Autism Spectrum Disorder and Childhood Apraxia of speech: early language-related hallmarks across structural MRI study

Eugenia Conti[1], Alessandra Retico[3], Letizia Palumbo[3], Giovanna Spera[3], Paolo Bosco[1], Laura Biagi[1], Simona Fiori[1], Michela Tosetti[1], Paola Cipriani[1], Giovanni Cioni[1,2], Filippo Muratori[1,2], Anna Chilosi[1*], Sara Calderoni[1,2*]

[1]*IRCCS Fondazione Stella Maris, Pisa, Italy,*

[2]*Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy,*

[3]*INFN, Pisa Division, Pisa, Italy*

Supplementary Material

S1. FreeSurfer analysis of brain MRI data

The FreeSurfer (FS, v.6.0) analysis pipeline (https://surfer.nmr.mgh.harvard.edu/) [1][2], has been implemented to carry out the segmentation of brain MRI in substructures and to compute a set of neuroanatomical descriptive features for each subject.

Firstly, we have converted 3D T1-weighted MR brain images from DICOM format to Nifti format.

Secondly, we have used the FS pre-processing workflow, known as *recon-all* analysis pipeline, which runs the input structural MRI scan across several FS functions performing all cortical reconstruction through 31 processing steps. The *recon-all* syntax is: *recon-all -i <input MRI scan> -s <subject id that you make up> -sd <directory to put the subject folder in> -all*

Lastly, we have extracted the volumes (mm^3) and thickness (mm^2) from the *aseg.stats* and *h.aparc.stats recon-all* output files, using *asegstats2table* and *aparcstats2table* FS scripts. These software generate tables of segmented subcortical volume and cortical parcellation statistics for each structure. The execution of this workflow is made automated for all MRI scans using a bash script.

A complete list of the FreeSurfer features considered in this analysis is provided in table S1.

S1. Statistical analysis

The statistical analysis of brain features carried out to identify significant differences between patients with ASD, CAS and TD children has been conducted according to the ANOVA test [3] for normally distributed features, whereas the Kruskal-Wallis [4] test in case the features were not normally distributed. The normality was evaluated by the Shapiro-Wilk test [5]. The statistical analysis was carried out with Matlab R2018a (The MathWorks, Inc.) through in-house built scripts and functions exploiting the *anova1*, *kruskalwallis* and *multcompare* matlab functions.

The Bonferroni method was used to correct the results for multiple comparisons [6]. The effect sizes were evaluated in terms of the Cohen's d [7].

To evaluate whether neuroanatomical characteristics are predictive either of the ASD or the CAS conditions, we performed a machine learning (ML)-based multivariate analysis. We implemented Support Vector Machine (SVM) binary classifiers [8] through the Matlab R2018a function *fitcsvm*. In particular, linear-kernel SVM were implemented to evaluate the predictive power of neuroanatomical features in the binary classification of the ASD vs. TD, CAS vs. TD, ASD vs. CAS groups. We implemented a 5-fold cross-validation scheme [9] in this analysis to partition the available data in train and test sets and to evaluate the classifier performance. The classification performance was evaluated in terms of the mean and standard deviation of area of the ROC curve (AUC) [10].

Table S1. **FreeSurfer features considered in the analysis**. Cortical volumes of 34 structures of the left (L) and right (R) hemispheres; cortical thickness of 34 structures of the L and R hemispheres and the average cortical thickness; 12 volumes of either subcortical or cerebellar L and R structures; volumes of 16 other structures, including white matter structures, brainstem and global measures.

Cortical volumes (L and R)	**Cortical thickness (L and R)**	**Subcortical and cerebellum volumes (L and R)**
Bankssts volume	Bankssts thickness	Lateral ventricle volume
Caudal anterior cingulate volume	Caudal anterior cingulate thickness	Inferior lateral ventricle volume
Caudal middle frontal volume	Caudal middle frontal thickness	Cerebellum white matter volume
Cuneus volume	Cuneus thickness	Cerebellum cortex volume
Entorhinal volume	Entorhinal thickness	Thalamus proper volume
Fusiform (gyrus) volume	Fusiform (gyrus) thickness	Caudate volume
Inferior parietal volume	Inferior parietal thickness	Putamen volume
Inferior temporal volume	Inferior temporal thickness	Pallidum volume
Isthmus cingulate volume	Isthmus cingulate thickness	Hippocampal volume
Lateral occipital volume	Lateral occipital thickness	Amygdala volume
Lateral orbitofrontal volume	Lateral orbitofrontal thickness	Nucleus Accumbens Area volume
Lingual volume	Lingual thickness	Ventral DC (Diencephalon) volume
Medial orbital frontal volume	Medial orbital frontal thickness	
Middle temporal volume	Middle temporal thickness	**Other subcortical volumes and global measures**
Parahippocampal volume	Parahippocampal thickness	Third Ventricle volume
Paracentral volume	Paracentral thickness	Fourth Ventricle volume
Pars opercularis volume	Pars opercularis thickness	Brain stem volume
Pars orbitalis volume	Pars orbitalis thickness	Cerebrospinal fluid volume
Pars triangularis volume	Pars triangularis thickness	Optic chiasm volume
Pericalcarine volume	Pericalcarine thickness	Posterior cingulate cortex volume
Postcentral volume	Postcentral thickness	Middle posterior cingulate cortex volume
Posterior cingulate volume	Posterior cingulate thickness	Central cortex volume
Precentral volume	Precentral thickness	Middle anterior cingulate cortex volume
Precuneus volume	Precuneus thickness	Anterior cingulate cortex volume
Rostral anterior cingulate volume	Rostral anterior cingulate thickness	Cortex volume
Rostral middle frontal volume	Rostral middle frontal thickness	Cerebral white matter volume
Superior frontal volume	Superior frontal thickness	Subcortical gray matter volume
Superior parietal volume	Superior parietal thickness	Total gray matter volume
Superior temporal volume	Superior temporal thickness	Brain segmented volume without ventricles (BrainSegVolNotVent)
Supra-marginal volume	Supra-marginal thickness	Estimated Total Intracranial Volume (eTIV)
Frontal pole volume	Frontal pole thickness	
Temporal pole volume	Temporal pole thickness	
Transverse temporal pole volume	Transverse temporal pole thickness	
Insula volume	Insula thickness	
	Cortical thickness mean	

References

[1] Fischl B. FreeSurfer. Neuroimage 2012. https://doi.org/10.1016/j.neuroimage. 2012.01.021

[2] Klein A, Tourville J. 101 labeled brain images and a consistent human cortical labeling protocol. Front Neurosci 2012;6:1–12. https://doi.org/10.3389/fnins.2012.00171.

[3] Fisher R. "The Correlation between Relatives on the Supposition of Mendelian Inheritance." Transactions of the Royal Society of Edinburgh, 52(2), 1918. Pages 399-433.
[4] William H. Kruskal and W. Allen Wallis. Use of ranks in one-criterion variance analysis. Journal of the American Statistical Association 47 (260): 583–621, December 1952.
[5] Sam S. Shapiro, Martin Bradbury Wilk (1965). "An analysis of variance test for normality (complete samples)", Biometrika, 52, 3 e 4, pagine 591-611.
[6] McDonald, J. H. (2009). Handbook of biological statistics (Vol. 2, pp. 6-59). Baltimore, MD: sparky house publishing.
[7] Cohen, J. (1988). Statistical power analysis for the behavioral sciences, 2nd ed. Lawrence Erlbaum Associates, Publishers
[8] Vapnik, VN NV. The nature of statistical learning theory. Berlin, Heidelberg: Springer-Verlag (1995).
[9] Stone, M (1974). "Cross-Validatory Choice and Assessment of Statistical Predictions". Journal of the Royal Statistical Society: Series B (Methodological). 36 (2): 111–147.
[10] Metz CE. Receiver operating characteristic analysis: a tool for the quantitative evaluation of observer performance and imaging systems. J Am Coll Radiol (2006) 3:413–22.