Hormonal Differences in Peripheral Blood and Gene Profiling in the Liver and Lymphocytes in Japanese Black Cattle with Growth Retardation

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ABSTRACT. Japanese Black cattle occasionally demonstrate growth retardation despite sufficient nutrient intake. To clarify hormonal and transcriptional characteristics, we investigated differences in blood components, including hormones, and differences in exhaustive gene expressions in the liver and peripheral lymphocytes of six cattle with growth retardation (GR cattle) and eight control cattle of the same age and pedigree with normal growth. Hematocrit values and concentrations of hemoglobin, serum albumin, total cholesterol, insulin-like growth factor 1 (IGF-1), thyroxine and insulin in GR cattle were significantly lower than those in controls. GR cattle also excreted higher levels of GH. We used three GR and three control cattle for a microarray analysis in the liver and found that 279 gene expressions were significantly different. However, gene expressions related to the GH-IGF-1 axis, such as the GH receptor and IGF-1, were not significantly different from those of controls. Immune-related gene expressions were significantly lower. To clarify these gene expression levels, peripheral lymphocytes were used for real-time RT-PCR. The expression rates of genes that were significantly lower in the liver, such as chemokine ligand 8, interferon gamma receptor 1 and immunoglobulin light chain VJ region were also significantly lower in three GR cattle than those in the three control cattle. These results suggest that the cause of growth retardation in the present study was due to other factors, not abnormal gene expressions of factors related to the GH-IGF-1 axis in the liver, and that GR cattle were susceptible to infectious disease.

KEY WORDS: cattle, growth hormone, growth retardation, lymphocyte, microarray analysis.


Cryptogenic growth retardation is often observed in Japanese Black cattle [6–8, 12, 15, 22, 29, 30]. These cattle do not have any obvious diseases, such as hereditary renal tubular dysplasia or bovine viral diarrhea, and are in good condition with normal appetites and activities [7, 8, 22, 23, 26, 29, 30]. Nevertheless, their body weights and heights are morbidity lower than the criteria of the Japanese Feeding Standard for Beef Cattle [1], even from the juvenile period [22, 30]. Since this disease is spasmodic and its responsible gene has not been identified, it remains a serious problem for farmers and breeders because of economic losses and ethical issues. Therefore, it is necessary to determine the cause of retarded growth.

To determine the physiological mechanisms and clinical conditions of growth retardation, the growth hormone (GH)—insulin-like growth factor 1 (IGF-1) axis, other hormones, and nutrient factors have been investigated [7, 8, 22–25, 30]. Kitagawa et al. [7, 8] and Takasu et al. [22, 23] have already shown significantly higher secretion of GH in Japanese Black cattle with growth retardation. Despite high serum GH concentrations, serum IGF-1 concentrations in cattle with growth retardation were much lower [22, 24, 25, 30]. Also, it was reported that cattle with growth retardation have some abnormalities in efficient energy utilization [26]. Taken together, it has been suggested that the pathway from the GH receptor to IGF-1 production in the liver may not be functioning normally. However, detailed analyses at the transcriptional level in the liver have not been reported. Since there is the possibility that growth retardation is intimately related to pedigree and is hereditary [7, 8, 12, 15, 22], exhaustive analyses could help detect the growth retardation-generating gene and establish a diagnosis.

Hence, in the present study, to assess the liver malfunction of various genes, exhaustive gene expressions were analyzed using a microarray system. Because downregulation of immunity-related genes was conspicuously seen in...
the above-mentioned examination, we confirmed whether results in the liver were similar to those in peripheral blood leukocytes.

MATERIALS AND METHODS

Animals: Six Japanese Black cattle with growth retardation (GR cattle; 4 females, 1 castrated male and 1 male) and eight control Japanese Black cattle with normal growth and similar pedigree (control cattle; 1 female and 7 castrated males) were utilized for clinical screening, standard blood examinations and serum chemistry and hormonal tests. We performed an exhaustive microarray analysis of liver tissues in 2010 and examined gene expressions of peripheral lymphocytes using real-time RT-PCR in 2011. Because the effective time of the study was different, it was not possible to use the same cattle for examining gene expressions of peripheral lymphocytes. Three GR cattle (GR-1 group; cattle Nos. #1 to #3) and three control cattle were used for microarray analysis of liver tissues. Three GR cattle (GR-2 group; Nos. #4 to #6) and three control cattle were used for real-time RT-PCR of peripheral lymphocytes. The ages of the cattle in the GR-1 group were 10, 12 and 11 months, and the ages of the cattle in the GR-2 group were 12, 8 and 7 months (Table 1). Two control cattle and five of the six GR cattle (except No. #6) were used for the GH assay. The cattle used in the present study were cared for at the Large Animal Clinical Center for the School of Veterinary Medicine, Kitasato University. All procedures, including animal housing and care, were conducted according to the Guidelines for the Care and Use of Laboratory Animals, School of Veterinary Medicine, Kitasato University (Nos. 09-004 and 10-018).

GR cattle had low birth weights, hypodynamia, anorexia, dysstasia, and diarrhea with infectious enteritis in the neonatal period. They had a diagnosis of weak calf syndrome (WCS) and were treated by local veterinarians. They recovered from WCS, but lost economic value because of their growth retardation. For this reason, they were contributed to our animal clinical center at the age of 2–6 months, were kept healthy, and were used for the present study.

The target daily gain (DG) of these cattle was set at 1.0–1.1 kg/day during the experimental period of 7–12 months, and the amount fed was adjusted every month to meet the Japanese feeding standard for beef cattle [1]. GR cattle had good or slightly low appetites during the present study. All GR cattle were free from renal tubular dysplasia (claudin-16 deficiency) [16] and bovine viral diarrhea (BVD) virus infection. One of the GR cattle (No. #4) had chronic bloat.

Regarding the family of Nos. #1, #2, #4 and #6, either YF (bull of Tajiri strain) or KT (bull of Tajiri strain) was present in the third degree of both kinships of the paternal and maternal side. In the family of No. #3, YF was present in the fourth degree of both kinships of the paternal and maternal side. In the family of No. #5, the paternal grandfather was KT, and the maternal father was MTF, who was the son of TFD. TFD was a son of YTD (bull of Tajiri strain), who was the father of YF and KT. Consequently, the GR cattle in this study were produced by inbreeding of the Tajiri strain, especially by crossbreeding between a bull and a dam of the YTD line (Fig. 1). The strains of control cattle were the same pedigrees of GR cattle. Their father was YSK or FYT. YSK was a son of HS (bull of Kedaka strain), and the maternal father was YF. FYT was a son of FS (bull of Tajiri strain), whose father was YF, and the maternal father was YH (bull of Tajiri strain). YH was a son of YF (a different cattle with the same name, the paternal father was TYD, whose grandson was YTD), and the maternal father was YF. Their maternal grandfathers were also sons of YF, YH or MTF. As

<table>
<thead>
<tr>
<th>Age (Months)</th>
<th>GR cattle (n=6)</th>
<th>Control cattle (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>145 ± 18</td>
<td>361 ± 12 †</td>
</tr>
<tr>
<td>BWI (b) (%)</td>
<td>52 ± 4 ***</td>
<td>101 ± 3 †</td>
</tr>
<tr>
<td>Body height (cm)</td>
<td>97 ± 4</td>
<td>120 ± 2 †</td>
</tr>
<tr>
<td>BHI (%)</td>
<td>87 ± 2 ***</td>
<td>101 ± 1 †</td>
</tr>
</tbody>
</table>

Table 1. Age and body growth characteristics of control and growth retarded (GR) cattle

Individual values and means ± SEM are indicated. Significantly different from control cattle (*** P<0.001). †: n=5. a) BWI (body weight index): percentage of the standard body weight for Japanese Black beef cattle of the same age and sex. b) BHI (body height index): percentage of the standard body height for Japanese Black beef cattle of the same age and sex.

Fig. 1. Family trees of six growth retarded (GR) cattle. ●: female. ■: male; #2 was a steer.
described above, all GR and control cattle had YTD as a bull on the paternal and maternal side of their ancestors.

Body weights and heights were measured monthly using a weighing machine and withers height scale, respectively. Body weight index (BWI) and body height index (BHI) were calculated from reference values in the Japanese feeding standard for beef cattle [1].

Blood samples for standard blood examinations and serum chemistry tests were collected before feeding between 08:00 and 09:00 hr from the jugular vein into vacuum tubes containing a clot activator, heparin, and dipotassium EDTA. After isolation of serum from the tubes with the clot activator and gel and isolation of plasma from heparin-containing tubes, sera and plasmas were stored at −80°C until analysis. Blood with dipotassium EDTA was used for the blood cell count, hematocrit value (Ht) and hemoglobin concentration (Hb) analysis. A biochemical autoanalyzer (Olympus AU640, Tokyo, Japan) was used to determine the concentrations of serum total protein (TP), albumin (Alb), total cholesterol (TC), urea nitrogen (UN) and plasma glucose and serum activities of aspartate amino transferase (AST) and γ-glutamyltranspeptidase (GGT). Serum thyroxine (T4) was determined by an electrochemiluminescence immunoassay [19]. Serum IGF-1 concentrations were estimated by an immunoradiometric assay [23]. Serum insulin concentrations were measured by a chemiluminescent enzyme immunoassay [14]. The measurement kits used for T4, IGF-1 and insulin were designed for humans; therefore, we confirmed that these kits could be used for cattle [30].

**GH measurement:** Peripheral blood samples (10 ml) were collected from the jugular vein with an indwelling needle (18 gauge and 51 mm in length; Terumo, Tokyo, Japan) from 08:00 to 18:00, every 30 min. Blood samples were placed in tubes containing clot activator. After centrifugation, serum was separated and stored at −80°C until assayed for GH. The GH assay was done as described previously [10, 21]. GH concentrations were measured by a competitive solid-phase immunoassay using europium (Eu)-labeled synthetic GH and polystyrene microtiter strips (Nalge Nunc International, Tokyo, Japan) coated with anti-rabbit gammaglobulin. The intra- and interassay coefficients of variation (CV) were 1.8 and 5.3%, respectively.

**Microarray analysis:** Biopsy samples of the liver were taken from between the right eighth rib and tenth rib by ultrasound-guided biopsy, frozen in liquid nitrogen, and stored at −80°C until analyzed. Total RNA was isolated from the liver with TRizol (Invitrogen, Carlsbad, CA, U.S.A.) according to the manufacturer’s instructions. A customized bovine oligonucleotide microarray with 15,268 unique genes (GPL9284) was used to detect genes expressed in six liver samples (three GR cattle and three controls). The oligo microarray produced by Agilent Technologies (Santa Clara, CA, U.S.A.) was used in this study. All total RNA tested, including those examined by real-time RT-PCR, had their quality and quantity confirmed using either a Bioanalyzer (Agilent Technologies) or NanoDrop instrument (Thermo Fisher Scientific, Waltham, MA, U.S.A.). We performed a one-color microarray analysis [20]. Microarray data from each sample were imported into GeneSpring GX11 (Agilent Technologies) for further data characterization.

**Real-time RT-PCR:** Since there were many immunological and inflammation-related factors whose gene expressions were reduced in liver tissues of GR cattle, we next measured mRNA expression rates in peripheral lymphocytes from three GR cattle (GR-2 group) and three control cattle of the same age using real-time RT-PCR analysis. Lymphocytes were collected from blood samples by gradient centrifugation (Lymphoprep, Axis-Shield, Norway) according to the manufacturer’s protocol. Blood samples (6 ml) were mixed with the same volume of saline, the Lymphoprep solution, PBS and 0.9% NH₄Cl solution. After centrifugation at 800 × g for 30 min, we collected 1.0 × 10⁷ of lymphocytes per tube. Total RNA was isolated from these cells with TRizol (Invitrogen, Carlsbad, CA, U.S.A.) according to the manufacturer’s instructions.

Procedures for real-time RT-PCR were previously described [27]. Primers were designed using the Primer Express computer software program (Applied Biosystems) based on bovine sequences. Primer sequences for each gene are given in Table 2. Each real-time RT-PCR reaction utilized 0.4 μl forward primer (10 μM), 0.4 μl reverse primer (10 μM), 10 μl Power SYBR Green PCR Master Mix (Applied Biosystems) and 7.2 μl nuclease-free water. Thermal cycling conditions included one cycle at 50°C for 2 min, one cycle at 95°C for 10 min and 40 cycles at 95°C for 15 sec
and 60°C for 1 min. Each cDNA template was analyzed for quantitation in duplicate. Relative differences in the initial amount of each cDNA were determined by comparing cycle threshold values. The one-peak melting curve for detecting the SYBR Green-based objective amplicon was confirmed.

**Statistical analyses:** GH pulses were identified by means of the ULTRA algorithm using the values of intra- and interassay CVs for corresponding peptides [5, 28]. Parameters of GH pulses calculated for each sampling session were as follows: pulse frequency, mean number of pulses in 10 hr, pulse amplitude, and the area under the curve above the baseline (AUC). Data of GH hormone could not be applied to statistical analysis, so we only showed the average values.

Differences between values of BWI, BHI, and blood examination between GR cattle and control cattle were analyzed using the Student’s t-test for homoscedastic items and Welch’s t-test methods for items with different variances. Significance was assumed if the P value was <0.05.

**RESULTS**

**Characteristics of growth and blood examination of GR cattle:** GR cattle were thin and showed a significantly lower BWI (Ave. 52%) and shorter BHI (Ave. 87%) than those in control cattle (Table 1). Blood count tests are shown in Table 3. Biochemical and hormonal tests are shown in Table 4. In blood tests, Ht values and concentrations of Hb, serum Alb and TC in GR cattle were significantly lower than those in control cattle. In the hormonal assay results, IGF-1, T4 and insulin levels in GR cattle were significantly lower than those in control cattle.

**GH hormone analysis of GR cattle:** GH profiles in control and GR cattle are shown in Table 5. In control cattle, GH was secreted in a pulsatile manner, which involved the recurrence of obvious peaks followed by troughs of virtually no GH secretion. In GR cattle, the GH baseline, pulse amplitude and AUC were relatively higher than those in control cattle, while the pulse width and frequency did not change.

**Liver microarray analysis of GR cattle:** A total of 279 genes were differentially higher or lower expressed in GR cattle by more than 2-fold that in controls. Using microarray data of these 279 genes, we constructed a map (Fig. 2), and gene symbols and descriptions are listed in Table 6. Most downregulated genes were related to immunological functions and the migration of leukocytes [haptoglobin, heat shock proteins, immunoglobulin light chain VJ region (LOC404062), IgG1 heavy chain constant region (IGG1C), interferon gamma (IFNG), interferon gamma receptor 1 (IFNGR1), major histocompatibility complex (MHC) class II, DR alpha (Bola-DRA), MHC class II, DR B3 (Bola-DRB3) and chemokine C-C motif ligand 8 (CCL8)]. Gene expression of Shwachman-Bodian-Diamond syndrome (SBDS), which is known to be one of the human hereditary diseases, was downregulated more in GR cattle than in control cattle. On the other hand, there were no significant differences in transcriptional expressions of factors related to GH-IGF-1 axis (GH receptor, IGF-1 isoforms and IGF-1 receptors) in the liver.

**Transcriptional expression of GR-related genes in lymphocytes:** Five genes (CCL8, SBDS, IFNG, LOC404062 and IFNGR1) that had significant differences in microarray results in liver tissues were selected to measure mRNA
Fig. 2. Microarray analysis of 279 differentially expressed genes in control (Cont) and growth retarded (GR) cattle. Genes were enhanced between at least two groups by more than 2-fold. The red scale indicates relatively higher expression levels, and the green scale indicates relatively lower expression levels. Expression levels were transformed to log values. Dendrograms of sample axes and gene axes represent overall similarities in gene expression profiles. 

- LOC504548: Ubiquitin D
- LOC404062: Immunoglobulin light chain VJ region
- HP: Haptoglobin
- LOC513144: Similar to metallopeptidase
- HSPA1A: Heat shock 70kDa protein 1A
- HSPH1: Heat shock 105kDa/110kDa protein 1
- BP10366: Similar to Bcl-2-binding protein BIS
- grp78: Heat shock 70kDa protein 5
- SCIN: Scinderin
- DNAJA1: DnaJ (Hsp40) homolog, subfamily A, member 1
- CLEC6A: C-type lectin domain family 6, member A
- CCL8: Chemokine (C-C motif) ligand 8
- SBDS: Shwachman-Bodian-Diamond syndrome
- U63639: Immunoglobulin heavy constant gamma 3
- IFNγ: Interferon gamma
- IGG1C: IgG1 heavy chain constant region
- B2M: Beta-2-microglobulin
- BE667821: Similar to IQ motif containing E
- 5hr2b: Serotonin receptor 2B
- LOC505478: Similar to Immunoglobulin lambda-like precursor
- CD48: CD48 molecule
- BOLA-DRA: Major histocompatibility complex, class II, DR alpha
- IFNGR1: Interferon gamma receptor 1
- CTSS: Cathepsin S
- BoLA-DRB3: Major histocompatibility complex, class II, DRB3
- LOC535947: Cytochrome P450, family 26, subfamily A, polypeptide 1
- AB099095: Mitochondrial RNA, similar to D-loop, clone: ORCS10746.
- AB099097: Mitochondrial RNA, similar to D-loop, clone: ORCS11619.
- LOC518033: Similar to SEC14p-like protein TAP3
- TXNRD3: Thioredoxin reductase 3
- BID36316: Similar to stress-activated protein kinase-3
- MGC133968: Calcium binding tyrosine-(Y)-phosphorylation regulated
- BI537255: Similar to Uroporphyrinogen-III synthase
- LOC519345: Similar to solute carrier family 17
- LOC614315: Kv channel interacting protein 4
- DNAJA1: DnaJ (Hsp40) homolog, subfamily A, member 1
expression rates in peripheral lymphocytes. Figure 3 shows the results of real-time RT-PCR analysis of these five representative genes. Four genes, excluding IFNG, were significantly lower in GR cattle than in control cattle (P < 0.05), and IFNG tended to be lower in GR cattle than in control cattle (P = 0.081).

DISCUSSION

Growth retardation of Japanese Black cattle is reportedly dependent on some degree of pedigree [7, 8, 15, 22]. KT, YF, HDSK and YTD were reported to be the paternal or maternal fathers or grandfathers of the cattle with growth retardation [7, 8, 15, 22]. Moreover, KT and YF were sons of YTD, and HDSK was a son of YF. In this study, GR cattle were produced by inbreeding of the Tajiri strain (bulls KT, YF, HDSK and YTD). This suggested the role of heredity in GR cattle. Unfortunately, cattle with growth retardation are still sporadic, and the responsible gene has not been identified.

In the infant period of growth, the role of the protein as a physical component is very important. Therefore, when cattle suffer from malnutrition, weight gain becomes compromised and "the weight ratio for the height" decreases [11]. In clinical screening, GR cattle were thin and showed significantly lower BWIs and shorter BHIs than those of control cattle, but reductions were seen more conspicuously in BWI than BHI. This symptom is similar to that of a malnourished infant, but the GR cattle in this study had sufficient nutrition intake.

Nutrition-associated factors (serum Alb, TC, T4, IGF-1 and insulin) in GR cattle were significantly lower than in control cattle. These symptoms have also been reported in cattle with growth retardation in previous studies [23, 30].

Table 6. Differentially expressed genes in the liver of growth retarded (GR) cattle relative to control cattle

<table>
<thead>
<tr>
<th>Ratio of GR cattle to control cattle</th>
<th>Accession No.</th>
<th>Gene symbol</th>
<th>Gene name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Downregulated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01</td>
<td>AJ271156</td>
<td>HP</td>
<td>Haptoglobin</td>
</tr>
<tr>
<td>0.04</td>
<td>AF109697</td>
<td>LOC404062</td>
<td>Immunoglobulin light chain VJ region</td>
</tr>
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<td>0.15</td>
<td>XM_580689</td>
<td>LOC504548</td>
<td>Ubiquitin D</td>
</tr>
<tr>
<td>0.16</td>
<td>BP102075</td>
<td>LOC513144</td>
<td>Similar to metalloproteinase</td>
</tr>
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<td>0.17</td>
<td>NM_174007</td>
<td>CCL8</td>
<td>Chemokine (C-C motif) ligand 8</td>
</tr>
<tr>
<td>0.18</td>
<td>NM_001034479</td>
<td>CLEC6A</td>
<td>C-type lectin domain family 6, member A</td>
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<tr>
<td>0.19</td>
<td>U63639</td>
<td>U63639</td>
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<td>S82409</td>
<td>IG1IC</td>
<td>IgG1 heavy chain constant region</td>
</tr>
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<td>Scinderin</td>
</tr>
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<td>0.21</td>
<td>AJ491864</td>
<td>Shn2b</td>
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<td>Interferon gamma</td>
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<td>AJ586431</td>
<td>grp78</td>
<td>Heat shock 70 kDa protein 5 (glucose-regulated protein, 78 kDa)</td>
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<td>0.26</td>
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<td>BP103686</td>
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<td>B1538308</td>
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<td>2.42</td>
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<td>Similar to solute carrier family 17 (sodium phosphate)</td>
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<td>B1536316</td>
<td>B1536316</td>
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Genes emphasized in a bold-face were used to measure mRNA expression rates in peripheral lymphocytes.
Fig. 3. Real-time RT-PCR analysis of five representative genes (CCL8, SBDS, IFNG, LOC404062 and IFNGR1) using peripheral lymphocytes from control (Cont) and growth retarded (GR) cattle. These genes were expressed at low levels in GR cattle in the liver microarray analysis. Expression of mRNA was normalized to the expression of GAPDH measured in the same RNA preparation. Each column and vertical bar represents the mean and SEM (n=3), respectively. Asterisks indicate significant differences (*P<0.05).

The results suggested that GR cattle experienced states of malnutrition despite sufficient energy and protein intake. Moreover, it has been suggested that serum insulin, IGF-1 and T4 concentrations in cattle with growth retardation are directly or indirectly related to growth and anabolic capacity [6–8, 12, 23–25]. Therefore, low levels of these hormones in this study may be due in part to a decline in anabolic capacity. Takasu et al. [28] reported that the digestibility of cattle with growth retardation was not significantly different from that of control cattle, which suggests that cattle with growth retardation had an abnormality in the assimilation capacity of nourishment. If there were assimilation abnormalities in GR cattle, we can explain their state of malnutrition in spite of sufficient nutritional intake.

As for cattle with growth retardation, higher serum GH concentrations and lower IGF-1 levels have been shown, although there were some variations based on pedigree [22]. We also confirmed the same hormonal differences in this study. Breier [4] reported that decreased nutrition and dietary protein restriction elevated GH secretion with reduced numbers of hepatic GH receptors and lowered levels of plasma IGF-1 with decreased expression of hepatic IGF-1 mRNA and serum Alb mRNA. Therefore, we had initially thought that low concentrations of serum IGF-1 in GR cattle were due to decreases in the number of GH receptors in the liver as a result of malnutrition. However, there were no significant differences in the transcriptional expression of factors related to GH-IGF-1 axis in the liver. This suggests the possibility of a more essential disorder related to production of IGF-1 and metabolic systems and that a disorder in the GH-IGF-1 axis was not an essential cause of retarded growth. Further study is needed to clarify this.

One of the famous short height diseases is Laron’s syndrome dwarfism, which is caused by a defect in the GH receptor or in post-receptor mechanisms [2, 9]. GR cattle seem to have similar characteristics to this syndrome, but there are two big differences. One is the gene expression levels described above, and the other is physical appearance. While Laron’s syndrome dwarfism presents with pygmy type dwarfism, leading to the formation of a small-sized body, the GR cattle in this study were thin, but were not pygmies.

The expression levels of 279 genes in GR cattle were higher or lower by more than 2-fold that of control cattle. Among them, haptoglobin expression was much lower in GR cattle. Haptoglobin is a positive acute phase protein that binds free hemoglobin and removes it from the circulation to prevent kidney injury and iron loss following hemolysis [17]. In addition, haptoglobin acts as a potent immunosuppressor of lymphocyte function and modulates the helper T-cell type 1 and type 2 (Th1/Th2) balance within the body [17]. Moreover, gene expressions of some regions of the immunoglobin light chain (LOC404062) and heavy chain (IGG1C), MHC class II (Bola-DRA and Bola-DRB3), chemokine (CCL8), IFNG and IFNGR1 ligands were also downregulated in GR cattle. These results suggest that development of an immunological response could be debilitated in GR cattle.

Besides these direct immunological factors, the Shwachman-Bodian-Diamond Syndrome (SBDS) gene was also expressed at significantly lower levels in the liver and peripheral lymphocytes of GR cattle. SBDS is a rare multisystem disorder characterized by exocrine pancreatic insufficiency, bone marrow dysfunction, and metaphyseal chondrodysplasia [3, 18]. Moreover, anemia and growth retardation were present in more than a third of SBDS patients [13]. Cattle with growth retardation have symptoms of early growth retardation and potential anemia, which are similar to those of SBDS patients. It has been speculated that low expression of the SBDS gene could also be one of the genetic risk factors of cattle with growth retardation.

Since the most significant differentially expressed genes were related to immunological and inflammation-related factors even in the liver, we hypothesized that these factors could also be changed in other tissues in peripheral leukocytes. The results of the real-time RT-PCR assay using peripheral leukocytes showed that the transcriptional expressions of LOC404062, CCL8 and IFNGR1 were significantly lower in GR cattle. These results suggested that cattle with growth retardation were susceptible to infectious disease. Furthermore, this data is consistent with the fact that the GR cattle in this study had contracted WCS with infectious enteritis in their neonatal periods and also with previous reports in which cattle with growth retardation tended to contract WCS [29, 30].

In conclusion, the results of this study suggested that GR cattle had some hereditary background and had compromised resistance to infection and abnormalities in nourishment assimilation ability. However, the real reasons for malnutrition and low concentrations of serum IGF-1 in GR cattle could...
not be determined. Further study is necessary to clarify the cause of growth retardation.

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REFERENCES


