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ORIGINAL ARTICLE

Circulating levels of neurotrophic factors in autism spectrum disorders

David Henrique Rodrigues [1,2], Natália Pessoa Rocha [1], Larissa Fonseca da Cunha Sousa [1], Izabela Guimarães Barbosa [1,3], Arthur Kummer [1,4], Antônio Lúcio Teixeira [1,3]

1 Interdisciplinary Laboratory for Medical Research, School of Medicine, Federal University of Minas Gerais, Brasil
2 Department of Health and Basic Sciences, Federal University of Juiz de Fora, Brasil
3 Neuropsychiatry Branch, Neurology Division, University Hospital, Federal University of Minas Gerais, Brasil
4 Department of Mental Health, School of Medicine, Federal University of Minas Gerais, Brasil

Correspondence to: David Henrique Rodrigues, PhD.
Interdisciplinary Laboratory for Medical Research,
School of Medicine, Federal University of Minas Gerais, Brasil.
TEL: +5531 8829-3033; E-MAIL: dhenrodrigues@gmail.com

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Abstract

OBJECTIVES: Evaluate the levels of a neurotrophic factor and some neurotrophins in the plasma of patients with Autism Spectrum Disorders (ASD).
DESIGN: This study enrolled 30 children with ASD and 19 healthy children. Plasma levels of the neurotrophins BDNF, NGF, NT3, NT4 and of the neurotrophic factor GDNF were measured by Enzyme-Linked Immunosorbent Assay.
SETTING: The etiopathogenesis of ASD is largely unknown, but it seems to involve dysfunction in several biological systems. One of these systems comprises the neurotrophic factors, which are molecules involved in many processes in the central nervous system, including neuronal survival, synaptogenesis and synaptic plasticity. Recent studies have shown association between neurotrophic factors and ASD.
RESULTS: No differences in plasma BDNF, NGF, NT3, NT4 and GDNF were found between ASD and control. Neurotrophic factors are not altered in ASD.
CONCLUSIONS: These molecules may play a minor role in ASD.

INTRODUCTION

Autism spectrum disorders (ASDs) are neurodevelopmental diseases characterized by restricted interests, repetitive behaviors, and deficient language and social skills. Initially, autism was considered a rare disorder, and even in the 1980's its prevalence was considered of 5 per 10,000 persons (Newschaffer *et al.* 2007). However, recent studies have shown that its prevalence may be of 1 child in every 110 (Mulvihill *et al.* 2009), even though this increasing prevalence could be attributable to factors such as new administrative classifications, policy and practice changes, and increased awareness (Levy *et al.* 2009). There is a lack of data concerning prevalence in Brazil, but it is estimated that autism affects more than 1 million Brazilians (Paula *et al.* 2011).

It is believed that early therapy intervention may be efficacious in ASD. But as reviewed by Rogers and Vismara (2008), many studies show insufficient improvement in autistic patients even after early therapy. Thus, the search for new therapies has increased the number of papers investigating ASDs. Of note, many authors are trying to unravel the mechanism that leads to autistic disorder. It is clear that neurodevelopment is disrupted in ASDs as several studies show changes in white matter structures when comparing children with ASDs and control children (Barnea-Goraly *et al.* 2010; Cheng *et al.* 2010). There is also evidence for incorrect neural connections between regions of the central nervous system in ASDs. As reviewed by Wass (2010), some regions in the brain of ASD children exhibit overconnectivity while others present underconnectivity of neurons. In this context, the investigation of molecules involved in neurodevelopment may be central to better understand the etiopathogenesis of ASDs. Among many molecules possibly involved in neurodevelopment, neurotrophins and neurotrophic factors are some of the most studied factors.

Neurotrophins are central to many processes in the central nervous system, from differentiation and neuronal survival to synaptogenesis and activity-dependent forms of synaptic plasticity (Lu *et al.* 2005). Up to date, there are four described neurotrophins: brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF) neurotrophin 3 (NT-3) and neurotrophin 4/5 (NT-4/5). They bind to two distinct classes of transmembrane receptor: the p75 neurotrophin receptor ($p75^{NTR}$) and the Trk family of receptor tyrosine kinases, which includes TrkA, TrkB and TrkC (Lu *et al.* 2005). These receptors can associate with other membrane bound molecules, such as sortilin and LINGO-1 and the binding of neurotrophins to their receptors may elicit several biological responses. Some of these responses are opposite as neurotrophins can promote either survival or cell death, either myelination or inhibition of neurite outgrowth (Lu *et al.* 2005). Besides neurotrophins, molecules classified as neurotrophic factors may be also relevant for the etiology of autism. These molecules act through binding to glycosylphophatidylinositol anchored receptors, and the glial derived neurotrophic factor (GDNF) is an example of neurotrophic factor (Pascual *et al.* 2011). GDNF is important in the neuronal survival, though its exact function is still under investigation (Pascual *et al.* 2011).

There is evidence that brain of ASD children may present underconnectivity of neurons when compared to healthy children (Cherkassky *et al.* 2006). As neurotrophins and neurotrophic factors are important in the formation of brain connections, we hypothesize that there is change in the circulating levels of these factors in ASDs. Thus, in this study, we measured plasma levels of four neurotrophins and of the neurotrophic factor GDNF from ASD children and compared them with control children.

Tab. 1. Socio-demographic and clinical feature of patients with autism spectrum disorders and controls.

	Controls (n=19)	ASD (n=30)	*p*-value
Male %	78.90	83.90	0.66[a]
Age in years – median (range)	8.0 (5.0-15.0)	8.0 (3.5–23.0)	0.91[b]
Maternal age in years – median (range)	26.0 (17.5-49.0)	30.0 (19.0-43.0)	0.06[b]
Social Responsiveness Scale – mean (±SE)	60.89 (±1.83)	101.20 (±3.44)	<0.0001[b]

[a]Pearson chi-square test; [b]Mann-Whitney

MATERIAL AND METHODS

Subjects

In this study 30 patients diagnosed with ASD and 19 children without psychiatric diseases and above 4 years of age were enrolled. Patients were recruited at the Child Psychiatry Clinic from the University Hospital, Universidade Federal de Minas Gerais, Brazil. All children fulfilled the DSM-IV-TR diagnostic criteria for autism. The principal investigators of the manuscript invited parents of controls (children without psychiatric diseases) and their children to participate and give their consent. Written informed consent was obtained from all participants. Patients were excluded if their medical histories included any of the following: severe auditive and visual deficiencies, neuroectodermosis, neoplasias of the nervous system, degenerative diseases, demyelinating diseases and inflammatory diseases. Patients and controls were matched for age, gender, maternal age at child birth (Table 1). The local institutional review board approved the study, which is in accordance with the Helsinki Declaration of 1975.

Samples

Ten milliliters of blood were drawn from each subject by venipuncture into a sodium heparin tube (Vacuplast, Huangyn, China) on the same day of the clinical assessment. All procedures were performed between 8 and 10 a.m. to minimize biological differences due to circadian rhythms (Choi *et al.* 2011). The blood was immediately centrifuged at 3000×g for 10 min, 4 °C, twice. The plasma was collected and stored at –80 °C until assayed. Plasma levels of BDNF, NGF, NT-3, NT4/5 and GDNF were measured by enzyme-linked immunosorbent assay (ELISA), according to the procedures supplied by the manufacturer (DuoSet, R&D Systems, Minneapolis, MN, USA). All samples were assayed in duplicate. Detection limits were defined at 5 pg/mL. Concentrations are expressed as pg/mL.

Social responsiveness scale

The Social Responsiveness Scale (SRS) (Constantino *et al.* 2003) is a 65-item rating scale that ascertains

autistic symptoms across the entire range of severity in which they occur in nature. It is a parent- and/or teacher-report measure of children's social impairment in naturalistic social settings. Each item on the scale inquires about an observed aspect of reciprocal social behavior that is rated on a scale from "0" (never true) to "3" (almost always true). The SRS was applied to parents from patients and control children as a measure of severity of autistic symptoms.

Statistical analysis

Medians were compared using Mann-Whitney U test. Spearman's correlation analyses were performed to examine the relation of BDNF, NGF, NT3, NT4/5 and GNDF levels with age and maternal age. Pearson chi-square test was used to evaluate proportion of males between controls and ASD patients. All statistical tests were two-tailed using a significance level of α=0.05. Statistical analyses were performed using SPSS software version 19.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

From the 30 patients enrolled, 15 were taking antipsychotics, 8 antiepileptic drugs and 5 methylphenidate, while 8 were not taking any drug at the moment that blood was collected. The other drugs used were: benzodiazepines (4), imipramine (1), and methylphenidate (5).

As it is shown in Figure 1, no differences were found in plasma levels of the four neurotrophins analysed when comparing ASDs and healthy children. There was not either any difference in the plasma levels of fhe neurotrophic factor GDNF when comparing ASDs and healthy children (Figure 2). ASDs and healthy children differed in SRS scores but no correlation was found between any neurotrophin and SRS, gender or maternal age at child birth.

DISCUSSION

To the best of our knowledge, this is the first comprehensive assessment of neurotrophins and GDNF in ASD. We did not find any difference between healthy children and children with ASD. This may sound paradoxical as neurotrophins are molecules involved in the generation of connections between neurons in the central nervous system (CNS) and the putative physiopathology of autism involves the occurrence of abnormal connections (Neul 2011). Conversely, changes in the levels of neurotrophic factors in the brain may not lead to changes in these same molecules in the blood (Lanz *et al.* 2012).

Studies involving neurotrophins, neurotrophic factors and autism have presented strikingly different results, with neurotrophin levels being lower, similar or higher in ASD when compared to controls. The neurotrophic factor NT4/5 and BDNF were found to be

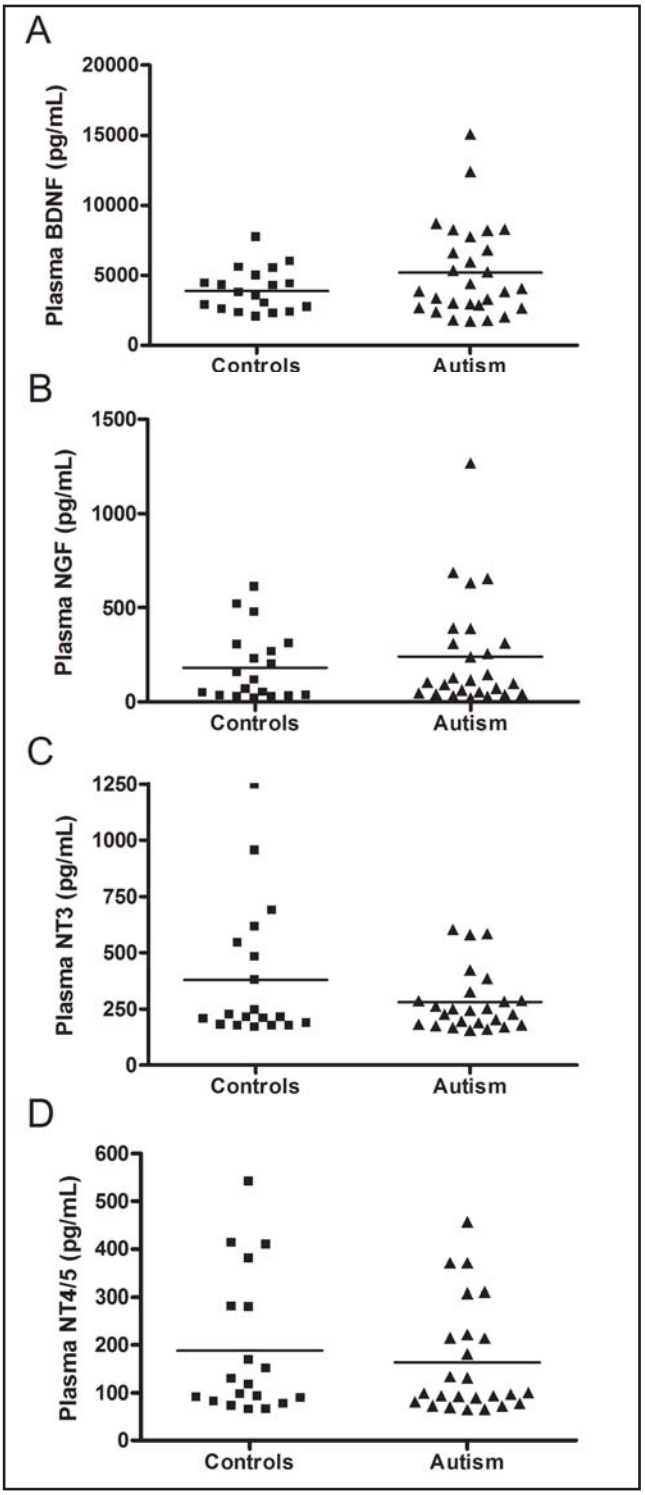

Fig. 1. Plasma levels of neurotrophins in children with autism and controls. A) Levels of BDNF were not different (p=0.28) in children with autism (median [range]; 3 962 pg/mL [1 726–15 071]) when compared to children without autism (3 730 pg/mL [1 987–7 680]). B) Levels of NGF were not different (p=0.40) in autistic children (110 pg/mL [23.40–1 269]) when compared with children without autism (113 pg/mL [17.04–607.6]). C) Levels of NT3 were not different (p=0.74) in autistic children (243.3 pg/mL [156.1–603.0]) when compared with children without autism (208.9 pg/mL [165.1–1245]). D) Levels of NT4/5 were not different (p=0.88) in autistic children (99.15 pg/mL [64.63–456.9]) when compared with children without autism (116.4 pg/mL [63.78–539.8]). Bars represent means.*p<0.05 **p<0.01 ***p<0.001

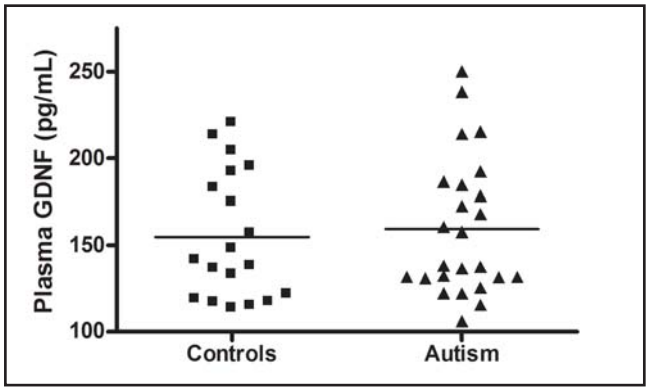

Fig. 2. Plasma levels of the neurotrophic factor GDNF in children with autism and controls. Levels of GDNF were not different (p=0.75) in children with autism (median [range]; 138.2pg/mL [106.0–250.1]) when compared with children without autism (141.5 pg/mL [113.5–220.6]).

elevated in children with ASD when compared to control children or children with cerebral palsy (Nelson *et al.* 2001). However, NT3 and NGF were not different in the cohort investigated by Nelson *et al.* It is relevant to state that blood was taken from these children at the moment of birth, thus, before the development of autistic symptoms. A study using a similar approach (Abdallah *et al.* 2013) found decreased levels of BDNF and NT4/5 at the moment of birth from children who became autistic. This raises the question of the role of these neurotrophic factors during development and the consequences of their dysfunction. As we found no difference in children with autism in our study when compared to controls, it may be speculated that the differences found in early life of children who will become autistic vanish after the first years of development. Besides, NT4/5 promotes the development and survival of selected peripheral and CNS neurons (Fan *et al.* 2000), and its functions overlap with those of BDNF. Even though no differences were found among the neurotrophins evaluated, our data are in agreement with a pilot study from Miyazaki *et al.* (2004), in which no differences were found in serum levels of BDNF and NT4/5 between ASD children and controls.

Studies investigating only BDNF have also found conflicting results. Al-Ayadhi (2012) used a cohort of patients similar to our work but found an increase in serum BDNF from patients with mild autism. Interestingly, when considering only patients with severe autism, there was no change in serum BDNF levels when compared to control children. However, in a study with adults, ASD group had lower levels of BDNF when compared to controls (Hashimoto *et al.* 2006). A possible explanation for such distinct results may be a change in the levels of BDNF through development. Katoh-Semba (2007) stratified patients from 0–9 years old and from 10–19 years old and found an interesting change in the profile of BDNF serum levels. While BDNF levels from ASD were lower in children from 0–9 years when compared to age matched controls, BDNF levels were higher in children from 10–19 with ASD when compared to controls. In this context, as the median of our group was 8 years, it is possible that our data reflects the turning point, that is, the moment when BDNF levels from ASD children are increasing compared to controls, but not yet significantly elevated.

The role of NT-3 in the brain overlaps those of other neurotrophins, which makes it another important neurotrophin to investigate. Levels of NT-3 in neonatal blood from ASDs were not different from controls (Nelson *et al.* 2001). However, NT-3 levels in the cerebellum of ASD children are elevated when compared to controls (Sajdel-Sulkowska *et al.* 2009). There is also an increase in NT-3 levels in specific regions of the brain: in dorsolateral prefrontal cortex in the case of older autistic cases and Wernicke's area and cingulated gyrus in the younger cases (Sajdel-Sulkowska *et al.* 2011). In this sense, our findings seem controversial as we could not detect differences in the levels of plasma NT-3, but it is relevant to state that changes in the levels of these neurotrophins in the brain may not necessarily induce changes in their levels in plasma.

NGF was also investigated by Nelson (2001) in neonatal blood of ASDs, and the levels of this neurotrophin were not different between ASDs and controls. In a work that investigated frozen samples from patients with Rett syndrome, a disorder of the autism spectrum, cerebrospinal fluid (CSF) levels of NGF were decreased when compared to controls (Riikonen 2003). However, the serum levels in the same study were not different between patients with Rett syndrome and controls. The lack of any difference in the levels of plasma or serum NGF may indicate that this neurotrophin plays a minor role in ASD.

The neurotrophic factor GDNF was also investigated by Riikonen (2003) in CSF from patients with Rett syndrome, but no difference was found between patients and controls.

The high variability of neurotrophin and neurotrophic factors results is a challenge in these studies. Even though it is logical to consider these molecules as essential in understanding the pathophysiology of ASDs, it is also possible that their importance is being overestimated. This would explain why these studies present such variable results. Another possibility is that the levels of these molecules vary considerably with the age and studies have not yet analyzed narrow age ranges. The fact that these molecules present overlapping functions may also contribute to this high variability.

There are two main limitations in our study. First, most patients were medicated. Many of these patients present a severe form of autism and are followed in a tertiary center. It would be unethical to keep these patients without medication. As our sample came from a tertiary health care unit, this may prevent the generalization of the results.

Further studies involving larger samples from clinical and community settings are warranted.

CONCLUSION

In conclusion, we found that there is no difference in the plasma levels of neurotrophins and GDNF between ASD patients and healthy children. In this sense, we suggest that the levels of neurotrophic factors in the plasma of ASD may not be an adequate parameter to study autism.

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