The Effect of Growth Hormone on mRNA Expression of the GABA\textsubscript{B1} Receptor Subunit and GH/IGF Axis Genes in a Mouse Model of Prader-Willi Syndrome

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\textbf{Purpose:} Growth hormone (GH) therapy substantially improves several cognitive functions in PWS. However, the molecular mechanisms underlying the beneficial effects of GH on cognition remain unclear in PWS. In this study, we investigated the effects of recombinant human GH on the gene expression of GABAB receptor subunits and GH/insulin-like growth factor (IGF) axis genes in the brain regions of PWS-mimicking mice (\textit{Snord116del}).

\textbf{Methods:} \textit{Snord116del} mice were injected subcutaneously with 1.0 mg/kg GH or saline, once daily for 7 days. The collected brain tissues were analyzed for mRNA content using quantitative PCR (qPCR) in the cerebellum, hippocampus, and cerebral cortex.

\textbf{Results:} GH increased the mRNA expression level of the GABA\textsubscript{B1} receptor subunit (GABA\textsubscript{B1R}) and IGF-1R in the cerebellum. Furthermore, a significant positive correlation was found between the level of GABA\textsubscript{B1R} mRNA and the expression of the IGF-1R transcript. GH also induced an increase in the mRNA expression of IGF-2 and IGF-2R in the cerebellum.

\textbf{Conclusion:} These data indicate that GH may provide beneficial effects on cognitive function through its influences on the expression of GABA\textsubscript{B1R} and GH/IGF-1 axis genes in PWS patients.

\textbf{Keywords:} Prader-Willi syndrome, \textit{Snord116del} mice, Cognitive impairment, Growth hormone, GABA\textsubscript{B} receptor subunit

\section*{Introduction}

Prader-Willi syndrome (PWS) is a complex neurogenetic disorder caused by loss of paternally expressed imprinted genes on human chromosome 15q11–q13\textsuperscript{1}. PWS is characterized by neurobehavioral abnormalities, cognitive impairment, and hypothalamic dysfunction including growth hormone (GH) deficiency (GHD) with short stature\textsuperscript{2}. GH replacement therapy has been reported to improve cognitive development in infants and adults\textsuperscript{3–5}, and to prevent cognitive deterioration and improve cognitive skills in children with PWS\textsuperscript{6}. However, the physiological and molecular mechanisms underlying the improvements in cognitive function after GH treatment remain unclear in PWS.

The GH/insulin-like growth factor (IGF)-1 axis is important for the growth, development, and function of the central nervous system (CNS)\textsuperscript{7}. GH deficiency in adults is characterized by cognitive impairment, which can be ameliorated by GH treatment\textsuperscript{8}. GH administration attenuates cognitive deficits and improves memory in hypophysectomised rodents\textsuperscript{9}. Another mediator of GH effects, IGF-2, has been proposed as a novel cognitive enhancer\textsuperscript{10}. The presence of binding sites for GH and IGF-1 in the brain has been suggested as evidence that GH crosses the blood–brain barrier\textsuperscript{11,12}, although the mechanisms behind the actions of GH on brain function remain unclear.

Gamma-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the CNS, acts via GABA\textsubscript{A} and GABA\textsubscript{B} recep-
tors. The functional $\text{GABA}_B$ receptors consist of two subunits, $\text{GABA}_{B1}$ and $\text{GABA}_{B2}$, which are responsible for the neuro-modulatory effect of $\text{GABA}$\textsuperscript{14,15}. Recently, exogenous GH has been reported to increase the abundance of the $\text{GABA}_B$ receptor in rat brain\textsuperscript{16} and $\text{GABA}_{B1}$ gene expression in hypophysectomised rat\textsuperscript{17}. These findings suggest a possible correlation between GH-induced cognitive function and the $\text{GABA}_B$ receptor. The Snord116 deletion (Snord116del) mouse, a PWS-mimicking model, is a dwarf strain caused by the deletion of the Snord116 C/D box snoRNA cluster. Since GH is an important regulator of developmental and cognitive functions in the CNS, we investigated the effects of GH on the expressions of $\text{GABA}_B$ receptor subunits as well as the GH/IGF axis gene in specific brain regions known to be affected by GH treatment\textsuperscript{18,19} in Snord116del mice.

**Materials and Methods**

1. **Animals and drug treatment**

All animal experiments were carried out in accordance with a protocol approved by the Institutional Animal Care and Use Committee, Laboratory Animal Research Center, Samsung Biomedical Research Institute (Seoul, Korea). Snord116del mice (B6(Cg)-Snord116tm1.1Uta/J) were obtained from The Jackson Laboratory (Bar Harbor, Maine, USA). At the start of the experiment, the mice were 3 weeks old (n=6–8 for each group). Male Snord116del mice were injected subcutaneously with 1.0 mg/kg GH (Growthtropin, provided from Dong-A Pharmaceutical Co., Yongin-si, Korea) or saline, once daily for 7 days. All animals were weighed every day to monitor their biological response in weight gain. On day 8 of the experiment, the mice were sacrificed, and the cerebellum, hippocampus, and cerebral cortex were dissected using a brain matrix.

2. **RNA extraction and cDNA synthesis**

Brain tissues were prepared for RNA extraction using the RNeasy Lipid Tissue Mini Kit (QIAGEN, MD, USA), according to the protocol provided by the manufacturer. The conversion of total RNA to cDNA was performed using the High Capacity RNA-to-cDNA kit (Applied Biosystems, Foster City, CA, USA).

3. **Quantitative polymerase chain reaction**

The expression of six genes ($\text{Gabbr1}$, $\text{Gabbr2}$, $\text{Igf-1}$, $\text{Igf-1r}$, $\text{Igf-2}$, and $\text{Igf-2r}$) was quantified using a TaqMan® Gene Expression Assay (Applied Biosystems), which included a TaqMan® real-time quantitative polymerase chain reaction (qPCR) in the cerebellum, hippocampus, and cerebral cortex. Predesigned gene-specific primers and probes were used to detect each gene (Applied Biosystems), as presented in Table 1. The amount of each transcript was normalized to the amount of GAPDH expressed in the same sample.

4. **Statistical analysis**

All statistical analyses were performed using GraphPad Prism.

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**Table 1. List of genes and assays for real-time PCR**

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Gene ID</th>
<th>ABI assay number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin-like growth factor 1</td>
<td>$\text{lgf-1}$</td>
<td>Mm00439560_m1</td>
</tr>
<tr>
<td>Insulin-like growth factor 1</td>
<td>$\text{lgf-1r}$</td>
<td>Mm00802831_m1</td>
</tr>
<tr>
<td>Insulin-like growth factor 2</td>
<td>$\text{lgf-2}$</td>
<td>Mm00439564_m1</td>
</tr>
<tr>
<td>Insulin-like growth factor 2</td>
<td>$\text{lgf-2r}$</td>
<td>Mm00439576_m1</td>
</tr>
<tr>
<td>Gamma-aminobutyric acid B receptor 1</td>
<td>$\text{Gabbr1}$</td>
<td>Mm00444578_m1</td>
</tr>
<tr>
<td>Gamma-aminobutyric acid B receptor 2</td>
<td>$\text{Gabbr2}$</td>
<td>Mm01352554_m1</td>
</tr>
<tr>
<td>Glyceroldehydrate-3-phosphate dehydrogenase</td>
<td>$\text{Gapdh}$</td>
<td>Mm99999915_g1</td>
</tr>
</tbody>
</table>

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**Fig. 1. Effects of GH treatment on body weight.** Mice received daily subcutaneous injections of GH (1.0 mg/kg) or saline injection for 7 days. Body weights were measured over 8 days. Data represent mean ± S.E.M. n=7–8/group. *P<0.05, **P<0.01, ***P<0.001. GH, growth hormone; Del, Snord116del mice injected with saline; Del+GH, Snord116del mice injected with recombinant growth hormone; WT, wild-type mice injected with saline.
5.0b (GraphPad Software, Inc., La Jolla, USA). The weight measurements were analyzed using two-way repeated ANOVA. The results from the qPCR were analyzed using one-way ANOVA with a post hoc Student–Newman–Keuls test for the statistical analysis of the differences between the groups. The correlations were tested by simple regression analysis. Values are presented as mean±SEM, and P-values less than 0.05 were considered significant.

**Results**

Compared to the WT mice, the *Snord116del* mice with GHD exhibited reduced body weight, and GH treatment significantly increased gains in body weight (Fig. 1). This indicates that the administered GH was physiologically active and had an expected systemic effect on body growth.

The expression of six genes (*Gabbr1, Gabbr2, Igf-1, Igf-1r, Igf-2*, and *Igf-2r*) in the cerebellum, hippocampus, and cerebral cortex was analyzed in *Snord116del* mice treated with GH (Del+GH) or saline (Del) and wild-type (WT) mice with saline. The results from the gene expression analysis of *Gabbr1* and *Gabbr2* in the cerebellum, hippocampus, and cerebral cortex are displayed in Fig. 2. In the cerebellum, there were significant differences between the treatment groups regarding the mRNA expression of *Gabbr1* (*P*<0.05) where both the Del+GH and WT groups showed increased *Gabbr1* mRNA expression compared with the Del group, but no effect on the *Gabbr2* expression was observed. However, the administration of GH did not alter the expression of *Gabbr1* or *Gabbr2* in the hippocampus and cerebral cortex.

The results from the gene expression analysis of *Igf-1, Igf-1r, Igf-2*, and *Igf-2r* in the cerebellum, hippocampus, and cerebral cortex are shown in Figs. 3 and 4. There was a significant difference between the treatment groups regarding the *Igf-1r, Igf-2*, and *Igf-2r* expression in the cerebellum. A significant decrease of
Igf-1r mRNA expression was found in the Del group compared to the WT group, and GH administration induced an increase of Igf-1r expression (P<0.05). In addition, alterations of Igf-2 and Igf-2r mRNA expression were found; the Del+GH group showed increased Igf-2 and Igf-2r expression (P<0.05). However, GH administration did not alter the expression of Igf-1, Igf-1r, Igf-2, or Igf-2r in the hippocampus or cerebral cortex (Figs. 3 and 4).

**Discussion**

We demonstrated both that the expression of GABA<sub>B1</sub> and Igf-1R transcripts are markedly decreased in the cerebellum of Snord116del mice compared to WT mice and that GH increases the expression of GABA<sub>B1</sub> and Igf-1R transcripts. GABA<sub>B</sub> receptor has been reported to be important for neuronal excitability and plasticity and is suggested to be involved in the regulation of long-term potentiation, which is the cellular mechanism for learning and memory<sup>15,20</sup>. GH treatment affects the functional density and metabolism of GABA<sub>B</sub> receptors in the area of the brain associated with cognition<sup>16</sup>. Other studies have revealed that GH treatment up-regulated the expression of GABA<sub>B1</sub> transcript in rat brain, suggesting a connection between GH and the GABA<sub>B</sub> system<sup>17,23</sup>.

The present study showed a significant positive correlation between the mRNA level of IGF-1R and GABA<sub>B1</sub> in the cerebellum. This finding is in agreement with a recent observation that the activation of the GABA<sub>B</sub> receptor induces IGF-1R transactivation leading to survival signaling in the cerebellum<sup>20</sup>. Moreover, several studies have suggested that GABA<sub>B</sub> receptor protects the brain from ischemic damage and improves memory<sup>23-25</sup>, providing evidence that stimulation of the GABA<sub>B</sub> receptor may be involved in a mechanism by which GH regulates brain function, including a cognitive and neuroprotective effect.

In addition, GH increased the gene expression for IGF-2 and IGF-2R in the cerebellum. IGF-2, another mediator of GH action, is known to be important for brain development and to have neurotrophic or neuroprotective properties<sup>26,27</sup>. IGF-2 signaling has been implicated in cognitive function, and it has been suggested that the effect of IGF-2 as a memory enhancer is selectively mediated by IGF-2R. IGF-2 has been shown to promote IGF-2R-dependent, persistent long-term potentiation, demonstrated by memory improvement<sup>20</sup>. Our data suggest the possibility that IGF-2/IGF-2R signaling could have an important role in GH-induced cognitive function in Snord116del mice.

However, the expression of GABA<sub>B1</sub>, GABA<sub>B2</sub>, Igf-1, Igf-1R, IGF-2, and IGF-2R in the hippocampus and cerebral cortex was unaffected by GH administration. The GH activity may be different regionally, because the brain is highly heterogeneously functional. Several potential mechanisms, such as differences in blood–brain barrier permeability and the distribution of GH receptor (GHR) and GH binding protein (GHBP), may account for differences in the effects of GH on GABA<sub>B</sub> receptor subunits and GH/IGF axis expression in specific brain regions of Snord116del mice. The local expression of GH and the presence of GHR in the cerebellum indicate that the cerebellum is an autocrine and/or paracrine site of GH action<sup>28</sup>. As it is known that GH and IGF-I increase brain growth, myelination, and has neuroprotective properties<sup>29-31</sup>, we could speculate that if the GH treatment had any effect of the brain, it would have a positive effect in terms of brain normalization.

Overall, the findings of the present indicated that GH restores the gene expression of GABA<sub>B1</sub> and Igf-1R and increases IGF-2 and IGF-2R in the cerebellum of Snord116del mice. The alterations of GABA<sub>B1</sub> and Igf-1R observed in Snord116del mice
could, at least partly, account for cognitive impairment. Because GHD during early life can impair proper brain development, thereby leading to cognitive deficits, it is suggested from our study that GH may provide beneficial effects on cognitive function through its influences on the expression of GABABR1 and GH/IGF-1 axis genes in PWS patients.

References