Urinary Metabolomic Profile in Autism Spectrum Disorder: a comparison between patients, siblings and healthy controls.

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Background:

Despite the progress in understanding the neurobiology of Autism Spectrum Disorder (ASD), the causes remain still unknown. A complex relationship between genetic, epigenetic and environmental factors contributes to ASD etiopathogenesis and it is responsible of the clinical heterogeneity.

Metabolomics explores the molecular complexity of ASD and the relationships among phenotypes related to external agents. As an emerging tool of network medicine, metabolomics provides a direct functional read-out of the phenotype by the detection, identification, and quantification of metabolites in biological fluids in order to recognize metabolic alteration between comparative samples. Recent evidences show a different urinary metabolomic profile between ASD children and their unaffected siblings[1-4]. More in detail, a high level of mammalian-microbial cometabolites, an alteration in nicotinic-acid metabolism, a mitochondrial dysfunction, and a dysregulation of antioxidant status and amino acid metabolism have been observed in ASD individuals.

Methods:

We enrolled 105 children (age range 2–11 years): 38 ASD, 35 unaffected siblings, 32 typical developing children without familiarity for ASD (Figure1).

Morning urine samples were collected for all participants. Urinary metabolites were analysed and quantified by Gas chromatography–mass spectrometry (GC-MS).

Medical history was collected with a focus on comorbidities such as gastrointestinal (GI) disorders, epilepsy, sleep problems. Standardized neuropsychological assessment was performed for ASD patients and their affected siblings (Table1-2).

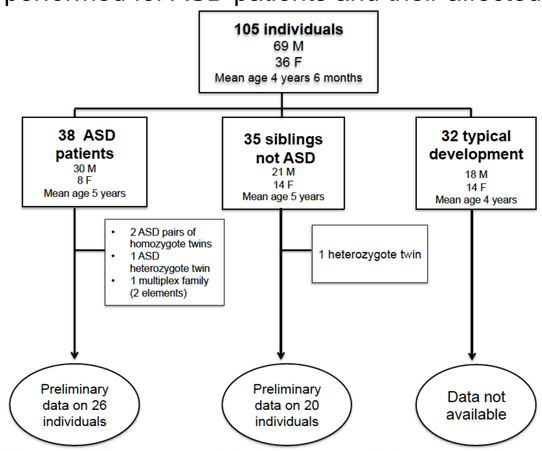

Figure.1.Demographic features of the sample

Table 1. ***ASD Clinical Assessment***

Development	Adaptive Skills	Core Symptoms	Behavior
PEP-3	Vineland-R	ADOS-2	CBCL
			SCQ
			SRS
			RBS
			ABC

PEP-3 =Psychoeducational Profile; CBCL = Child Behavior Checklist; SCQ = Social Communication Questionnaire; SRS = Social Responsiveness Scale; RBS = Repetitive Behavior Scale; ABC = Aberrant Behavior Scale.

Table 2. ***Clinical characteristics of ASD children***

ASD Group *N* = 38	Age* average (SD)	Gender male/female	DQ average (SD)	CSS average
	5.0 (2.4)	30/8	53.9 (14.8)	7

* **years**
DQ = Developmental Quotient
CSS = Calibrated Severity Score

Objectives:

To identify urinary metabolic pathways involved in the etiopathogenesis of ASD, in 3 different samples: ASD children, siblings and a control group (typical development).

To describe a specific urinary metabolomic profile related to the clinical phenotype (core symptoms, developmental quotient, adaptive skills, disruptive behaviors, medical comorbidity such as sleep and gastrointestinal problems).

Results:

Our preliminary results on 46 children (26 ASD and 20 unaffected siblings) (Figure 1) show two distinct urinary metabolomic profiles in the ASD population compared to their unaffected siblings (OPLS-DA R_2 0.5, Q_2 0.02, with a valid permutation test=0.049). The loading plot analysis reveals the different clusterization among the two groups (Figure 2). The main metabolic findings in ASD children include high concentrations of mammalian-microbial metabolites (lyxose, xylose, glucose, arabitol, sorbitol, threitol, fucose, p-cresol) and an alteration of the tryptophan pathway (kynurenic acid, xanturenic acid, quinic acid, ribonic acid) (Figure 3).

The metabolites most notably changed were compared with data obtained by Human Metabolome Database.

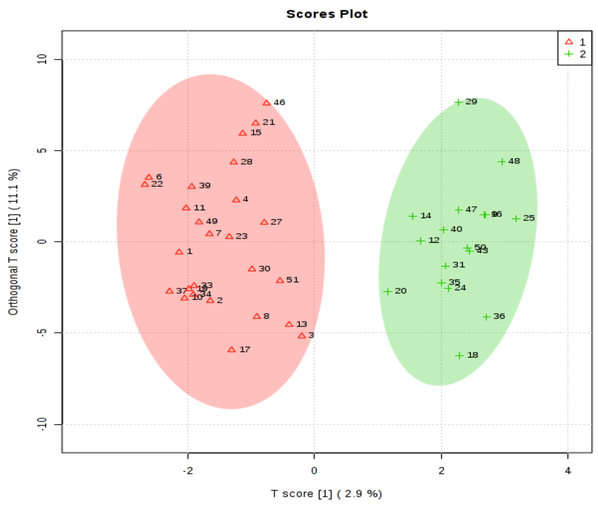

Figure.2 OPLS-DA orthogonal-projection on latent structures-discriminant analysis (Red = ASD; Green = Unaffected Siblings)

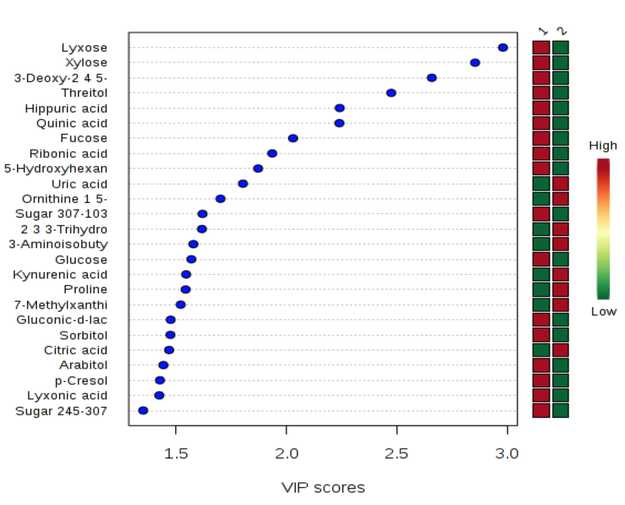

Figure 3. The 25 metabolites most discriminant amongst ASD children (Class1) and unaffected siblings (Class 2).

Conclusion:

High levels of mammalian-microbial metabolites (lyxose, xylose, glucose, arabitol, sorbitol, threitol, fucose, p-cresol), suggesting GI dysbiosis, can be related to the GI symptoms described in autistic children. Notably, the high urinary levels of p-cresol can be due to proliferation of gut bacteria, constipation and increased oral exploration of objects.

Low concentrations of kynurenic acid are associated with dysregulation of the tryptophan pathway, excitatory/inhibitory imbalance, increased oxidative stress. Furthermore, reduced levels of uric acid are related with perturbation of antioxidative system. Our preliminary findings suggest a potential role of GI dysbiosis, perturbation of antioxidant status, excitatory/inhibitory imbalance in the etiopathogenesis of ASD comorbidity such as GI disorders, epilepsy and sleep problems. The subsequent association with ASD clinical phenotype (core symptoms, developmental quotient, adaptive behavior) could allow to outline a specific clinical-metabolomic profile within ASD population.

References:

1. Gevi F, Zolla L, Gabriele S, Persico A. Urinary metabolomics of young Italian autistic children supports abnormal tryptophan and purine metabolism. Molecular Autism. 2016.
2. Mussap M, Noto A, Fanos V. Metabolomics of autism spectrum disorders: early insights regarding mammalian-microbial cometabolites. Expert Rev Mol Diagn. 2016.
3. Noto A, Fanos V, Barberini L et al. The urinary metabolomics profile of an Italian autistic children population and their unaffected siblings. J Matern Fetal Neonatal Med. 2014;27 Suppl 2:46-52.
4. Yap IK, Angley M, Veselkov K et al. Urinary Metabolic Phenotyping Differentiates Children with Autism from Their Unaffected Siblings and Age-Matched Controls. J Proteome Res. 2010;9(6):2996-3004.