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Insulin-Like Growth Factor Binding Protein-3 Deficiency Leads to Behavior Impairment with Monoaminergic and Synaptic Dysfunction

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Insulin-like growth factor binding protein (IGFBP)-3 regulates IGF bioactivity, induces apoptosis, and inhibits cell growth independent of IGFs, but the functional role of IGFBP3 in the brain is not clear. In the present study, we revealed the effect of IGFBP3 on the brain by characterizing the phenotype of *Igfbp3*-null mice. Compared with wild-type mice, *Igfbp3*-null mice had significantly decreased IGF-1 content in the brain but no change in weights of brain and body. In *Igfbp3*-null mice, the number of dendritic spines was significantly reduced, and the dendritic diameter was thickening. In addition, in *Igfbp3*-null mice, a decrease in phosphorylated Akt and ERK1/2 significantly reduced PSD-95 expression, and GAD65/67 expression was significantly decreased. These results indicate that IGFBP3 deficiency impairs neuronal structure and signaling. In behavioral studies, *Igfbp3*-null mice were hyperactive, and a Y-maze alternation test revealed impaired spatial working memory but no anxiety-like behavior. Monoaminergic analysis using high-performance liquid chromatography indicated that *Igfbp3*-null mice had lower levels of dopamine and serotonin compared with wild-type mice, suggesting an abnormal monoaminergic neurotransmission. In conclusion, our studies found that the deletion of IGFBP3 results in behavioral impairments that are associated with abnormal synaptic function and monoaminergic neurotransmission, which helps to characterize the critical role of IGFBP3 in the brain. *(Am J Pathol 2017, 187: 390–400; http://dx.doi.org/10.1016/j.ajpath.2016.10.011)*

Insulin-like growth factor (IGF)-1 plays an essential role in the development and growth of the brain.[1–4] The effects of IGF-1 are modulated by insulin-like growth factor binding proteins (IGFBPs), which can prolong IGF-1 half-life, thereby regulating the availability and bioactivity of IGF-1.[5] A total of six high-affinity binding proteins have been identified to date as IGFBP1 through IGFBP6. Among them, IGFBP3 is a major IGFBP isoform that binds 90% of circulating IGF-1 to control the actions of IGF-1 by regulating its distribution. IGFBP3 also regulates IGF-1 activity by interacting with its receptor, the IGF-1 receptor.[6] The effect of IGFBP3 on IGF-1 can be stimulatory or inhibitory, depending on the expression of proteases, which can degrade IGFBP3 and release IGF-1 from the IGF-1–IGFBP3 complex.[5,6] In addition, IGFBP3 has highly important roles in cell growth and brain development. IGFBP3 stimulates growth in MCF-10A human breast epithelial cells via increased epidermal growth factor receptor phosphorylation and activation of p44/42 and the p38 mitogen-activated protein kinase signaling pathway.[7] Our previous study indicated that in the brain, IGFBP3 expression is highest during the embryonic period, decreases during the postnatal period, and is lowest in the adult.[8] High concentrations of IGF-1 and IGFBP3 were also observed in the cerebral spinal fluid of children younger than 6 months, suggesting that these proteins might participate in the active processes of myelination and synapse formation in the developing nervous system.[2] In the developing brain, IGF-1 is also important for neuronal proliferation, maturation, survival, and growth.[9–12] IGF-1

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deficiency in the developing brain impairs neurogenesis, synaptogenesis, and myelination, resulting in neuronal death.[13] IGFBP3 specifically regulates the IGF-1–mediated neural progenitor cell proliferation via down-regulation of phosphorylated Akt and cyclin D1.[14] The *Igfbp3* transgenic mouse has a decreased brain weight and a reduction in neural proliferation in the periventricular zone, indicating a potential role for IGFBP3 in brain growth and neurogenesis.[15,16]

Moreover, *IGFBP3* is downstream of *MECP2*. *MECP2*, the causative gene of the neurodevelopmental disorder Rett syndrome, directly binds to the promoter of *IGFBP3* and regulates its expression. *MECP2* deficiency in patients and mice with Rett syndrome causes an increased expression of IGFBP3.[8] On the other hand, *MECP2* duplication syndrome and *Mecp2*-overexpressed mice have the similar phenotypes of Rett syndrome, such as mental retardation and autistic behavior.[17,18] We can speculate IGFBP3 dose-dependent psychomotor phenotypes, although the IGFBP3 effect caused by excess MECP2 is not yet known. IGFBP3 functional characterization in the brain may be of benefit to understand the pathogenesis of some neurologic diseases, such as *MECP2*-related disorders. In the present study, we clarified the effect of IGFBP3 on the brain by investigating the phenotypic character of *Igfbp3*-null mice.

Materials and Methods

Preparation of *Igfbp3*-Null Mice

Cryopreserved mouse sperm with the *Igfbp3*$^{tm1(KOMP)Vlcg}$ allele of the C57BL/6N strain completely replaced with

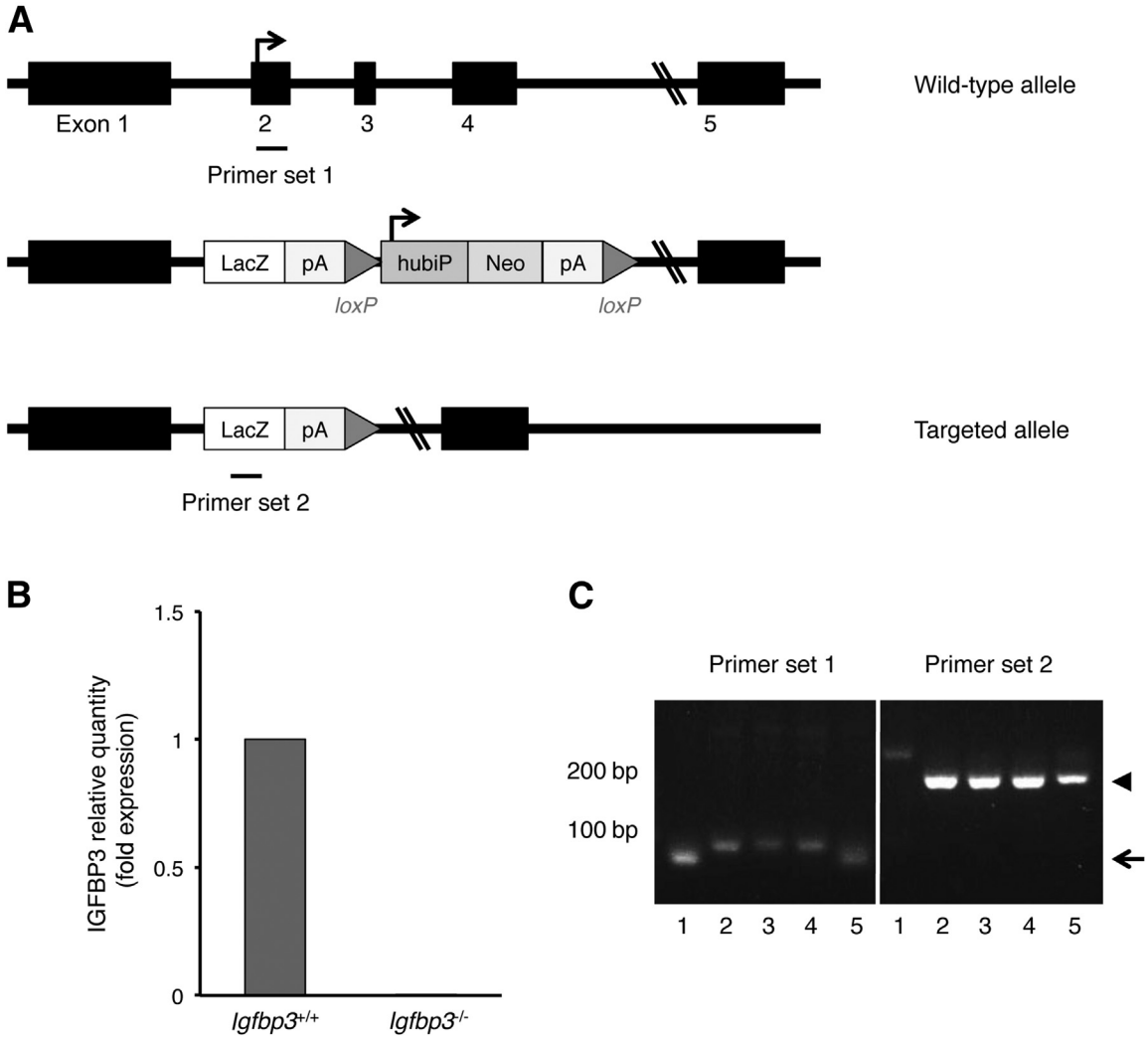

Figure 1 Construction and identification of insulin-like growth factor binding protein-3 (*Igfbp3*)–targeted alleles for generation of *Igfbp3*-null mice. **A:** Transcription start sites for *Igfbp3* are shown before exon 2 disruption. LoxP sites are denoted as triangles. Relative locations for PCR primer set are indicated. Crossing of *Igfbp3* conditional mice with Nestin-Cre deleter mice results in the excision of the transcriptional start site of *Igfbp3* and the creation of the *Igfbp3*-null allele. **B:** Quantitative PCR of *Igfbp3* indicates no expression in the targeted mice. **C:** *Igfbp3* wild-type and targeted alleles as differentiated using two sets of PCR. Primer set 1 shows the wild-type allele (**arrow**) as the lower bands of lane 1 and 5. Primer set 2 shows the targeted alleles (**arrowhead**) of lane 2, 3, 4, and 5. hubiP, promoter from the human ubiqutin C gene; LacZ, β-galactosidase cording sequence from the *E. coli* LacZ gene; Neo, neomycin; pA, polyadenylation signal.