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

A. Mezzelani[1], M. E. Raggi[2], A. Marabotti[2,3], L. Milanesi[1]

[1]Institute of Biomedical Technologies, National Research Council, Via Fratelli Cervi 93, 20090 Segrate, MI, Italy, [2]Scientific Institute, IRCCS Eugenio Medea, Bosisio Parini, Lecco, Italy, [3]Department 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 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

Correspondence to: A Mezzelani, Institute of Biomedical Technologies, National Research Council, Via Fratelli Cervi 93, 20090 Segrate, MI, Italy. Email: alessandra.mezzelani@itb.cnr.it

DOI 10.1179/1476830515Z.000000000186

appealing was neuroligin4X (NLGN4X), a cell adhesion postsynaptic protein that plays a crucial role in the formation and maintenance of glutaminergic synaptic transmission. Genetic alterations of this gene (missense mutations, exon deletions, and splice variants) were found in ~1% of autistic subjects,[5] 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.[6] A similar association has been found in an ASD Italian cohort.[7]

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[8] and mycotoxin-producing molds[9] 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.[10] This gene is involved in the development of the male phenotype and, interestingly, up-regulation of SOX9 was detected in autistic cases.[11]

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[12–14] some of which are dysregulated in autism and psychiatric diseases, such as mir-132 and mir-29,[15] 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 dysregulates the expression of the DiGeorge syndrome critical region 8 gene (DGCR8), located on 22q11.2 chromosome[12,14,16] (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[16] or to dysregulation of methyl CpG binding protein 2 (MeCP2) gene, which directly binds to DGCR8, as happens in Rett syndrome and ASD.[17]

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[18] and suffering from dysbiosis and leaky gut.[4]

GENE	X	ENVIRONMENT
genes located on X-chromosome: ↓ **neuroligin4X (NLGN4X)** • is involved in synaptic plasticity • is mutated in ~1% of autistic patients • can have some 3'UTR SNPs that create binding sites for illegitimate microRNAs	X	neurotoxic mycotoxins: ↓ **ochratoxinA (OTA)** • is produced by strains that can over-growth in case of gut dysbiosis • is one of major food contaminating mycotoxin • causes epato, nefro 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.

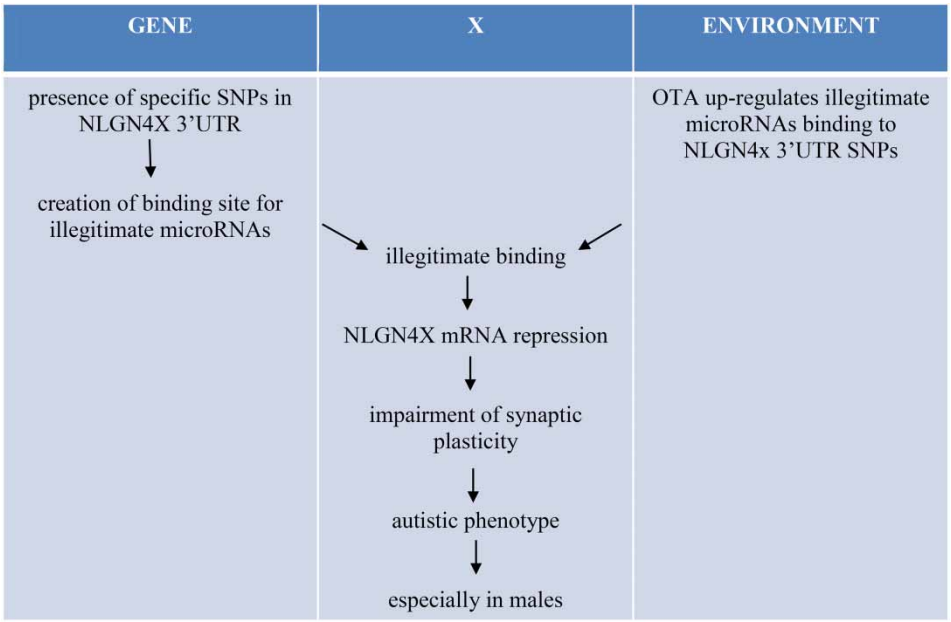

Figure 2 OTA up-regulates illegitimate microRNAs that bind to NLGN4X 3′UTR carrying specific SNPs. This could cause the suppression of NLGN4X mRNA leading to impairment of synaptic plasticity and to autistic phenotype especially in males.

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

Acknowledgments

We thank John Hatton of Institute of Biomedical Technologies (CNR-ITB) for proofreading the manuscript, Italian Ministry of Health 'GR-2009-1570296' project, Italian Ministry of Education, University, and Research 'InterOmics' Flagship project.

Disclaimer statements

Contributors None.

Funding None.

Conflicts of interest None.

Ethics approval None.

References

1 Mezzelani A, Landini M, Facchiano F, Raggi ME, Villa L, Molteni M, *et al.*. Environment, dysbiosis, immunity and sex-specific susceptibility: a translational hypothesis for regressive autism pathogenesis. Nutr Neurosci [Epub ahead of print]. PMID: 2014, 24621061.

2 Sarachana T, Zhou R, Chen G, Manji HK, Hu VW. Investigation of post-transcriptional gene regulatory networks associated with autism spectrum disorders by microRNA expression profiling of lymphoblastoid cell lines. Genome Med 2010;2(4):23.

3 Chevillet JR, Lee I, Briggs HA, He Y, Wang K. Issues and prospects of microRNA-based biomarkers in blood and other body fluids. Molecules 2014;19(5):6080–105.

4 Mundalil Vasu M, Anitha A, Thanseem I, Suzuki K, Yamada K, Takahashi T, *et al.*. Serum microRNA profiles in children with autism. Mol Autism 2014;5:40. DOI 10.1186/2040-2392-5-40.

5 Li X, Zou H, Brown WT. Genes associated with autism spectrum disorder. Brain Res Bull 2012;88(6):543–52. DOI 10.1016/j.brainresbull.2012.05.017.

6 Qi H, Xing L, Zhang K, Gao X, Zheng Z, Huang S, *et al.*. Positive association of neuroligin-4 gene with nonspecific mental retardation in the Qinba Mountains Region of China. Psychiatr Genet 2009;19(1):1–5. DOI 10.1097/YPG.0b013e3283088e54.

7 Landini M, Raggi ME, Merelli I, Galluccio N, Ciceri F, Villa L, *et al.*. A significant association of non coding variants in neuroligin3 and 4X genes with autism spectrum disorder in an Italian cohort. Submitted to Eur J Hum Genet.

8 Bolte ER. Autism and *Clostridium tetani*. Med Hypotheses 1998;(51):133–44.

9 Proietti M, Del Buono A, Pagliaro G, Del Buono R, Di Rienzo C. The intestinal permeability syndrome, celiac disease, gluten sensitivity, autistic spectrum, mycotoxins and immunological tolerance. Mediterr J Nutr Metab 2013;6:99–104. DOI 10.1007/s12349-013-0125-3.

10 Ueta E, Kodama M, Sumino Y, Kurome M, Ohta K, Katagiri R, *et al.*. Gender-dependent differences in the incidence of ochratoxin A-induced neural tube defects in the Pdn/Pdn mouse. Congenit Anom (Kyoto) 2010;(50):29–39.

11 Ghahramani Seno MM, Hu P, Gwadry FG, Pinto D, Marshall CR, Casallo G, *et al.* Gene and miRNA expression profiles in autism spectrum disorders. Brain Res 2011;1380:85–97. DOI 10.1016/j.brainres.2010.09.046.

12 Stachurska A, Ciesla M, Kozakowska M, Wolffram S, Boesch-Saadatmandi C, Rimbach G, *et al.*. Cross-talk between microRNAs, nuclear factor E2-related factor 2, and heme oxygenase-1 in ochratoxin A-induced toxic effects in renal proximal tubular epithelial cells. Mol Nutr Food Res 2013;57(3):504–15. DOI 10.1002/mnfr.201200456.
13 Dai Q, Zhao J, Qi X, Xu W, He X, Guo M, *et al.*. MicroRNA profiling of rats with ochratoxin A nephrotoxicity. BMC Genomics 2014;15:333. DOI 10.1186/1471-2164-15-333.
14 Qi X, Yang X, Chen S, He X, Dweep H, Guo M, *et al.*. Ochratoxin A induced early hepatotoxicity: new mechanistic insights from microRNA, mRNA and proteomic profiling studies. Sci Rep Article number 2014;5163(4). DOI 10.1038/srep05163.
15 Sarachana T, Zhou R, Chen G, Manji HK, Hu VW. Investigation of post-transcriptional gene regulatory networks associated with autism spectrum disorders by microRNA expression profiling of lymphoblastoid cell lines. Genome Med 2010;2(4):23. DOI 10.1186/gm144.
16 Sellier C, Hwang VJ, Dandekar R, Durbin-Johnson B, Charlet-Berguerand N, Ander BP, *et al.*. Decreased DGCR8 expression and miRNA dysregulation in individuals with 22q11.2 deletion syndrome. PLoS One 2014;9(8):e103884. DOI 10.1371/journal.pone.0103884.
17 Cheng TL, Wang Z, Liao Q, Zhu Y, Zhou WH, Xu W, *et al.*. MeCP2 suppresses nuclear microRNA processing and dendritic growth by regulating the DGCR8/Drosha complex. Dev Cell 2014;28(5):547–60. DOI 10.1016/j.devcel.2014.01.032.
18 Tordjman S, Somogyi E, Coulon N, Kermarrec S, Cohen D, Bronsard G, *et al.*. Gene × environment interactions in autism spectrum disorders: role of epigenetic mechanisms. Front Psychiatry 2014;5:53. DOI 10.3389/fpsyt.2014.00053.