Abstract
Introduction
Clinical interventions against autism spectrum disorder are currently limited and also not very rewarding. This paper is based on a serendipitous finding that valproate, that is known to cause autism-like symptoms in children with foetal valproate syndrome, increases the binding of GATA transcription factor with its promoter.

Additional study revealed that valproate causes the nuclear translocation of GATA3 transcription factor in neuron-like PC12 cells.

Hypothesis
Increased GATA3 activity may cause excessive differentiation of naïve T helper cells and the neuronal precursors causing immune deregulation and brain dysfunction in subjects with autism.

Conclusion
GATA3 is involved in the differentiation of several cell types including naïve T cells into Th2 type. GATA3 is required for the differentiation of serotoninergic, dopaminergic and noradrenergic neurons.

Therefore, heightened GATA3 may cause excessive differentiation of naïve Th cells and the above mentioned neurons in subjects with autism, causing symptoms that are consistent with the immune dysfunctions and aberrant functions of these neurons.

Introduction
Autism is a neurodevelopmental disorder that manifests as a wide spectrum of behavioural abnormalities (restricted and repetitive), problems with communication (verbal, non-verbal and social), and in some cases mental retardation and epilepsy. More males are diagnosed with autism than female. The precise cause of autism is not known. The current notion is that there is no single cause of autism.

Genetic studies point to autism-sensitive loci in all human chromosomes, and it is now considered that multiple loci in concert may increase the genetic susceptibility to autism spectrum disorder (ASD). The rapid rise in autism cases in recent years and the lack of conclusive evidence of genetic studies, however, advocate the conviction that maternal exposure to environmental factors during gestation and/or early life of subject may cause autism. The list of environmental triggers that may cause autism include valproic acid (VPA), thalidomide, alcohol, congenital infection with cytomegalovirus, rubella, MMR vaccination and ultrasound. Nevertheless, the fact that widespread environmental factors such as viral infection or immune challenge do not cause autism in all cases, a genetic predisposition for the environmental trigger in the aetiology of autism is likely (Figure 1). Indeed, a recent study with rodents showed that the maternal genotype determines the effects of prenatal immune challenge on the severity of autism-like behaviour in the offspring.

The autism-like behavioural phenotypes in rodent models caused by pre- or post-natal VPA exposure to environmental triggers are providing valuable information in unraveling the mysteries of autism. Of several rodent models of autism-like behaviours, the pre- and post-natal VPA exposure model of autism is promising, because children exposed to VPA in foetal life develop foetal valproate syndrome with symptoms similar to autism, including deficits in language and communication, the appearance of stereotypic behaviour, hyperexcitability and global delays in behavioural development. Prenatal exposure to VPA in rodents causes autism-like behavioural phenotypes, reduces the number of Purkinje cells, a common feature in autistic subjects, and also shows improvements with the enrichment of behaviour that is witnessed with behavioural intervention in children diagnosed with autism. Moreover, the postnatal exposure to VPA in rats causes behavioural problems similar to autistic regression.

VPA inhibits cell proliferation and migration, accelerates differentiation and changes expression of genes and adhesion molecules. A multiplex transcription factor binding assay with PC12 cells in culture revealed that GATA-binding transcription factors may be the most sensitive of the total 50 different transcription factors assayed simultaneously. Of six GATA isoforms that bind to a common consensus DNA sequence WGATAR (W=A or T and R=A or G), only GATA2 and GATA3 are expressed in the brain with the latter functioning downstream of the former. Additional experiments showed that VPA increased the DNA-binding activity of GATA3 by increasing its nuclear levels in PC12 cells. VPA also increases GATA3 mRNA in the foetal brain of C57BL/6J mice (unpublished observation). GATA3 induces differentiation of several cell types. It is expressed in neurons of foetal brain and peripheral nervous system, suggesting that the
prenatal VPA or VPA-induced GATA3 activity may cause excessive differentiation of these neurons. VPA treatment accelerates neurogenesis of foetal cortical neurons (Figure 2) and alters the pattern of differentiation of neuron-like PC12 cells (Figure 3). This suggests that the prenatal VPA or VPA-induced GATA3 activity may alter the morphology (cytoskeleton) and cause excessive differentiation of neurons. The excessive differentiation of neurons may result in insufficient pruning and superfluous synaptogenesis.

**Hypothesis**

Increased GATA3 activity may cause excessive differentiation of naïve T helper cells and the neuronal precursors causing immune deregulation and brain dysfunction in subjects with autism.

**Evaluation of hypothesis**

The authors have referenced some of their own studies in this hypothesis. These referenced studies have been conducted in accordance with the Declaration of Helsinki (1964) and the protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed. All human subjects, in these referenced studies, gave informed consent to participate in these studies. GATA3 supports the differentiation of naïve CD+ T cells into Th2 type while inhibiting the differentiation of Th1 type in the blood21. Th1 cells produce interferon-gamma (IFN-γ), interleukin-2 (IL-2) and tumour necrosis factor-β (TNF-β) that are responsible for eradicating intracellular microorganisms (protozoa, bacteria or viruses), whereas Th2 cells express IL-4, IL-5 and IL-13 when immune cells recognize extracellular parasites such as helminthes22. Altered Th1 function is pathogenic in many autoimmune diseases, such as Type 1 diabetes mellitus, rheumatoid arthritis or inflammatory bowel disease, and upregulated Th2 function causes allergic inflammation and ulcerative colitis23. Th2 cytokines, IL-4 and IL-13 regulate the expression of IgE from B lymphocytes, while IL-5 regulates eosinophilic inflammation. Elevated IgE levels and eosinophil counts are hallmarks of worm infection and allergic asthma24. Eosinophils are a constitutive component of the columnar gastrointestinal tract and play a significant role in the allergic responses and parasitic infections. The eosinophilic gastrointestinal diseases (EGIDs) with eosinophilic inflammation of the intestine are strongly associated with food allergies (egg, milk and fish) and family histories of allergic diseases. While food and aeroallergens have crucial role in EGID, the interplay of genetic and environmental factors in the aetiology is now recognized, as several patients with EGID have an immediate family member with an EGID25,26. A rapid increase in EGID cases since the first report (1937) and the associated male predominance is intriguing in the context of autism, which is also known for its male predominance and gastrointestinal disorders27. Currently, studies linking gastrointestinal worms, gut eosinophilia and GATA3 expression in autism are lacking.

In children with autism, the numbers of CD4+ and CD8+ T cells that produce IFN-γ and IL-2 [Th1 and type 1 CD8(+) T cells, respectively] in the blood are significantly reduced, and the number of CD4+ and CD8+ T cells that produce IL-4 [Th2 and type 2 CD8(+) T cells, respectively] are elevated28. The elevated Th2 cell activity causes autoantibody production from B cells29. All these are consistent with higher GATA3 expression in the lungs of foetal (PN7 and PN15) brains, GATA3 mRNA is detected in the brains of the offspring of mice administered with VPA30 are caused by VPA-induced higher levels of GATA3. Neurons of rhombomere-4 that extend axons to the sensory epithelia of the ears in developing mice also express GATA3, and mice with compromised GATA3 activity display unusual axonal projections and problems with the semicircular canals30. Thus, heightened GATA3 activity during development of the ear may cause excessive differentiation of neurons involved in hearing or sound perception causing enhanced sensitivity to sounds in autistic individuals30. GATA3 regulates the transcriptional activity of tyrosine hydroxylase (TH), involved in serotonin biosynthesis20. While food and aeroallergens have crucial role in EGID, the interplay of genetic and environmental factors in the aetiology is now recognized, as several patients with EGID have an immediate family member with an EGID25,26. A rapid increase in EGID cases since the first report (1937) and the associated male predominance is intriguing in the context of autism, which is also known for its male predominance and gastrointestinal disorders27. Currently, studies linking gastrointestinal worms, gut eosinophilia and GATA3 expression in autism are lacking.

In children with autism, the numbers of CD4+ and CD8+ T cells that produce IFN-γ and IL-2 [Th1 and type 1 CD8(+) T cells, respectively] in the blood are significantly reduced, and the number of CD4+ and CD8+ T cells that produce IL-4 [Th2 and type 2 CD8(+) T cells, respectively] are elevated28. The elevated Th2 cell activity causes autoantibody production from B cells29. All these are consistent with higher GATA3 expression in the lungs of foetal (PN7 and PN15) brains, GATA3 mRNA is detected in the brains of the offspring of mice administered with VPA30 are caused by VPA-induced higher levels of GATA3. Neurons of rhombomere-4 that extend axons to the sensory epithelia of the ears in developing mice also express GATA3, and mice with compromised GATA3 activity display unusual axonal projections and problems with the semicircular canals30. Thus, heightened GATA3 activity during development of the ear may cause excessive differentiation of neurons involved in hearing or sound perception causing enhanced sensitivity to sounds in autistic individuals30. GATA3 regulates the transcriptional activity of tyrosine hydroxylase (TH), involved in serotonin biosynthesis. GATA3 is expressed in the pretectal regions, midbrain and most of the raphe nucleus30. GATA3 expression in the human brain is not known. The effects of prenatal VPA or immune challenge on the GATA3 expression in foetal or early postnatal brain in animal models are also not known.

In the dorsal raphe nucleus of PN15 animals, about 80% neurons that express GATA3 also express tryptophan hydroxylase 2 (an enzyme involved in serotonin biosynthesis)30. GATA3 regulates the development of serotonergic neurons in the caudate raphe nucleus and deletion of GATA3 alters the cytoarchitecture of serotonergic neurons57. It may be that the abnormal serotonergic neurons in the brains of the offspring of mice administered with VPA are caused by VPA-induced higher levels of GATA3. Neurons of rhombomere-4 that extend axons to the sensory epithelia of the ears in developing mice also express GATA3, and mice with compromised GATA3 activity display unusual axonal projections and problems with the semicircular canals30. Thus, heightened GATA3 activity during development of the ear may cause excessive differentiation of neurons involved in hearing or sound perception causing enhanced sensitivity to sounds in autistic individuals30. GATA3 regulates the transcriptional activity of tyrosine hydroxylase (TH), involved in serotonin biosynthesis20.
Gene-Environment Interactions and Autism

Figure 1: Schematics (cartoon) of current view on the gene–environment interaction in the aetiology of ASD. The environmental factors may act during prenatal and/or early postnatal period of the subjects diagnosed with ASD. The genetic predisposition may include both paternal and maternal genes. The intensity of environmental factors, genetic predisposition and the behavioural outcomes are shown by the shade of the horizontal bars representing these factors.

Figure 2: VPA changes the neuritogenesis of foetal cortical neurons in culture. Dissociated neuron preparations from E16.5 Long–Evans rats cultured for 24 h on laminin (10 µg/ml) coated wells (a). In some wells, VPA (200 µM) was added. At 24 h of incubation, images of untreated (control) and VPA-treated neurons were captured. (b) Neurite lengths per image were measured. Mean and standard error of means of neurite lengths of nine images each of control and treated neurons were plotted (c). Inset in (b) shows GATA3 specific band in three different nuclear extracts of foetal cortical neuron detected using purified GATA3 antibody (BD Pharmingen, San Jose, CA). Neuron isolation and neuritogenesis methodology are described earlier68. Arrows in (a) show neurites. *p < 0.05. Bar: 100µm.

Licensee OA Publishing London 2013. Creative Commons Attribution Licence (CC-BY)

Hypothesis

Competing interests: none declared. Conflict of Interests: none declared.

All authors contributed to the conception, design, and preparation of the manuscript, as well as read and approved the final manuscript.

All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.


GATA3 Multitasking

Figure 3: VPA changes the pattern of PC12 cell differentiation in culture. PC12 cells were cultured on laminin (10 µg/ml)-coated plates to examine the effects of VPA (200 µM). Panels show PC12 cells at 10 h and 7 days in culture, respectively, in the absence (panels a and c) or presence (panels b and d) of sodium valproate (100 µM). PC12 cell culture and VPA treatments were conducted as described earlier\textsuperscript{13}. Bar: 100 µm.

Figure 4: The cause and repercussions of heightened GATA3 that relates to autism. Details are in the text.

droxylase (TH) and dopamine-β-hydroxylase (DBH)\textsuperscript{10,41}, and targeted disruption of GATA3 causes severe deformities in brain\textsuperscript{42}. Gene manipulation studies further show that GATA3 is involved in the development of central nervous system (CNS)\textsuperscript{15} and induction of DBH in primary neural crest cells\textsuperscript{10}. Excessive dopaminergic activity causes hypervigilance, during which monkeys fixate on inanimate objects, and stereotypic behaviours such as rocking, whirling, grimacing and blinking\textsuperscript{43}. In animal models, dopamine agonists decrease social play and increase social isolation and stereotypic behaviour\textsuperscript{45}, and drugs that escalate dopaminergic activities exacerbate these symptoms in autistic subjects\textsuperscript{44}. Therefore a hyperdopaminergic state in autism may occur due to heightened GATA3 activity due to genetic predisposition and/or environmental insults (Figure 1).

In the GATA3 null mice, the reduced accumulation of TH and DBH mRNA and reduced noradrenaline in the sympathetic nervous system seem to be one of the reasons for brain abnormalities and death, as some mutants could be saved by feeding catecholamine intermediates to pregnant mothers or restoring the GATA3 activity in the sympathetic neurons\textsuperscript{16}. Brain catecholamine dysfunction in autism is reported earlier\textsuperscript{45}, and DBH gene is associated with the intelligence quotient of autistic children\textsuperscript{46}. Individuals with acquired prefrontal cortex (PFC) damage and individuals with ASD have some common symptomatology (e.g., repetitive behaviours, difficulty in task switching), and it is proposed that disruptions of the PFC may contribute to the cognitive and social difficulties experienced by individuals with ASD\textsuperscript{47}. The norepinephrine (NE) serves as an important neurotransmitter within the CNS, with noradrenergic neurons projecting from the locus coeruleus to several cortical regions, preferably the PFC\textsuperscript{48}, and changes in the central levels of NE impact PFC and the ex-
cutive function\(^5\). Low levels of NE enhances PFC functioning by focusing attention and improving executive function\(^6\), and hyperactivation of NE system deranges PFC function\(^5\). The higher than normal NE levels in individuals with ASD\(^2\) may result from excessive GATA3 activity that may disturb the PFC function. GATA3 is also expressed in the intestine\(^3\) and excessive gut-derived NE due to increased GATA3 activity may increase the pathogenic potential of gut bacteria aggravating the symptoms of autism\(^4\).

GATA3 binds to two cis-regulatory elements located within the estrogen receptor alpha (Era) gene, and the Era directly stimulates the transcription of GATA3 gene, indicating that these two factors are involved in positive cross-regulatory loop\(^5\). Thus, the overexpression of GATA3 would increase the synthesis of Era, an intracellular hormone receptor and transcription factor increasing signalling mediated by estrogen (E2) (17α-estradiol). The estrogen receptor is highly indiscriminate in binding several environmental agents, including phytosterogens, mycoestrogens and man-made xenoestrogens, such as bisphenol A of plastics and insecticide methoxychlor\(^5\). Estrogens, such as bisphenol A of plastics and environmental agents, may increase GATA3 activity causing immunological abnormalities, roles of GATA3 in the differentiation of several cell types including Th2 cells, immunological abnormalities in subjects with autistic mothers, connection between brain and immune systems, VPA-mediated increased GATA3 activity, expression of GATA3 in dopaminergic, seroergic and noradrenergic neurons and hyperactivity of these neurons in autism strongly suggest that higher GATA3 expression may cause and/or aggravate the symptoms of ASD. Further studies are now warranted to target this key molecule for reducing the incidence and/or severity of autism.

**Discussion**

Obviously, GATA3 plays a central role in several cellular processes in the body that relate to ASD (Figure 4). A recent study also reported higher GATA3 transcript levels in the lymphoblastoid cell lines derived from the lymphocytes of autistic subjects compared to their non-autistic siblings\(^3\), suggesting that GATA3 may be a biomarker for autism diagnosis. The good news is that the GATA3 expression and activity may be manipulated in the body with the existing knowledge and technologies. These may include manipulating Notch1 and β-catenin signalling that are influenced by VPA and also regulate the expression of GATA3\(^6\), nasal administration of antisense GATA3 oligonucleotide that suppresses the expression of GATA3 and significantly attenuates the allergic airway inflammation in the animal model of asthma\(^5\), inhalation of corticosteroids (fluticasone) that suppresses nuclear import of GATA3 and expression of Th2 cytokine in the peripheral blood lymphocytes of patients\(^5\). Drugs such as Mepolizumab (Bosatria), a humanized monoclonal antibody against IL-5, and Omalizumab (Xolair), a humanized IgG1k that binds both fluid- and membrane-bound IgE and lowers eosinophil counts\(^5\), may reduce allergen-aggravated immune reactions, gastrointestinal disorders and possibly autistic symptoms caused by Th2/Th1 imbalance associated with hyper-GATA3 activity. The expression of GATA3 may also be manipulated by antisense nucleotides, application of mimics for micro-RNAs that regulate GATA3 transcript levels and by regulating the expressions of protein/s that either downregulate (Rog, Fog and Pu.1) or potentiates (Pias1) GATA3 functions\(^5\). The abovementioned molecular intervention and drugs such as propranolol (a β1 and β2 adrenergic antagonist), that causes post-synaptic downregulation of the non-adrenergic recep-


Hypothesis

Hypothesis

[Text about the hypothesis]

Licensee OA Publishing London 2013. Creative Commons Attribution Licence (CC-BY)


All authors contributed to the conception, design, and preparation of the manuscript, as well as read and approved the final manuscript.

Competing interests: none declared. Conflict of interests: none declared.

All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.