., each ASD subgroup had its own (atypical)
470 neurobiological profile. These results highlight the significant cross-sectional and longitudinal
471 neurobiological and associated clinical (adaptive) heterogeneity, both between neurotypicals and
472 ASD as a whole group and within the autism spectrum. This has implications for future clinical
473 trials; especially given that adaptive behaviour has been recommended (by researchers and
474 stakeholders (8)) – and is increasingly used (67, 68) – as a treatment endpoint in intervention
475 studies. For example, our results suggest that future clinical trials which use adaptive outcome as
476 an endpoint should consider stratifying their participants into neurobiologically and or clinically
477 homogeneous subgroups. By using our results (once they are validated), these studies could parse
478 ASD heterogeneity to identify groups of interest (e.g., those individuals less likely to improve
479 regardless of interventions) and thereby advance 'precision medicine'.
480
481 Notably, the specificity of our results (i.e., the identified regions and associated genes) to adaptive
482 (vs other cognitive-behavioural) outcomes remains to be explored. Specifically, we observed
483 neuroanatomical differences in large brain regions, many of which have been linked not only to
484 adaptive behaviour and ASD, but also to other cognitive functions. This included differences in
485 the anterior cingulate cortex, which has also been implicated in repetitive behaviour (69), a core
486 symptom of ASD. Similarly, we observed differences in the cuneus and the lateral occipital cortex,
487 which have been linked to sensory (e.g., visual) processing (70). A potential explanation for this
488 observation is that adaptive outcome is underpinned by networks of brain regions that subserve
489 not only social-communication processing but also other (ASD-related) features. This is in line
490 with the fact that, although adaptive behavior has been strongly associated with social

communication, it is a composite measure that also incorporates aspects such as motor function, sensory processing, restricted and repetitive behaviors, and symptoms of psychiatric conditions (e.g., inattention and hyperactivity in attention-deficit/hyperactivity disorder [ADHD]) (71). Alternatively, our findings may reflect that, during the observed time period, autistic individuals changed not only in adaptive behaviour but also in other (related) cognitive-behavioural features; and each of these outcomes may also be associated with a neuroanatomical profile. This is in line with our secondary findings that neuroanatomical differences between the 'original' subgroups overlapped spatially with differences between subgroups derived using alternative clinical and behavioural features, e.g., restricted/repetitive behaviours. Nonetheless, additional research is required to determine the specificity of our observed neuroanatomical differences to variation in adaptive outcome. Similarly, it is unclear if the genomic factors associated with these neuroanatomical differences are specific to adaptive outcome-related neuroanatomy. For instance, we identified enrichment for genes related to migrating and maturing excitatory cells and to GABAergic pathways. However, previous studies have shown that excitatory pyramidal cells represent the majority (~75-89%) of neurons in the cortex (72) and may therefore be implicated in ASD regardless of the specific clinical outcome. Similarly, altered excitation-inhibition (e.g., glutamatergic-GABAergic) systems are thought to be a central element in ASD pathophysiology (20, 73, 74, 75, 76); and may therefore also underpin a broad range of functions other than adaptive behaviour. In fact, this prior work, together with the known interaction between different behavioural domains/cognitive functions (and the spatial overlap in the associated neuroanatomical profiles we detected), suggest that it is unlikely that genetically determined mechanisms underpinning differences in neurodevelopment are specific to adaptive outcome in ASD.

Our results need to be considered in view of several methodological considerations and limitations that need to be addressed before our results can be applied in the clinic. Principal among these is age. Our sample included individuals ranging from childhood to adulthood. Selecting such a broad age-range was a conscious decision made for the following reason: unlike previous (longitudinal) studies of neuroanatomy (and associated genetic variation) that were restricted to individual age groups (e.g., (63)), including individuals from childhood to adulthood provided us with the unique opportunity to capture the relationship between neuroanatomical and clinical ASD phenotypes *across different* developmental stages. Also, using a dimensional approach to study the impact of age helped us avoid potential pitfalls of a categorical approach. For instance, the latter relies on (arbitrary) age-cutoffs at the group-level, which may not relate to the developmental status of individuals. Nonetheless, we acknowledge that, given the developmental nature of ASD, the relationship between adaptive outcome and neuroanatomy may be age-dependent; for instance, it is possible (and perhaps expected) that a developmental period of 1-2 years may hold a different significance in a 6-year-old compared to a 30-year-old person. To account for this, we rigorously corrected our analyses for (linear and quadratic) age, follow-up duration, and their interaction. Also, to examine the age-dependency of our discovered effects further, we stratified our sample by age-groups (children, adolescents, and adults). However, these results should be interpreted with caution: this is because our stratification yielded unbalanced samples. Hence, it is unclear if our results reflect real biological developmental differences (i.e., the fact that between-group differences are differently prominent in younger/older participants); or if they stem from differences in sample sizes and resulting differences in variance.

Second, the investigated follow-up duration was limited to 12-24 months. This opportunity to examine neuroanatomical and clinical development in ASD longitudinally (i.e., using repeated-measures within the same individuals) was unprecedented, given the scarcity of other comparable datasets and the challenges inherent to collecting large-scale longitudinal samples (e.g., cost, logistics, participant drop-out etc.). Nonetheless, in view of the developmental nature of ASD, longer follow-up periods would be desirable to further trace developmental trajectories in this condition. To address this limitation, we are currently collecting additional follow-up data from a third time point.

Further steps that will move us towards being able to apply our results in the clinic include a replication of our results in an independent sample. The main reason for why we have not yet been able to do this is the specific design of our study (longitudinal collection of multimodal data) and our sample (a heterogeneous group of neurotypical and autistic individuals [men and women] across age, cognitive abilities [e.g., including intellectual disability], and with a range of co-occurring conditions). Specifically, while the study design and sample represent a strength of our project (as they enabled us to answer a novel question in a uniquely suited dataset), they also prevented us from identifying a comparable dataset to attempt a replication of our findings. We aim to do this once suitable datasets become available.

Taken together, these future steps will help consolidate our results in different subgroups along the autism spectrum and thereby establish the context of use in which our results may be applicable (e.g., in children/adults) in the clinic. Combined, such studies will provide a basis for the future

development of clinical interventions that target the mechanisms associated with specific (e.g., relatively poor adaptive) clinical outcomes.

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Conflicts of interest: C.H.C. is a full-time employee of F. Hoffmann-La Roche, Ltd. J.T. is a consultant to F. Hoffmann-La Roche Ltd. T.C. has served as a paid consultant to F. Hoffmann-La Roche Ltd and Servier. He has received royalties from Sage Publications and Guilford Publications. J.B. has been in the past three years a consultant to / member of advisory board of / and/or speaker for Takeda/Shire, Roche, Medice, Angelini, Janssen, and Servier. He is not an employee of any of these companies, and not a stock shareholder of any of these companies. He has no other financial or material support, including expert testimony, patents, royalties. A.M.P. receives royalties from Hogrefe and S.E.U., and has received support and/or been a speaker for Servier and Sanofi. T.Ba. served in an advisory or consultancy role for eye level, Infectopharm, Lundbeck, Medice, Neurim Pharmaceuticals, Oberberg GmBH, Roche, and Takeda. He received conference support or speaker's fee by Janssen, Medice, and Takeda. He received royalties from Hogrefe, Kohlhammer, CIP Medien, and Oxford University Press. The present work is unrelated to these relationships. C.M.F. receives royalties for books on ASD, ADHD, and MDD. D.M. has served as a paid consultant to F. Hoffmann-La Roche Ltd and Servier. The remaining authors declare no competing interests.

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Conceptualization: C.M.P., D.G.M.M. Methodology: C.M.P., D.L.F., T.S., A.B., C.G., M.V.L., C.H.C., J.T., T.C., M.A., D.G.M.M., and C.E. Software: C.M.P., T.S., M.V.L., C.E. Validation: C.M.P., M.V.L., C.E. Formal analysis and investigation: C.M.P., T.S., M.V.L., M.A., and C.E. Resources: EU-AIMS/AIMS-2-TRIALS Consortium. Data curation, collection, and pre-processing: C.M.P., D.L.F., T.S., A.B., C.G., J.T., T.C., E.J., S.A., T.Bu., G.D., F.C., C.S.L., E.L., B.O., J.K.B., S.B.-C., C.F.B., A.M.P., T.Ba., S.D., C.M.F., EU-AIMS/AIMS-2-TRIALS

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Code availability:

To examine genetic enrichment (as described in the Methods), we used a script that is available at github.com/mvlombardo/utils/blob/master/genelistOverlap.R.

Supplementary information is available at MP's website.

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Figure Legends

Fig. 1 Neuroanatomical differences between neurotypicals and those individuals whose adaptive behavioural scores increased. Each row displays random field theory (RFT)-corrected t-values. Abbreviations: L, left; R, right.

Fig. 2 Neuroanatomical differences between neurotypicals and those individuals whose adaptive behavioural scores did not change. Each row displays random field theory (RFT)-corrected t-values. Abbreviations: L, left; R, right.

Fig. 3 Neuroanatomical differences between neurotypicals and those individuals whose adaptive behavioural scores decreased. Each row displays random field theory (RFT)-corrected t-values. Abbreviations: L, left; R, right.

Fig. 4 Genetic correlates of neuroanatomical variability: Enrichment analyses for cortical phenotypes (y-axis, rows) by ASD-associated gene lists (x-axis, columns). Tile colours indicate FDR q-values. Tile labels indicate enrichment odds ratios. Abbreviations: CT, cortical thickness; Δ, change between T1 and T2; DG, Decreasers; IG, Increasers; NCG, No-changers; SA, surface area; T1, time point 1; T2, time point 2.

Tables

Table 1 Demographics (at T1, unless otherwise specified) and total brain measures. Data are expressed as mean ± standard deviation (n, unless as specified at the top of the column). Abbreviations: ADI, autism diagnostic interview (comm: communication subscale; rrb: restricted and repetitive behaviour subscale; social: social subscale); ASD, autism spectrum disorder; CSS, autism diagnostic observation schedule calibrated severity score (sa: social affect subscale; rrb: restricted and repetitive behaviour subscale; total: overall score); CT, cortical thickness; F, female; FSIQ, full-scale IQ; ID, intellectual disability; M, male; SA, surface area; T1, measure at timepoint 1; T2, measure at timepoint 2; V, Vineland Adaptive Behaviour Scale (comm: communication domain; daily living: daily living domain; social: social domain; standard: composite score); Δ, measurement of change between timepoint 1 and 2. P-values are not corrected for multiple comparisons.

Measure	Decreasers n = 53	No-changers n = 42	Increasers n = 66	Test Statistic (ASD subgroups)		ASD N = 161	Neurotypicals N = 172	Test statistic (ASD vs Neurotypicals)	
ADI social	16.21 ± 7.3	17.93 ± 5.7	16.29 ± 6.9 (65)	$F_{2,157}$=0.962	p=.384	1.69 ± 6.7 (160)			
ADI comm	13.26 ± 5.8	14.64 ± 5.7	12.89 ± 5.6 (65)	$F_{2,157}$=1.258	p=.287	13.48 ± 5.7 (160)			
ADI RRB	3.98 ± 2.8	5.17 ± 2.6	3.52 ± 2.2 (65)	$F_{2,157}$=5.459	p=.005	4.11 ± 2.6 (160)			
Age (Years)	17.07 ± 6.7	14.68 ± 4.3	18.10 ± 4.7	$F_{2,158}$=5.337	p=.006	16.87 ± 5.5	16.35 ± 5.7	$F_{1,331}$=0.727	p=.394
CSS total	5.35 ± 2.9 (52)	5.60 ± 2.8 (40)	4.83 ± 2.5 (63)	$F_{2,152}$=1.090	p=.339	5.20 ± 2.74 (155)			
CSS SA	6.02 ± 2.8 (52)	6.25 ± 2.6 (40)	5.48 ± 2.5 (63)	$F_{2,152}$=1.187	p=.308	5.86 ± 2.7 (155)			
CSS RRB	4.77 ± 2.8 (52)	4.63 ± 2.7 (40)	4.29 ± 2.9 (63)	$F_{2,152}$=0.450	p=.638	4.54 ± 2.8 (155)			
FSIQ	95.75 ± 18.9	105.06 ± 22.6	104.63 ± 17.8	$F_{2,158}$=3.832	p=.024	101.82 ± 19.8	107.05 ± 16.5	$F_{1,331}$=6.888	p=.009
ID	9	5	5	χ^2_2=2.499	p=.287	19	11	χ^2_1=2.965	p=.085
Mean CT (mm)	2.68 ± 0.1	2.71 ± 0.1	2.67 ± 0.1	$F_{2,158}$=1.586	p=.208	2.69 ± 0.1	2.69 ± 0.1	$F_{1,331}$=0.012	p=.912
Sex	25 F, 28 M	6 F, 36 M	19 F, 47 M	χ^2_2=12.103	p=.002	50 F, 111 M	64 F, 108 M	χ^2_1=1.399	p=.250
Time (yrs)*	1.60 ± 0.3	1.60 ± 0.3	1.64 ± 0.2	$F_{2,158}$=0.494	p=.611	1.62 ± 0.3	1.59 ± 0.3	$F_{1,331}$=1.041	p=.308
Total SA (cm^2)	2230.11 ± 271.08	2349.98 ± 159.96	2308.22 ± 228.0	$F_{2,158}$=3.459	p=.034	2293.40 ± 232.0	2316.47 ± 225.0	$F_{1,331}$=0.848	p=.358
T1 V Comm	81.60 ± 18.3	77.00 ± 12.5	73.74 ± 13.5	$F_{2,158}$=4.031	p=.020	77.18 ± 15.3			
T1 V Daily living	77.98 ± 18.7	76.90 ± 15.4	71.86 ± 12.4	$F_{2,158}$=2.642	p=.074	75.19 ± 15.6			
T1 V Social	73.38 ± 14.9	71.98 ± 11.2	70.55 ± 15.4	$F_{2,158}$=0.582	p=.560	71.85 ± 14.2			
T1 V Standard	75.60 ± 15.2	73.31 ± 10.1	69.50 ± 11.0	$F_{2,158}$=3.717	p=.026	72.50 ± 12.5			
Δ V Comm	-15.06 ± 13.1	-2.55 ± 6.8	9.15 ± 13.0	$F_{2,158}$=62.752	p<.001	-1.87 ± 15.6			
Δ V Daily living	-10.40 ± 8.5	0.14 ± 7.4	8.59 ± 8.7	$F_{2,158}$=76.666	p<.001	0.14 ± 11.6			
Δ V Social	-7.83 ± 9.9	2.45 ± 7.8	12.36 ± 10.1	$F_{2,158}$=66.828	p<.001	3.13 ± 12.8			
Δ V standard	-11.23 ± 8.0	0.05 ± 2.0	9.86 ± 5.5	$F_{2,158}$=187.437	p<.001	0.36 ± 10.8			
T2 V Comm	66.55 ± 22.1	74.45 ± 11.3	82.89 ± 15.1	$F_{2,158}$=13.710	p<.001	75.31 ± 18.3			
T2 V Daily living	67.58 ± 16.9	77.05 ± 16.8	80.45 ± 12.9	$F_{2,158}$=10.668	p<.001	75.33 ± 16.3			
T2 V Social	65.55 ± 19.9	74.43 ± 11.0	82.91 ± 13.7	$F_{2,158}$=18.497	p<.001	74.98 ± 17.1			
T2 V Standard	64.38 ± 18.7	73.36 ± 10.8	79.36 ± 11.0	$F_{2,158}$=16.961	p<.001	72.86 ± 15.3			

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