Molecular Autism

SHORT REPORT   **Open Access**

Insulin-like growth factor-1 rescues synaptic and motor deficits in a mouse model of autism and developmental delay

Ozlem Bozdagi[1,2], Teresa Tavassoli[1,2] and Joseph D Buxbaum[1,2,3,4,5,6*]

Abstract

Background: Haploinsufficiency of *SHANK3*, due to either hemizygous gene deletion (termed 22q13 deletion syndrome or Phelan-McDermid syndrome) or to gene mutation, accounts for about 0.5% of the cases of autism spectrum disorder (ASD) and/or developmental delay, and there is evidence for a wider role for SHANK3 and glutamate signaling abnormalities in ASD and related conditions. Therapeutic approaches that reverse deficits in *SHANK3*-haploinsufficiency may therefore be broadly beneficial in ASD and in developmental delay.

Findings: We observed that daily intraperitoneal injections of human insulin-like growth factor 1 (IGF-1) over a 2-week period reversed deficits in hippocampal α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) signaling, long-term potentiation (LTP), and motor performance that we had previously reported in *Shank3*-deficient mice. Positive effects were observed with an IGF-1 peptide derivative as well.

Conclusions: We observed significant beneficial effects of IGF-1 in a mouse model of ASD and of developmental delay. Studies in mouse and human neuronal models of Rett syndrome also show benefits with IGF-1, raising the possibility that this compound may have benefits broadly in ASD and related conditions, even with differing molecular etiology. Given the extensive safety data for IGF-1 in children with short stature due to primary IGF-1 deficiency, IGF-1 is an attractive candidate for controlled clinical trials in *SHANK3*-deficiency and in ASD.

Keywords: Pharmacotherapy, Personalized medicine, Individualized medicine, 22q13 deletion syndrome, Phelan-McDermid syndrome

Findings

SHANK proteins are master scaffolding proteins of the postsynaptic density (PSD) of glutamatergic synapses and are critical determinants of glutamate transmission and synaptic spine dynamics [1]. Loss of one functional copy of *SHANK3* accounts for about 0.5% of the cases of autism spectrum disorder (ASD) and/or developmental delay [2], and there is likely a wider role for SHANK3 and glutamate signaling abnormalities in ASD and related neurodevelopmental disorders [3,4]. Targeted disruption of the full-length form of *Shank3* (sometimes called Shank3a) in mice leads to deficits in hippocampal

AMPA signaling, long-term potentiation (LTP), and motor performance [5-7], likely reflecting delayed synaptic development as shown by the reduced AMPA signaling [5] and decreased levels of PSD-95 (unpublished results). IGF-1, which enters the central nervous system (CNS) through an interaction with lipoprotein-related receptor 1 (LRP1) [8], has multiple effects on neuronal and synaptic development and function, including effects on neurogenesis and synaptogenesis [9]. IGF-1 treatment also enhances the PSD as measured both by PSD length and by levels of PSD-95 [10,11]. Recombinant human IGF-1 has substantial human safety data and is approved for use in children, making IGF-1 an attractive compound for evaluation in neurodevelopmental disorders.

To investigate whether IGF-1 could reverse deficits in a preclinical model of *SHANK3*-haploinsufficiency, we made use of a mouse with hemizygous loss of full-length *Shank3* due to targeted disruption of the ankyrin repeat

---

* Correspondence: joseph.buxbaum@mssm.edu
[1]Seaver Autism Center for Research and Treatment, Icahn School of Medicine at Mount Sinai, New York, NY, USA
[2]Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York, NY, USA
Full list of author information is available at the end of the article

domain (ARD) [5]. This isoform has been directly implicated in ASD, language delay, and intellectual disability (ID), as there exist disruptive *de novo* point mutations in ARD in patients with ASD and ID [12,13]. In all studies, we compared heterozygous mice with wild-type littermates using heterozygote × heterozygote mating. Consistent with previous results from our group [5], LTP induced by high-frequency stimulation was reduced in the heterozygous mice compared to wild-type littermates in the current experiments (Figures 1a and 2a) (for
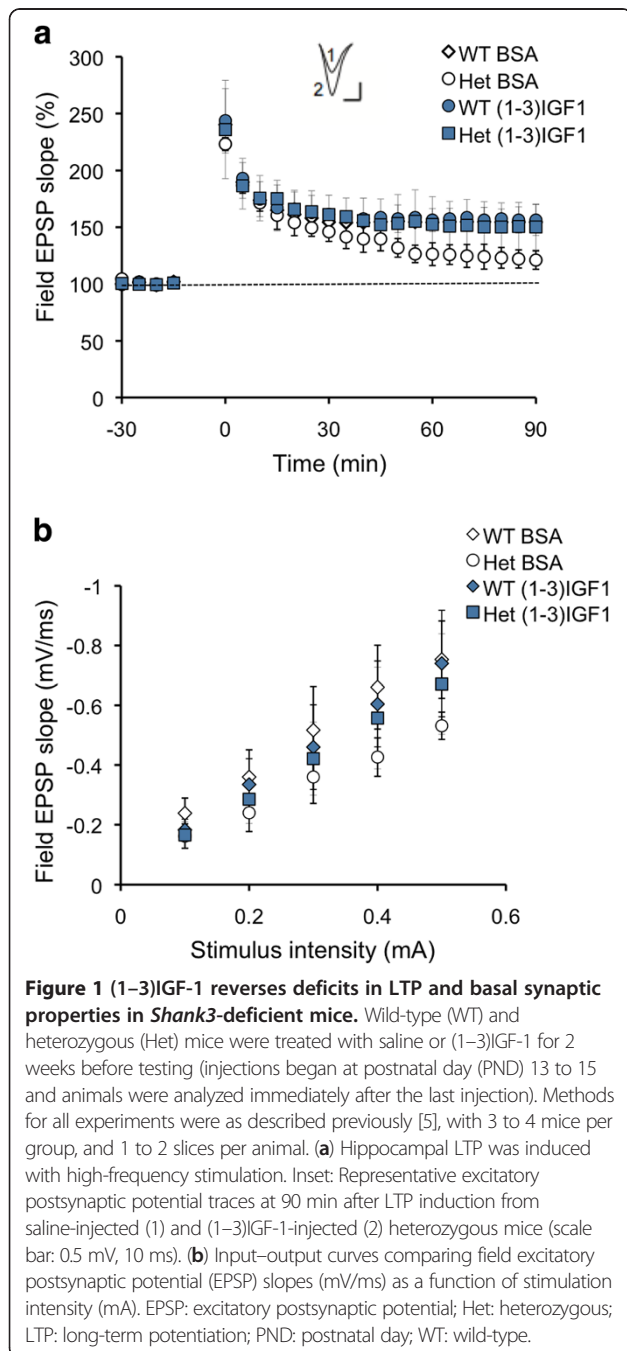
example, in Figure 2a, repeated measures ANOVA was used for analysis of the last five time points, $F(1,6) = 33.71$, $P = 0.001$).
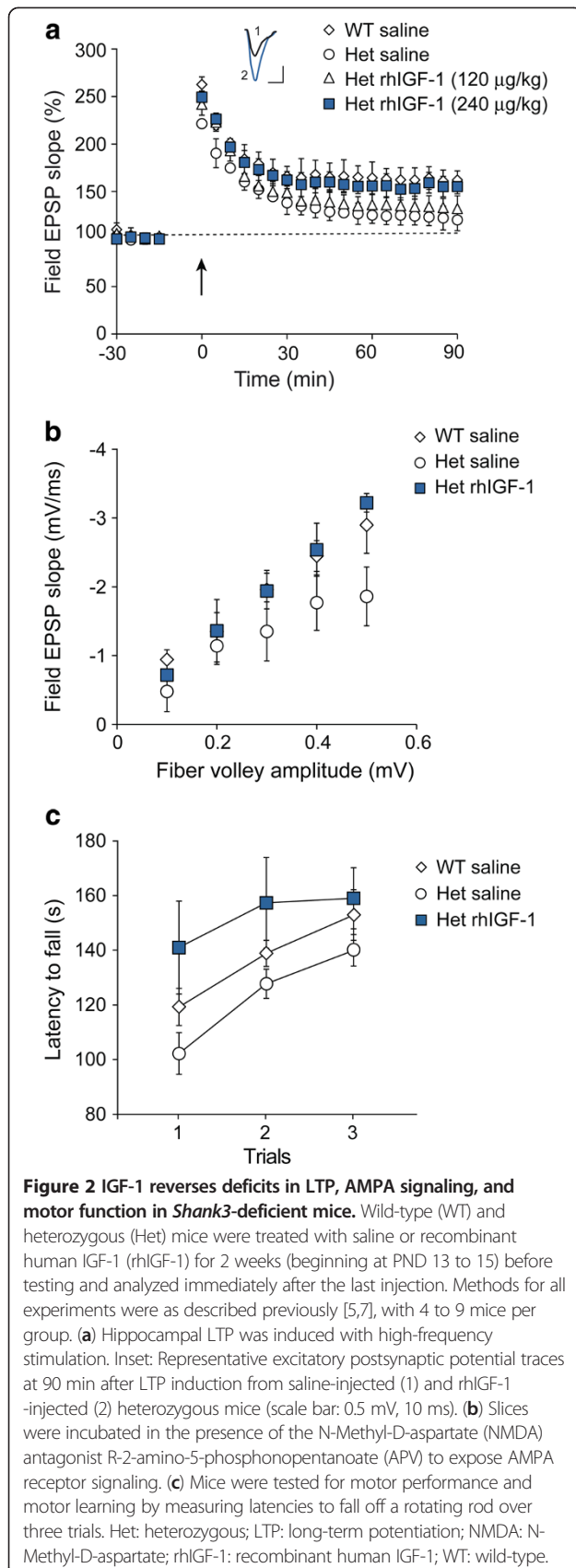
We first tested an active peptide derivative of IGF-1, (1–3)IGF-1, which has been shown to cross the blood–brain barrier and rescue Rett syndrome symptoms in *Mecp2*-deficient mice [11]. We observed that intraperitoneal injections at 10 μg/g/day for 2 weeks restored normal hippocampal LTP in *Shank3* heterozygous mice but had no effect on wild-type mice (repeated measures ANOVA was used to analyze the last five time points, $F(3,11) = 6.07$, $P = 0.011$). In *post hoc* analyses, vehicle-treated heterozygous mice were significantly different from wild-type mice ($P = 0.004$), while (1–3)IGF-1 treated heterozygous mice were not ($P = 0.66$). Furthermore, peptide treatment reversed deficits in the mean slope of the input/output (I/O) function (Figure 1b) (one-way ANOVA, $F(3,19) = 4.25$, $P = 0.02$). Vehicle-treated heterozygous mice were significantly different from vehicle-treated wild-type mice ($P = 0.001$), while (1–3)IGF-1 treated heterozygous mice were not different from vehicle-treated wild-type ($P = 0.89$), and there were no significant differences between vehicle-treated wild-type mice and wild-type mice treated with IGF-1 ($P = 0.812$), so further studies used just three conditions.

We next administered full-length IGF-1, like that used in children with short stature due to primary IGF-1 deficiency, by intraperitoneal injection at 240 μg/kg/day, starting at PND 13 to 15 and continuing for 2 weeks (Figure 2a). This dose, chosen because it represents the maximum dose according to the current FDA label for IGF-1, was effective in rescuing deficits in LTP (repeated measures ANOVA was used to analyze the last five time points, comparing heterozygous mice with and without IGF-1, $F(1,6)=28.04$, $P=0.002$). In contrast, lower dose IGF-1 (120 μg/kg/day for 2 weeks) was associated with more modest reversal of deficits in LTP (for the last five time points: $F(1,6)=2.62$, $P=0.012$), showing a dose–response effect and providing preclinical dosing information.

Specific deficits in the glutamate AMPA receptor component of neural signaling [5] were also reversed by a 2-week treatment of 240 μg/kg/day full-length IGF-1 (Figure 2b). The mean slope of the I/O function was 0.50 ± 0.14 for wild-type, 0.34 ± 0.06 for *Shank3* heterozygotes and 0.61 ± 0.059 for IGF-1 injected heterozygotes (one-way ANOVA, $F(2,9) = 8.62$, $P = 0.008$). In *post hoc* analyses, vehicle-treated heterozygous mice were significantly different from vehicle-treated wild-type mice ($P = 0.039$), while IGF-1-treated heterozygous mice were not different from vehicle-treated wild-type mice ($P = 0.12$).

Patients with *SHANK3*-haploinsufficiency frequently present with hypotonia and motor deficits of variable severity, and we have observed subtle motor deficits in *Shank3*-heterozygous mice [5,7]. After treating male

Figure 1 (1–3)IGF-1 reverses deficits in LTP and basal synaptic properties in *Shank3*-deficient mice. Wild-type (WT) and heterozygous (Het) mice were treated with saline or (1–3)IGF-1 for 2 weeks before testing (injections began at postnatal day (PND) 13 to 15 and animals were analyzed immediately after the last injection). Methods for all experiments were as described previously [5], with 3 to 4 mice per group, and 1 to 2 slices per animal. (**a**) Hippocampal LTP was induced with high-frequency stimulation. Inset: Representative excitatory postsynaptic potential traces at 90 min after LTP induction from saline-injected (1) and (1–3)IGF-1-injected (2) heterozygous mice (scale bar: 0.5 mV, 10 ms). (**b**) Input–output curves comparing field excitatory postsynaptic potential (EPSP) slopes (mV/ms) as a function of stimulation intensity (mA). EPSP: excitatory postsynaptic potential; Het: heterozygous; LTP: long-term potentiation; PND: postnatal day; WT: wild-type.

Figure 2 IGF-1 reverses deficits in LTP, AMPA signaling, and motor function in *Shank3*-deficient mice. Wild-type (WT) and heterozygous (Het) mice were treated with saline or recombinant human IGF-1 (rhIGF-1) for 2 weeks (beginning at PND 13 to 15) before testing and analyzed immediately after the last injection. Methods for all experiments were as described previously [5,7], with 4 to 9 mice per group. (**a**) Hippocampal LTP was induced with high-frequency stimulation. Inset: Representative excitatory postsynaptic potential traces at 90 min after LTP induction from saline-injected (1) and rhIGF-1-injected (2) heterozygous mice (scale bar: 0.5 mV, 10 ms). (**b**) Slices were incubated in the presence of the N-Methyl-D-aspartate (NMDA) antagonist R-2-amino-5-phosphonopentanoate (APV) to expose AMPA receptor signaling. (**c**) Mice were tested for motor performance and motor learning by measuring latencies to fall off a rotating rod over three trials. Het: heterozygous; LTP: long-term potentiation; NMDA: N-Methyl-D-aspartate; rhIGF-1: recombinant human IGF-1; WT: wild-type.

heterozygous mice with 240 μg/kg/day for 2 weeks, we observed enhanced motor performance following treatment (Figure 2c) ($F(2,20) = 3.98$, $P = 0.03$).

Our results provide preclinical evidence for a beneficial role for IGF-1 in *SHANK3*-haploinsufficiency. Moreover, as there is emerging evidence that the SHANK3 pathway and the postsynaptic density, which it helps sculpt, play a role in many neurodevelopmental disorders, as evidenced by large-scale genetic, proteomic, and gene expression studies [3,4,14], therapies for *SHANK3* deficiency and synaptic development represent important targets that could have a widespread positive impact for neurodevelopmental disorders. The beneficial effects of IGF-1 in models of Rett syndrome [11,15] are consistent with this hypothesis.

There are some limitations to the current study. We, and others working with similar *Shank3*-deficient mice, see only limited behavioral abnormalities, with none except for rotarod deficits at the ages where we carried out the IGF-1 treatments and electrophysiological studies. For this reason, the phenotypes we measure are somewhat limited. In addition, a mechanistic understanding of the neuronal effects of IGF-1 has eluded the neuroscience community and we cannot precisely explain how IGF-1 reverses the deficits observed. We do hope, however, that our findings, together with those on IGF-1 in Rett syndrome models, may help spur further research on the action of IGF-1 in the CNS. We did not see any effect produced by the (1–3)IGF-1 peptide on control animals but we did not test the effects of full-length IGF-1 on wild-type mice. There could be enhanced LTP or rotarod performance in control animals following treatment with full-length IGF-1. Many drugs have effects on both healthy and non-healthy individuals and there is hence no *a priori* reason to assume that IGF-1 has no effect on control animals. In fact, given the positive effects of IGF-1 in Rett syndrome models it is likely that IGF-1 has a general effect on CNS function, which might also be observed in controls.

In summary, our results show that IGF-1, approved for use in children, can lead to functional improvements in a mouse model of ASD and developmental delay, representing an important preclinical step towards novel therapeutics. Clinical trials of IGF-1 in *SHANK3*-deficient individuals and in ASD are now underway (ClinicalTrials.gov Identifier NCT01525901).

Abbreviations
ARD: Ankyrin repeat domain; ASD: Autism spectrum disorder; AMPA: α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; CNS: Central nervous system; EPSP: Excitatory postsynaptic potential; Het: Heterozygous; ID: Intellectual disability; IGF-1: Insulin-like growth factor 1; I/O: Input/output; LRP1: Lipoprotein-related receptor 1; LTP: Long-term potentiation; NMDA: N-Methyl-D-aspartate; PND: Postnatal day; PSD: Postsynaptic density; rhIGF-1: Recombinant human IGF-1; WT: Wild-type.

Author details
[1]Seaver Autism Center for Research and Treatment, Icahn School of Medicine at Mount Sinai, New York, NY, USA. [2]Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York, NY, USA. [3]Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY, USA. [4]Department of Genetics and Genomics Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA. [5]Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA. [6]Mindich Child Health and Development Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA.

References
1. Boeckers TM, Bockmann J, Kreutz MR, Gundelfinger ED: **ProSAP/Shank proteins - a family of higher order organizing molecules of the postsynaptic density with an emerging role in human neurological disease.** *J Neurochem* 2002, **81**(5):903–910.
2. Abrahams BS, Geschwind DH: **Advances in autism genetics: on the threshold of a new neurobiology.** *Nat Rev Genet* 2008, **9**(5):341–355.
3. Sakai Y, Shaw CA, Dawson BC, Dugas DV, Al-Mohtaseb Z, Hill DE, *et al*: **Protein interactome reveals converging molecular pathways among autism disorders.** *Sci Transl Med* 2011, **3**(86):86ra49.
4. Darnell JC, Van Driesche SJ, Zhang C, Hung KY, Mele A, Fraser CE, *et al*: **FMRP stalls ribosomal translocation on mRNAs linked to synaptic function and autism.** *Cell* 2011, **146**(2):247–261.
5. Bozdagi O, Sakurai T, Papapetrou D, Wang X, Dickstein DL, Takahashi N, *et al*: **Haploinsufficiency of the autism-associated Shank3 gene leads to deficits in synaptic function, social interaction, and social communication.** *Mol Aut* 2010, **1**(1):15.
6. Wang X, McCoy PA, Rodriguiz RM, Pan Y, Je HS, Roberts AC, *et al*: **Synaptic dysfunction and abnormal behaviors in mice lacking major isoforms of Shank3.** *Hum Mol Genet* 2011, **20**(15):3093–3108.
7. Yang M, Bozdagi O, Scattoni ML, Wohr M, Roullet FI, Katz AM, *et al*: **Reduced excitatory neurotransmission and mild autism-relevant phenotypes in adolescent Shank3 null mutant mice.** *J Neurosci* 2012, **32**(19):6525–6541.
8. Nishijima T, Piriz J, Duflot S, Fernandez AM, Gaitan G, Gomez-Pinedo U, *et al*: **Neuronal activity drives localized blood–brain-barrier transport of serum insulin-like growth factor-I into the CNS.** *Neuron* 2010, **67**(5):834–846.
9. O'Kusky JR, Ye P, D'Ercole AJ: **Insulin-like growth factor-I promotes neurogenesis and synaptogenesis in the hippocampal dentate gyrus during postnatal development.** *J Neurosci* 2000, **20**(22):8435–8442.
10. Shi L, Linville MC, Tucker EW, Sonntag WE, Brunso-Bechtold JK: **Differential effects of aging and insulin-like growth factor-1 on synapses in CA1 of rat hippocampus.** *Cereb Cortex* 2005, **15**(5):571–577.
11. Tropea D, Giacometti E, Wilson NR, Beard C, McCurry C, Fu DD, *et al*: **Partial reversal of Rett syndrome-like symptoms in MeCP2 mutant mice.** *Proc Natl Acad Sci USA* 2009, **106**(6):2029–2034.
12. Moessner R, Marshall CR, Sutcliffe JS, Skaug J, Pinto D, Vincent J, *et al*: **Contribution of SHANK3 mutations to autism spectrum disorder.** *Am J Hum Genet* 2007, **81**(6):1289–1297.
13. Hamdan FF, Gauthier J, Araki Y, Lin DT, Yoshizawa Y, Higashi K, *et al*: **Excess of** *de novo* **deleterious mutations in genes associated with glutamatergic systems in nonsyndromic intellectual disability.** *Am J Hum Genet* 2011, **88**(3):306–316.
14. Laumonnier F, Cuthbert PC, Grant SG: **The role of neuronal complexes in human X-linked brain diseases.** *Am J Hum Genet* 2007, **80**(2):205–220.
15. Marchetto MC, Carromeu C, Acab A, Yu D, Yeo GW, Mu Y, *et al*: **A model for neural development and treatment of Rett syndrome using human induced pluripotent stem cells.** *Cell* 2010, **143**(4):527–539.