www.jpnim.com Open Access eISSN: 2281-0692
Journal of Pediatric and Neonatal Individualized Medicine 2018;7(2):e070205
doi: 10.7363/070205
Received: 2018 May 02; accepted: 2018 May 10; published online: 2018 May 14

Editorial

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

Vassilios Fanos[1], Antonio Noto[2], Michele Mussap[3]

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

"We are such stuff as dreams are made on"
William Shakespeare*, The Tempest, Act IV, Scene 1*

Keywords

Autism spectrum disorder, autism, metabolomics, microbiomics, acetaminophen, systems biology.

Corresponding author

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

How to cite

Fanos V, Noto A, Mussap M. The juniper bush of autism spectrum disorder (ASD): metabolomics, microbiomics, acetaminophen. What else? J Pediatr Neonat Individual Med. 2018;7(2):e070205. doi: 10.7363/070205.

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 (^{1}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 ^{1}H 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 microflora 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. siraeum*, and *Bifidobacterium spp.* As a result, in these children the urine metabolome is marked by alterations in hippuric acid, p-hydroxyphenyl-acetic acid and 3-(3-hydroxyphenyl)-3-hydroxy-propanoic 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

[33] 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 antidepressant 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

1. Kanner L. Autistic disturbances of affective contact. Nerv Child. 1943;2:217-50.
2. American Psychiatric Association, American Psychiatric Association DSM-5 Task Force. Diagnostic and Statistical Manual of Mental Disorders: DSM-5. 5th ed. Washington, DC: American Psychiatric Publishing, 2013.
3. Harris J. Leo Kanner and autism: a 75-year perspective. Int Rev Psychiatry. 2018;30(1):3-17.
4. Baxter AJ, Brugha TS, Erskine HE, Scheurer RW, Vos T, Scott JG. The epidemiology and global burden of autism spectrum disorders. Psychol Med. 2015;45:601-13.
5. Lyall K, Croen L, Daniels J, Fallin DM, Ladd-Acosta C, Lee BK, Park BY, Snyder NW, Schendel D, Volk H, Windham GC, Newschaffer C. The Changing Epidemiology of Autism Spectrum Disorders. Annu Rev Public Health. 2017;38:81-102.
6. Baio J, Wiggins L, Christensen DL, Maenner MJ, Daniels J, Warren Z, Kurzius-Spencer M, Zahorodny W, Robinson Rosenberg C, White T, Durkin MS, Imm P, Nikolaou L, Yeargin-Allsopp M, Lee LC, Harrington R, Lopez M, Fitzgerald RT, Hewitt A, Pettygrove S, Constantino JN, Vehorn A, Shenouda J, Hall-Lande J, Van Naarden Braun K, Dowling NF. Prevalence of Autism Spectrum Disorder Among Children Aged 8 Years – Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2014. MMWR Surveill Summ. 2018;67(6):1-23.
7. Lai MC, Lombardo MV, Baron-Cohen S. Autism. Lancet. 2014;383(9920):896-910.
8. Chen JA, Peñagarikano O, Belgard TG, Swarup V, Geschwind DH. The emerging picture of autism spectrum disorder: genetics and pathology. Annu Rev Pathol. 2015;10:111-44.
9. Jiang YH, Yuen RK, Jin X, Wang M, Chen N, Wu X, Ju J, Mei J, Shi Y, He M, Wang G, Liang J, Wang Z, Cao D, Carter MT, Chrysler C, Drmic IE, Howe JL, Lau L, Marshall CR, Merico D, Nalpathamkalam T, Thiruvahindrapuram B, Thompson A, Uddin M, Walker S, Luo J, Anagnostou E, Zwaigenbaum L, Ring RH, Wang J, Lajonchere C, Wang J, Shih A, Szatmari P, Yang H, Dawson G, Li Y, Scherer SW. Detection of clinically relevant genetic variants in autism spectrum disorder by whole-genome sequencing. Am J Hum Genet. 2013;93(2):249-63.
10. Brandler WM, Antaki D, Gujral M, Noor A, Rosanio G, Chapman TR, Barrera DJ, Lin GN, Malhotra D, Watts AC, Wong LC,

Estabillo JA, Gadomski TE, Hong O, Fajardo KV, Bhandari A, Owen R, Baughn M, Yuan J, Solomon T, Moyzis AG, Maile MS, Sanders SJ, Reiner GE, Vaux KK, Strom CM, Zhang K, Muotri AR, Akshoomoff N, Leal SM, Pierce K, Courchesne E, Iakoucheva LM, Corsello C, Sebat J. Frequency and Complexity of De Novo Mutation in Autism. Am J Hum Genet. 2016;98(4): 667-79.

11. Nygren G, Sandberg E, Gillstedt F, Ekeroth G, Arvidsson T, Gillberg C. A new screening programme for autism in a general population of Swedish toddlers. Res Dev Disabil. 2012;33(4):1200-10.
12. Elder JH, Kreider CM, Brasher SN, Ansell M. Clinical impact of early diagnosis of autism on the prognosis and parent-child relationships. Psychol Res Behav Manag. 2017;10:283-92.
13. Hazlett HC, Gu H, Munsell BC, Kim SH, Styner M, Wolff JJ, Elison JT, Swanson MR, Zhu H, Botteron KN, Collins DL, Constantino JN, Dager SR, Estes AM, Evans AC, Fonov VS, Gerig G, Kostopoulos P, McKinstry RC, Pandey J, Paterson S, Pruett JR, Schultz RT, Shaw DW, Zwaigenbaum L, Piven J; IBIS Network; Clinical Sites; Data Coordinating Center; Image Processing Core; Statistical Analysis. Early brain development in infants at high risk for autism spectrum disorder. Nature. 2017;542(7641):348-51.
14. Bjørklund G, Kern JK, Urbina MA, Saad K, El-Houfey AA, Geier DA, Chirumbolo S, Geier MR Mehta JA, Aaseth J. Cerebral hypoperfusion in autism spectrum disorder. Acta Neurobiol Exp. 2018;78:21-9.
15. Zamboni N, Saghatelian A, Patti GJ. Defining the Metabolome: Size, Flux, and Regulation. Mol Cell. 2015;58(4):699-706.
16. Zampieri M, Sekar K, Zamboni N, Sauer U. Frontiers of high-throughput metabolomics. Curr Opin Chem Biol. 2017;36: 15-23.
17. Faa G, Manchia M, Pintus R, Gerosa C, Marcialis MA, Fanos V. Fetal programming of neuropsychiatric disorders. Birth Defects Res C Embryo Today. 2016;108(3):207-23.
18. Fanni D, Gerosa C, Rais M, Ravarino A, Van Eyken P, Fanos V, Faa G. The role of neuropathological markers in the interpretation of neuropsychiatric disorders: Focus on fetal and perinatal programming. Neurosci Lett. 2018;669:75-82.
19. Borre YE, O'Keeffe GW, Clarke G, Stanton C, Dinan TG, Cryan JF. Microbiota and neurodevelopmental windows: implications for brain disorders. Trends Molec Med. 2014;20(9):509-18.
20. Manchia M, Fanos V. Targeting aggression in severe mental illness: The predictive role of genetic, epigenetic, and metabolomic markers. Prog Neuropsychopharmacol Biol Psychiatry. 2017;77:32-41.
21. Hagenbeek FA, Kluft C, Hankemeier T, Bartels M, Draisma HH, Middeldorp CM, Berger R, Noto A, Lussu M, Pool R, Fanos V, Boomsma DI. Discovery of biochemical biomarkers for aggression: A role for metabolomics in psychiatry. Am J Med Genet B Neuropsychiatr Genet. 2016;171(5):719-32.
22. Rajula HSR, Mauri M, Fanos V. Scale-free networks in metabolomics. Bioinformation. 2018;14(3):140-4.
23. Mussap M, Noto A, Fanos V. Metabolomics of autism spectrum disorders: early insights regarding mammalian-microbial cometabolites. Expert Rev Mol Diagn. 2016;16(8):869-81.
24. Gevi F, Zolla L, Gabriele S, Persico AM. Urinary metabolomics of young Italian autistic children supports abnormal tryptophan and purine metabolism. Mol Autism. 2016;7:47.
25. Bitar T, Mavel S, Emond P, Nadal-Desbarats L, Lefèvre A, Mattar H, Soufia M, Blasco H, Vourc'h P, Hleihel W, Andres CR. Identification of metabolic pathway disturbances using multimodal metabolomics in autistic disorders in a Middle Eastern population. J Pharm Biomed Anal. 2018;152:57-65.
26. Noto A, Fanos V, Barberini L, Grapov D, Fattuoni C, Zaffanello M, Casanova A, Fenu G, De Giacomo A, De Angelis M, Moretti C, Papoff P, Ditonno R, Francavilla R. The urinary metabolomics profile of an Italian autistic child population and their unaffected siblings. J Matern Fetal Neonatal Med. 2014;27(Suppl 2):46-52.
27. Lussu M, Noto A, Masili A, Rinaldi AC, Dessì A, De Angelis M, De Giacomo A, Fanos V, Atzori L, Francavilla R. The urinary ^{1}H-NMR metabolomics profile of an Italian autistic children population and their unaffected siblings. Autism Res. 2017;10(6):1058-66.
28. Frye RE, Rose S, Slattery J, MacFabe DF. Gastrointestinal dysfunction in autism spectrum disorder: the role of the mitochondria and the enteric microbiome. Microb Ecol Health Dis. 2015;26:27458.
29. Siddiqui MF, Elwell C, Johnson MH. Mitochondrial Dysfunction in Autism Spectrum Disorders. Autism Open Access 2016;6(5): 1000190.
30. Morris G, Berk M. The many roads to mitochondrial dysfunction in neuroimmune and neuropsychiatric disorders. BMC Med. 2015;13:68.
31. Griffiths KK, Levy RJ. Evidence of Mitochondrial Dysfunction in Autism: Biochemical Links, Genetic-Based Associations, and Non-Energy-Related Mechanisms. Oxid Med Cell Longev. 2017;2017:431402.
32. Vignais ML, Caicedo A, Brondello JM, Jorgensen C. Cell Connections by Tunneling Nanotubes: Effects of Mitochondrial Trafficking on Target Cell Metabolism, Homeostasis, and Response to Therapy. Stem Cells Int. 2017;2017:6917941.
33. Curatolo P, Noto A, Siracusano M, Riccioni A, Mazzone L, Barberini L, Fanos V, Fattuoni C. Autism from conventional diagnosis to metabolomics profile in a population of children with autism spectrum disorders. Selected lectures 13th International Workshop on Neonatology. Lect 27. J Pediatr Neonat Individ Med. 2017;6(2):e060235.
34. Mussap M, Moretti C, Noto A, Fanos V. Metabolomics and autism. Selected lectures 12th International Workshop on Neonatology. Lect 62. J Pediatr Neonat Individ Med. 2016;5(2):e050246.
35. Kim N, Yun M, Oh YJ, Choi HJ. Mind-altering with the gut: Modulation of the gut-brain axis with probiotics. J Microbiol. 2018;56(3):172-82.
36. Iovene MR, Bombace F, Maresca R, Sapone A, Iardino P, Picardi A, Marotta R, Schiraldi C, Siniscalco D, Serra N, de Magistris

L, Bravaccio C. Intestinal Dysbiosis and Yeast Isolation in Stool of Subjects with Autism Spectrum Disorders. Mycopathologia. 2017;182(3-4):349-63.

37. Stromland K, Nordin V, Miller M, Akerström B, Gillberg C. Autism in thalidomide embryopathy: a population study. Dev Med Child Neurol. 1994;36(4):351-6.
38. Faa G, Gerosa C, Fanni D, Nemolato S, van Eyken P, Fanos V. Factors influencing the development of a personal tailored microbiota in the neonate, with particular emphasis on antibiotic therapy. J Matern Fetal Neonatal Med. 2013;26(Suppl 2): 35-43.
39. Boukhris T, Sheehy O, Mottron L, Bérard A. Antidepressant Use During Pregnancy and the Risk of Autism Spectrum Disorder in Children. JAMA Pediatr. 2016;170(2):117-24.
40. Mezzacappa A, Lasica PA, Gianfagna F, Cazas O, Hardy P, Falissard B, Sutter-Dallay AL, Gressier F. Risk for Autism Spectrum Disorders According to Period of Prenatal Antidepressant Exposure. A Systematic Review and Meta-analysis. JAMA Pediatr. 2017;171(6):555-63.
41. Christensen J, Grønborg TK, Sørensen MJ, Schendel D, Parner ET, Pedersen LH, Vestergaard M. Prenatal valproate exposure and risk of autism spectrum disorders and childhood autism. JAMA. 2013;309:1696-703.
42. Nicolini C, Fahnestock M. The valproic acid-induced rodent model of autism. Exp Neurol. 2018;299(Pt A):217-27.
43. Masarwa R, Levine H, Gorelik E, Reif S, Perlman A, Matok I. Prenatal Exposure to Acetaminophen and Risk for Attention Deficit Hyperactivity Disorder and Autistic Spectrum Disorder: A Systematic Review, Meta-Analysis, an Meta-Regression Analysis of Cohort Studies. Am J Epidemiol. 2018 Apr 24. [Epub ahead of print].
44. Parker W, Hornik CD, Bilbo S, Holzknecht ZE, Gentry L, Rao R, Lin SS, Herbert MR, Nevison CD. The role of oxidative stress, inflammation and acetaminophen exposure from birth to early childhood in the induction of autism. J Int Med Res. 2017;45(2):407-38.
45. [No author listed]. Progress in the USA for autistic spectrum disorder. Lancet. 2018;391:1750.