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Research Article

Increased Glycogen Synthase Kinase 3 Alpha (GSK3A) in Children with Autism

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Received: November 21, 2019; **Published:** January 07, 2020

Abstract

Autism spectrum disorders (ASDs) are characterized by problematic social interactions, verbal and nonverbal communication, and stereotyped and repetitive behaviors. Glycogen synthase kinase 3-alpha (GSK3A) is a multifunctional protein serine kinase which controls several regulatory proteins and has been associated with the etiology of ASDs. In this study, cellular, phosphorylated GSK3a concentration was measured in autistic children and neurotypical, age and gender similar controls using an Immuno-array assay. We found cellular GSK3a levels in autistic children, significantly higher than neurotypical controls. These results suggest that therapies designed to lower GSK3a levels in individuals with autism may improve aberrant behaviors.

Keywords: *Autism; ASD; GSK3a*

Abbreviations

ASD: Autism Spectrum Disorders; GSK3a: Glycogen Synthase Kinase 3 Alpha

Introduction

Autism spectrum disorders (ASDs) are characterized by problematic social interactions, verbal and nonverbal communication, and stereotyped and repetitive behaviors [1]. They are among the most common behavioral disabilities diagnosed in children aged 3 - 5 [2].

Glycogen synthase kinase 3-alpha is a multifunctional protein serine kinase which controls several regulatory proteins including the phosphorylation of glycogen synthase [3] and over 50 other substrates which are involved in the regulation of many fundamental processes, including development, cell structure, microtubule dynamics, gene expression, and cell survival [4,5].

Mice with deletion of the fragile X mental retardation 1 (*Fmr1*) gene, which are often used to model autism behaviors [6-8], demonstrate GSK3 hyperactivity. Inhibition of GSK3 by lithium administration improves some of the autism-like behavioral impairment, such as sensitivity to audiogenic seizures, hyperactivity, and impaired passive avoidance memory, in these mice [9].

This suggests a role for GSK3 in social behaviors and implicates inhibition of GSK3 as a potential therapeutic modality for autism.

Aim of the Study

The aim of this study is to measure phosphorylated GSK3a levels in individuals with autism and neurotypical controls.

Materials and Methods

Subjects

Cellular phosphorylated GSK3a was measured in 26 autistic children and 12 age and gender similar neurotypical, controls.

The diagnostic criteria used in this study were defined by DSM-IV criteria. In 2012, the separate diagnostic labels of Autistic Disorder, Asperger's Disorder, and Pervasive Developmental Disorder-not otherwise specified (PDD-NOS) were replaced by one umbrella termed "Autism Spectrum Disorder".

White blood cells from consecutive individuals with diagnosed autism (n = 26; 20 male; mean age 10.7 years) and controls (n = 12; 10 male; mean age 9.8 years) were obtained from patients presenting at the Health Research Institute (HRI)[1] over a two year period. All autistic individuals who presented to HRI were asked to participate, and patients who participated in this study were randomly chosen from all patients who volunteered. The autistic individuals in this study met the DSM-IV criteria and many were diagnosed using The Autism Diagnostic Interview-Revised (ADI-R) (17) before presenting to the HRI.

Patient consent was obtained from all patients involved in this study and this study was approved by the IRB of the HRI.

Cellular phosphorylated ERK 1 and 2 concentration was measured using an Immuno-array assay described below.

Buffy coat white blood cells

All experimental and control cells were separated from plasma in whole blood using centrifugation and were treated in an identical fashion-refrigerated (4°C) immediately after collection and cell/serum separation. Frozen plasma and buffy coat samples were placed at -70°C and used for Immunoassay analysis.

Immuno-array assays

Immuno-array assays were performed by RayBiotech, Inc, Peachtree Corners, GA. 30092.

Blocking and incubation

Add 1 ml Blocking Buffer and incubate at room temperature with gentle shaking for 1 hour to block membranes. Decant Blocking Buffer from each container. Add 1.0 ml of sample into each array membrane, and cover with the lid. Incubate at room temperature for 2 hours. Dilute sample using Blocking Buffer. Decant the samples from each container and wash 3 times with 2 ml of 1X Wash Buffer I at room temperature with shaking. 3 minutes per wash.

Remove each array membrane and place all of membranes into a plastic container with a minimum of 20 ml of 1X Wash Buffer I. Rinse the 8-Well Multi-dish with deionized or distilled water and dry thoroughly. Wash array membranes with 1X Wash Buffer with shaking. Repeat 2 times for a total of 3 washes. 5 minutes per wash.

Wash 3 times with a minimum of 20 ml of 1X Wash Buffer II at room temperature with shaking. 5 minutes per wash.

*The Health Research Institute is a comprehensive treatment and research center, specializing in the care of individuals with neurological disorders, including autism.

Remove each array membrane from the container, return it to the 8-well tray.

Add 1 ml of diluted Cocktail of Biotin-Conjugated Anti-GSK3a Antibody to each membrane. Incubate at room temperature with gentle shaking for 2 hours.

Wash as directed above.

Add 1.5 ml of 1X HRP-conjugated streptavidin to each membrane.

Incubate at room temperature for 2 hours.

Wash as directed in steps 5 and 6.

Add 250 µl of Detection Buffer C and 250 µl of Detection Buffer D for one membrane; mix both solutions; Drain off excess wash buffer by holding the membrane vertically with forceps. Place membrane protein side up ("-" mark is on the protein side top left corner) on a clean plastic sheet (provided in the kit). Pipette the mixed Detection Buffer on to the membrane and incubate at room temperature with gentle shaking for 2 minutes. Ensure that the detection mixture is completely and evenly covering the membrane without any air bubbles.

Drain off excess detection reagent by holding the membrane vertically with forceps and touching the edge against a tissue.

Gently place the membrane, protein side up, on a piece of plastic sheet. Cover the array with another piece of plastic sheet. Gently smooth out any air bubbles. Avoid using pressure on the membrane.

Detect signal directly from membrane using a Kodak X-OmatTM AR film developer. Expose the membranes for 40 Seconds. Then re-expose the film according to the intensity of signals. If the signals are too strong (background too high), reduce exposure time (e.g. 5 - 30 seconds). If the signals are too weak, increase exposure time (e.g. 5 - 20 minutes or overnight). Or re-incubate membranes overnight with 1X HRP-conjugated streptavidin, and repeat detection on the second day.

Statistics

Unpaired t-test and odds ratios with 95% confidence intervals was used for statistical analysis. Correlations were performed using Pearson Moment analysis also with 95% confidence intervals for determining statistical significance.

Results

In this study, using an immune-array assay, we found that cellular levels of phosphorylated GSK3a in children with autism were significantly higher than GSK3a levels of neurotypical controls (p = 0.005).

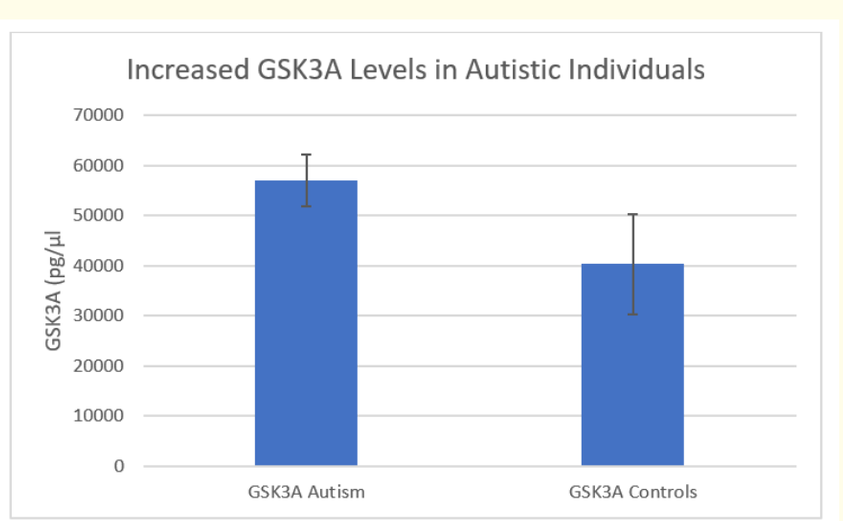

Figure 1: *Cellular levels of GSK3a in children with autism were +/-significantly higher (m = 56919 +/- 5152 pg/µl) than neurotypical controls (m = 40318 +/- 10020 pg/µl) (p = 0.005).*

Discussion

Among the potential candidate genes identified in ASD to date, those involved in Akt/mammalian target of rapamycin (mTOR) signaling and the downstream effects of this pathway are highly represented including *FMR1, PTEN, TSC1* and *TSC2* [10].

The Akt/mTOR pathway is involved in many cellular processes associated with ASD symptoms. For example, it is believed to be important in the process of learning and memory formation by augmenting long-term potentiation (LTP) of synapses [11].

Impaired inhibitory regulation of GSK3 in Fmr1 knockout mice may contribute to some socialization deficits and lithium treatment can ameliorate autism related socialization impairments. This suggests a role for GSK3 in social behaviors and implicates inhibition of GSK3 as a potential therapeutic approach to the curbing of autistic behaviors [12].

AKT deletion evokes a change in behavior reflecting the psychiatric appearance reminiscent of schizophrenia, anxiety and depression [13]. In the Akt intracellular pathway GSK3a is downstream from Akt and is inhibited by Akt. Akt deletion, therefore likely contributes to increased GSK3a levels. Our lab has previously found that Akt levels are decreased in individuals with autism [14] and these levels normalized after zinc therapy [15].

The mood stabilizer lithium has been used for the treatment of schizophrenia, depression, and other mental illnesses, and has been shown to inhibit the GSK3 signaling [16].

In humans GSK3a contributes to insulin regulation of glycogen synthesis by phosphorylating and inhibiting GYS1 activity and hence glycogen synthesis [17,18]. To our knowledge, there are no other reports of GSK3a associated with autism.

This research, therefore, supports the possibility that inhibitors of GSK3a may be helpful in altering aberrant behaviors seen in ASDs.

This study is limited by the number of autistic individuals and controls and should be repeated with larger sample sizes.

Conclusion

We found that GSK3a levels were significantly higher in individuals with autism. This suggests that therapies designed to lower GSK3a levels may normalize behaviors associated with autism.

Authors' Contributions

AR carried out the immunoassays, participated in the design of the study and performed the statistical analysis. AR, AM, JB conceived of the study and participated in its design and coordination. AM, JB provided clinical records of patients and controls. AR, AM, JB drafted and approved the final manuscript.

Competing Interests

The authors have no competing interests.

Acknowledgements

The author would like to acknowledge the financial support from the Autism Research Institute.

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Volume 9 Issue 2 February 2020