CANDY

Comorbid Analysis of Neurodevelopmental Disorders and epilepsY

Deliverable D4.3

Report on status of posting results

CANDY-PIP

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1 Executive summary

The Preschool Brain Imaging and Behaviour (PIP) study is the first longitudinal, cross-condition preschool investigation to adopt a transdiagnostic framework. Its aim is to uncover the underlying mechanisms that drive differences in the development of clinical features (and functional outcomes) among neurodivergent and neurotypical preschool children, and to harness this knowledge to improve individual predictions and tailor support needs more effectively.

The study is exploratory in scope, acknowledging that prognostic markers may emerge as either diagnosis-specific or transdiagnostic in nature. To establish the added value of this novel approach over traditional methods, we first undertook conventional between-group comparisons for each measure (Goodwin et al., in prep). This deliverable 4.3 presents these results across various data domains including neurocognitive testing, eye-tracking, and brain structure (MRI, DTI), with EEG analyses currently underway.

Deliverable 4.5 highlights illustrative examples of prognostic biomarker identification, showcasing the potential of this approach to transform early detection and intervention in preschool populations.

2 Introduction

The Preschool Brain Imaging and Behaviour Project (PIP) was first designed under the auspices of AIMS-2-TRIALS to identify mechanisms underpinning diverse developmental trajectories in autistic and neurotypical children. CANDY offered the opportunity to extend the study to include children with Developmental Delay, ADHD, and epilepsy to establish whether any biomarkers identified in this study may be specific for autistic (sub-populations), diagnosis specific or transdiagnostic.

Between January 2020 and October 2024, 432 children were enrolled into the PIP study at five sites across Europe: King's College London (United Kingdom), Ghent University (Belgium), Karolinska Institute (Sweden), Radboud University Medical Centre (The Netherlands) and Assistance Publique-Hôpitaux de Paris (France). Demographic characteristics for the study sample at baseline are shown in Table 1.

Autistic children and children with developmental delay were enrolled between 3 and 4.5 years old. To cover both the expected chronological and mental ages of neurodivergent children, neurotypical children were enrolled between a broader age bracket of 2.5 and 4.5 years old. Children with ADHD were enrolled between 4 years and 5.75 years old, due to ADHD generally being diagnosed at a later age. Neurotypical children, autistic children and children with developmental delay were assessed at three longitudinal timepoints with a target interval of 12-15 months between assessments. The first two assessment time points (Wave 1, and Wave 2) comprised a multi-modal protocol incorporating comprehensive clinical, behavioural, neurocognitive, neurobiological measures, as well as biological samples. The final assessment, Wave 3, included parent-report clinical questionnaires and interviews (online) assessments only. To commensurate the older age at

enrolment, children with ADHD underwent two assessment time points (Wave 2 and 3), rather than 3.

Clinical characterisation of the cohort is summarised in the WP4 report for Period-4. In brief, clinical characterisation of the PIP cohort attests significant clinical heterogeneity within each condition (autism, ADHD and ID) and substantial overlap between conditions, lending support for the need of novel transdiagnostic approaches. Autistic core features are strongly correlated in both neurodivergent and neurotypical children, which may indicate common (transdiagnostic) processes. Autistic and ADHD features on the other hand are moderately to highly correlated.

3 Main section

3.1 Case control comparisons, by modality

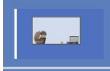
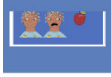
3.1.1 Neurocognitive measures (KCL)

Between-group (autism versus NT) comparisons for executive function and reward learning

Data acquisition rates ranged from 64% to 97%. Significant group differences were found in sustained attention, reward learning (both on primary dependent variable), inhibitory control (albeit on a secondary dependent variable – reaction time variability – only), and the two false belief tasks. No group differences were found on a novel social reward learning task, an emotion recognition and 'understanding desires' task. A more detailed summary of results of the two Executive function and two reward learning tasks – the priority of CANDY – is given in the WP2 periodic report 4.

Table 1: *Summary of completion rates, usable data after QC steps, results of group comparisons (here: Autism and neurotypical) and split-half reliability at Wave 1. A. Executive function and reward learning. B. Social and emotional processing.*

	Sample	Age (months)	Completion rates	Data usable after QC	Group comparison	Split-half reliability (Spearman-Brown)
Inhibitory Control 	n=132 Autism 150 Neurotypical	**43.50**±7.21	64% Autistic 87% Non-autistic	62% Autistic 80% Non-autistic	- **Yes**, significant difference in **RTV** (β=-0.05, CI=[-0.08, -0.01], p<0.05) - ns for other DVs	0.91
Sustained attention 	n=114 Autism 143 Neurotypical	**43.29**±7.60	76% Autistic 85% Non-autistic	69% Autistic 87% Non-autistic	- **Yes**, significant difference in **accuracy**(β=2.1, CI=[0.17, 4.0], p<0.05) - ns for other DVs	0.87
Non-social reward learning 	n=144 Autism 155 Neurotypical	**43.47**±7.55	85% Autistic 97% Non-autistic	85% Autistic 97% Non-autistic	- **Yes**, significant difference on the **non-social RL learning criterion**(β=28, CI=[20, 37], p<0.001), **learning rate**(β=3.8, CI=[0.32, 7.3], p<0.05) and **accuracy** (β=49, CI=[36, 63], p<0.001)	NA
Social reward learning 	n=125 Autism 146 Neurotypical	**43.55**±7.11	68% Autistic 90% Non-autistic	80% Non-autistic 92% Non-autistic	- ns for all DVs	NA

	Sample	Age (months)	Completion rates	Data usable after QC	Group comparison	Split-half reliability
Emotion Recognition 	n=90 Autistic 124 NT	51.21 ±11.9	75% Autistic 84% Non-autistic	82% Autistic 84% Non-autistic	**No,** similar median accuracy (~90%), and similar overall performance. p= 0.424.	0.82
False Belief 	n=88 Autistic 132 NT	53.8 ±8.4	93.1% Autistic 97% Non-autistic	75% Autistic 75% Non-autistic	**Yes** (1) Test Question : ASD :**14%**, TD**: 37.2%,** Chi-squared = 11.47, p-value = **0.00071** (2)Control Question :ASD:84.1%, TD : 76.7% Chi-squared = 1.27, p-value = 0.26).	NA
Verbal False Belief 	n=101 Autistic n=108 NT	53.71 ±8.9	96% Autistic 97% Non-autistic	86%Autistic 75%Non-autistic	**Yes** Test Question correct rate: ASD :64%, TD: 88% **p= 0.00027**	NA
Understanding Desire 	n=130 Autistic n=178 NT	48.9 ±11.0	87% Autistic 87% Non-autistic	81%Autistic 86%Non-autistic	**No** Correct Rate median: TD: 81%, ASD: 75%. P=: 0.237.	0.71

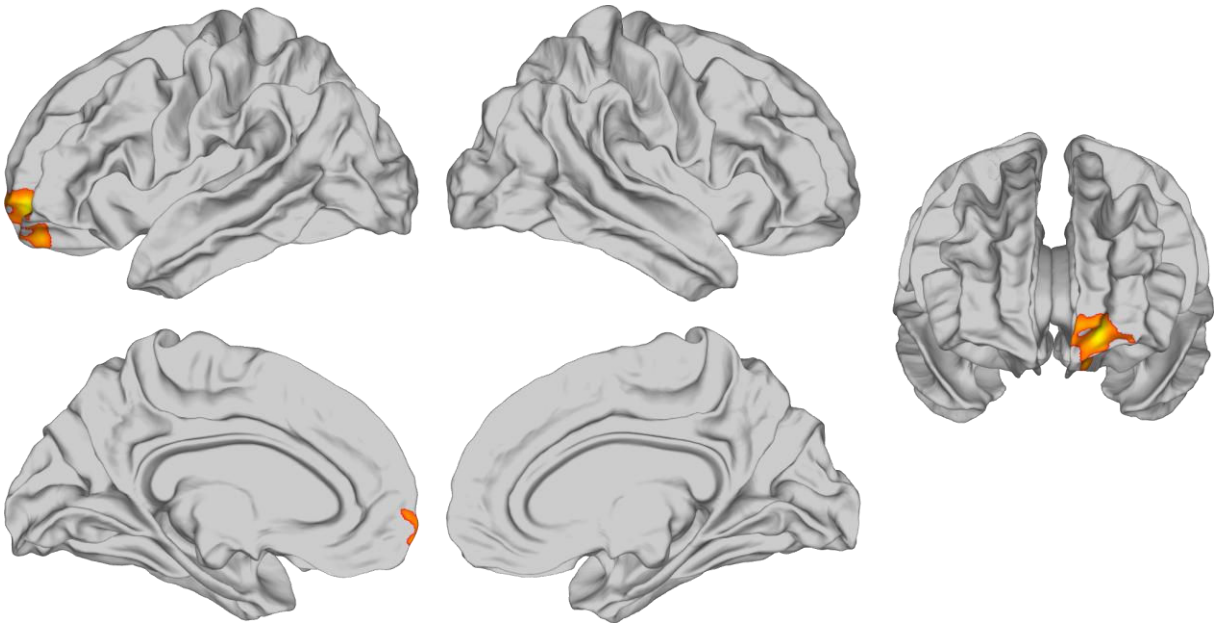
3.1.2. Eye-tracking (KCL)

Preliminary results of eye-tracking analyses of the 50 faces (face/ body and eyes-mouth) ratio revealed no significant group differences.

3.1.3. **Brain anatomy - MRI (KCL,** in collaboration with the group of Christine Ecker at Goethe University Frankfurt – J. Koziel is a joint PhD student in E. Loth and C. Ecker labs)

As detailed during the previous reporting period, we developed a data pre-processing pipeline to maximise data quality, including a) adjusting parameters to achieve less aggressive skull-stripping, particularly the watershed parameters, b) incorporating both the T1-weighted and T2 weighted images for multi-channel segmentations, c) and use of a novel AI tool, SynthSR (Iglesias et al. 2023), which employs a convolutional neural network (CNN) to enhance scan quality, effectively correcting motion artifacts within the sample.

Figure 1:scan data preprocessing pipeline and novel AI tool (SynthR)



Participant characteristics (Autism and neurotypical) at W1.

Acquisition rates for the structural modality were 49.6% for autistic children (n=164) and 59.3% for neurotypical children (n=168). This did not yield a significant group difference in acquisition rates ($\chi 2 = 2.37$, p=0.12).

The final sample consisted of 131 children who had available pre-processed and good quality MRI data and a reliably derived Developmental Quotient (DQ). Participant characteristics are summarized in Table 2. There were no significant group differences in Gender ($\chi 2 = 2.30$, p<0.13), mean cortical thickness (CT) (W=2009, p=0.58) or total surface area (SA) (W=2433, p=0.16). Autistic children were significantly older than the neurotypical peers (t128.94t128.94 =2.68, p<0.01) and had significantly lower DQ (W=603, p<0.001).

Table 2. Demographic Sample Characteristics and Between-Group Comparison

Group	**N**	**Autism** N = 60[1]	**Neurotypical** N = 71[1]	=Statistical Test
Gender	131			
Female		16 (27%)	29 (41%)	$\chi 2 = 2.30$, p<0.13
Male		44 (73%)	42 (59%)	
Age (months)	131	47.02 (±6.48)	43.75 (±7.51)	
Developmental Quotient	131	73.17 (±30.59)	109.72 (±13.10)	W=603, p<0.001
Mean CT (mm)	131	2.82 (±0.23)	2.84 (±0.22)	W=2009, p=0.58

Average SA (cm)	131	2,144.18 (±231.29)	2,076.52 (±177.45)	W=2433, p=0.16

[1]n (%); Mean (±SD)

[2]*p<0.05; **p<0.01; ***p<0.001

Between-group comparisons on cortical thickness and surface area

The between-group analyses yielded no significant group differences in cortical thickness (see Figure 2A). There was a significant group difference in SA, with increases in the autistic group rostral middle frontal gyrus and frontal pole. This pattern of vertex-wise differences was retained while repeating the analysis after excluding children with severe Intellectual disability from analyses (see Figure 2 in the Supplement).

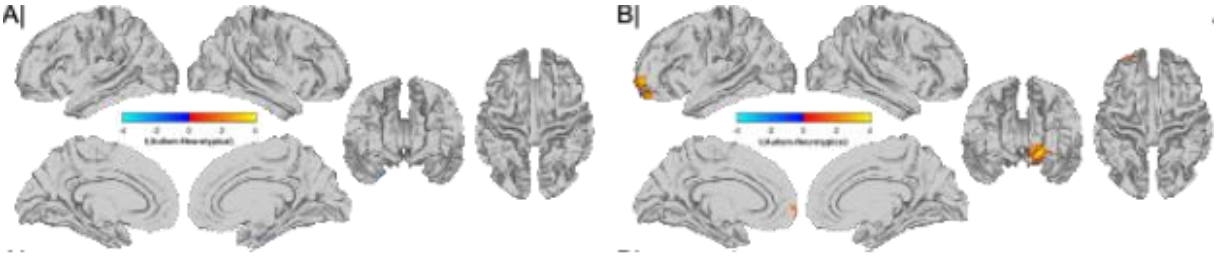


Figure 2: *The cluster-corrected differences in A) cortical thickness and B) surface area between autistic and neurotypical children. Relative decreases for the autism group are presented in blue and increases in red.*

Investigation of neuroanatomical differences between autistic children with and without ADHD traits and neurotypical children

We subsequently compared the neurotypical children against autistic children with and without co-occurring ADHD traits. The comparison between Autism+ADHD and neurotypical groups showed widespread differences in both cortical thickness and surface area (see Figure 3 for all comparisons). The autism+ADHD group had decreases in CT in prefrontal cortices such as rostral middle frontal gyrus, frontal pole, as well as in a large cluster subsuming both frontal and parietal cortices, and including paracentral lobule, precentral gyrus, precuneus cortex, posterior-cingulate cortex and left postcentral gyrus.

In terms of SA, children with autism+ADHD showed increases relative to neurotypical children which were present in lateral orbital frontal cortex, rostral middle frontal gyrus, middle temporal gyrus, inferior temporal gyrus, lateral occipital cortex and inferior parietal cortex.

Contrastingly, the Autism (only) group showed differences in CT relative to neurotypical children which were subsumed to decreases in the fusiform gyrus, the parahippocampal gyrus, entorhinal cortex and increases in parietal and frontal cortices. They showed decreases in SA in left caudal middle frontal gyrus. They also showed increases in right parietal and temporal regions.

Finally, while comparing autistic children with and without co-occurring ADHD traits, we found that the co-occurring group had relative CT decreases in frontal and cingulate areas. In terms of SA, they showed decreases in bilateral parietal and insular regions, as well as postcentral regions. They also showed increases in the right frontal areas.

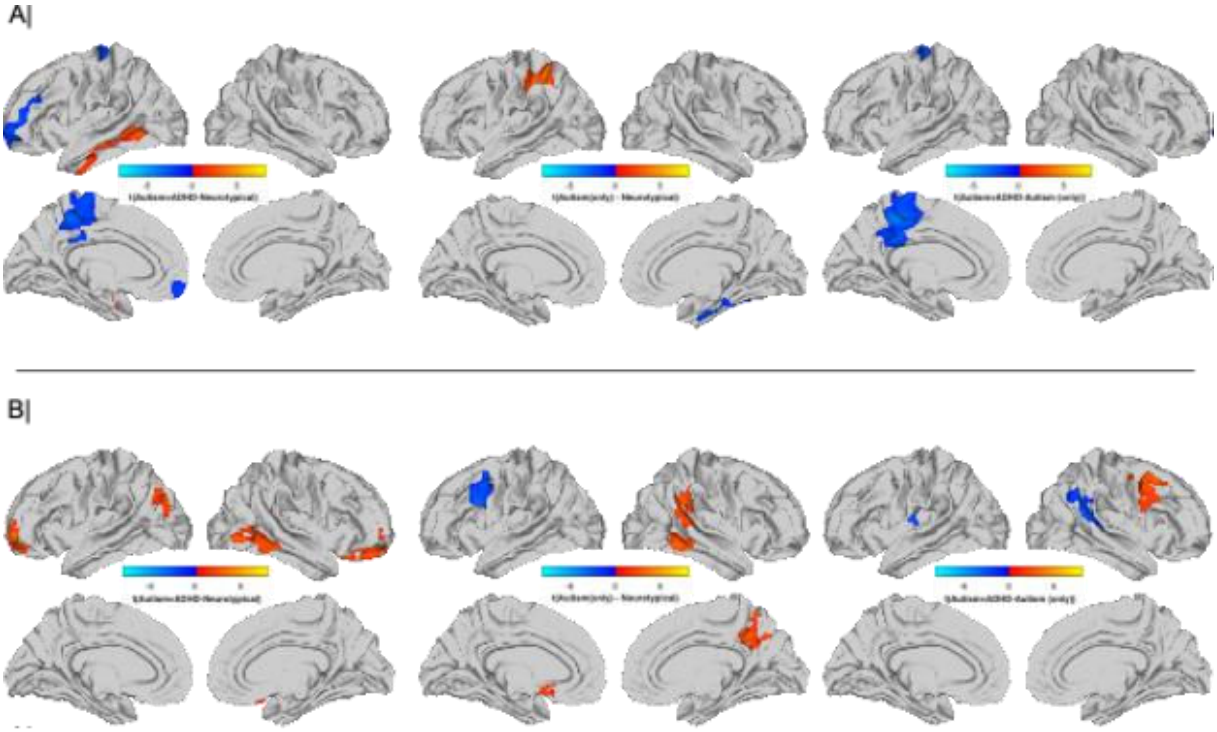


Figure 3: *The details of the cluster-corrected differences in A) cortical thickness and B) surface area between all pairwise comparisons. Decreases are presented in blue and increases in red.*

Table 3. Regional CT/ SA differences for pairwise comparisons.

	H	Region	BA	Vertices	Talairach	$p_{cluster}$
Autism+ADHD < Neurotypical	L	Paracentral lobule, precentral gyrus, precuneus cortex, posterior-cingulate cortex, postcentral gyrus	4/6, 4, 7, 23/31, 1	5227	-17, -35, 49	3.649641e-03
	L	rostral middle frontal gyrus, frontal pole	46, 10	3727	-31 44 14	3.633093e-02
Autism+ADHD>Neurotypical	L	middle temporal gyrus, inferior temporal gyrus	21, 37	2612	-50 -60 -1	4.881227e-02
Autism < Neurotypical	R	fusiform gyrus, parahippocampal gyrus, entorhinal cortex	37, 27,28	1895	22 -9 -26	5.632494e-05
Autism > Neurotypical	L	superior parietal cortex, postcentral	5, 1, 40	3434	-33 -46 49	3.434377e-02

		gyrus, inferior parietal cortex				
Autism+ADHD<Autism	L	paracentral lobule, precentral gyrus, precuneus cortex, postcentral gyrus, isthmus-cingulate cortex, posterior-cingulate cortex	4/6, 4, 7, 1, 23/31	7523	-23 -26 49	1.491642e-05
Surface area						
Autism+ADHD > Neurotypical	R	lateral orbital frontal cortex, rostral middle frontal gyrus	47, 10	1619	17 56 -15	1.555039e-04
	L	rostral middle frontal gyrus, lateral orbital frontal cortex	10, 47	1401	-18 52 -7	2.450471e-04
	R	middle temporal gyrus, inferior temporal gyrus, lateral occipital cortex	21, 37, 19	2161	57 -42 -7	1.485892e-03
	L	inferior parietal cortex	40	1357	57 -42 -7	1.115607e-02
Autism < Neurotypical	L	caudal middle frontal gyrus	6	2019	-33 12 23	2.166692e-03
Autism > Neurotypical	R	inferior parietal cortex, supramarginal gyrus, banks superior temporal sulcus	40, 42	2724	49 -49 28	4.750837e-05
	R	precuneus cortex	7	1804	11 -41 37	1.575451e-03
	R	middle temporal gyrus, inferior temporal gyrus	21, 37	1323	57 -48 -1	3.538395e-02

Autism+ADHD<Autism	R	inferior parietal cortex, banks superior temporal sulcus	40, 42	2804	49 -41 17	4.918964e-05
	L	insula, postcentral gyrus, precentral gyrus	13, 1, 4	1494	-42 -7 15	7.114686e-03
Autism+ADHD>Autism		caudal middle frontal gyrus, precentral gyrus	6, 4	3538	38 22 42	1.994497e-05

Autism + DD vs DD only

Additional analyses focused on the comparison of autistic children with developmental delay and children with developmental delay only were carried out at Goethe University Frankfurt (not part of CANDY).

Table 4. Demographics and CT/ SA descriptives

Variable	**Autism**		**non Autism**		**t-value**	**p-value**
	N = 7		N = 10			
	Mean	SD	Mean	SD		
Age (days)	1386	132	1362	142	0.35	0.73
DQ	50.76	33.8	56.81	27.7	-0.4	0.69
Mean cortical thickness (mm)	2.93	0.08	2.93	0.19	-0.05	0.96
Total surface area (mm2)	229428	18018	207191	28945	1.79	0.09

Comparison of autistic children plus DD versus children with DD without autism (controlling for age, sex, site, DQ and the total brain measure) consistently revealed one cluster in both surface area and cortical thickness. The autism group showed reductions in SA in the precentral gyrus and postcentral gyrus. and lower precentral and postcentral cortical thickness than the developmentally delayed children.

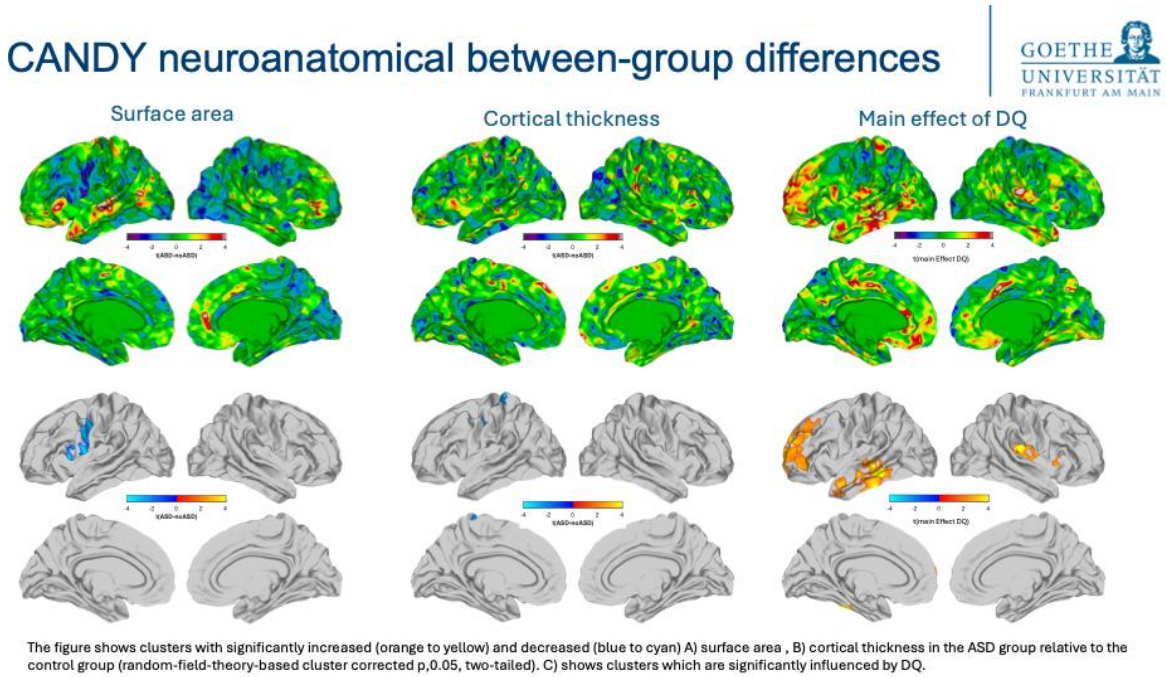


Figure 4: *Analyses also revealed a main effect of DQ but no correlation with autistic features (based on ADOS).*

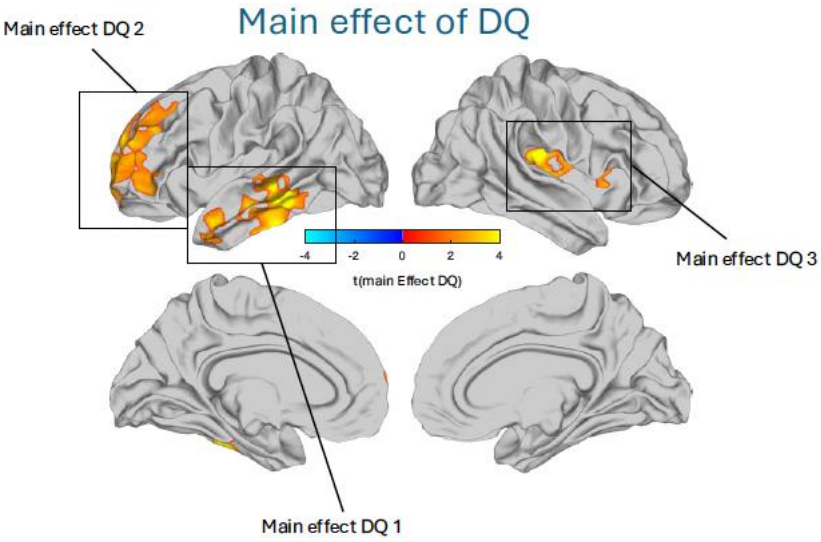


Figure 5: *Effect of developmental delay*

Dimensional analyses (collapsed across groups) revealed significant main effects of developmental delay in three clusters including 1) left middle and inferior temporal gyrus, left superior temporal gyrus, fusiform gyrus, 2) left rostral middle frontal gyrus and superior frontal gyrus, and 3 insula. These results however should be considered as preliminary given the small sample sizes.

Comparison of the MRI data of the autism and NT groups with those of ADHD children at wave 2 is pending.

3.1.4. DTI (KCL, dell' Acqua).

KCL (dell' Acqua) created a data analysis pipeline for analyses of white matter tracts. Results from group comparisons autism vs neurotypical are presented below. Data from CANDY DD and ADHD are yet to be added to the same data analysis framework.

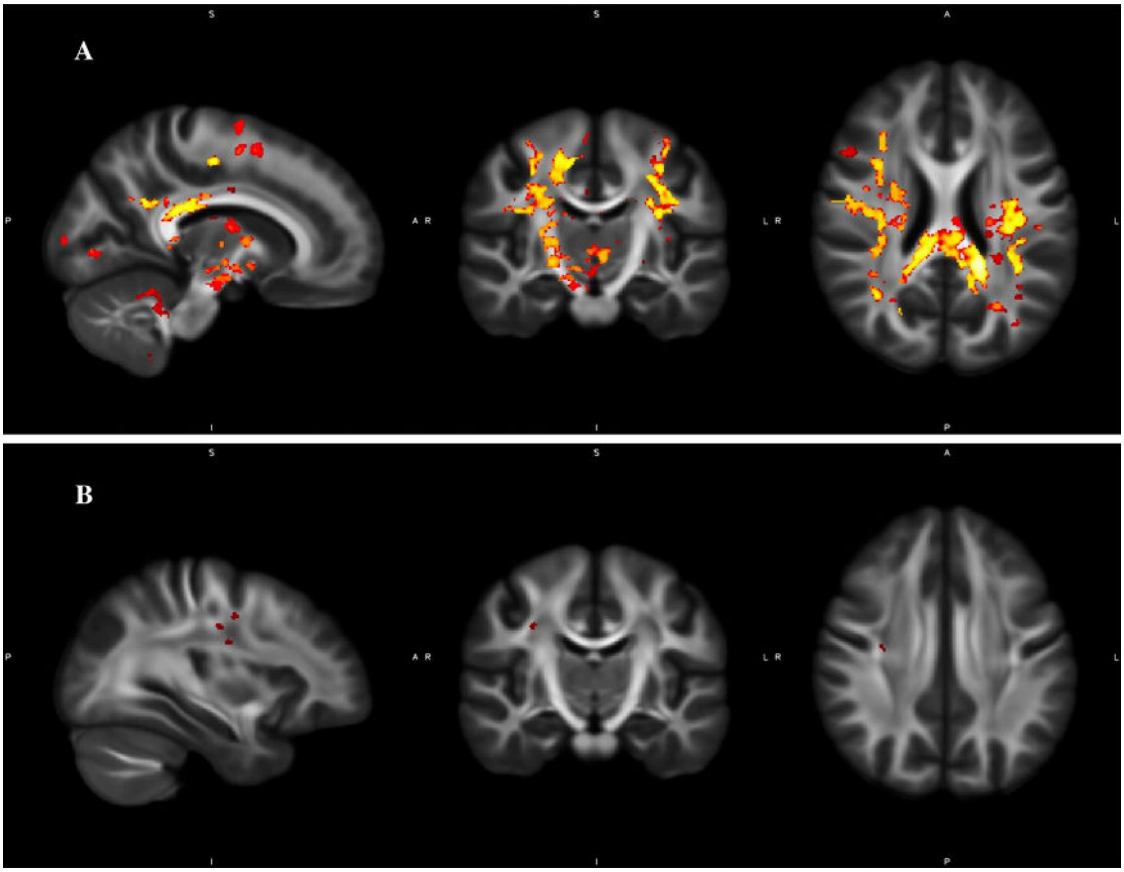


Figure 6A: analyses of white matter tracts - group comparison Autism vs NT

A) FA decreased in autistic children in: genu, body and splenium of corpus callosum, fornix, bilateral inferior cerebellar peduncle, bilateral anterior limb of internal capsule, bilateral superior corona radiata, bilateral superior longitudinal fasciculus, right posterior limb of internal capsule, and right retrolenticular part of internal capsule.

B) RD increased in autistic children in: right superior longitudinal fasciculus.

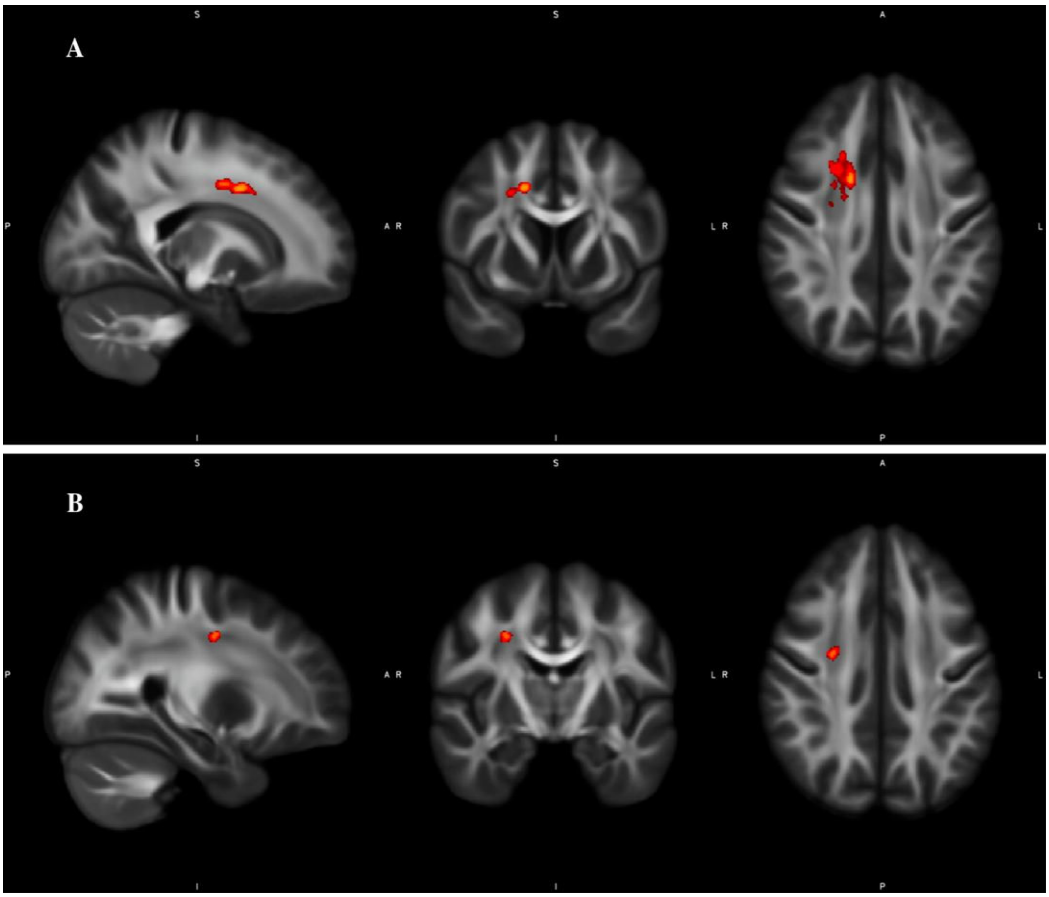


Figure 7B: analyses of white matter tracts - group comparison Autism vs NT

A) MD correlation with MSEL scores were stronger in neurotypical children than in those with ASD in the right anterior corona radiata.

B) AD correlation with MSEL scores were stronger in neurotypical children than in those with ASD in the right anterior corona radiata.

3.1.5. EEG (KCL)

EEG data was acquired from 237 preschoolers at Wave 1. During the current reporting period, we have developed centralised pre-processing and data cleaning pipelines for the preschool EEG data (**KCL,** Mason**).** In line with recommendations from the EMA, we are finalising a pre-registration to replicate the LEAP N170 biomarker findings (EEG and clinical) in our preschool PIP sample (**KCL:** Goodwin, Fritz, Loth, Mason; **BC:** Jones). Analysis of DD and ADHD groups will ascertain the specificity of the candidate N170 prognostic biomarker for autism vs. broader neurodevelopmental conditions.

Preliminary analyses of the pre-registered N170 candidate biomarker project are summarized in D4.5.

4 Repository for primary data and posting of the results

Participant data from individual sites is held locally at the involved sites (Radboud, KCL, UGent, KI, APHP). Pseudonymised data from all sites are held centrally in the CANDY internal database (OWEY). For participants who consented to this, PIP data will be released externally to the CANDY consortium via ELIXIR-LU in the near future (once all data waves have been completed and QC'd).

5 Conclusions

Between-group (autism versus NT) comparisons of different biomarker modalities partially replicate earlier findings. We demonstrate mean-group differences in executive function, reward learning and theory of mind. Against expectations, we found no significant group differences in social attention or social reward learning. There were modest group differences in brain structure and white matter tracts. Effect sizes are broadly consistent with those reported in current literature in older children, adolescents and adults (and effect sizes observed in our AIMS-2-TRIALS LEAP project) but may be weaker-than-expected given the young age of diagnosis of participants. As described in WP2, we found significant differences in the DD group across the EF and reward learning measures at W2.

These between-group differences provide a 'reference' point for on-going and planned biomarker analyses that will also involve longitudinal data (wave-1 and wave-2) and data of the ADHD group (collected at wave-2) (see also D4.5).

6 Glossary

Abbreviation / acronym	Description
ADHD	Attention-Deficit/Hyperactivity Disorder
CNN	Convolutional Neural Network
CT	Cortical Thickness
DQ	Developmental Quotient
EEG	Electro Encephalogram
QC	Quality Control
SA	Surface Area