Ochratoxin A as possible factor trigging autism and its male prevalence via epigenetic mechanism

A. Mezzelani¹, M. E. Raggi², A. Marabotti²-³, L. Milanesi¹

¹Institute of Biomedical Technologies, National Research Council, Via Fratelli Cervi 93, 20090 Segrate, MI, Italy, ²Scientific Institute, IRCCS Eugenio Medea, Bosisio Parini, Lecco, Italy, ³Department of Chemistry and Biology, University of Salerno, Via Giovanni Paolo II 132, 84084 Fisciano, SA, Italy

The role of dysbiosis causing leaky gut with xenobiotic production and absorption is increasingly demonstrated in autism spectrum disorder (ASD) pathogenesis. Among xenobiotics, we focused on ochratoxin A (one of the major food contaminating mycotoxin), that in vitro and in vivo exerts a male-specific neurotoxicity probably via microRNA modulation of a specific target gene. Among possible targets, we focused on neuroligin4X. Interestingly, this gene carries some single nucleotide polymorphisms (SNPs) already correlated with the disease and with illegitimate microRNA binding sites and, being located on X-chromosome, could explain the male prevalence. In conclusion, we propose a possible gene–environment interaction triggering ASD explaining the epigenetic neurotoxic mechanism activated by ochratoxin A in genetically predisposed children. This mechanism offers a clue for male prevalence of the disease and may have an important impact on prevention and cure of ASD.

Keywords: Autism, Male-prevalence, Environment, Microbiota, Epigenetics, Ochratoxin A

This is a revised and up-to-date hypothesis of our previous proposal in which we discuss a possible causative role of dysbiosis, with neurotoxic mycotoxin production and/or adsorption, in the pathogenesis of autism spectrum disorder (ASD)¹. With reference to more recent articles, we not only confirm our previous hypothesis but try to explain the possible epigenetic mechanism that trigger the disease.

ASD is an increasing neurodevelopmental disorder with a broader phenotype, appearing by 3 years of age that often shows co-morbid situations, such as mental retardation, epilepsy, and recurrent gastrointestinal abnormalities. Moreover, male prevalence is one of the most known characteristics of autism and in an inexplicit manner may hold the key for understanding the cause of its onset.

Genetics play a relevant role in etiology of ASD and hundreds of genes have been correlated to the disease, but only ~30% of autistic patients carry a causative genetic variation. Epigenetic factors have also been investigated and dysregulation of gene expression and microRNAs (short non-coding RNA ~22 nt that negatively regulate the expression of target genes binding to their 3'UTR region) have been related to autism.² MicroRNAs are constantly involved in differentiation, development, and proper function of tissues and organs with a complex spatio-temporal expression.

Dysregulated microRNAs were detected in a plethora of diseases and microRNA signatures can define specific pathological conditions, progression evaluation, and therapeutic response. Recently, microRNAs have been found within body fluids (serum, plasma, saliva, urine, and milk), becoming reliable and non-invasive diagnostic and prognostic biomarkers for many diseases,³ including ASD.⁴

As in other diseases, in which an interplay between a specific gene and a specific environment factor (the so called 'gene × environment' interplay) triggers the disease, there are known proposals where the cause of ASD in genetically susceptible subjects could be due to pathogens or xenobiotics (heavy metals, pesticides, neurotoxins, and drugs). Along these lines, we focused on a specific gene and on a specific environmental factor as co-responsible for idiopathic autism pathogenesis.

Considering the male prevalence, we explored the autism-related genes located on the X-chromosome excluding those related to other X-linked pathologies with autistic traits, such as Rett syndrome and Fragile-X syndrome. Among these genes, the most
appealing was neuroligin4X (NLGN4X), a cell adhesion postsynaptic protein that plays a crucial role in the formation and maintenance of glutamnergic synaptic transmission. Genetic alterations of this gene (missense mutations, exon deletions, and splice variants) were found in ~1% of autistic subjects, meaning that a mutation in NLGN4X is sufficient to lead to autistic phenotype. Moreover, SNPs in NLGN4X 3′UTR that create binding sites for illegitimate microRNAs have already been related to a non-specific mental retardation in the Qinba Mountains Region of China. A similar association has been found in an ASD Italian cohort.

From an environmental point of view, dysbiosis represents one of the most interesting and emerging environmental causes that could lead to leaky gut and to xenobiotics production and absorption. Recently, attention has been directed to neurotoxin-producing bacteria and mycotoxin-producing molds that can infect the intestinal tract and/or contaminate food, especially cereals. Indeed most of these may cause leaky gut, exert important immunosuppressive activity, and produce neurotoxins.

Among the possible microbial toxins, we focused on ochratoxin A (OTA). OTA has a nephrotoxic, hepatotoxic, immunotoxic, and genotoxic effect and induces carcinogenicity, teratogenicity, and mutagenicity. Moreover, OTA is produced by strains of Aspergillus and Penicillium that can overgrow in case of gut dysbiosis and are also the major food contaminating molds. Interestingly, in literature, we found that, in mice models, this molecule caused a male-dependent neurological damage, with neural tube defects, probably due to the dysregulation of several gene expression including the up-regulation of SOX9. This gene is involved in the development of the male phenotype and, interestingly, up-regulation of SOX9 was detected in autistic cases.

In order to clarify the molecular mechanisms of OTA toxicity, some authors also investigated the role of OTA on microRNA expression. Recent articles reported that in vitro and in vivo experiments, this toxin dysregulates a list of microRNAs some of which are dysregulated in autism and psychiatric diseases, such as mir-132 and mir-29, or bind to the wild type NLGN4X target such as mir-200c, as predicted by the bioinformatics tool ‘microrna’ (http://www.microrna.org). OTA also regulates the expression of the DiGeorge syndrome critical region 8 gene (DGCR8), located on 22q11.2 chromosome (Fig. 1). This gene, together with DROSHA Class 2 RNase III enzyme, forms the microprocessor complex DROSHA/DGCR8 that plays a key role in the biogenesis of microRNAs. In humans, decreased expression of DGCR8 gene leads to severe neurodevelopmental disorders and can be due to: 22q11.2 Deletion Syndrome (also known as DiGeorge Syndrome or Velocardiofacial syndrome), causing wide clinical manifestations with neurological dysfunctions such as learning disabilities and behavioral disorders or to dysregulation of methyl CpG binding protein 2 (MeCP2) gene, which directly binds to DGCR8, as happens in Rett syndrome and ASD.

Our hypothesis is that NLGN4X 3′UTR SNPs create binding sites for illegitimate microRNAs, thus causing the genetic susceptibility to ASD. On the other hand, illegitimate microRNAs, up-regulated by OTA effect, could inhibit NLGN4X expression by binding to NLGN4X mRNA. This could lead to synaptic plasticity impairment and to autistic phenotype. Moreover, OTA effect should be more common in males, as they carry only one copy of NLGN4X, in comparison to heterozygous females (Fig. 2), thus explaining the male prevalence of ASD.

In conclusion, SNPs in NLGN4X 3′UTR and illegitimate microRNA inducing OTA could be a possible biological mechanism reflecting the gene × environment interaction for those patients without causative mutations and suffering from dysbiosis and leaky gut.

Seek of further analyses is necessary to demonstrate this hypothesis and understand the effects of OTA and

| GENE | ENVIRONMENT |
|------|-------------|
| genes located on X-chromosome: | neurotoxic mycotoxins: |
| neuroligin4X (NLGN4X) |  
| - is involved in synaptic plasticity | - is produced by strains that can over-growth in case of gut dysbiosis |
| - is mutated in ~1% of autistic patients | - is one of major food contaminating mycotoxin |
| - can have some 3′UTR SNPs that create binding sites for illegitimate microRNAs | - causes epato, nephro and neurotoxicity |
| | - exerts a male-specific neurotoxicity |
| | - dys-regulates microRNAs and microRNA targetgene expression some of which are involved in autism (including DGCR8) |

Figure 1 Roles of neuroligin4X and ochratoxin A.
other toxins on the general population in order to establish the risk assessment and the daily tolerable intake. Therefore, we recommend to pay further attention to variations in regulating sequences, such as splitting sites or promoter and 3′UTR regions as well to epigenetic factors such as microRNAs. Indeed, genetic variations could predispose to a harmful microRNA cross talk between a specific genotype and a specific environmental agent in regulating gene expression.

On the other hand, the sex-dependent activity of candidate environmental factors, including their sex-specific toxicokinetics and toxicodynamics, should be investigated to discover new possible targets that result useful in understanding the gene × environment interplay.

The discovery of genetic and environmental biomarkers could aid the development of diagnostic tests allowing to design prevention plans and personalized interventions.

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Disclaimer statements

Contributors

A. Mezzelani searched and interpreted the references, designed the hypothesis and wrote the letter. A. Marabotti, MER and LM assisted in discussing the hypothesis and in writing the manuscript.

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Conflicts of interest

None.

Ethics approval

None.

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