Increased MKK3 Correlates with GSK3A and P38 Concentrations in Children with Autism

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Abstract

GSK3A (Glycogen synthase kinase 3-alpha), MKK3 (mitogen-activated protein kinase kinase 3) and P38 (P38 mitogen-activated protein kinases) all function in glucose metabolism. GSK3 acting upon glycogen synthase. MKK3 catalyzes the concomitant phosphorylation of a threonine and a tyrosine residue in the MAP kinase P38 and P38 regulates the expression of GLUT (glucose transporter). In this study we measured the concentration of MKK3, P38 and Insulin in an autistic group and controls and compared these concentrations to GSK3A and the concentrations of each other. We found that, like GSK3A, MKK3 is significantly higher in the autistic group and MKK3 levels correlate significantly with the GSK3A levels. We also found that both MKK3 and GSK3A concentrations correlated significantly with P38 levels in these patients. These results support a role for glucose membrane transport dysfunction in the etiology of autism.

Keywords: GSK3A (Glycogen Synthase Kinase 3-Alpha); MKK3 (Mitogen-Activated Protein Kinase Kinase 3); P38 (P38 Mitogen-Activated Protein Kinases); GLUT (Glucose Transporter)

Introduction

Autism spectrum disorder (ASD) is characterized by persistent deficits in sociability and communication, as well as restricted and repetitive patterns of behavior and interests [1-3]. Mitochondria are known to be affected by many of the same endogenous and exogenous risk factors of ASD, such as toxins, drugs, immune activation, and metabolic disturbances [4] and mitochondrial dysfunction has also been observed in animal models of ASD induced by environmental risk factors such as maternal immune activation and exposure to propionic acid or valproic acid (VPA) [5,6].

GSK3A (Glycogen synthase kinase 3-alpha), MKK3 (mitogen-activated protein kinase kinase 3) and P38 (P38 mitogen-activated protein kinases) all function in glucose metabolism.

The protein kinases MKK3 belongs to the MAP kinase kinase family. It is activated by cytokines, as well as mitogenic and environmental stress [7]. It phosphorylates P38 by catalyzing a threonine and tyrosine residue [8,9]. This kinase can be activated by insulin and is necessary for the expression of glucose transporter [10], which is ultimately responsible for getting glucose across the cell membrane.

GSK3 is a Ser/Thr kinase implicated in the insulin signaling pathway, controlling glycogen metabolism by acting upon glycogen synthase, but it is now also recognized as a multifunctional kinase regulating an array of additional cellular functions [11]. There is much evidence to support that Akt inhibits both GSK3A and GSK3B. GSK3B becomes inactivated by phosphorylation in a PKA (cAMP-protein kinase

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A)-dependent manner, which in turn leads to increased activity of the MKK3/6-p38 MAPK signaling module [12], but there is evidence to support that while Akt, inhibits both GSK3A and GSK3B, p38 MAPK does not phosphorylate GSK3A [13]. The role of insulin is critical. Insulin stimulates glucose uptake and activates glycogen synthase which promotes the conversion of glucose to glycogen. Insulin activates glycogen synthase by inducing its dephosphorylation at a cluster of C-terminal residues (Ser641, Ser645, Ser649 and Ser653), which are phosphorylated by GSK3A and GSK3B [14].

P38s are a class of mitogen activated protein kinases (MAPKs) that are responsive to stress stimuli, including cytokines, UV radiation, and heat shock. They are involved in cell death and differentiation [8]. P38 MAPKs are involved in regulating the expression of GLUT (glucose uptake) proteins (especially GLUT 1 and 4) [15,16]. In fact, inhibition of p38 MAPK altered the expression levels of Glut1 and Glut4, and decreased glucose uptake [17].

In this study we measured the concentration of MKK3, P38 and Insulin in an autistic group and controls, and compared these concentrations to GSK3A and the concentrations of each other. We hypothesized that high concentrations of GSK3A would correlate with both MKK3 and P38 concentrations in the autistic group.

Materials and Methods

Subjects

Cellular phosphorylated MKK3, Insulin and P38 were measured in 26 autistic children and 12 age and gender similar neurotypical, controls.

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)* over a two year period. All HRI patients in this study were randomly chosen from all patients who volunteered. The autistic individuals were diagnosed using The Autism Diagnostic Interview-Revised - ADI-R and met the DSM-IV criteria.

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

Cellular phosphorylated concentrations were measured using an Immuno-array assay described below.

Buffy coat white blood cells

All experimental and control cells were obtained from whole blood using centrifugation and were all treated identically then refrigerated (4°C). Plasma and buffy coat samples were frozen at -70°C and used for ELISAs and Immunoassay analysis.

Immuno-array assays

Immuno-arrays were performed by RayBiotech, Inc, Peachtree Corners, GA 30092 and described previously [18]. Cellular phosphorylated MKK3, Insulin and P38 were measured in both the autistic and control groups.

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

We found that dual specificity mitogen-activated protein kinase kinase 3 (MAP2K3) or (MKK3) was significantly higher in the autistic population compared to controls (Figure 1).

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

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Figure 1: We measured phosphorylated MKK3 in WBCs of 27 individuals with autism (22 males mean age 10.7 years) and 12 neurotypical controls (8 males mean age 12.2 years) and found phosphorylated MKK3 concentration to be 65382 +/- 3880.6 pg/µl in the autistic group and 48771.5 +/- 3 4261.2 ng/µl in the control group (p = < 0.001).

We found that MKK3 levels correlated significantly with P38 levels in this group (Figure 2).

Figure 2: MKK3 levels correlate significantly with P38 levels (r = 0.36; p = 0.03).

We previously reported that GSK3A levels were significantly higher in the autistic group. We also found that these GSK3A levels correlated significantly with P38 levels in these autistic individuals (Figure 3).

![Figure 3: GSK3A levels correlate significantly with P38 in this autistic group (r = 0.40; p = 0.03).](image)

GSK3A levels also correlate with the MKK3 levels in this autistic group (Figure 4).

![Figure 4: GSK3A levels correlate significantly with MKK3 levels in the autistic group (r = 0.5; p = 0.008).](image)
We did not find that Insulin concentrations in the autistic group were different from controls (p = 0.76), and we did not find that P38 levels were different in the autistic group compared to controls (p = 0.13).

**Discussion**

We previously reported that GSK3A levels were significantly higher in this group of autistic individuals [18]. The data presented here shows a strong correlation between GSK3A and MKK3 in individuals with autism, as well as each having a significant correlation with p38 suggests a role for dysfunctional glucose transport in the etiology of autism. The fact that we did not find Insulin or P38 levels to be different in the autistic group, suggests that the dysfunction lies in the presence of excess GSK3A and MKK3 in individuals with autism.

**Conclusion**

Our data, which shows significant correlations between GSK3A, MKK3 and P38, support a role for glucose membrane transport dysfunction in the etiology of autism.

**Acknowledgement**

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