Original Research

Folate Receptor Alpha Autoantibodies Modulate Thyroid Function in Autism Spectrum Disorder

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The folate receptor alpha (FRα) is essential for folate transportation across the blood-brain barrier and is closely associated with cerebral folate deficiency, a syndrome that commonly presents with autism spectrum disorder (ASD) features. FRα autoantibodies (FRAAs) interrupt FRα function and have a high prevalence in children with ASD. Since the FRα is also located on the thyroid, FRAAs could also interfere with thyroid function. Interestingly, ASD has been inconsistently associated with hypothyroidism. The aim of this study was to determine if thyroid dysfunction in ASD could be related to FRAAs. To this end we investigated the relationship between serum FRAA titers (both blocking and binding) and thyroid stimulating hormone (TSH) in 32 children with ASD. Blocking, but not binding, FRAAs were found to be related to TSH levels. Higher FRAAs were significantly correlated with higher TSH concentrations (r = 0.36, p = 0.025), while ASD children who were positive for blocking FRAAs demonstrated a significantly higher serum concentration of TSH than children who were negative for FRAAs (t(31) = 2.07, p = 0.02). These results are consistent with the notion that blocking FRAAs are associated with reduced thyroid function and suggest that thyroid function should be examined in children with ASD who are positive for the blocking FRAAs.

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INTRODUCTION

Folate is a water-soluble B vitamin that is essential for numerous physiological systems and is critical for neurodevelopment.[1,2] Folate is transported across cellular membranes using several mechanisms, including the folate receptor alpha (FRα), the folate receptor beta,[3] the reduced folate carrier (RFC)[4] and the proton-coupled folate transporter.[5] The FRα is located on the endothelium of the choroid plexus,[6] thyroid cells,[7] the microvillus plasma membrane of the placenta,[8] as well as the epithelium of the fallopian tubes, uterus, and epididymis, acinar cells of the breast, submandibular salivary and bronchial glands and the alveolar lining including pneumocytes.[9] The folate receptor beta appears to be important in the placental transport of folate,[3] while the proton-coupled folate transporter is critical for gastrointestinal tract folate transport.[5] The RFC is a transmembrane protein that is expressed in a wide range of tissues, including the placenta, kidney, intestine and both the basolateral and apical surface of the choroid plexus.[10] The RFC is unique as it allows bidirectional transport of folate across the cellular membrane,[11] is responsible for folate transport across the blood-brain barrier when extracellular folate concentrations are high[6] and has a higher affinity for reduced forms of folate as compared to the oxidized form commonly known as folic acid.[4]

Autoantibodies that bind to the FRα and greatly impair its function were described approximately a decade ago when they were linked to cerebral folate deficiency (CFD).[7] CFD is a neurometabolic disorder characterized by severe neurodevelopmental symptoms. CFD is defined by below normal concentrations of 5-methyltetrahydrofolate (5MTHF) in the cerebrospinal fluid despite normal systemic folate levels. The FRα is located on both sides of the endothelial surface of the choroid plexus and is believed to be the primary transportation mechanism for folate across the blood-brain barrier.[6] As such CFD is believed to be due to impaired FRα function, in large part due to FRα autoantibodies (FRAAs). FRAAs have been linked to CFD in cases with[12] and without[7] ASD. Recently, Frye et al. measured FRAA titers on 93 children with ASD as part of a medical workup.[13] Overall, 60% and 44% were positive for the blocking and binding FRAAs, respectively; 29% children were positive for both FRAAs; 46% were positive for only

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one FRAA and 75% were positive for at least one FRAA. The prevalence of blocking FRAA (60%) was much higher than the prevalence reported in the general population in Spain (7.2%),[14,15] Ireland (12.6%)[16] and the general U.S. population (10-15%, unpublished data from Dr. Quadros' laboratory). In addition, a recent study from Belgium has verified the high prevalence of the blocking FRAA in ASD children.[17] In this study 47% of ASD children were found to be positive for the blocking FRAA as compared to only 3.3% of developmentally delayed non-ASD controls.[17]

Given the fact that the FRα is important for the transportation of folate into other organs it is possible FRAAs could interfere with the function of other organs that use the FRα. FRα is essential for folate transportation into thyroid cells and, interestingly, there have been inconsistent reports of thyroid dysfunction in ASD. One study of 5 children with ASD reported that 3 of them had congenital hypothyroidism and two of the mothers in the study had probable hypothyroidism during pregnancy.[18] The basal and thyrotropin-releasing hormone stimulated peak thyroid stimulating hormone (TSH) levels were shown to be lower in 41 autistic boys as compared to children with mental retardation, minimal brain dysfunction and typically developing controls in a Japanese study.[19] In another small study, young adults with ASD were generally found to have higher TSH levels but the magnitude of the difference between the ASD and control participants was dependent on the time of day due to the significant diurnal variation in TSH levels.[20] The largest study examined thyroxin levels at birth in 784 children with ASD and 554 matched control children born in California in two groups, a group born in 1994 and a group born in 1995. The study found that very low thyroxin levels at birth increased the risk of developing ASD but only in the 1995 study group.[21] Interestingly, one study of 308 children with ASD reported that autoimmune thyroid disorders in family members was associated with ASD regression in the child (OR=1.89; 95% CI=1.17-3.10).[22] One way in which thyroid dysfunction could adversely affect neuronal migration is via the regulation of reelin as this is dependent on adequate levels of triiodothyronine.[23] However, several early studies have not found a relationship between abnormal concentrations of TSH, thyroxin or triiodothyronine and ASD.[24,25] One of the reasons that studies are inconsistent is that there may be only a subgroup of children with ASD who have abnormal thyroid function and it may be difficult to detect this subgroup when looking at the whole population of children with ASD. Thus, it is possible that thyroid dysfunction may be related to the subgroup of ASD children with FRAAs.

To determine if there is a relationship between FRAAs and thyroid function, we examined the relationship between blocking and binding FRAAs and thyroid stimulating hormone (TSH) concentrations in a case-series of patients diagnosed with ASD.

METHODS

Two of the authors (REF; DAR) offered FRAA testing as part of the workup for medical conditions associated with ASD. Approximately 1ml of serum was collected and sent to the laboratory of Dr. Edward Quadros, PhD, at the State University of New York, Downstate (Brooklyn, NY). The assay for both the blocking and binding FRAAs has been described previously.[7,16] Blocking FRAAs were expressed as pmoles of folic acid blocked from binding to FRα per ml of serum and binding FRAAs were expressed as pmoles of IgG antibody per ml of serum.

Thirty-two children with ASD who had FRAA and TSH testing were included in this study. All children met the Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition – Text Revision[26] criteria for ASD and had previously been diagnosed with ASD by a developmental pediatrician, pediatric neurologist or clinical psychologist. Review of each child's medical record was obtained through an Institutional Review Board approved protocol. Patient laboratory values such as the TSH concentration were abstracted from the medical record. For TSH interpretation, the National Academy of Clinical Biochemistry standard for children was used as a reference range, which is 0.4 to 5.0 mIU/L.

Statistical Analysis

The relationship between FRAAs and TSH was investigated in two ways for both the blocking and binding FRAAs separately. First, the Pearson correlation between the FRAA titers and TSH was calculated. Second, patients were divided into two groups, FRAA positive and FRAA negative, and the two groups were compared. TSH was found to be log distributed and was log transformed before analysis. An alpha of 0.05 was used as a one-tailed test, since the relationships between FRAAs and TSH were predicted to be in one particular direction. Specifically, it is hypothesized that higher FRAA titers would be related to higher TSH concentrations and FRAA negative patients would have lower TSH values than FRAA positive patients.

RESULTS

Subject Characteristics

The average age of the participants was 7y 2m (SD = 2y 8m) and 91% were male.

Folate Receptor Alpha Autoantibody Titers

56% of the patients were positive for the blocking FRAA and 50% of the patients were positive for the binding FRAA. 28% were negative for both FRAAs, 44% were positive for only one FRAA and 28% were positive for both FRAAs. The average blocking FRAA titer was 0.44 (SD = 0.48) and ranged from 0 to 1.44. The average binding FRAA titers was 0.48 (SD = 0.65) and ranged from 0 to 3.45.

Thyroid Stimulating Hormone Concentrations

The average TSH concentration was 2.76 (SD = 3.42) and ranged from 0.81 to 20.8. Using the standard reference range, 0% of the ASD children demonstrated an abnormally low TSH and 3% of the ASD children demonstrated an abnormally elevated TSH.

Relationship Between Folate Receptor α Autoantibodies and Thyroid Stimulating Hormone

No significant relationship was found between the binding FRAA and TSH. However, a higher blocking FRAA titer was significantly related to a higher TSH concentration [r = 0.36, p = 0.025; See **Figure 1A**]. Patients who were positive for the blocking FRAA were found to have a significantly higher TSH concentration as compared to patients who were negative for the blocking FRAA [t(31) = 2.07, p = 0.02; see **Figure 1B**].

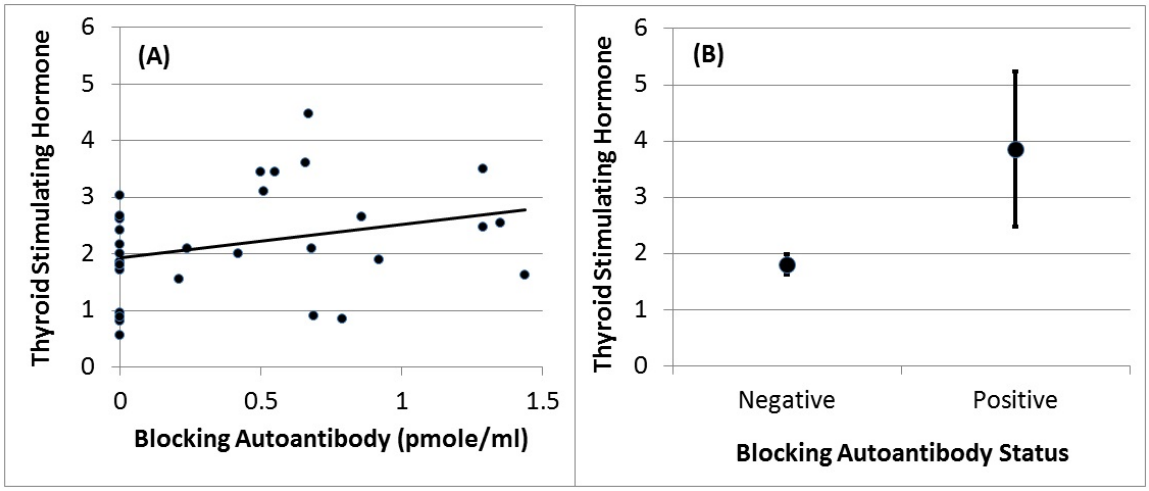

Figure 1. The relationship between the blocking folate receptor alpha autoantibody and thyroid stimulating hormone. (A) The blocking folate receptor alpha autoantibody titer is correlated with serum concentrations of thyroid stimulating hormone such that higher titers are related to higher thyroid stimulating hormone levels (note that one individual with a very high thyroid stimulating hormone level is not included in this graph). (B) Thyroid stimulating hormone levels are significantly higher for children with ASD who were positive for the blocking folate receptor alpha autoantibody as compared to children with ASD who were negative for the blocking folate receptor alpha autoantibody.

DISCUSSION

This study has demonstrated a potential relationship between the blocking FRAA and TSH in children with ASD, suggesting that autoantibodies to the FRα could modulate thyroid function in children with ASD. Several studies have demonstrated that blocking FRAA titers have a relationship to central 5MTHF concentrations, presumably by blocking the transportation of folate through the FRα. This study suggests that a disruption of folate transportation into thyroid cells through the FRα may similarly disrupt folate levels in thyroid cells leading to thyroid dysfunction. Since thyroid dysfunction would presumably decrease the amount of thyroxin or triiodothyronine produced, an increase in TSH, that has been shown to be related to blocking FRAAs in this study, would follow.

We have demonstrated that TSH is significantly elevated in ASD children who are positive for the blocking FRAA as compared to children who are negative for the blocking FRAA. However, for the most part, all TSH levels were within the range considered normal for children. There are several possibilities why more children with abnormally elevated TSH were not found. First, it is believed that the current upper limit of the TSH reference range is too high to detect subclinical hypothyroidism in adults[27] and that high normal TSH levels are associated with increased risk of metabolic abnormalities.[28] Thus, some investigators have advocated reducing the reference range to detect these subclinical cases of hypothyroidism in adults.[27] However, this has not been studied in children to provide a basis to lower the upper limit of the TSH reference range for children. Second, iodine deficiency has been reported in some children with ASD[29] and some investigators think it may play a role in ASD causation,[30] especially in combination with pesticide exposure.[31] Iodine deficiency has been implicated as a cause of thyroid dysfunction in children with ASD[32,33] and had been correlated with ASD severity in one study.[32] It is possible that abnormalities in folate transport may synergistically combine with other factors such as iodine deficiency to result in thyroid dysfunction in children with ASD. Third, since thyroid dysfunction in children with ASD is highly correlated with thyroid dysfunction in their mothers, abnormalities in thyroid function in children with ASD may simply be an epiphenomenon of thyroid dysfunction during gestation which can cause significant neurodevelopmental consequences.[32] Lastly, subtle abnormalities in thyroid function during childhood could be a marker for more severe thyroid dysfunction earlier in life when the sensitivity of neurodevelopment to thyroid function is more critical.

Clearly there are several unanswered questions that require further research. Unfortunately the current study did not measure thyroxin or triiodothyronine levels to more accurately determine thyroid function in this series of children with ASD. Future studies to examine the FRAAs should consider investigating thyroid function and studies that investigate thyroid function should consider measuring FRAA as a cause of thyroid dysfunction. As this is a limited sample of children, it is difficult to make firm conclusions of the exact relationship between FRAAs and thyroid function. Indeed, larger cohorts are needed to confirm and extend the preliminary findings of this study.

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CONFLICT OF INTEREST

Drs. Frye and Rossignol have no conflict of interest to declare. Two of the authors (J.M.S. and E.V.Q.) are inventors on a US patent for the detection of FR autoantibodies issued to the Research Foundation of the State University of New York.

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