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Genome-wide linkage scan localizes the harlequin locus in the Great Dane to chromosome 9

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ABSTRACT

Harlequin is a coat pattern of the Great Dane characterized by ragged patches of full color on a white background. Harlequin patterning is a bigenic trait, resulting from the interaction of the merle allele of *SILV*, and a dominant modifier locus, *H*. Breeding data suggest that *H* is embryonic recessive lethal and that all harlequins are *Hh*. To identify linkage with the harlequin phenotype, 46 Great Danes from 5 pedigrees were genotyped for 280 microsatellite markers in a whole genome screen. One marker on the telomeric end of chromosome 9 was suggestive of linkage. Fine mapping of this region using additional microsatellite markers and 10 Great Danes from a sixth pedigree resulted in significant LOD scores for 2 markers. Reported herein is linkage mapping of the *H* locus to a 3.27 Mb region of chromosome 9 containing approximately 20 genes.

1. Introduction

The harlequin coat pattern, named for its large ragged patches of color, is a popular variety of the Great Dane breed. Harlequin is a bigenic trait, resulting from the interaction of the harlequin locus (*H*) and the merle locus (*M*) (Sponenberg, 1985). Merle is a dominant coat pattern characterized by patches of full pigment on a dilute background (Fig. 1A). Harlequin is a dominant modifier of merle that removes the dilute pigment, leaving the background white (Fig. 1B). The patches of a harlequin are generally larger than those of a merle. Harlequin has no effect on the coats of non-merle dogs.

The merle phenotype results from a SINE insertion in *SILV* (Clark et al., 2006), a gene with a central role in melanogenesis (Theos et al., 2005). The insertion displaces the intron splice acceptor site and the resulting use of a cryptic site causes partial exonization of the SINE (Clark et al., 2008). Shortening of the unstable poly-A tail of the SINE during migration of melanoblasts permits proper splicing and results in random patches of color (Clark et al., 2008). *Mm* dogs are healthy, but *MM* dogs are predominantly white and have auditory and ocular defects. *MM* Great Danes are termed merlequins if patches of merling are present or white if no patches exist. Harlequin was once thought to be an allele of *M*, but studies of breeding records show that it is a separate locus (Sponenberg, 1985).

The merle pattern in the Great Dane is prohibited from the show ring. As such, breeders have selected for harlequin and against merle for decades, yet the merle pattern persists. All harlequin matings produce merle offspring, indicating that harlequins are heterozygous for the *H* locus (Sponenberg, 1985). The absence of dogs homozygous for harlequin suggests that the *HH* genotype is lethal (Sponenberg, 1985). Observations of statistically smaller litter sizes from harlequin to harlequin matings support this conclusion (O'Sullivan and Robinson, 1989).

No direct phenotypic homologue of harlequin has been described for the human, although harlequin and merle dogs both exhibit characteristics resembling those of Waardenburg syndrome type 2 (WS2), a heterogeneous auditory-pigmentary disorder. Abnormalities of WS2 are melanocyte-specific, and most prominent of these are sensorineural hearing loss and hypopigmentation; other clinical subtypes also involve musculoskeletal, intestinal, or other systems (Hornyak, 2006). WS2 results from mutations in genes important in melanocyte development. These genes include *MITF*, a regulator of *SILV*, and *SNAI2/SLUG* (Baxter and Pavan, 2003; Du et al., 2003; Sanchez-Martin et al., 2002; Tassabehji et al., 1994). *PAX3*, which is causative for WS1 (Tassabehji et al., 1992), was recently proposed as a candidate gene for harlequin based on genotype data (Schmutz and Berryere, 2007).

Abbreviations: LOD, logarithm of the odds; PCR, polymerase chain reaction; SINE, short interspersed element; SOLAR, Sequential Oligogenic Linkage Analysis Routines; TAMU, Texas A&M University; WS2, Waardenberg syndrome type 2.

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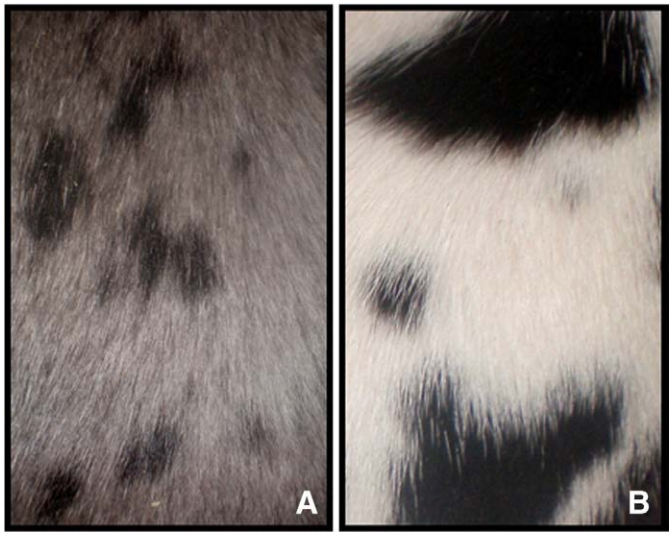

Fig. 1. The merle coat pattern has a dilute background (A) while the harlequin coat pattern has a white background (B).

Because there are numerous candidate genes for harlequin, a whole genome screen was performed to localize the *H* locus. Small pedigrees of harlequin and merle Great Danes were assembled and data were collected for a set of microsatellite markers spanning all canine chromosomes. Significant linkage (LOD = 3.44) was detected for a single marker on chromosome 9. The 7.6X dog genome assembly (CanFam2.0) was used to identify additional microsatellite markers flanking this region. A maximum LOD score of 4.07 was obtained for 56 Great Danes from 6 families. Reported herein is mapping of the harlequin locus to a 3.27 Mb segment near the telomeric end of canine chromosome 9. This region exhibits conservation of synteny with human chromosome 9q and does not harbor any genes known to influence coat patterning.

2. Materials and methods

2.1. Sample collection

Great Dane litters having both harlequin and merle puppies were recruited for this study. Whole blood or buccal cells were collected from all puppies and both parents, when available. Genomic DNA was isolated using the Puregene DNA Isolation kit (Gentra Systems, Minneapolis, MN) and adjusted to a concentration of 50 ng/μl.

2.2. Genotyping

Multiplex PCR was performed using fluorescently-labeled primers for markers of the minimal screening set -2 (Clark et al., 2004; Guyon et al., 2003). Primers for two additional microsatellites on chromosome 9 were designed using CanFam2.0 (GAAA1 forward = GCACCTTCTGAATAAG-CACA, GAAA1 reverse = AAACTTTACTCCTAAGCCCT; GAAA2 forward = ATCT-TAGGGTTGTGAGACTGAG, GAAA2 reverse = ACTCGGTGGAGATTCTGCTT). All PCRs were carried out as previously described (Clark et al., 2004). Products were resolved using an ABI Prism 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA) with an internal size standard (GeneScan 500 LIZ, Applied Biosystems). Genotypes were determined using Genemapper® Software v3.5 (Applied Biosystems).

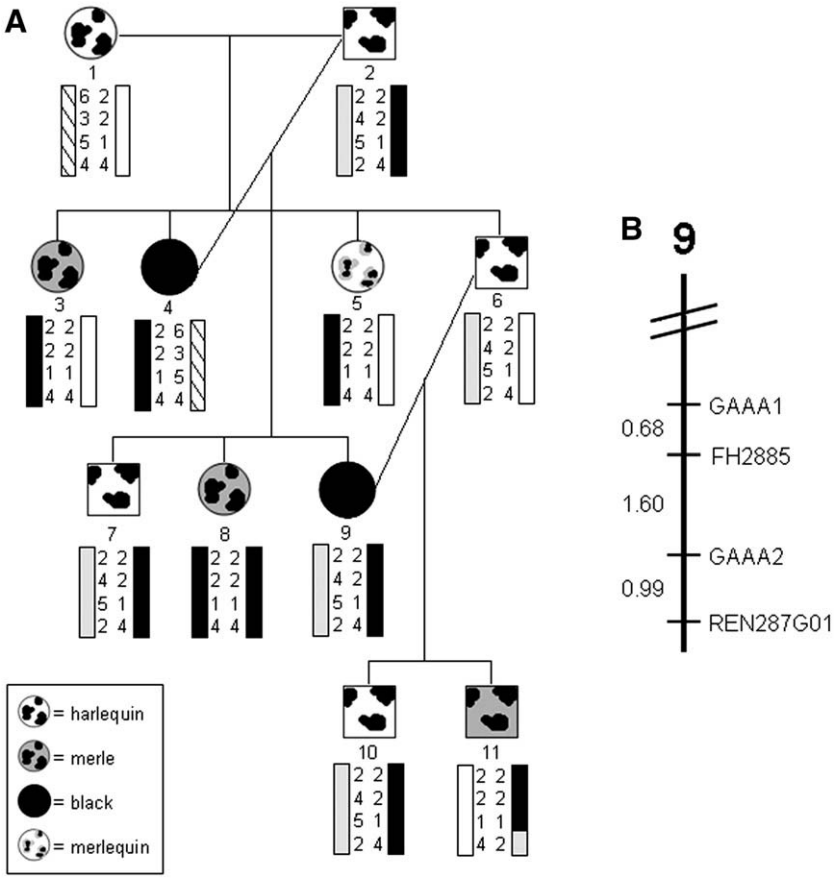

Fig. 2. A: Representative pedigree used in linkage analysis showing haplotypes for four microsatellite markers. The harlequin chromosome is striped for dog 1 and grey for dog 2. Dog 11 has a recombination event between the *H* locus and REN287G01. B: Schematic representation of the telomeric end of chromosome 9 showing the distance (Mb) of the four markers from A.

2.3. Linkage analysis

Calculation of two-point LOD scores was completed using the SOLAR (Sequential Oligogenic Linkage Analysis Routines) software package v2.1.4 (Almasy and Blangero, 1998). For analysis, only overt harlequins were assigned the harlequin phenotype. Black dogs, which can carry harlequin but cannot show the phenotype without the merle allele, were classified as unknown. Merles were assigned a non-harlequin phenotype. Based on breeding records, merlequin dogs were classified as non-harlequin and white dogs were classified as unknown. White dogs are often bred to black dogs to produce litters of roughly 50% harlequins and 50% merles, and it is unclear which parent gives the harlequin allele.

3. Results

3.1. Assembly of six pedigrees

Five unrelated pedigrees were assembled from 44 dogs in the general pet population. An additional four-generation family of 12 Great Danes was obtained from colleagues at Texas A&M University (TAMU) who designed an experimental pedigree for the investigation of dilated cardiomyopathy in the breed. Our population of Great Danes was comprised of 30 harlequins, 17 merles, 6 blacks, 2 merlequins, and 1 white.

3.2. Linkage analysis

Initially, 46 Great Danes from 5 families (4 pet families and the TAMU family) were genotyped for 280 microsatellite markers that cover the 38 autosomes of the dog. Four positive two-point LOD scores did not reach significance. Only one marker, FH2885, was suggestive of linkage with a LOD score of 2.40. Genotype data for this marker were generated for 10 Great Danes from a sixth family that was collected after completion of the initial screen. Subsequent recalculation of the LOD score revealed significant linkage (LOD = 3.44). FH2885 is located near the telomere of canine chromosome 9.

Two GAAA microsatellites flanking FH2885 were identified and genotyped for all 56 Great Danes. One marker, termed GAAA1, is located 685 Kb centromeric to FH2885, and was monomorphic in two kindred and did not yield a positive LOD score. The second marker, GAAA2, is 1.6 Mb telomeric to FH2885, was polymorphic in all families, and yielded a LOD score of 4.07. Alleles of both FH2885 and GAAA2 segregated perfectly with harlequin in all families.

3.3. Haplotype analysis

To fine map the harlequin locus, haplotypes were determined for GAAA1, FH2885, GAAA2, and Ren287G01; the latter marker is located 992 Kb telomeric to GAAA2 (Fig. 2). One recombination event was detected between the *H* locus and GAAA1, while three recombination events were detected between the *H* locus and Ren287G01. These markers flank a 3.27 Mb region containing approximately 20 genes.

4. Discussion

The *H* locus interacts with the *M* allele of *SILV* to produce complete hypopigmentation in areas that are normally dilute in merle dogs. Candidate genes for harlequin are those implicated in pigmentary anomalies of other species, such as the phenotypically similar WS2 of the human. Most WS2 cases have not been described at the genetic level, suggesting numerous genes that have an effect on development of melanocytes remain unidentified (Hornyak, 2006). The linkage data presented herein reveal that the *H* locus maps to a 3.27 Mb region of chromosome 9, which has conservation of synteny with human chromosome 9q32–34. No genes implicated in coat color or patterning are located in this area, suggesting that the *H* locus may be a novel pigmentation gene.

Haplotype analyses show the pattern of transmission of the harlequin chromosome. All harlequin dogs in this study population

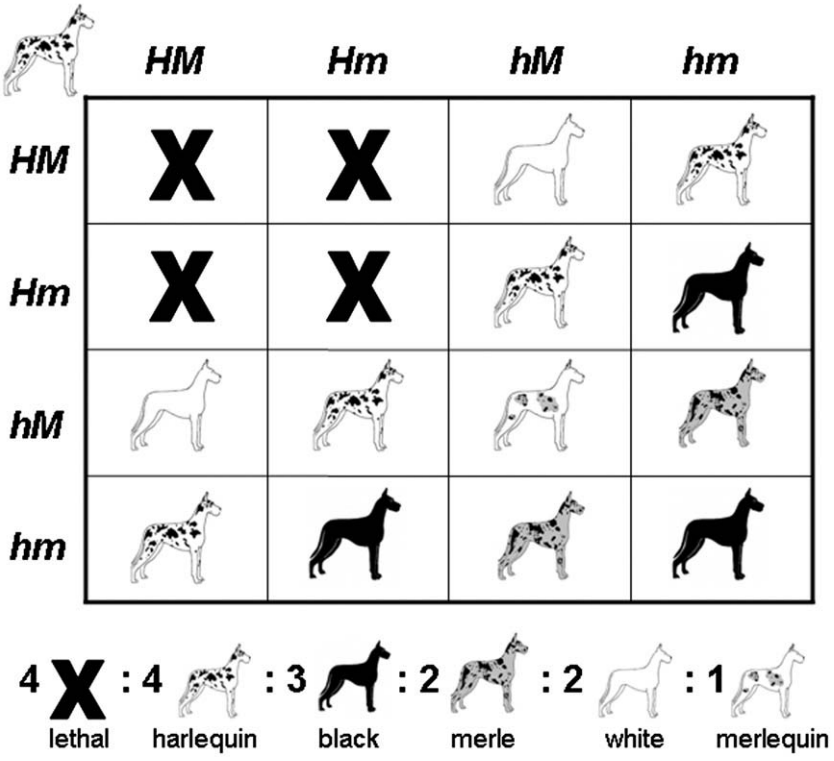

Fig. 3. Punnett square showing the phenotypic ratios of coat patterns resulting from a harlequin to harlequin mating.

are heterozygous for the harlequin chromosome. The absence of homozygous animals supports the hypothesis that the *H* locus is embryonic lethal in the homozygous state. Similarly, when homozygous mutations are present in other genes known to be important in pigmentation and development of melanocytes (*e.g.*, *SOX10* and *PAX3*), they are also embryonic lethal (Tachibana et al., 2003).

MM dogs are termed merlequin or white based on phenotypic characteristics, but they are generally not considered to be different genetically. Haplotype analyses herein revealed that two unrelated merlequins did not receive the harlequin chromosome from either parent. Conversely, the white dam gave the harlequin chromosome to all of her harlequin progeny; the black sire of her litter did not carry harlequin. These data suggest that merlequins are *MMhh* and whites are *MMHh*.

Based on the above, a harlequin to harlequin mating predicts the following phenotypic ratios: 1/4 lethal, 1/4 harlequin, 1/8 black (harlequin), 1/8 white, 1/8 merle, 1/16 merlequin, 1/16 black (non-harlequin) (Fig. 3). The study population is insufficient to confirm expected frequencies of coat patterns; however, Sponenberg (1985) and O'Sullivan and Robinson (1989) previously analyzed breeding records for Great Danes. They observed a deficiency of white offspring from harlequin to harlequin matings and postulated that the *MMHh* genotype has low viability (O'Sullivan and Robinson, 1989; Sponenberg, 1985). Our data predict that 16.7% of puppies born to harlequin parents will be white. This percentage is in agreement with those observed in the aforementioned studies (mean white births from harlequin to harlequin matings = 16%) and suggests that the *MMHh* genotype is not lethal.

One of the genes in the mapped region is *HSPA5* (also termed *BiP*, *GRP78*), which encodes an ER molecular chaperone of the highly conserved HSP70 family (Ting and Lee, 1988). *HSPA5* is a striking candidate for harlequin for two reasons: 1) it is a constituent protein of melanosomes (Chi et al., 2006) that may be involved in trafficking of SILV (Valencia et al., 2007) and 2) targeted disruption of *HSPA5* in a murine model causes homozygous mutant embryos to degenerate and resorb early in pregnancy (Luo et al., 2006.). Sequence analysis of *HSPA5* in the harlequin Great Dane will be the focus of further studies.

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