Carrier Rate of Factor XI Deficiency in Stunted Japanese Black Cattle

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ABSTRACT. Blood examinations and genotyping of Factor XI (F11) were performed in growth retardation Japanese Black cattle and their dams. Genotyping of F11 revealed that the recessive homozygous and heterozygous genotype frequencies were 5.2% and 50.0% in the Claudin-16 (CL-16) deficiency group (n=58), 0% and 14.2% in the renal dysplasia group (n=7), 0% and 26.1% in the non-CL-16 deficiency nephritis group (n=23), 8.9% and 46.7% in the hypogenesis syndrome group (n=45), 6.2% and 25.0% in the neonatal weak calf syndrome group (n=32), 9.1% and 38.6% in the respective dams group (n=44), 0% and 23.1% in the normal cattle group (n=13), and 5.9% and 38.2% in total (n=222), respectively. These results showed that the carrier rate of F11 deficiency was high in Japanese Black cattle, and that the CL-16 deficiency, hypogenesis syndrome, neonatal weak calf syndrome, and dams groups had a large amount of recessive homozygous genotype than the other groups. No abnormal bleeding was observed clinically in the present study, and 4 of the recessive homozygous dams showed normal growth and parturition.

KEY WORDS: claudin-16 deficiency, factor XI deficiency, growth retardation, hypogenesis, Japanese Black cattle.

Calves with abnormal development caused by Claudin-16 (CL-16) deficiency, which is known to cause hereditary renal failure [7, 12–15, 18, 19, 28, 29], non-CL-16 deficiency nephritis, which genotyping is not CL-16 deficiency in spite of similarity to CL-16 deficiency in its clinical symptoms, hematological aspects, and pathological findings [21, 22], renal dysplasia, which is suspected to be a hereditary disease [23], neonatal weak calf syndrome [15], hypogenesis syndrome, and other defects are frequently seen in Japanese Black cattle. It has been suggested that neonatal weak calf syndrome is associated with asiderotic anemia and immunodeficiency [16], and that hypogenesis syndrome is associated with growth hormone (GH) deficiency [17], insulin like growth factor-1 (IGF-1) deficiency in contrast with hypersecretion of GH [5, 6, 11, 26], primary or secondary underactive thyroid function [5, 6, 24, 30] and other factors.

Factor XI (F11) is a factor of intrinsic blood coagulation that is produced in the liver. Factor XI deficiency is inherited in an autosomal recessive or dominant manner [1], and it causes a congenital bleeding disorder characterized by minor bleeding episodes and severe protracted bleeding after trauma or surgical procedures in humans [20].

The first case of F11 deficiency in Holstein Friesian cattle was reported by Kociba et al. [8], and in that case, the inheritance of F11 activity in the Holstein Friesian cattle was autosomal dominant, with severe defects in homozygotes and partial deficiency in heterozygotes [2]. Recently, a causative mutation for F11 deficiency in Japanese Black cattle was identified by Kunieda et al. [9], making genotype analysis of the F11 gene possible. Factor XI deficiency with mild bleeding and severe deficiency of F11 activity were detected in Japanese Black cattle [9, 25].

The authors studied the hematological aspects and genotype frequencies of F11 deficiency in abnormally developed cattle, dams, and normal cattle groups, and attempted to unravel the associations between retarded growth and F11 deficiency in Japanese Black cattle.

MATERIALS AND METHODS

Animals: Cattle with abnormal growth (n=165) were divided into 5 groups for this study based on clinical symptoms, hematological characteristics, pathological findings, and genotyping of CL-16 deficiency as follows: CL-16 deficiency (n=58), renal dysplasia (n=7), non-CL-16 deficiency nephritis (n=23), hypogenesis syndrome (n=51), and neonatal weak calf syndrome (n=26). In addition, 2 groups, dams of stunted cattle (n=44) and normal cattle (n=13), were examined for hematological aspects and the genotype of F11 deficiency (Table 1).

CL-16 deficiency was examined using the DNA-based tests developed by Hirano et al. [4]. Renal dysplasia was diagnosed from pathological findings. Non-CL-16 deficiency nephritis was used as the category for cases that were not CL-16 deficiency according to the DNA-based tests despite the similarity to CL-16 deficiency in clinical symptoms, hematological characteristics, and pathological findings. Hypogenesis syndrome was used as the category for cases of normal appearance without congenital ateliosis. The cattle used were determined to be free of BVD-MD infections and did not show symptoms of the aftereffects of diseases such as pneumonia and diarrhea. A number of the cattle with hypogenesis syndrome cattle had ongoing neona-
tal weak calf syndrome. Neonatal weak calf syndrome was used as the category for cases of weakness of unknown cause in the neonatal period, and in some animals in this category, anorexia, depression, staggering, diarrhea, and anemia were observed. However, several cases of neonatal weak calf syndrome have been found with ongoing weakness until 2–3 months of age. Therefore, this syndrome was diagnosed during the nursing period in this study.

Clinical screening: We investigated the form of manifestation of the defects and performed familial screening for these affected cattle. Body weight index (BMI) was expressed as a percentage of the standard body weight of Japanese Black cattle [27] in the same month.

Blood samples were collected from the jugular vein and mixed with a small volume of heparin sodium for laboratory tests, complete blood work using an automatic blood cell counter, and biochemistry tests using an autoanalyzer (Hitachi AU550, Tokyo) for total protein (TP), albumin (Alb), total cholesterol (T. chol), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), urea nitrogen (UN), creatinine (Cre), calcium (Ca), inorganic phosphorus (iP), and magnesium (Mg).

Genotype analysis: Heparinized blood samples stored at −40°C were used for genotyping. Genotype analyses for $F11$ deficiency were performed using the DNA–based tests developed by Kunieda et al. [9].

Statistics: The differences between each group and the normal cattle in the blood examination were compared using the Student’s $t$ test for the homoscedastic items, and using the Cochran-Cox methods for items with different variences. A p-value of less than 0.05 was considered to indicate a significant difference.

RESULTS

Clinical screening: The cattle and findings of clinical screening used in the present study are listed in Table 1. Clinical symptoms included insufficient growth, anorexia, depression, and long hooves in the majority of cattle with chronic interstitial nephritis in the $CL-16$ deficiency and non-$CL-16$ deficiency nephritis groups. The mean body weight index (BMI) for both groups was 66–73%, which was higher than that in the other stunted groups (39–66%), and several cattle in the $CL-16$ deficiency group had almost normal BWI. Most of the cattle in the $CL-16$ deficiency and non-$CL-16$ deficiency nephritis groups first showed failure to thrive at 5 months old or later, and thus the age at which this abnormality was identified was later than in the other stunted groups. All calves in the renal dysplasia group had marked ateliosis (mean BWI: 39%), depression, and inability to nurse with congenital marked dysplasia of the kidneys (0–60% of the weight of normal kidneys), and most of these calves also had shortening of the lower jaw. The cattle in the hypogenesis syndrome group had no anamnesis, but did show insufficient growth (mean BWI: 57%). In the neonatal weak calf syndrome group, clinical symptoms included weakness, depression, inability to nurse or anorexia, and diarrhea (partly).

In the present study, no cattle with abnormal bleeding were clinically observed in spite of the fact that 13 cattle had $F11$ deficiency, and their dams displayed normal growth, reproduction, and parturition. The cattle in the $CL-16$ deficiency, hypogenesis syndrome, neonatal weak calf syndrome, and dams groups were within the same family as the cattle in the $F11$ deficiency group.

Table 1. Characterization of the stunted cattle groups, their dams and normal cattle

<table>
<thead>
<tr>
<th>Cattle group</th>
<th>Female heads</th>
<th>Male heads</th>
<th>Total heads</th>
<th>Mean month age (ranges)</th>
<th>Mean BWI (ranges)</th>
<th>Main symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Claudin-16 deficiency</td>
<td>34</td>
<td>24</td>
<td>58</td>
<td>13.8 (1–38)</td>
<td>72.7 (31–93)</td>
<td>Anorexia, depression, long hooves (about 65%), chronic interstitial nephritis</td>
</tr>
<tr>
<td>Renal dysplasia</td>
<td>4</td>
<td>3</td>
<td>7</td>
<td>0.9 (0–3)</td>
<td>38.7 (21–53)</td>
<td>Ateliosis, depression, inability to nurse, renal dysplasia, shorting of lower jaw (about 60%)</td>
</tr>
<tr>
<td>Non-Claudin-16 deficiency nephritis</td>
<td>12</td>
<td>11</td>
<td>23</td>
<td>13.5 (1–27)</td>
<td>66.3 (37–87)</td>
<td>Anorexia, depression, long hooves (about 50%), chronic interstitial nephritis</td>
</tr>
<tr>
<td>Hypogenesis syndrome</td>
<td>26</td>
<td>19</td>
<td>45</td>
<td>11.6 (3–31)</td>
<td>56.6 (26–79)</td>
<td>No sign without lower growth</td>
</tr>
<tr>
<td>Neonatal weak calf syndrome</td>
<td>16</td>
<td>16</td>
<td>32</td>
<td>1.5 (0–3)</td>
<td>48.8 (17–82)</td>
<td>Weakness, depression, inability to nurse or anorexia, diarrhea (partly)</td>
</tr>
<tr>
<td>Dams of stunted cattle</td>
<td>44</td>
<td>0</td>
<td>44</td>
<td>63.8 (24–118)</td>
<td>98.2 (88–114)</td>
<td>No sign</td>
</tr>
<tr>
<td>Normal cattle</td>
<td>9</td>
<td>4</td>
<td>13</td>
<td>17.2 (1–29)</td>
<td>97.6 (86–120)</td>
<td>No sign</td>
</tr>
<tr>
<td>Total</td>
<td>145</td>
<td>77</td>
<td>222</td>
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a) Body Weight Index; Percentage to the standard body weight of Japanese Black cattle in the same month.

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In the blood examinations, the number of red blood cells (RBC) and hematocrit (Ht) values were lower in the \textit{CL-16} deficiency and neonatal weak calf syndrome groups (Table 2). Plasma UN, Cre, and iP were significantly higher, and plasma Ca was significantly lower in the \textit{CL-16} deficiency, renal dysplasia, and non-\textit{CL-16} nephritis groups compared with the normal cattle. Plasma T. chol was significantly higher in the \textit{CL-16} deficiency group. Plasma Alb was significantly lower in the hypogenesis syndrome group, and TP and Alb were significantly lower in the neonatal weak calf syndrome group.

Genotype analysis: As shown in Table 3, genotyping of \textit{F11} revealed that the recessive homozygous and heterozygous genotype frequencies were 5.2% and 50.0% in the \textit{CL-16} deficiency group (n=58), 0% and 14.2% in the renal dysplasia group (n=7), 0% and 26.1% in the non-\textit{CL-16} nephritis group (n=23), 8.9% and 46.7% in the hypogenesis syndrome group (n=45), and 5.9% and 38.2% in the total cattle (n=222), respectively.

These results show that the rate of carriers for \textit{F11} deficiency was high in Japanese Black cattle, and that the \textit{CL-16} deficiency, hypogenesis syndrome, neonatal weak calf syndrome, and their dams groups had higher rates of recessive
homzygous genotype than the other groups.

DISCUSSION

In this study, it was demonstrated that the rate of carriers for F11 deficiency is high in Japanese Black cattle. The reason for this high rate of carriers for F11 deficiency in the CL-16 deficiency, hypogenesis syndrome, neonatal weak calf syndrome, and dams groups was thought to be that the families of these groups were within the same family as the F11 deficiency. This family of affected cattle is known for its excellent beef marbling score and meat grade, and for that reason, the semen of the sires in this family is widely used for artificial insemination in Japan. In fact, the affected cattle were inbred in this family, and consequently, it is suggested that these diseases occur often in the population of Japanese Black cattle.

In humans, F11 deficiency is inherited in an autosomal recessive or dominant manner [1]. In cattle, a mutation that prevents homodimer formation usually results in recessive inheritance, since significant amounts of normal homodimer can be formed by the products of the wild-type alleles in the heterozygous genotype, and mutations in the catalytic domain of proteins usually result in dominant inheritance by formation of heterodimers of the mutant and wild-type polypeptides [9].

Kunieda et al. [9] reported that Japanese Black cattle with F11 deficiency showed a marked reduction in F11 activity (less than 10% of the normal activity) with prolonged bleeding time and abnormal plasma coagulation. Takasu et al. [25] also reported an F11 deficiency case in a 1-month-old Japanese Black calf showing growth retardation, hip dysplasia and abnormal bleeding with prolongation of activated partial thromboplastin time (APTT) and low F11 activity.

In the present study, 13 heads of cattle, including 4 dams, were identified as having the F11 deficiency recessive homozygous genotype; however, none of them had shown abnormal bleeding in the past. Therefore, it is possible that the cattle with F11 deficiency normally show only mildly abnormal bleeding.

On the other hand, Canadian Holstein cattle with F11 deficiency showed a lower rate of fetal and calf survival, higher susceptibility to infectious disease, and higher frequency of being repeat breeders [10]. Furthermore, one Holstein cow with repeat breeding has been found to have the F11 deficiency heterozygous genotype in Japan [3]. However, no dams showed repeat breeding or abnormal bleeding at parturition in the F11 deficiency dams in the present study, and the rate of carriers was very high in the Japanese Black cattle. Therefore, F11 deficiency might have little effect on the reproductive ability of Japanese Black cattle. However, further investigations will be necessary to determine the differences of symptoms between Japanese Black and Holstein F11 deficiency cattle.

The percentages of cattle with the normal homozygous F11 deficiency genotype in the hypogenesis syndrome and neonatal weak calf syndrome groups were 45% and 69%, respectively. For this reason, it seems that F11 deficiency is not directly associated with these syndromes.

The cattle with F11 deficiency in combination with CL-16 deficiency, hypogenesis syndrome and/or neonatal weak calf syndrome may display more severe symptoms. Therefore, the use of semen from non-carriers or sires of other families should be considered for the prevention of hereditary diseases when mating with dams that are either carriers of an F11 and/or CL-16 deficiency or members of a family of such carriers.

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REFERENCES


