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Dance Round

WE DANCE ROUND IN A RING AND SUPPOSE,
BUT THE SECRET SITS IN THE MIDDLE AND KNOWS.
ROBERT FROST

AUTISM SPECTRUM DISORDERS: NEUROTROPHINS ENTER THE DANCE

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INTRODUCTION

Autism spectrum disorders (ASD) is a group of lifelong neurodevelopmental disorders characterized by impairment in social interaction and communication, delayed and disordered language, restricted and stereotypic patterns of behaviour, interests and activities, and onset before 3 years of age. They are classified according to ICD 10 (1) (Table 1).

Autism spectrum disorders continue to increase at an alarming rate with the most recent statistics released by the Centers for Disease Control (CDC) indicating an incidence of 1.14%, or one in every 88 neurotypical children (2). Its ethiopathogenesis is poorly understood. Although a genetic origin has been recognized, it has been hypothesized a role for environmental factors, immune dysfunctions, and alterations of neurotransmitter systems. Sex-bias of ASD has a male to female ratio of approximately 4:1 (3). Recent findings from a study of more than 6 million ASD patients indicate that the prevalence of ASD seems to be increasing linearly from year to year (4).

Table 1. *F84 Pervasive developmental disorders*

F84.0	Childhood autism
F84.1	Atypical autism
F84.2	Rett's syndrome
F84.3	Other childhood disintegrative disorder
F84.4	Overactive disorder associated with mental retardation and stereotyped movements
F84.5	Asperger's syndrome
F84.8	Other pervasive developmental disorders
F84.9	Pervasive developmental disorder, unspecified

The neurobiological basis for autism remains elusive. A variety of findings suggest disturbances of brain development as a key feature of pathophysiology (5). Recent studies show that the abnormal levels of neurotrophins, such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and their relatives, might represent one of the aspects implicated in the pathogenesis of ASD (5-7). Studies on infants suggest that babies later diagnosed with ASD have smaller brain size at birth but undergo a rapid increase in brain volume during the first years of their life (8, 9). It has been hypothesized that

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the formation of neuronal connections and the elimination of inappropriate connections happens not in a physiological manner. Neuropathological studies on ASD show smaller neuronal sizes, decreased dendritic branches and alterations in dendritic spine density and shape in cortical projection neurons (10). Likewise, increased inhibitory synaptic transmission (11) and decreased Purkinjie cells in vermis and hemispheres of the cerebellum (12). There are some dysregulations also in the reelin, a glycoprotein secreted by neurons that is critically involved in the corticogenesis (13). Reelin also promotes maturation of dendrites and dendritic spines (14). Some aspects of the relationship between developmental dendritic pruning and elevated mTORC1 signaling (15), and macroautophagy in ASD have been recently demonstrated (4).

To make the *dance* even more complicated, considerable attention has been recently centred upon the functionality and plasticity of glial cells, particularly astrocytes (16). These cells participate in normal brain development and also in neuropathological processes. Data from an ASD animal model highlights that the astrocytic clearance and destination of glutamate in the synaptic cleft in tripartite synapses might be altered in autism, pointing out important aspects to be considered from both pathophysiologic and pharmacological approaches in ASD (17). The evidence also implicates dysfunctional signaling *via* Ca^{2+}-dependent mechanisms, extracellular signal-regulated kinases (ERK)/ phosphatidylinositol-3-kinases (PI3K) and neuroligin–neurexin–SHANK as convergent molecular mechanisms in ASD (14).

Whatsoever, the molecular mechanisms underlying these changes are yet not fully understood. As mentioned above, neurothrophic factors are one of the most prominent vectors influencing the development and maintenance of the central and peripheral nervous system. They are a family of proteins that induce the survival, development, and function of neurons. They exert a key role in brain development and maintenance of neurons and are able to critically influence the formation and elimination of neuronal connections. Given these properties they have been recently discussed as promising candidates playing a role in autism pathobiology and being responsible at least partially for the alterations in ASD (5-7).

The neurotrophin family of proteins consists of NGF, pro-NGF, BDNF, pro-BDNF, neurotrophin-3 (NT-3), NT-4/5, NT-6, NT-7 (18). Here we will *dance round* all these factors. Besides the neurotrophins, there are other protein families exerting neurotrophic actions, such as glial cell line-derived neurotrophic factor (GDNF), ciliary neurotrophic factor (CNTF) and insulin-like growth factors (IGF), which are out of the scope of present *Dance round*.

NGF

It has been hypothesized that abnormal levels of serum NGF may represent a serological marker for autistic children who may develop cognitive impairment, regression and finally epilepsy. The objective of a preliminary study was to measure serum NGF concentrations of autistic children and compare these levels with those of healthy children (19). Consecutive children who were referred to a pediatric unit were investigated and serum samples analyzed for NGF levels. Forty-nine autistic children and an equal number of healthy children (control group) were included in the study. Serum NGF concentrations were significantly higher in the study group compared with the control group. This preliminary findings suggested that enhanced serum NGF concentration could be used as a potential diagnostic tool in ASD.

In another study with rats, where a model of maternal infection was simulated, NGF expression was significantly increased in neonatal cortex, which was speculated to represent a potential mechanism through which maternal infection increased the risk for neurodevelopmental disorders (20). In a third study the level of NGF plasma levels were lower in patient with Rett syndrome with prolonged corrected QT interval in comparison with those with the same syndrome but normal QT (21). On the other hand, a previous study (22) detected normal levels of NGF in the cerebrospinal fluid in autism and low to negligible levels in Rett syndrome, which was in agreement with the different morphological and neurochemical findings (brain growth, affected brain areas, neurotransmitter metabolism) in the two syndromes. A suggestion was made to use cerebrospinal fluid NGF as a biomarker for differentiation of patients with autism from those with Rett syndrome.

In another study the level of autoantibodies to nerve growth factor was evaluated in blood serum of 163 children with different forms of mental dysontogenesis of different origin (23). Significant elevation of the level of autoantibodies was found in all forms of psychic dysontogenesis. The most significant elevation of the level of autoantibodies, as compared with the controls (45 children), was characteristic for endogenic forms of dysontogenesis (schizophrenia, early children's autism, schizotypic diathesis). The level of autoantibodies was also found as an indicator of the acuteness

of the pathologic state. Besides, its elevation was observed 1-2 weeks prior to the onset of the clinical exacerbation.

BDNF

In a commonly used animal model of autism a transient increase of both $mRNA^{BDNF}$ and BDNF protein levels was detected in the embryonic mouse brain (24). This model was created by the administration of valproic acid to pregnant animals at gestational days 12.5 (E12.5) or E13.5 which leads to autistic-like symptoms in the offspring. Of the nine 5'-untranslated exons of the mouse BDNF gene, only expression of exons I, IV and VI was stimulated by valproic acid *in utero*. The conclusion of the study was that in light of the well-established role of BDNF in regulating neurogenesis and the laminar fate of postmitotic neurons in the developing cortex, an aberrant increase in BDNF expression in the fetal brain may contribute to valproic acid-induced cognitive disorders by altering brain development.

BDNF elevation in newborn blood serum was found to predict intellectual/social developmental abnormalities. Thus, the circulating BDNF levels and IgG/IgM autoantibodies to BDNF were measured in children with autism, healthy children, and children with non-neurological illness (25). Mean BDNF levels were elevated in children with autism compared to healthy or children with non-neurological illnesses. Mean IgG and IgM BDNF autoantibodies were significantly elevated in children with autism compared to healthy children but not to children with non-neurological illness. The conclusion of the study was that children with autism have higher autoantibodies to BDNF compared to controls. The presence of both BDNF autoantibodies and elevated BDNF levels in some children with autism suggests a previously unrecognized interaction between the immune system and BDNF.

Although this observation is in harmony with results from other studies showing high concentrations of BDNF in autistic children (26), in another study the opposite result is reported (27). The level of serum BDNF was investigated as well as its age-related changes in healthy controls in comparison to autistic subjects. The concentration of BDNF was measured after its gradual released from platelets at 4° C. In healthy controls, the serum BDNF concentration increased over the first several years, then slightly decreased after reaching the adult level. There were no sex differences between males and females. In the autism cases, mean levels were significantly lower in children 0–9 years old compared to teenagers or adults, or to age-matched healthy controls, indicating a delayed BDNF increase with development.

In another study (28), subjects were recruited from a hospital in the Netherlands - 37 ASD patients [age around 10 years; body mass index (BMI) = 18.0 ± 3.7 kg/m^2] and 37 controls (age around 10 years; BMI = 17.6 ± 3.0 kg/m^2). It has been noted that there were not any age-related changes in the serum levels of BDNF as in other studies (27).

In a population of Chinese children aged around 4 years the potential role of BDNF was explored (29). Level of BDNF was assayed with enzyme-linked immunosorbent assay methods, and severity of ASD was evaluated with the Childhood Autism Rating Scale (CARS) Score. The results indicated that the median serum BDNF levels were significantly higher in children with ASD as compared to normal cases. The investigators found that an increased risk of ASD was associated with BDNF levels more than 15.0 ng/ml. This study demonstrated that serum BDNF levels were associated with ASD in a Chinese population, and higher levels could be considered as an independent contributing factor for ASD.

Differences in the analytic platforms used in the above cited studies, assay and sample test methods, and subject populations may explain the inconsistencies among studies (30, 31). Furthermore, circadian and/or seasonal and sex dependent changes in the level of BDNF is recognized in some studies (27, 32-34).

NEUROTROPHINS-3, -4/5

A paucity of cerebellar Purkinje cells and abnormalities in the inferior olive are among the best-documented changes in brain structure in autism (12). NT-3 plays an important role in glutamatergic synapse development *in vitro* (35) and this could be a mechanism influencing brain development in autism.
Valproic aicd exposure *in utero* (in a rat model of autism) induced small increases in the expression of $mRNA^{NT-3}$ (2.5-fold) and $mRNA^{NT-4/5}$ (2-fold) (24). Expression of the neurotrophin receptors, TrkA, TrkB and TrkC were minimally affected, while levels of the low-affinity panneurotrophin receptor, $p75^{NTR}$, doubled. In contrast to this investigation, in a study of Nelson *et al* (31), using a double-antibody immunoaffinity assay (Luminex) and ELISA technology, the concentrations of certain neurotrophins was measured in pooled samples eluted from archived neonatal blood of children with later diagnosed autism, Down syndrome, very preterm birth, or term control infants. Concentrations in control subjects differed by age: BDNF rose markedly with age, while NT-3 and NT-4/5 concentrations were lower in adults than in

Table 2. *A summary of the studies on neurotrophobiology of autism spectrum disorders*

Neurotrophin	Target population	Sample used	Main findings	Reference
NGF	Autistic and healthy children	Blood serum	↑ serum level of NGF in autistic compared to healthy children	19
NGF	Neurodevelopmental disorders	Neonatal cortex	Increased expression	20
NGF	Rett syndrome with prolonged corrected QT interval	Plasma	↓ Serum level of NGF compared with Rett syndrome with normal corrected QT interval	21
NGF	Autism and Rett syndrom	CSF	Normal CSF NGF level in autism ↓in Rett syndrom	22
Autoantibodies to NGF	mental dysontogenesis of endogenic, residual-organic, psychogenic and deprivative origin	Blood serum	↑ level of autoantibodies compared to controls	23
NGF, BDNF, NT-3, NT-4/5	autistic spectrum disorders, mental retardation without autism and cerebral palsy, and control children	Neonatal blood	No significant changes	7
BDNF	Offsprings in an animal model of autism	Brain	↑BDNF 5–6-fold	24
BDNF	Children with autism, childhood disintegrative disorder, healthy children, non- neurological illnesses	Blood serum	↑ in autism and disintegrative disorders compared to healthy children and non-neurological illnesses	25
Ig G, Ig M BDNF autoantibodies	Children with autism, childhood disintegrative disorder, epilepsy, healthy children, non- neurological illnesses, epilepsy	Blood serum	↑ in autism, disintegrative disorders and epilepsy compared to healthy children and non- neurological illnesses	25
BDNF	Pregnant women, children with autism, mental retardation and typical development	blood	No change in mid-pregnancy and neonatal specimens	30
BDNF	Autistic and healthy children	In serum, gradually released from platelets	Circadian but not seasonal changes. ↓ in autistic children 0–9 years old compared to teenagers or adults, or to age-matched healthy controls	27
BDNF, NT-4	autism and mental retardation, or healthy controls	Blood serum	↑ BDNF in autistic group (and the mental retardation compared to the control group. ↑ serum NT-4 concentration in the mental retardation group	6
BDNF	ASD and controls	Blood serum	No age dependent changes	28
BDNF	ASD and controls	Blood serum	↑ in autistic children	29
NT-3, NT-4/5	autism, Down syndrome, very preterm birth, or term control infants	Neonatal blood pooled samples	↓ NT-3, NT-4/5 in autistic children compared to controls	31
IGF-1,-2	Autism and controls	CSF	↓ IGF-1 in autistic children compared to controls, IGF-2 no difference between groups	37
IGF-1	Autism and controls	CSF	↓ IGF-1 in autistic children compared to controls	36
Glutamat uptake	Male rats in an animal model of autism	Hippocampus	unchanged at day 15 ↑160% at day 120	17
Glutamine synthetase activity	Male rats in an animal model of autism	Hippocampus	↑ 43% at day 15 ↓ 28% at day120	17
Glutathione content	Male rats in an animal model of autism	Hippocampus	unaltered at day15 ↑27% at day 120	17

newborn infants. In samples from autistic subjects, NT-3 levels were significantly lower than controls and an increase in VIP approached statistical significance. Concentrations of NT-4/5 were correlated in infants with autism but not in controls. Some of these results differ from earlier findings using a single-antibody recycling immunoaffinity chromatography (RIC) system. The mean concentration of NT-3 tended to be lower in children with later-diagnosed autism than in control subjects. The difference was of borderline statistical significance. Values for NT-4/5 did not distinguish children with autism from control infants. Thus measurement by Luminex again did not confirm the previous observations by RIC (7). There were no changes again in the level of NT-4/5 also in another study, in which the BDNF level was markedly increased (6). Meanwhile, for the possible involvement of insulin-like growth factor-I (IGF-I) in the development of autism, see (36, 37).

CODA

The present *Dance round* demonstrates that blood serum and/or cerebrospinal fluid levels of the neurotrophins NGF, BDNF, NT-3 and NT-4/5 may be associated with the pathogenesis of ASD (Table 2). Indeed, much more research is necessary in order to draw final conclusions about the possible link between the amount of neurotrophins and ASD in different population and age groups, and the methodology and biological samples which could be used for this purpose.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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