

Brief communication

Linkage of dermatomyositis in the Shetland Sheepdog to chromosome 35

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Abstract Dermatomyositis is an inflammatory disease of the skin and muscle and is most commonly found in the Shetland sheepdog. Both the clinical presentation and the age of onset of dermatomyositis vary widely, and the inability to diagnose dermatomyositis before clinical symptoms ensue has made control of the disease difficult. Identification of a genetic marker that cosegregates with dermatomyositis would facilitate the development of a DNA-based test for the early detection of affected dogs. We report the use of linkage disequilibrium (LD) mapping to identify linkage to phenotypic dermatomyositis in the Shetland sheepdog. One marker, microsatellite marker FH3570 on canine chromosome 35, had evidence of LD ($P = 0.00002$). Further studies are necessary to narrow the region harbouring the dermatomyositis locus, identify candidate genes and determine mode of inheritance.

Dermatomyositis is a devastating disease of the skin and/or muscle affecting the Shetland sheepdog and collie breeds.^{1–3} In our experience, Shetland sheepdogs predominantly have skin lesions. The earliest clinical signs of dermatomyositis are small focal areas of crusting and scaling that most commonly develop on the face and lower extremities. Over time, the crusts become more extensive and alopecia develops. In the late stages of the disease there is dermal scarring, associated with erythema and mottled pigmentation. The severity of the skin disease can vary from mild, resolving within a month, to severe, with signs lasting for months, years or the dogs' lifetime. Lesions may also wax and wane and are thought to be exacerbated by stressful periods such as estrus. Some dogs may also develop a concurrent pyoderma and secondary pruritus. Although muscle lesions are rare, when present, they can be the most severe clinical presentation of dermatomyositis as evidenced by difficulty in drinking and eating, diminished gag reflex and an abnormal high-stepping gait. Megaesophagus, which may be followed by aspiration pneumonia, may also occur. The quality of life of affected dogs can be so diminished that euthanasia is necessary.^{1,3}

Inheritance studies of dermatomyositis in the collie suggest that it may be an autosomal dominant trait with incomplete penetrance.² The mode of inheritance of dermatomyositis in the Shetland sheepdog has not

been investigated. Control of the disease has been difficult because of the variability in expression and age of onset, as well as the inability to diagnose dermatomyositis prior to the development of symptoms.

The objective of this study was to conduct a whole genome scan to identify a marker that cosegregates with dermatomyositis. Identification of a genetic marker linked to a disease allele can facilitate the diagnosis of affected and/or carrier dogs and the positional cloning of candidate genes. To detect linkage, we utilized an approach termed linkage disequilibrium (LD), which requires small numbers of unrelated affected and unaffected individuals. LD mapping is recognized as an effective tool for the identification of linkage in purebred dog populations.^{4,5}

Linkage studies are based on the principle that neighbouring loci are co-inherited. LD mapping operates on the assumption that the mutation causative for a disease occurred as a founder event and that affected individuals in subsequent generations inherit both the disease allele and the proximal DNA markers from this ancestor. With each generation, recombination events cause the LD to decay so that only markers that are very close to the disease locus will continue to be co-inherited. In LD, an allele for a DNA-marker is found more often among the affected (or normal) dogs than in the alternative group.

Microsatellites, tandem repeats of one to six base pairs, are DNA markers widely used to map disease traits. The most comprehensive screening set of microsatellite markers available for linkage studies in the dog is termed the minimal screening set-2 (MSS-2) and

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Table 1. Variables associated with the eight microsatellite markers that had increased *P* values when recalculated with additional genotypes

| Microsatellite | <i>P</i> value | No. of alleles observed | Allele of interest | Canine chromosome | Human chromosome |
|----------------|----------------|-------------------------|--------------------|-------------------|------------------|
| FH3570 | 0.00002 | 9 | 324 | 35 | 6p |
| FH2171 | 0.006 | 10 | 621 | 15 | 12q |
| REN213G21 | 0.008 | 3 | 269 | 19 | 2q |
| REN153O21 | 0.01 | 3 | 220 | 12 | 6p |
| C22.279 | 0.02 | 5 | 119 | 22 | 13q |
| AHT130 | 0.02 | 7 | 110 | 18 | 11q |
| REN94K23 | 0.04 | 5 | 238 | 35 | 6p |
| FH2145 | 0.04 | 5 | 297 | 3 | 15q |

provides an average of 9 Mb coverage of the canine genome.⁶

Sixty-one Shetland sheepdogs were evaluated for participation in the study. Skin punch biopsy samples were collected using routine procedures⁷ from dogs having clinical signs consistent with dermatomyositis and were used to histologically confirm disease status. Twenty-two Shetland sheepdogs, ages 3 months to 5 years, were biopsied and classified as affected. Dogs not exhibiting dermatomyositis symptoms were assumed to be unaffected. Thirty-nine dogs were classified as normal and ranged in age from 2 to 14 years, with a mean of 6 years. Younger dogs included in the study were from families with no history of dermatomyositis. Whole blood was collected from all dogs and used to isolate genomic DNA using the Flexigene DNA Kit (QIAGEN Inc., Valencia, CA, USA) or the Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, MN, USA).

Fluorescently labelled primers were synthesized, and multiplex polymerase chain reaction (PCR) was carried out for the MSS-2 as described in Clark *et al.*⁸ PCR products were resolved with an internal size standard (GeneScan 500 LIZ, Applied Biosystems, Foster City, CA, USA) using an ABI 3730XL DNA Analyser (PE Biosystems). Genotypes were determined using Genemapper® software version 3.5 (Applied Biosystems).

Analyses for LD were conducted by calculating the probability of an association between a given marker allele and the disease status. For each marker, the allele more often associated with affected dogs was identified. All other alleles were combined into a second independent class. Fisher's exact probability test for 2 × 2 tables was used to compare allelic frequencies between the normal and affected groups. A marker in LD with the dermatomyositis locus will not be in Hardy-Weinberg equilibrium. By convention, a *P* value of < 0.0001 provides evidence for LD and thus linkage between the marker and the locus affecting dermatomyositis.

Genotype data for 279 MSS-2 markers were generated for 19 affected and 29 normal Shetland sheepdogs. The remaining 48 MSS-2 markers either could not be amplified or were not able to be reliably genotyped. Additionally, 6 of the 279 genotyped markers showed only one allele and thus were uninformative in our population. Of the 273 markers analysed for LD with the dermatomyositis locus, 16 had an allele that appeared

to be more common among affected dogs. No significant *P* values were obtained for any of the 16 markers.

Because population size is important in linkage analysis, genotypes for an additional two affected and four unaffected Shetland sheepdogs were collected for the aforementioned 16 markers and *P* values were recalculated. Eight markers had increased *P* values (Table 1) and for one marker, FH3570, located on canine chromosome 35, there was evidence of LD (*P* value = 0.00002).

Primers for two microsatellite markers flanking FH3570, REN126G10 and FH3987 were synthesized and PCR products were analysed for all dogs. Unfortunately, both markers were predominantly mono-allelic and thus not informative for this study.

Our findings suggest that a locus affecting the dermatomyositis phenotype is located near marker FH3570 on chromosome 35, which lies in a region corresponding to 6p24.1–6p25.3 on human chromosome 6. Two dermatologic conditions have been mapped to this region: striate palmoplantar keratodema and skin fragility/woolly hair syndrome.⁹ Both diseases result from mutations in *desmoplakin* (*DSP*).⁹ *DSP* is the most abundant protein found in desmosomes, which functions to maintain the architecture and integrity of epidermal and cardiac tissue.⁹ Because it has been suggested that dermatomyositis is an autoimmune disorder,^{1,3} other genes in the area that are important to immune system function, such as *LY86* and *SERPINB9*, are also of interest.

Based on these data alone, it is not advisable to pursue sequencing candidate genes identified in this region because LD can extend for large distances in the pure-bred dog.^{4,5} Sutter *et al.* report that LD can extend from 400 to 700 kb in hugely popular breeds to 3 to 3.2 Mb in rarer breeds with smaller population sizes.⁵ The Shetland sheepdog has experienced many years of popularity in the USA, with 15 000 new registrations and a top 20 ranking by the American Kennel Club in 2004.¹⁰ These population statistics indicate that the extent of LD could be lower in this breed; however, LD is widely variable between breeds and loci.⁵ Therefore, it is necessary to further define the region of chromosome 35 harbouring the dermatomyositis locus by identifying other markers linked to the dermatomyositis phenotype. This may be accomplished through the identification of new microsatellites that are polymorphic in our population or using other marker types, such as single nucleotide polymorphisms.

Because the mode of inheritance of dermatomyositis in the Shetland sheepdog has not been determined, it is unknown if the locus on 35 is a major locus or a quantitative trait locus. Although the MSS-2 offers 9 Mb coverage, 54 markers were not genotyped or were uninformative in our study, reducing the actual coverage achieved. Data from new markers in regions having minimal coverage may reveal additional areas of interest. Multigenerational pedigrees of Shetland sheepdogs segregating dermatomyositis should be assembled for statistical evaluation to establish the mode of inheritance.

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Résumé La dermatomyosite est une maladie inflammatoire de la peau et des muscles, décrite principalement chez le Shetland. La présentation clinique et l'âge à l'apparition des symptômes varient nettement et il est impossible de diagnostiquer cette maladie avant que les symptômes ne soient apparus. L'identification d'un marqueur génétique faciliterait la mise au point d'un test ADN pour la détection précoce des chiens atteints. Nous rapportons ici l'utilisation d'une technique permettant d'identifier le lien au phénotype de la dermatomyosite chez le Shetland. Un marqueur, le FH3570 sur le chromosome 35 a été identifié ($P = 0.00002$). Des études supplémentaires sont nécessaires pour déterminer avec plus de précision la région du locus de la dermatomyosite, identifier les gènes candidats et déterminer le mode de transmission.

Resumen La dermatomiositis es una enfermedad inflamatoria de la piel y del músculo esquelético que ocurre con más frecuencia en el perro pastor de Shetland. Tanto el cuadro clínico como la edad de aparición de la dermatomiositis son muy variables, y la incapacidad para diagnosticarla antes de reconocer los signos clínicos ha hecho difícil el control de la enfermedad. La identificación de un marcador genético que segregue conjuntamente con el fenotipo de dermatomiositis facilitaría el desarrollo de una prueba de ADN para la detección temprana de los perros afectados. Aquí describimos el uso de mapeo mediante desequilibrio de ligamiento para identificar la relación con el fenotipo de dermatomiositis en el perro pastor de Shetland. Un marcador, el microsatélite FH3570 en el cromosoma canino 35, presentó evidencia de desequilibrio de ligamiento ($P = 0.00002$). Estudios más detallados serán necesarios para precisar la región que contiene el locus de dermatomiositis, identificar genes candidatos, y determinar el modo de herencia.

Zusammenfassung Dermatomyositis ist eine entzündliche Erkrankung der Haut und Muskulatur, die am häufigsten bei Shelties gefunden wird. Die klinische Präsentation sowie das Alter bei Beginn der Dermatomyositis zeigen eine große Variation. Zusätzlich erschwert die Unmöglichkeit, Dermatomyositis vor dem Erscheinen von klinischen Symptomen zu diagnostizieren, die Kontrolle der Krankheit. Die Identifizierung eines genetischen Markers, der mit Dermatomyositis gekoppelt ist, würde die Entwicklung eines auf DNS basierenden Tests für eine Früherkennung von betroffenen Hunden erleichtern. Wir beschreiben die Verwendung von Linkage Disequilibrium Mapping, um die Verbindung zum Phänotyp der Dermatomyositis beim Sheltie zu identifizieren. Ein Marker, der Mikrosatellitenmarker FH3570 auf dem caninen Chromosom 35, zeigte Linkage Disequilibrium ($P = 0.00002$). Weitere Studien sind notwendig, um die Region einzugrenzen, die den Dermatomyositis Locus beherbergt, um Anwärtergene zu identifizieren und um den Erbgang zu bestimmen.