The juniper bush of autism spectrum disorder (ASD): metabolomics, microbiomics, acetaminophen. What else?

Vassilios Fanos¹, Antonio Noto², Michele Mussap³

¹Department of Surgical Sciences, Neonatal Intensive Care Unit, Neonatal Pathology and Neonatal Section, University of Cagliari, Cagliari, Italy
²Department of Medical Sciences and Public Health, University of Cagliari, Cagliari, Italy
³Department of Surgical Sciences, University of Cagliari, Cagliari, Italy

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Corresponding author

Antonio Noto, Department of Medical Sciences and Public Health, University of Cagliari, Cagliari, Italy; email: antonotocagliari@gmail.com.

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“We are such stuff as dreams are made on”
William Shakespeare, The Tempest, Act IV, Scene 1
When in 1943 Leo Kanner published the first systematic description of early infantile autism, suggesting a non-restricted genetic etiology [1], he would never have believed that about 80 years later we are still unable to definitively understand the multifactorial origin and pathogenesis of the complex spectrum of neurodevelopmental disorders currently defined as autism spectrum disorder (ASD) [2, 3]. Likewise, he would never have believed the extraordinary increase over time of ASD prevalence worldwide, documented both by data from the World Health Organization (WHO) and data obtained from systematic reviews and public surveys. Concisely, WHO estimated 0.76% of the world’s children with ASD in 2010 [4]; similar data (0.70%) were obtained elsewhere [5] even though epidemiological estimates can considerably vary by demographic factors, namely ethnicity and socioeconomic status, as well as diagnostic criteria. In the United States, a very recent survey performed in 11 states on children aged 8 years [6] assessed an overall prevalence of 16.8 autistic children every 1,000 non-ASD children (equal to 1 child every 59), with a higher prevalence in boys (26.6 per 1,000) than in girls (6.6 per 1,000). Worldwide, autism affects 2 to 3 times more males than females [7].

Actually, very few human diseases like ASD can be considered the result of interplay between a multitude of factors: genetics, epigenetics, environment, socioeconomic status, maternal and neonatal infections, prenatal nutrients (i.e. folic acid), immune system, gut microbiota composition, maternal exposure to potentially toxic drugs (e.g. thalidomide) and environmental toxicants, and formula feeding (instead of breastfeeding) [8]. Taken individually, each of these factors may be considered a potential risk factor for developing ASD. However, the wide range of symptoms and disabilities depicting ASD as a “galaxy of social and communication difficulties” takes place through the combination of two or more factors cited above; notably, the role of each (e.g. genetics) cannot be dissociated from the context of epigenetic mechanisms and specific interactions. Consequently, the identification of common inherited genetic variants by whole-genomic sequencing [9, 10] and the clinician’s appraisal of symptoms through parental interview, observation, and standardized behavioral scales [2] remain the current standards for ASD diagnosis. Unfortunately, in children aged under two and a half years a definite clinical diagnosis is highly unlikely [11], even in the presence of early disturbances in sleeping, crying and feeding; indeed, autism does not rely on pathognomonic symptoms. Despite a worldwide agreement on the urgent need for a timely identification of ASD as early in life as possible [12], most children with ASD are diagnosed far too late. The delay in diagnosis hampers initiating effective measures for managing cognitive impairment and adopting educational training both for parents and preschool staff.

Despite this wholly unsatisfactory scenario, encouraging perspectives are emerging from new insights into non-genetic factors involved in the origin of ASD and from advanced diagnostic tools, namely metabolomics. We are aware that in the second decade of the third millennium the cornerstone of ASD diagnosis is (and will be) based on the system biology approach: no longer the equation “one symptom one biomarker”, but the detection of the individual molecular phenotype and its changes over time, just like a fingerprint. The molecular phenotype, closely reflecting the result of interplay between genomics, transcriptomics, proteomics, environmental factors and gut microbiota, should thus be associated with the type and degree of the behavioral/cognitive impairment and with functional neuroimaging [13, 14]. Phenotype is represented by metabolites, low-molecular-weight end-products of cellular metabolic pathways, which in turn are influenced by genetic and non-genetic factors. Metabolomics allows the systematic identification and quantification of the global collection of all metabolites, namely the metabolome, recognizable either in biological fluids (e.g. urine) or in tissues [15]. Since metabolites have no set of codons, they can be sequenced neither like genes, encoded by 4 nucleotides, nor like proteins, constituted by 20 amino acids; therefore, metabolites can be identified by methods able to characterize their elemental composition, molecular charge and mass, stereochemical orientation, and order of atoms [16]. Today, high throughput technologies like proton nuclear magnetic resonance (H NMR) spectroscopy, liquid chromatography and gas chromatography coupled with mass spectrometry (LC-MS and GC-MS, respectively) and further sophisticated analytical methods are outstanding tools that allow researchers to accurately explore the metabolome and its variations over time in various perinatal conditions involved in ASD etiology, for example perturbations of the gut-brain axis, due to gut dysbiosis, and to the lack of the intestinal mucosal barrier, caused by inflammation.
This means a great opportunity to establish an early diagnosis of ASD, to assess the risk of developing postnatal ASD and to search for new highly sensitive and specific biomarkers.

Like other neuropsychiatric diseases, ASD may be closely correlated with fetal and perinatal programming of the brain, consisting of maternal and fetal epigenetic factors that influence brain development and maturation [17, 18]. The temporal profile of neurodevelopmental sequences from perinatal age to old age is closely associated with the age degree of microbiota stability/diversity throughout life; in particular, perturbations of the developing gut microbiota during fetal and perinatal life can impact neurodevelopment and potentially lead to adverse mental health outcomes [19]. Changes occurring in the fetal and perinatal ages can be accurately recognized either by targeted or untargeted metabolomic approaches [20]; moreover, metabolomics allows the discovery of new biomarkers for an early diagnosis and monitoring of fetal and perinatal programming [21]. Notably, metabolomics accurately identifies those metabolites that are involved in the same pathway as well as the metabolic network shaped by nodes (metabolites) and their interactions (scale-free network models) [22].

Several metabolomic studies have explored the urine (and very few the blood plasma) metabolome in ASD children: their main findings have recently been summarized in a comprehensive review [23]. Despite a considerable heterogeneity in study design, population age and technologies, most of these studies and further studies published later [24, 25] have found abnormalities in gut bacterial-derived compounds, tryptophan, vitamin B6, and purine metabolic pathways, phenylalanine and tyrosine biosynthesis, unbalanced concentration of intermediary compounds of the tricarboxylic acid cycle (TCA), also known as the citric-acid or Krebs cycle, and finally diet-derived metabolites. By using 1H NMR spectroscopy, our group found a combination of increased and decreased concentrations of: hippurate, glycine, creatine, tryptophan, D-threitol, and glutamate, creatinine, lactate, valine, betaine, and taurine, respectively [26, 27]. These findings strongly suggest a crucial role of oxidative stress and gut microbiota in ASD development. In children with ASD, gut dysbiosis is characterized by the increase in Clostridium, Alistipes, Akkermansia, Caloramator, Sarcina spp., and by the reduction in Prevotella spp., E. siraueum, and Bifidobacterium spp. As a result, in these children the urine metabolome is marked by alterations in hippuric acid, p-hydroxyphenylacetic acid and 3-(3-hydroxyphenyl)-3-hydroxypropanoic acid concentration. Moreover, propionic acid (PA), a short-chain fatty acid naturally present in many foods and extensively used in the food industry and agriculture, play a pivotal role in altering neurotransmitter pathways and acting as a mitochondrial toxin when its production increases dramatically following bacterial fermentation due to the increase in Clostridium spp. Indeed, the combination of a considerable amount of PA within the intestinal lumen and impairment of the intestinal mucosa permeability leads to the passage of this organic acid into the bloodstream and its immediate accumulation in the central nervous system (CNS). The most dangerous effects are: (a) PA interfering in the biosynthesis of neurotransmitters; (b) by closing gap junctions, PA hampers the passage of small molecules and ions between cells, which is vital for synchronizing neural electrical activity and crucial in early brain development. Moreover, a decrease in gap-junction coupling may also inhibit cortical pruning, a phenomenon consistent with the increased density of neurons found in ASD patients; (c) abnormal amounts of PA enter the TCA cycle, causing a shift in the cycle and thus leading to less NADH production with a deficiency of energy carriers, and to the blockage of fatty acid oxidation. Interestingly, in the urine of ASD children, we found an increase in citric acid and aconitate combined with a decrease in succinic acid [28, 29], thus confirming the association between ASD and mitochondrial dysfunction, which has been well documented in children with ASD [30, 31]. Recently, the strategic role of mitochondria has been revealed by the discovery of their transit through nanotubules that connect cells one to another, thus operating as primary messengers for inter-cellular communication [32]. Although the road ahead is still long, especially in knowledge translation from bench to bedside, it appears unequivocal that the challenge of precision medicine in autism is mainly based on metabolomics: for example, neither genomics nor proteomics can assess changes in PA concentration and its dangerous effects on several human cellular pathways. Metabolomics can lead to the discovery of dozens of biomarkers strongly implicated in the pathogenesis of ASD (i.e. mannitol, L-threonic acid, fucose, glycine, serine, and many others), as recently confirmed by new preliminary results.
and can help in reducing time lags in translational medicine even by facilitating new patent applications for using metabolomics methods and reagents easily adaptable to routine clinical practice [34]. Nevertheless, the metabolomics approach to the study of ASD cannot be dissociated from the investigation of gut microbiota, especially in the perinatal age, with the aim of adjusting in a very short time any bacterial dysbiosis by therapeutic interventions [35]. Interestingly, new insights are emerging on the role of intestinal tract yeast colonization [36].

Encouraging perspectives in reducing non-genetic risk factors for autism consist of a close monitoring of maternal lifestyle and changes in the placental and fetal microbiota during pregnancy. A predominant role in determining the prenatal risk of ASD is interpreted by maternal drug ingestion/administration. Besides the well-known deleterious effect of thalidomide [37], prolonged assumption of antibiotics during pregnancy can significantly alter the homeostatic maintenance of a balanced intestinal bacterial flora, thus leading to dysbiosis [38]. The (ab)use of antidepressants drugs during the preconception period and over gestation is also highly dangerous owing to the harmful effect of selective serotonin reuptake inhibitors [39, 40]. Both human and animal epidemiological studies have demonstrated that valproic acid consumption during pregnancy is associated with ASD [41, 42]. Finally, the potential toxicity of acetaminophen (paracetamol), a very common analgesic and antipyretic drug widely used during pregnancy, should be carefully considered [43]. This drug easily crosses the placental barrier and induces neurodevelopmental impairment by interfering with fetal hormones and signaling pathways. Even worse, the exposure to acetaminophen after birth and in early childhood may be considered a potential risk factor for the development of ASD, as recently described [44]. While we should move with caution before definitively establishing that a drug or an environmental product can be considered a risk factor for ASD, we cannot rest on our laurels. We must promote further research on ASD based on the system biology approach. Putting ourselves in the shoes of parents who have just been told that their child has autism, we cannot say to them “unfortunately your problem is very common, your family belongs to around 16.8 per 1,000 families with an autistic child” [45].

Please, let’s roll up our sleeves and build a multidisciplinary network of scientists and physicians in search of tools that offer new, favorable perspectives to parents of autistic children, thus reducing the strong negative social impact of autism.

Declaration of interest

The Authors declare that there is no conflict of interest.

References


