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Canine Leukocyte Adhesion Deficiency: Presence of the Cys36Ser β-2 Integrin Mutation in an Affected US Irish Setter Cross-Breed Dog and in US Irish Red and White Setters

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Canine leukocyte adhesion deficiency (CLAD) is a primary immunodeficiency disease characterized by recurrent bacterial infections in the presence of marked leukocytosis. The disease was 1st described in the mid-1980s in a cross-breed Irish Setter Dog in the United States. It results from a defective β-2 subunit of heterodimeric leukocyte adhesion proteins. The causative mutation for CLAD in Irish Setter Dogs from Europe has been identified as a missense mutation at base pair position 107 in the β-2 integrin subunit gene (ITGB2) that results in an amino acid change from cysteine to serine at amino acid 36 (Cys36Ser) in the β-2 integrin subunit protein. In the current work, the originally described dog with CLAD has been genetically tested and shown to have the same mutation as the European Irish Setters. This suggests that the mutation has been in the Irish Setter population for many generations spanning more than 2 decades. A related breed, the Irish Red and White Setter, has a history of interbreeding with Irish Setters and shares a common ancestry with the Irish Setter breed. DNA from Irish Red and White Setters residing in the United States was screened either by sequencing or by the newly developed restriction enzyme test for the Irish Setter Cys36Ser CLAD mutation. Seven of 54 dogs tested (13%) were found to be carriers of the Irish Setter CLAD mutation. Five of these were directly related to a sire from the UK, demonstrating the importation of an allele from another continent and establishing the need for genetic testing in this breed in the United States.

Key words: Genetic disease; Hereditary disease; Leukocyte adhesion deficiency; Mutation screening.

Leukocyte adhesion deficiency is a primary immunodeficiency characterized by severe recurrent infections despite a marked leukocytosis. It is caused by a deficiency of the common β-2 integrin subunit (CD18) of the major heterodimeric leukocyte adhesion proteins including, but not limited to, CD11a/CD18 (LFA-1), CD11b/CD18 (MAC-1/CR3), and CD11c/CD18 (CR4/gp 150,95). These proteins are critical in neutrophil cell adhesion, extravasation from blood vessels, migration to sites of infection, and phagocytosis of C3b-opsonized bacteria. Clinical signs include frequent bacterial infections beginning shortly after birth and are accompanied by severe leukocytosis and a lack of purulent exudate. Omphalophlebitis, gingivitis, osteomyelitis, and pyoderma are frequent sequelae.[1–3]

Canine leukocyte adhesion deficiency (CLAD) was 1st characterized in a cross-breed Irish Setter[4] shortly after the disease was described in humans. At that time, it also was recognized that the previously reported canine granulocytopathy syndrome[5] in Irish Setters probably was the same disease. A bovine leukocyte adhesion deficiency was subsequently characterized in Red Holstein Cattle.[2] Recently, CLAD has been reported in a group of Irish Setters from Sweden[6,7] and the UK,[8] and the causative molecular defect in those dogs has been characterized[8] as a guanine to cytosine transition at nucleotide position 107 in the β-2 integrin subunit gene (ITGB2) coding for the β-2 subunit of leukocyte integrins.[8] This missense mutation results in the substitution of a cysteine for a serine at position 36 (Cys36Ser) in the β-2 integrin subunit protein. Genetic screening studies have recognized carriers of this missense mutation in Irish Setters from other European countries and Australia.[9]

We report that the original Irish Setter cross-breed dog from the United States in which the disease was 1st characterized has the same mutation as reported in the Swedish Irish Setters. These results establish that the gene defect has a worldwide distribution and has been present in the Irish Setter population for many generations.

We also have found this mutation in a rare breed with shared ancestry to the Irish Setter, the Irish Red and White Setter. The Irish Red and White Setter is believed to be the ''original'' Irish Setter. In the late 1800s, both the red Irish and the Irish Red and White Setters were shown together in the breed ring, but the red Irish Setters soon gained popularity, and the red and white dogs became rare. The breed was revived in the 1950s by crossing the few remaining Red and White Setters with field-bred red Irish Setters. There is a strong possibility, therefore, that this breed also could carry the mutation for CLAD. We have confirmed this suspicion in the population of Irish Red and White Setters in the United States.

Materials and Methods

Animals

Samples were solicited from Irish Red and White Setters owned by members of the Irish Red and White Setter Association and the Irish Red and White Setter Club of America. All dogs were living in the United States at the time of sampling. Cheek swabs were received from 54 dogs. There are approximately 450 Irish Red and White Setters in the United States today, and an estimated 10–25% of these dogs have been or will be bred. Not all of the dogs tested are breeding animals, and we estimate that we have tested about 50% of the breeding population in the United States. DNA was extracted from buccal mucosal cells by standard procedures (Gentra Systems).[a]

Medical records were reviewed from the affected female spayed, cross-breed Irish Setter 1st described with CLAD in 1987.[4] DNA was

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extracted by standard procedures (Qiagen)[b] from lymphocyte and fibroblast cultures collected at the time of euthanasia. DNA was obtained from EDTA blood collected from a Miniature Schnauzer and a Newfoundland Dog to serve as unaffected control samples.

DNA Sequencing

The portion of the β-2 integrin gene flanking position 107 in exon 1 was amplified by standard polymerase chain reaction (PCR) techniques with gene-specific primers (lowercase) tagged with M13 complementary sequences (uppercase) at the 5′ ends as follows (5′ to 3′): M13CLADF: CACGACGTTGTAAAACGACcgtcctgccaggagtgcacca agta; and M13CLADR: GGATAACAATTTCACACAGGgcttctggcacc aggcgcagccg. Two rounds of PCR were carried out in 50-μL reaction volumes with 2–3 μL DNA in the initial reaction and 1 μL of the previously amplified product in the 2nd reaction. Cycling conditions for both rounds were 95°C for 30 seconds, followed by 72°C for 1 minute for a total 30 cycles per round. The amplified products, 126 bp in length, were gel purified by standard methods (Qiagen).[c] Bidirectional sequence analysis was performed using a Licor system[d] with dye-primer chemistry and standard 800 and 700 infrared (IR)2-labeled M13FORWARD and M13REVERSE primers[10] with absorbance measured at 795 and 695 nm.

Enzyme Digest Test

DNA was extracted from cells collected from cheek swab samples as described by Richards et al.[11] Thirty-microliter PCR reactions were prepared containing 0.5 μM primers, 2 mM deoxyribonucleotide triphosphate (dNTP), 1.5 U Hot Start Polymerase (Qiagen),[e] Hot Start Polymerase Buffer (Qiagen),[f] and 3 μL DNA. Primer sequences were as follows: Forward: CTTAGCCTCCTGCCAGGACTGCACCAA; and Reverse: TTCAGCTTCTGGCACCAGGCGCAGCCGGGCCCC AA.

PCR cycling conditions were as follows: incubation at 95°C for 15 minutes and 94°C for 1 minute, followed by 35 cycles of 92°C for 1 minute, 68°C for 30 seconds, and 72°C for 30 seconds, with a final extension of 75°C for 3 minutes. A 3.5-μL aliquot of Reaction Buffer 2 (New England Biolabs)[g] was added directly to the PCR reaction, as was 1.5 μL of the restriction enzyme *Hin*fI.[h] After incubation at 37°C for 1 hour, 5 μL loading dye (glycerol and bromophenol blue) was added, and the samples were electrophoresed for 45 minutes at 85 V in a 3% agarose gel with Tris-borate-EDTA as the running buffer. All samples were assayed in duplicate from independent DNA preparations, and appropriate positive (known carrier samples) and negative (no DNA) controls were included. Positive control samples were included with each batch because it is necessary to confirm the expected restriction enzyme activity.

The Forward primer adds nucleotides to the exon to make the amplified fragment 96 bp in length. The Reverse primer destroys an internal *Hin*fI site that is present in this amplified region. The CLAD mutation introduces a new restriction site at approximately the middle of the 96-bp amplified fragment. Amplified regions containing the mutant allele are cut into 2 fragments of 50- and 46-bp lengths that comigrate as a single band on an agarose gel. The PCR product bearing the normal allele remains uncut in the presence of *Hin*fI restriction enzyme activity. Thus, homozygous normals (''clear'' animals) will have one 96-bp band, homozygous affecteds will have one ~50-bp band, and carriers (heterozygotes) will show both bands on an agarose gel.

Results

Case Report

The clinical course, up to 18 months of age, of the female Irish Setter cross-breed dog in which CLAD was 1st described was detailed in a previous report.[4] This dog was the result of a mother-son mating in which the dam was a purebred Irish Setter, and the sire was her son from a mating with a mixed-breed dog. The dog survived for another 2.5 years, during which time she had a persistent severe leukocytosis (reaching 230,000/μL) that consisted of a mature neutrophilia, eosinophilia, monocytosis, and lymphocytosis. Antibiotic therapy was required throughout her life and was regularly changed on the basis of culture and sensitivity results. A spectrum of antibiotics was used. She developed skin papillomas believed to be viral in origin that spontaneously regressed and a number of serious bacterial infections, attributable to neutrophil dysfunction, including ascending osteomyelitis leading to amputation of the affected right hind limb, pyometra, chronic dermatitis with severe facial alopecia, and pneumonia. The complication of osteomyelitis of the remaining left hind limb occurred at 4 years of age and compelled the owner to elect euthanasia.

DNA Analysis

DNA obtained from 2 normal non-Irish Setter breed dogs (a Miniature Schnauzer and a Newfoundland) was amplified and sequenced for the region of the ITGB2 gene in which the missense mutation responsible for CLAD occurs.[8] As expected, both of these dogs had the normal (wild type) β-2 integrin subunit sequence. Amplified DNA of the same gene region from the cross-breed Irish Setter dog known to be affected with CLAD was found to have only the mutant sequence, a cytosine in the sense strand at position 107 in the ITGB2 gene (Fig 1). This is the same mutation as described in the CLAD-affected Irish Setters from Sweden[8] and indicates that this mutation has been present in the Irish Setter population on at least 2 continents for more than a decade.

Survey of Irish Red and White Setters

We also sought to evaluate the presence of this mutation in a related breed of dogs, the Irish Red and White Setter. All 54 Irish Red and White Setters from the United States were tested for the mutation at position 107 in ITGB2. Gene sequencing was used to evaluate 19 of these dogs. The remaining dogs were analyzed by a restriction enzyme–based procedure (Fig 2). Of the 54 Irish Red and White Setters tested, 7 were found to be carriers (heterozygotes) for the Cys36Ser mutation. No affected dogs were identified. Five of the carrier dogs were directly related to a particular sire from the UK. Although this dog is deceased and no samples were available for testing, 2 of his offspring were found to be carriers. Because the dam tested ''clear'' (homozygous wild type), the sire must be an obligate carrier for the CLAD mutation. A 3rd littermate tested clear. A 4th deceased littermate must also be an obligate carrier because he produced carrier offspring with a bitch that tested ''clear'' for CLAD (Fig 3). Pedigrees from the remaining 2 detected carriers were insufficient to determine their relationship to this kinship.

Discussion

In humans, several different mutations of the ITGB2 are known, and the clinical course varies depending on which

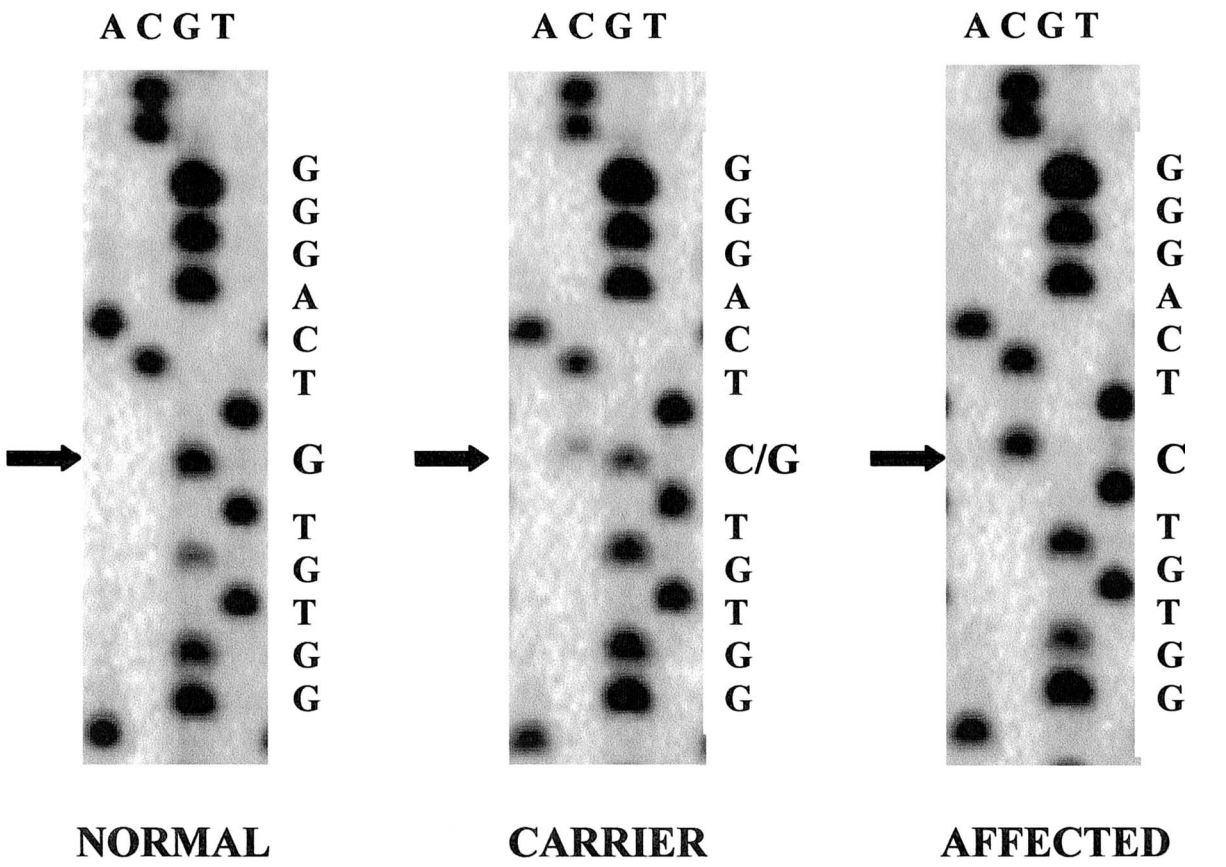

Fig 1. Sequencing gel showing the region of the β-2 integrin subunit gene (ITGB2) containing the canine leukocyte adhesion deficiency (CLAD) mutation. Arrows point to position 107. Each lane corresponds to 1 nucleotide, and the sequence is read sequentially. The normal β-2 integrin subunit allele has a G at position 107 that is part of a codon leading to a cysteine at position 36 in the resulting protein. The affected allele has a C in this position, leading to a serine at position 36 in the resulting protein. A carrier (heterozygotes) has both alleles.

mutation or mutations the patient carries. It was possible, therefore, that more than 1 mutation was responsible for CLAD in Irish Setters as well. The mutation in the ITGB2 gene responsible for CLAD in Irish Setters from Sweden and the UK has been identified.[8] We have shown that the cross-breed dog from the United States in which CLAD was 1st described in 1987 indeed had the same missense mutation that is responsible for the disease in the Swedish and English Irish Setters. It is likely that the Irish Setter dogs described by Renshaw et al[5] in the mid-1970s also had this disease and that the same missense mutation was responsible in those dogs as well. A survey of the mutant allele in Irish Setters throughout Europe and in Australia[9] supports a worldwide distribution of this mutation in Irish Setters. The probable longstanding presence of this allele in both the UK and the United States suggests that it has been present in Irish Setter populations for many generations and likely arose in the UK before the exportation of Irish Setters throughout the world.

We also demonstrated the presence of this allele in a related breed of dogs in the United States, the Irish Red and White Setter. This is one of several rare breeds (eg, Red Setters) developed in the UK that are closely related to Irish Setters and that are thought to be at risk for harboring the Cys36Ser mutation because of interbreeding and shared ancestry with Irish Setters. Seven of 54 dogs (13%) tested were found to be carriers of the same missense mutation at position 107 in the ITGB2 gene that is responsible for CLAD in Irish Setters. Because the survey was limited, and 5 of these dogs were directly related to one another, the true mutant allele frequency cannot be determined. With the recognition of the Cys36Ser mutation in the population of breeding US Irish Red and White Setters, however, breeders now have the ability to select for unaffected off-

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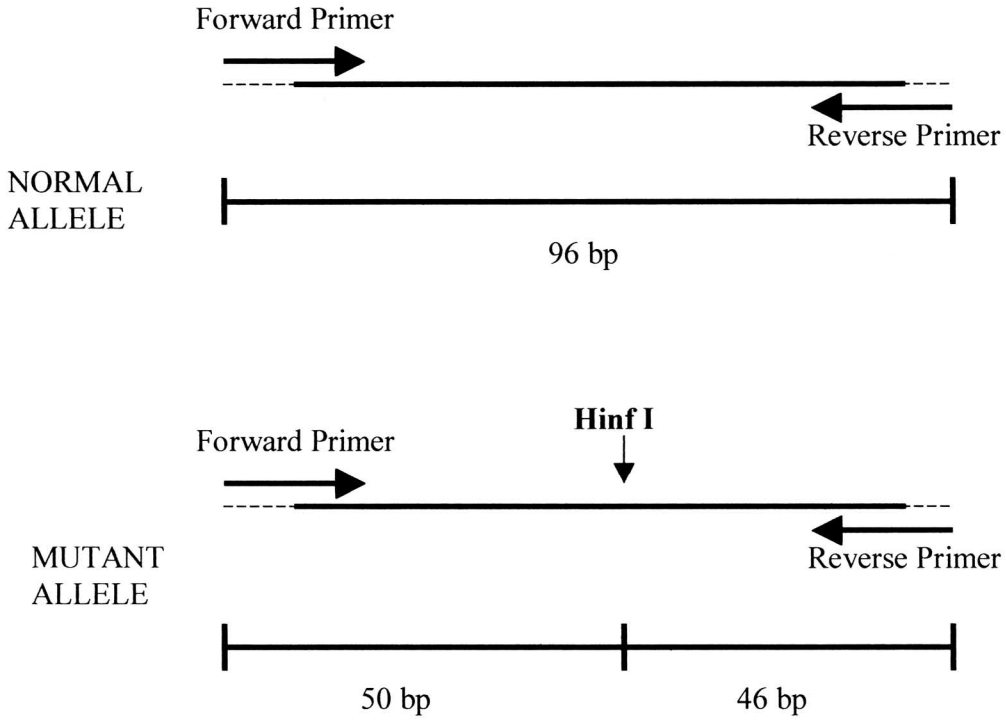
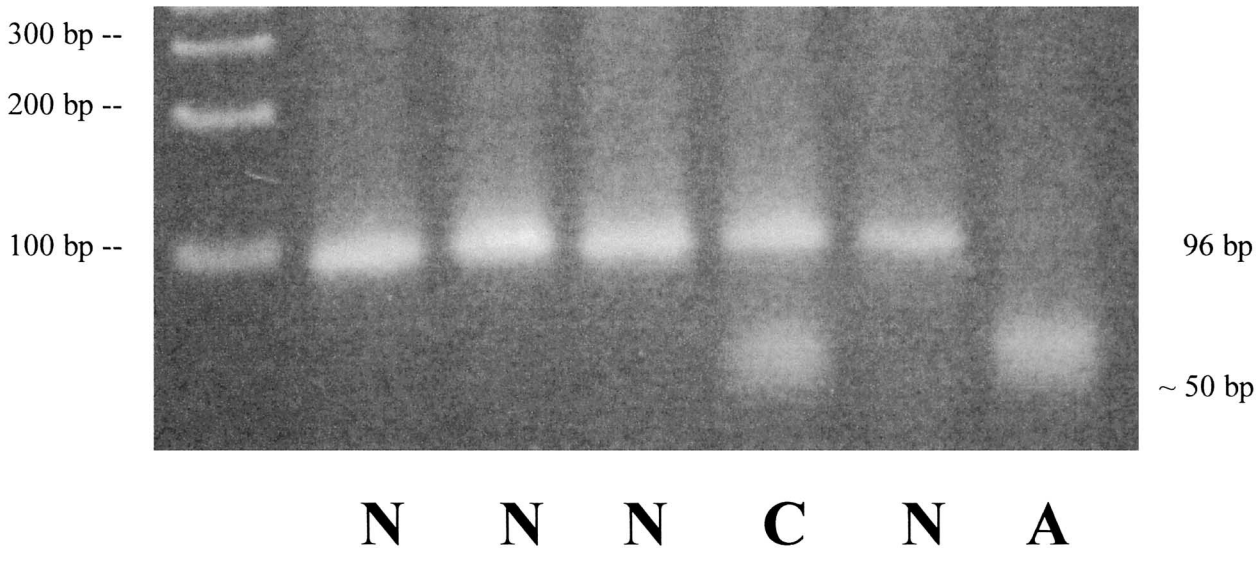
Fig 2. **(A)** Restriction enzyme procedure. The normal allele has no *Hin*fI restriction site in the 96-bp sequence amplified. Therefore, exposure to the restriction enzyme *Hin*fI has no effect on the length of the amplified segment. The mutation responsible for canine leukocyte adhesion deficiency (CLAD), however, results in a new *Hin*fI restriction site in approximately the middle of the amplified sequence. Exposure to *Hin*fI results in 50- and 46-bp bands that will comigrate on a 3% agarose gel. Carriers (heterozygotes) have both alleles. **(B)** Agarose gel. After amplification and *Hin*fI restriction enzyme digestion, normal individuals (N) are identified as having a single 96-bp band; affected individuals (A), by having only the approximately 50-bp band; and carriers (C), by the having both bands on a 3% agarose gel.

A.


Forward Primer
Reverse Primer
NORMAL ALLELE
96 bp
Hinf I
Forward Primer
Reverse Primer
MUTANT ALLELE
50 bp
46 bp

B.


300 bp --
200 bp --
100 bp --
96 bp
~ 50 bp
N N N C N A

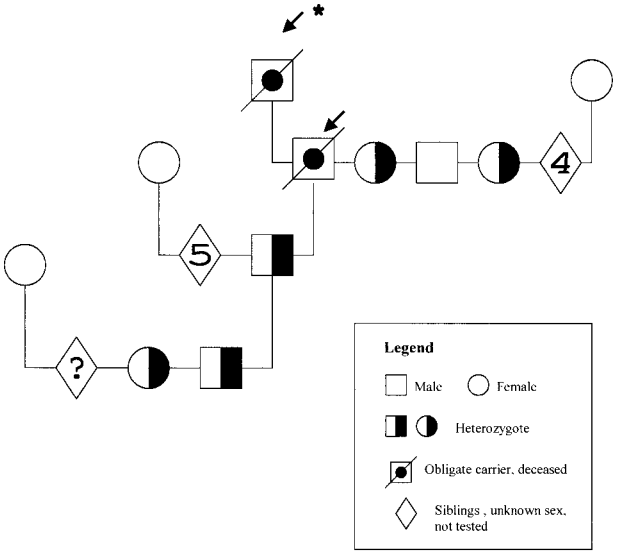


Fig 3. Pedigree of a family of Irish Red and White Setter dogs known to carry the canine leukocyte adhesion deficiency (CLAD) mutation. Arrows point to 2 deceased obligate carrier males. The asterisk (*) indicates the sire from the UK. Half-shaded circles and squares represent female and male carriers of the CLAD mutation, respectively. Open circles and squares indicate normal (clear) tested females and males, respectively. Numbers in diamonds refer to untested littermates.

spring. Because of the small gene pool in this breed, it might be advisable to adopt the strategy of not eliminating carriers with desirable traits, but rather opting to breed them only to normal (clear) animals and then either to test the progeny for the presence of the Cys36Ser mutation or choose to spay or neuter them before placement as pets. Following this strategy allows elimination of the CLAD mutation in a timely fashion without too severely restricting the breeding population, losing desirable characteristics, or producing additional CLAD-affected offspring.

Most of the Irish Red and White Setters in this study also have been tested for the presence of the mutation that causes type I rod-cone dysplasia (*rcd1*) in Irish Setters.[12] Approximately 70 dogs were tested between 1997 and 1998, and these represented most of the breeding stock in the United States at that time. None of these Irish Red and White Setters were found to carry the Irish Setter *rcd1* mutation. It would seem, then, that selective breeding efforts should focus on the eradication of the CLAD mutation in this breed.

The cross-breed dog in which CLAD was 1st described lived with an acceptable quality of life to 4 years of age, with intensive management by means of antimicrobial therapy. In contrast, the clinical course of the Irish Setters with CLAD from Sweden described by Trowald-Wigh et al[7] indicates that despite treatment with a variety of antibiotics, all of these dogs were euthanized by 6 months of age. Other affected dogs have survived as long as 14 months.[8]

Despite the longstanding presence of the CLAD mutation in the Irish Setter breed and the relatively high frequencies of the mutant allele in Irish Setters in Sweden and other parts of Europe,[9] few affected animals apparently are diagnosed and reported by veterinarians. This observation may be explained by CLAD-associated neonatal death (eg, omphalophlebitis) or by the lack of recognition of this hereditary disease by clinicians. Animals that have been reported with CLAD have had littermates that died soon after birth.[4,5,7] Because 25% of the offspring of a mating of 2 carrier parents would be expected to have CLAD, most affected dogs should have at least 1 other affected littermate (assuming an average litter size of 8 or more in Irish Setters). Thus, fading Irish Setter puppies with chronic or recurrent infections as well as their parents and littermates should be tested for CLAD.

Footnotes

[a] DNA extraction, Gentra Systems, Minneapolis, MN
[b] DNA extraction, Qiagen, Valencia, CA
[c] Gel purification, Qiagen, Valencia, CA
[d] Bidirectional sequence analysis, Licor, Lincoln, NE
[e] Hot Start Polymerase, Qiagen, Valencia, CA
[f] Hot Start Polymerase Buffer, Qiagen, Valencia, CA
[g] Reaction Buffer 2, New England Biolabs, Inc, Beverly, MA
[h] Restriction enzyme *Hin*fI, New England Biolabs, Inc, Beverly, MA

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References

1. Anderson DC, Smith CW. Leukocyte adhesion deficiency. In: Scriver CR, Beaudet AL, Sly WS, Valle D, ed. The Metabolic and Molecular Bases of Inherited Disease, 8th ed. New York, NY: McGraw-Hill; 2001:4829–4856.

2. Kehrli ME, Ackermann MR, Gilbert RO, Shuster DE. Leukocyte adhesion deficiency. In: Feldman BF, Zinkl JG, Jain NC, ed. Schalm's Veterinary Hematology. Philadelphia, PA: Lippincott Williams & Wilkins; 2000:995–1001.

3. Giger U, Greene CE. Immunodeficiencies and infectious disease. In: Green CE, ed. Infectious Diseases of the Dog and Cat. Philadelphia, PA: WB Saunders; 1998:683–693.

4. Giger U, Boxer LA, Simpson PJ, et al. Deficiency of leukocyte surface glycoproteins Mo1, LFA-1, and Leu M5 in a dog with recurrent bacterial infections: An animal model. Blood 1987;69:1622–1630.

5. Renshaw HW, Chatburn C, Bryan GM, et al. Canine granulopathy syndrome: Neutrophil dysfunction in a dog with recurrent infections. J Am Vet Med Assoc 1975;166:443–447.

6. Trowald-Wigh G, Hakansson L, Johannisson A, et al. Leucocyte adhesion protein deficiency in Irish setter dogs. Vet Immunol Immunopathol 1992;32:261–280.

7. Trowald-Wigh G, Ekman S, Hansson K, et al. Clinical, radiological and pathological features of 12 Irish setters with canine leucocyte adhesion deficiency. J Small Anim Pract 2000;41:211–217.

8. Kijas JMH, Bauer TR Jr, Gaefvert S, et al. A missense mutation in the β-2 integrin gene (ITGB2) causes canine leukocyte adhesion deficiency. Genomics 1999;61:101–107.

9. Kijas JMH, Juneja RK, Gaefvert S, et al. Detection of the causal mutation for canine leukocyte adhesion deficiency (CLAD) using pyrosequencing. Anim Genetics 2000;31:326–328.

10. Chadwick RB, Conrad MP, McGinnis MD, et al. Heterozygote and mutation detection by direct automated fluorescent DNA sequencing using a mutant *Taq* DNA polymerase. BioTechniques 1996;20: 676–683.

11. Richards B, Skoletsky J, Shuber AP, et al. Multiplex PCR amplification from the CFTR gene using DNA prepared from buccal brushes/swabs. Hum Mol Genetics 1993;2:159–163.

12. Suter ML, Pittler SJ, Qin N, et al. Irish setter dogs affected with rod/cone dysplasia contain a nonsense mutation in the rod cGMP phosphodiesterase β-subunit gene. Proc Natl Acad Sci USA 1993;90:3968–3972.