ABSTRACT: The main objective of this study was to study risk factors affecting metaphyseal irregularities (MI) in the distal radius and ulna of growing Newfoundland dogs. Risk factors studied included the genetic effects, effects of litters, BW, circumferences of the distal radius and ulna (CDRU), and total serum alkaline phosphatase (ALP) concentrations. The study included 118 Newfoundland dogs (60 females, 58 males), derived from 32 litters. Body weight, separated on sex and MI, was fitted to the Gompertz function. Occurrence of MI differed significantly between sexes, with 55% of males and 35% of females affected ($P = 0.03$). Growth curves for the 2 groups of dogs, with and without MI, diverged after 60 to 70 d, and dogs with irregularities were heavier at maturity than dogs of the same sex without irregularities. In univariate analysis, the litter effect was a significant predictor of MI, explaining 32% of total variability of the MI incidence, but the genetic effects were not significant. However, the latter were significant in bivariate analysis of MI and BW. In the bivariate analysis, the effects of litter on MI and BW were significantly correlated at all observational points except at birth, 180 d, and 536 d. Total ALP concentrations decreased with increasing age, and differences between groups diminished with increasing age, indicating a negative effect of total ALP on MI. Correlation between MI and total ALP concentrations of litters was estimated in a bivariate analysis. This correlation was significant and ranged between −0.34 and −0.62. Similarly, the genetic relationship between total ALP and MI from 120 d of age onward varied between −0.31 to −0.60. However, correlations were only significant at 356 d of age (genetic correlation = 0.60; $P = 0.01$). The mean CDRU increased from 90 d of age toward a peak at 180 d. Thereafter, CDRU declined and stabilized at about 1 yr of age. The mean CDRU between the groups of dogs with and without MI diverged most at 90 d of age, then was nearly stable until 180 d and gradually declined until 356 d, when the CDRU began to equalize. Metaphyseal irregularities and CDRU levels of litter were significantly correlated. Litter effect was a significant predictor of MI. The effects of litters and the genetic effects on BW and MI were correlated at most phases of the growth of the dog. Similar, but lower, correlations were found for CDRU and MI, and total ALP and MI.

Key words: alkaline phosphatase, body weight, canine, growth curve, metaphyseal irregularities

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doi:10.2527/jas.2006-838

INTRODUCTION

Metaphyseal irregularities (MI) were discovered on radiographs in young Newfoundland (NF) dogs during a large study. The metaphyseal changes occurred in a segment of the population and apparently did not have any clinical expression. Metaphyseal irregularities were characterized by islands of reduced opacity, outlined by thickened, radiopaque osseous trabeculae, which were aligned with the axis of stress. Radiographic examinations were reviewed and grouped according to common findings and apparent severity and longevity of skeletal changes. According to severity of MI, each animal was graded into 1 of 4 groups: grade 0, grade I, grade II, and grade III (Trangerud et al., 2005).

Metaphyseal osteopathy, enostosis, osteochondrosis, hip and elbow dysplasia, and pes varus, is a group of
developmental orthopedic diseases that cause skeletal abnormalities in young growing dogs (Demko and McLaughlin, 2005). Osteochondrosis and hip dysplasia have been related to fast growth (Hedhammar et al., 1974; Ekman and Carlson, 1998; Smith et al., 2006). The etiology of most developmental orthopedic diseases is considered to be multifactorial, attributable to both genetic and environmental factors. Several studies regarding the genetic heritability of hip and elbow dysplasia have been conducted and indicate that genetic factors play a significant role in disease development (Hedhammar et al., 1979; Wood et al., 2002; Janutta et al., 2006).

Radiographic appearance in dogs with MI diverges from normal development in growing dogs. The significance of MI is yet unknown. Despite increased occurrence of MI being found in the NF breed, there is presently no information available about the factors that might affect the occurrence and radiographic expression of MI. The aim of the current study was to describe how MI may be related to skeletal growth and maturation and to investigate the influence of genetic and environmental factors.

MATERIALS AND METHODS

Study Design

The experiment was carried out in agreement with the provisions enforced by the National Animal Research Authority. Newfoundland dogs (n = 118; 60 females and 58 males) participating in a large study were included in the present assessment. The 118 NF dogs derived from 32 different litters (each litter was assigned a number as identification – litter-number), each 1 with a housing and feeding regimen decided by its owner.

Inclusion of Animals

All dogs included in the study were born in Norway between November 1998 and June 2001. The breeding stock consisted of dogs born in Norway and imported dogs. These 118 dogs are approximately 25% of all NF dogs born in Norway during the designated period. Each breeder, dog owner, and veterinarian that participated in the project signed a written agreement of cooperation to comply with the project plan.

Inclusion of a litter in the project started when the bitch was mated. The puppies were registered in the Norwegian Kennel Club. The Kennel Club registration numbers of the parents and grandparents were recorded for every dog. Not all animals enrolled in the study continued to completion. These reasons included, but were not limited to, death of the dog and relocation of the owners during the study. Additionally, some dogs missed one or more of the examinations during the study for unknown reasons.

The study dogs had an international genetic background. All the dams were born in Scandinavia; 18 in Norway, 1 in Sweden, and 1 in Denmark. Fifty-four dogs (37.3%) had sires from outside Scandinavia, and 17 (38.6%) were diagnosed radiographically with MI. Thirty-three dogs (28%) had sires from outside Europe (United States and Australia), of which 15 (45.5%) had MI (Trangerud et al., 2005).

Clinical Registrations and Questionnaires

History, management, and clinical information were obtained from 3 sources: 1) the breeder of the litter, 2) the new owner of the puppy, and 3) the veterinarian that examined the dog. All 3 sources completed questionnaires and recorded information in a booklet prepared for each of them. All questionnaire sheets appeared in duplicate in the booklet, so that 1 sheet could be mailed to the researchers and a copy retained in the booklet. The breeder was asked to record the BW of each puppy at birth and on d 3 and 7, and then weekly until 8 wk. The feeding regimen of each litter was decided by the breeder. All puppies stayed with their mother from birth to approximately 8 wk, when they were delivered to the new owner. The new owners completed the questionnaires and reported information at the ages at 3, 4, 6, 12, and 18 mo (observational ages). The questionnaires can be found online (http://www.veths.no/templates/Page.aspx?id=9150).

The owner reported information regarding housing, exercise, nutrition, and health of their dogs. The owners agreed to have their dogs examined by a veterinarian and to have radiographs made according to a predetermined schedule (observational ages) to document bone formation and remodeling. Radiographic and clinical examinations were scheduled at observational ages, including a mediolateral radiographic projection of the right antebrachium with the elbow flexed, and made at a film-to-focus distance of 100 cm. Blood samples, measurements of BW, assessment of body condition, and measurements of circumferences of the distal radius and ulna (CDRU) were collected at the scheduled veterinary controls at observational ages. Dogs included in this study on MI had a radiograph of the radius and ulna at 6 mo and again at 4 or 12 mo of age.

Radiographic examinations were reviewed and grouped according to common findings and apparent severity and longevity of the changes in the skeleton. Grade I was given when only the ulna had changes. Grade II had changes in the radius, the ulna, or both bones that were more evident than grade I, and unlike grade I, the changes increased between 6 and 12 mo of age. Grade III changes were dramatic and distinct; they occurred in both the radius and ulna and persisted in some form in adult bone. All dogs that had irregularities during some phase of development consistently had changes at 6 mo of age (Trangerud et al., 2005).

Laboratory Analysis

Hematological tests were conducted and the blood serum was analyzed for total alkaline phosphatase
Factors affecting metaphyseal irregularities

(ALP). Total ALP was analyzed with the diethanolamine (DEA) method, as recommended by Scandinavian Committee on Enzymes. In the ALP (DEA) method, the sample is added to a p-nitrophenyl phosphate substrate and DEA buffer is used to maintain the reaction at between pH 9.7 and 9.8. Magnesium ions are added to the DEA buffer to activate and stabilize the enzyme. During the reaction, ALP hydrolyzes the p-nitrophenyl phosphate to form p-nitrophenol, which can be measured photometrically at 405 nm. The reaction rate follows zero-order kinetics. The units of activity can be calculated as micromoles of substrate hydrolyzed per second.

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Statistical Analysis

Body weight data separated on sex and the group with and without MI were fitted to a Gompertz function. A Gompertz function is a nonlinear, sigmoid function, with its point of inflection at 36.8% of mature BW. Growth was modeled with the following equation (Helmink et al., 2000), using the NLIN procedure (SAS Inst. Inc., Cary, NC):

\[ W_t = W_{max}\exp\{-e^{-(t-c)/b}\}, \]

where \( W_t = BW \) at time \( t \), \( W_{max} = mature BW \), \( b = \) proportional to duration of growth, \( c = \) the age at the point of inflection (i.e., when 36.8% of mature BW is reached), and \( t = \) the age in days.

Analyses were carried out separately for sex and groups with and without MI. Duration of growth was estimated by \((4b + c)\), which describes 98% of the growth duration (Helmink et al., 2000). The derivative of the Gompertz function describes the growth rate.

A univariate analysis of MI was conducted to estimate the genetic effects and the effects of litters on MI. The statistical model was

\[ MI = sexe + animal + error, \]

where sexe denotes the fixed effect of males and females; and animal denotes a random effect, with the covariance between animal i and j being \( A_{ij} \times \sigma^2_a \), where \( A_{ij} \) is the genetic relationship between animals i and j (e.g., the relationship between full sibs is \( \frac{1}{2} \) and \( \sigma^2_a \) is the variance due to genetic effects. Similarly, the effect of litters was estimated by the model: \( MI = sexe + litter + error \), where the litters are assumed to have independent random effects. Unfortunately, the data set was too small to estimate the effects of litter and genetics simultaneously in 1 model.

To investigate the relationship of MI with BW \( d \) (i.e., BW at day \( d \)), a bivariate analysis was conducted using the statistical model:

\[ \begin{pmatrix} MI \\ BW_d \end{pmatrix} = \begin{pmatrix} sexe_{MI} \\ sexe_{BW_d} \end{pmatrix} + \begin{pmatrix} litter_{MI} \\ litter_{BW_d} \end{pmatrix} + \begin{pmatrix} error_{MI} \\ error_{BW_d} \end{pmatrix} \]

where the effects of litter on MI and BW \( d \) were estimated simultaneously, and the correlation between the effects of litter on MI and BW \( d \) was estimated. This analysis was repeated for every day at which BW were available. A similar analysis was conducted in which the litter effect was replaced by an animal effect, which made it possible to estimate the genetic correlation between MI and BW. The same models were used on CDRU. The univariate and bivariate analysis was estimated using ASREML (Gilmour et al., 2000). Statistical differences were considered significant when \( P < 0.05 \).

RESULTS

Litter and Genetic Effect

In univariate analysis, the litter effect was a significant predictor of MI, explaining 32% (variance due to litter = 0.077; SE = 0.034; \( P < 0.05 \)) of total variability of the MI incidence. Genetic effects were not a significant predictor of MI incidence explaining <0.001% of total variability of the MI incidence.

Body Weight: Litter and Genetic Effect

Metaphyseal irregularities were more frequent in males (55%) than females (35%; \( P = 0.03 \); Table 1), and changes classified as grade I and III occurred more frequently in the males (Table 1).

Average daily gain and BW are shown in Figures 1 and 2. Estimated BW increased rapidly during the first 100 d after birth and then plateaued in all dogs, reaching maturity between 405 and 432 d (Figures 1 and 2). The growth curves for the 2 groups, with MI and without MI, diverged after 60 to 70 d, and dogs with MI were heavier at mature age than dogs of the same sex without MI (Figures 1 and 2). Estimated mean mature BW was 52.1 kg in females without MI and 56.4 kg in females with MI, and 57.2 kg in males without MI and 61.2 kg in males with MI (Table 2). Average daily gain, expressed by the derivative of the Gompertz function, reached its maximum value between 102 and 109 d (point of inflection), after which it gradually declined as mature BW was achieved (Figures 1 and 2, Table 2). Body condition did not seem to influence the difference in BW between the 2 groups. Average daily gain for males in the time period between 1 and 8 wk was significantly different (\( P < 0.001 \)) between the 2 groups [without MI, ADG = 128.8 (SE = 5.6), with MI, ADG = 155.6 (SE = 5.0)]. For females, ADG was significantly different (\( P = 0.03 \)) between the groups in the same time period [without MI, ADG = 133.1 (SE = 5.2), with
MI, ADG = 157.6 (SE = 9.3)]. In the period 1 wk to 4 mo, differences in ADG were only significant ($P < 0.001$) for the males [without MI, ADG = 188.4 (SE = 5.4), with MI, ADG = 219.5 (SE = 4.6)]. In the bivariate analysis, ADG is corrected by the litter effect; the correlations (between 1 and 8 wk = 0.02, and 1 wk and 4 mo = 0.42) between ADG and MI levels of litters were not significant. Average daily gain was not significant between the 2 groups with and without MI in either gender after 4 mo.

In the bivariate analysis, the effects of litters on MI and BW were significantly correlated at all observational points, except at birth, 180, and 536 d (Table 3). The correlation coefficients increased from birth to 56 d of age, at which time the correlation coefficient was 0.71 ($P < 0.0001$; Table 3). After this time point, the estimated correlation coefficients followed a decreasing trend. Correlation coefficients were lower, but had the same pattern of change when grade of MI was included in the analyses. There was a significant genetic correlation between BW and MI at all ages studied. The greatest correlation coefficients were found from 42 d of age onward (Table 4).

**Total Serum Alkaline Phosphatase: Litter and Genetic Effect**

The estimated total ALP concentrations decreased in both groups from 90 to 360 d of age, after which they stabilized at a mean concentration of 120 IU/L (Figure 3). The mean concentration of total ALP reached its maximum at 90 d of age, and this was the only age where the mean concentrations significantly differed ($P = 0.04$) between the 2 groups. Total ALP concentrations decreased, with increasing age, and differences between the groups diminished with increasing age (Figure 3).

The correlations between MI and total ALP levels of litters were estimated in a bivariate analysis. These correlations ranged between $-0.34$ and $-0.62$ (Table 5). Similarly, the genetic relationship between total ALP and MI from 120 d of age onward ranged from $-0.31$ to $-0.60$. However, only at 356 d of age was the correlation significant (genetic correlation = 0.60; $P = 0.01$).

**Metaphyseal Circumference of the Distal Radius and Ulna: Litter and Genetic Effect**

The estimated average CDRU increased from 90 d of age toward a peak at 180 d. Thereafter, CDRU declined and stabilized at about 1 yr of age (Figure 4). At comparable ages, males had a greater mean CDRU than females ($P < 0.001$). In males, animals with MI generally had greater CDRU than those without MI. The mean CDRU of the groups of males with and without MI diverged most at 90 d, and were significantly different at 90 d ($P = 0.002$) and 120 d ($P = 0.01$) (Figure 4), after which there was a decline toward 356 d of age, when
Factors affecting metaphyseal irregularities

Table 2. Least squares means for the variables\(^1\) of the Gompertz function by status on metaphyseal irregularities (MI) and sex

<table>
<thead>
<tr>
<th>Sex(^2)</th>
<th>MI(^3)</th>
<th>Wmax, kg</th>
<th>c, d</th>
<th>b, d</th>
<th>4b + c, d</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>0</td>
<td>57.16</td>
<td>0.54</td>
<td>109.2</td>
<td>1.60</td>
</tr>
<tr>
<td>M</td>
<td>1</td>
<td>61.08</td>
<td>0.48</td>
<td>104.3</td>
<td>1.35</td>
</tr>
<tr>
<td>F</td>
<td>0</td>
<td>52.07</td>
<td>0.43</td>
<td>102.1</td>
<td>1.49</td>
</tr>
<tr>
<td>F</td>
<td>1</td>
<td>56.42</td>
<td>0.61</td>
<td>104.3</td>
<td>2.06</td>
</tr>
</tbody>
</table>

\(^1\)Wmax = mature BW; c = age at point of inflection; b = proportional to duration of growth; and 4b + c = duration of growth.

\(^2\)M = male; F = female.

\(^3\)0 = without MI; 1 = with MI.

the mean CDRU of each group was more or less equal. In the females, there was no significant difference between animals with and without MI at any age.

The bivariate analyses showed that MI and CDRU levels of litter were correlated at 90 and 120 d (\(P < 0.01\)). The correlation coefficients ranged between 0.03 and 0.62 (Table 6). Similarly, there was a genetic relationship between CDRU and MI at 90 d (genetic correlation = 0.56; \(P = 0.01\)) and 120 d (genetic correlation = 0.55; \(P = 0.01\)).

**DISCUSSION**

Although it was not possible to distinguish between genetic and litter effects in the current study, the data conclude that litter was a significant predictor of MI, explaining a total of 32% of MI incidence variation in the univariate analysis. The fact that the litter effect on the correlation between BW and MI showed an increasing trend until weaning indicates that environmental factors after birth are perhaps more important for the development of MI than factors related to the uterine environment. However, the influence of environmental factors in the postweaning period appeared to be less important because the correlation coefficients decreased after weaning at 8 wk of age. This indicates that maternal milk production, milk quality, physical activity, and additional feeding are factors that should be considered when evaluating the etiology of MI.

The univariate analysis showed no genetic effect on the incidence of MI. However, there was a significant effect and genetic correlation between BW at all ages and MI in the bivariate analysis. Hence, the genetic correlation between BW and MI helped to statistically detect the genetic effect of MI. We could not account for the binary nature of the MI trait in the statistical analysis due to convergence problems in the single and multitrait analyses. Treating binary traits as linear is known to result in underestimates of heritability (Gianola, 1979), but this underestimation is small if the disease incidence is close to 50%, as was the case here. In any case, the heritability and fraction of the variance due to litters is expected to be underestimated due to this effect. In the data set, a few (6) dams had multiple litters, which is too few to estimate parity-of-dam effects, but it might have caused some underestimation of the variance due to litter because a few of the litters are related (i.e., whelped by the same dam).

Nielen et al. (2001) found high heritability estimates (>0.55) on birth weight and adult BW in Boxers, as well
Figure 3. The relationship between age and mean concentrations (±SE) of total alkaline phosphate (total ALP) for Newfoundland dogs with metaphyseal irregularities (—), and without metaphyseal irregularities (---).

Table 5. Correlation between metaphyseal irregularities and total alkaline phosphatase concentrations of litters

<table>
<thead>
<tr>
<th>Age, d</th>
<th>r²</th>
<th>SE</th>
<th>P-value</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>-0.37</td>
<td>0.25</td>
<td>0.064</td>
<td>113</td>
</tr>
<tr>
<td>120</td>
<td>-0.34</td>
<td>0.29</td>
<td>0.127</td>
<td>115</td>
</tr>
<tr>
<td>180</td>
<td>-0.39</td>
<td>0.28</td>
<td>0.082</td>
<td>117</td>
</tr>
<tr>
<td>356</td>
<td>-0.62</td>
<td>0.29</td>
<td>0.017</td>
<td>101</td>
</tr>
<tr>
<td>536</td>
<td>-0.54</td>
<td>0.37</td>
<td>0.072</td>
<td>83</td>
</tr>
</tbody>
</table>

as a litter effect on the same 2 traits. These findings can probably explain why, in the current study, there was a high genetic correlation between BW and MI at some ages, even though the genetic effect alone explained virtually no variation in MI incidence.

In the current study, heavier BW during growth increases the risk of MI. Because male NF are generally heavier and grow faster than females, this may partly explain the greater frequency of MI among males than among females (Trangerud et al., 2007). Heavier BW has also been demonstrated to be a trigger for other skeletal developmental diseases, such as osteochondrosis and hip dysplasia in Labrador retrievers (Kealy et al., 1997; Ekman and Carlson, 1998). As for MI, these diseases also occur more frequently in males than in females. However, it is not known whether BW itself, or other factors related to BW, are the real triggers for development of these diseases (Bohning et al., 1970; Ekman and Carlson, 1998; Wood et al., 2002). The high correlation between the litter effect and BW and MI indicate that environmental effects within litters, affecting BW and incidence of MI.

Metaphyseal irregularities were radiologically characterized by islands of reduced opacity outlined by thickened, radiopaque osseous trabeculae, which were aligned with the axis of stress (Trangerud et al., 2005). Seven of the dogs with MI had the most severe form, and 6 of these were males. The radiolucent areas in the metaphysis in dogs with MI indicate a reduced bone density. In humans, increased bone density can be achieved in patients with osteoporosis by moderate weight-bearing and resistance exercises for prevention and treatment (Compston, 2001; NIH Consensus Development Panel on Osteoporosis, 2001). Thus, it appears unlikely that BW per se is a main trigger for the development of MI. Based on the results from the current study, it seems appropriate to focus more strongly on factors related to growth in the search for etiological factors for MI.

The fact that the prevalence of MI differed between genders points to sex-related differences in growth and bone formation as important risk factors for development of MI. It is known that sex steroids secreted during adolescence affect bone mineral density (NIH Consensus Development Panel on Osteoporosis, 2001). Hitz et al. (2006) reported sex-dependent differences in bone density related to growth hormone deficiency in humans. Growth-hormone-deficient males had less bone density than normal males whereas growth-deficient females compared with normal females had identical values. The gender difference seemed to be caused by
the fact that females were given estrogen substitution therapy whereas the males were given testosterone to compensate for the lack of GH.

In the current study, mean total ALP concentrations were lower in dogs with MI than in dogs without MI. The concentration of total ALP is effected by the activity of osteoblasts, which build bone (Allen et al., 1998, 2000; Breur et al., 2001). Bone ALP, one of the ALP isoenzymes, is synthesized by and expressed on the external surface of the osteoblasts, and bone ALP is the main contributor to total ALP in dogs less than 1 yr of age (Allen et al., 2000). Although the etiology of the MI observed in the current study has not been elucidated, it could be that a lower activity of the osteoblasts is associated with the “mouldy” appearance of the skeletons in dogs with MI. A combination of low osteoblast activity and fast growth could possibly aggravate the radiographic findings in dogs with MI.

In the current study, CDRU was investigated as a clinical measure of metaphyseal circumference of distal radius and ulna. In the current study male dogs with MI had greater CDRU than normal males, whereas in females CDRU was not affected by presence or absence of MI. The interaction between MI and BW in the current study would certainly indicate a greater CDRU in the group with irregularities, and this corresponds well with the findings. However, the lack of a similar relationship in females suggests that the etiology of the observed MI is more complex than simply a change in bone remodeling, mediated via BW.

Litter effect was a significant predictor of MI, explaining 32% of total variability of the MI incidence. The effects of litters and the genetics of the dogs on BW and MI were strongly correlated at most phases of the growth curve. Similar, but lower, correlations were found for CDRU and MI, and total ALP and MI. The litter effect and the differences in occurrences and grade among sexes should be studied more thoroughly to elucidate the etiology of MI.

**Table 6.** Correlation between circumference of distal radius and ulna and metaphyseal irregularities incidence of litters

<table>
<thead>
<tr>
<th>Age, d</th>
<th>$r^2$</th>
<th>SE</th>
<th>P-value</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>0.62</td>
<td>0.24</td>
<td>0.005</td>
<td>106</td>
</tr>
<tr>
<td>120</td>
<td>0.59</td>
<td>0.25</td>
<td>0.008</td>
<td>110</td>
</tr>
<tr>
<td>180</td>
<td>0.32</td>
<td>0.33</td>
<td>0.161</td>
<td>110</td>
</tr>
<tr>
<td>356</td>
<td>0.40</td>
<td>0.31</td>
<td>0.102</td>
<td>94</td>
</tr>
<tr>
<td>536</td>
<td>0.03</td>
<td>0.53</td>
<td>0.480</td>
<td>73</td>
</tr>
</tbody>
</table>

**LITERATURE CITED**


