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Evidence of reactive astrocytes but not peripheral immune system activation in a mouse model of Fragile X syndrome

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ABSTRACT

Fragile X syndrome (FXS) is the most common form of inherited mental retardation and is one of the few known genetic causes of autism. FXS results from the loss of Fmr1 gene function; thus, Fmr1 knockout mice provide a model to study impairments associated with FXS and autism and to test potential therapeutic interventions. The inhibitory serine phosphorylation of glycogen synthase kinase-3 (GSK3) is lower in brain regions of Fmr1 knockout mice than wild-type mice and the GSK3 inhibitor lithium rescues several behavioral impairments in Fmr1 knockout mice. Therefore, we examined if the serine phosphorylation of GSK3 in Fmr1 knockout mice also was altered outside the brain and if administration of lithium ameliorated the macroorchidism phenotype. Additionally, since GSK3 regulates numerous functions of the immune system and immune alterations have been associated with autism, we tested if immune function is altered in Fmr1 knockout mice. The inhibitory serine phosphorylation of GSK3 was significantly lower in the testis and liver of Fmr1 knockout mice than wild-type mice, and chronic lithium treatment reduced macroorchidism in Fmr1 knockout mice. No alterations in peripheral immune function were identified in Fmr1 knockout mice. However, examination of glia, the immune cells of the brain, revealed reactive astrocytes in several brain regions of Fmr1 knockout mice and treatment with lithium reduced this in the striatum and cerebellum. These results provide further evidence of the involvement of dysregulated GSK3 in FXS, and demonstrate that lithium administration reduces macroorchidism and reactive astrocytes in Fmr1 knockout mice.
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1. Introduction

Fragile X syndrome (FXS) is caused by functional loss of the *fragile X mental retardation 1* (*Fmr1*) gene on the X chromosome, resulting in lack of the gene product, fragile X mental retardation protein (FMRP), an RNA binding protein that regulates translation [1,2]. FXS is the most common cause of inherited mental retardation and is the first identified autism-related gene because FXS patients have many characteristics commonly associated with autism spectrum disorders (ASDs), such as developmental delays, communication impairments, and anxiety [2–9]. These conditions are modeled in *Fmr1* knockout mice [10] that display several phenotypes of FXS and ASDs [11–20]. Thus, Fmr1 knockout mice provide an important animal model to study characteristics of FXS as well as autistic traits, and to test potential therapeutic interventions. Studies of pharmacological interventions in *Fmr1* knockout mice have identified therapeutic effects of antagonists of metabotropic glutamate receptor 5 (mGluR5) [1,21] and of lithium [13,14,20], an inhibitor of glycogen synthase kinase-3 (GSK3) [22,23].

FXS and autism are generally considered to be neuronal disorders because of the predominant behavioral and cognitive abnormalities. However, neuronal function can be modified by many types of cells, such as glia and immune cells, and there is substantial evidence that neuronal dysfunction can be caused by neuroinflammation [24,25]. Notably, treatment with minocycline, a tetracycline antibiotic that exerts anti-inflammatory effects, rescued some FXS-related impairments in Fmr1 knockout mice [26]. Neuroinflammation occurs in response to brain injury, degenerating cells, insults, or infection, as well as psychological stress, and is mediated by the immune resident cells in the brain, microglia and astrocytes, as well as by infiltration of peripheral immune cells [24,25,27]. Although a role for immune responses and associated inflammation in autism is controversial [28–30], there is some evidence of activated glia in autism [28,31–33] and altered plasma cytokines associated with FXS [34]. However, little is known about the immune system in *Fmr1* knockout mice.

GSK3 represents a potential link between FXS and inflammation. GSK3 is a partially constitutively active serine/threonine kinase that is predominantly controlled by inhibitory serine phosphorylation of its two isoforms, serine-9 in GSK3β and serine-21 in GSK3α [35–37]. Recently, we found that the inhibitory serine phosphorylation of both GSK3 isoforms is decreased in several brain regions of Fmr1 knockout mice compared with wild-type mice [13,20]. GSK3 has many regulatory influences on the immune system [38], particularly promoting inflammation both in the periphery [39] and in glia

Abbreviations: ASDs, autism spectrum disorders; FXS, Fragile X syndrome; *Fmr1*, *fragile X mental retardation 1*; FRMP, fragile X mental retardation protein; GFAP, glial fibrillary acidic protein; GSK3, glycogen synthase kinase-3; IFNγ, interferon-γ; IL-6, interleukin-6; LPS, lipopolysaccharide; mGluR5, metabotropic glutamate receptor 5; TNFα, tumor necrosis factor-α

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[40,41]. Additionally, administration of GSK3 inhibitors ameliorated a number of immune-mediated conditions in animal models, such as septic shock ([39], reviewed in [42]). The present study extended the examination of GSK3 serine phosphorylation to peripheral tissues, and tested if the hyperactive GSK3 in *Fmr1* knockout mice was associated with changes in the peripheral or central immune systems because GSK3 has widespread influences on immune function [38].

2. Materials and methods

2.1. Animals and in vivo tests

This study used adult, male C57Bl/6J littermates, ~3 months of age, with or without a disruption of the *Fmr1* gene (originally kindly provided by Dr. W. Greenough, University of Illinois). The *Fmr1* knockout mice were generated by breeding male C57BL/6J hemizygous *Fmr1* knockout mice and female C57BL/6J heterozygous *Fmr1* knockout mice to generate male homozygous *Fmr1* knockout mice and wild-type littermates. Genotype was confirmed by PCR using the Jackson Laboratory protocol for genotyping *Fmr1* mice. Mice were given water and food *ad libitum*. Lithium was administered in pelleted food containing 0.2% lithium carbonate (Harlan-Teklad) and mice were given 0.9% saline in addition to water to prevent hyponatremia. Protein-free *E. coli* (K235) LPS was prepared as described [39]. All mice were housed and treated in accordance with National Institutes of Health and the University of Alabama at Birmingham Institutional Animal Care and Use Committee guidelines.

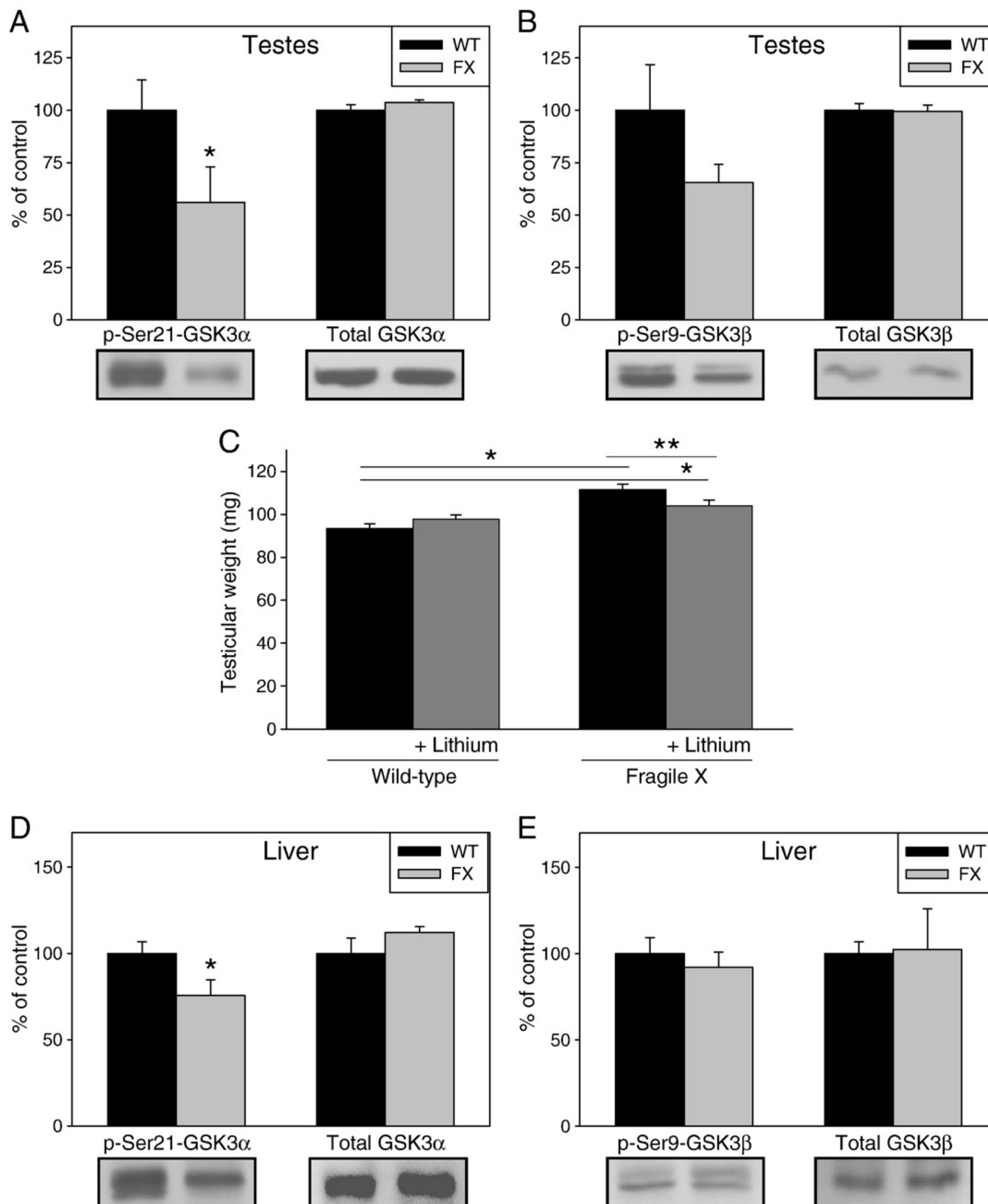

Fig. 1. Reduced serine phosphorylation of GSK3α in the testis and liver of FX mice. Immunoblots of protein extracts from testis (A and B) or liver (D and E) of *Fmr1* knockout (FX) or wild-type (WT) mice were probed with antibodies to (A and D) phospho-Ser21-GSK3α or total GSK3α, and (B and E) phospho-Ser9-GSK3β or total GSK3β. Immunoblots were quantified by densitometry and values shown are the percentage of wild-type values. Means ± SEM; $n = 5$ mice per group; *$p < 0.05$ compared to wild-type values (Student's *t*-test). (C) Testicular weights were measured from *Fmr1* knockout mice and wild-type mice with and without lithium treatment between the ages of 12–16 weeks of age and compared to untreated littermates. Means ± SEM; $n = 10$ mice per group; *$p < 0.05$ compared to wild-type, **$p < 0.05$ compared to untreated (one-way ANOVA).

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