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BRIEF REPORT

A study of Rhodesian Ridgeback dogs indicates that the duplication responsible for hair ridge is not identical with the hypothesized locus for dermoid sinus

SOURCE/DESCRIPTION

The dorsal hair ridge in Rhodesian and Thai Ridgeback dogs is caused by a dominant 133-kb duplication on dog chromosome (CFA) 18 involving three *fibroblast growth factor* (*FGF*) genes (Salmon Hillbertz et al., 2007). A study on 42 Rhodesian and 12 Ridgebacks proposed a genetic model where dogs homozygous for the 133-kb duplication on CFA18 are affected by dermoid sinus (DS) (Salmon Hillbertz et al., 2007). This condition is an inborn malformation most frequently seen in ridged dogs and characterized by a tubular skin indentation with extensions into underlying tissues (Miller & Tobias, 2003; Salmon Hillbertz, 2005; Salmon Hillbertz et al., 2007; Wagner et al., 2007). Inflammations of the dermoid sinus may cause pains and neurological signs when there is a communication with the dura mater of the spinal cord (Miller & Tobias, 2003; Salmon Hillbertz, 2005; Salmon Hillbertz et al., 2007; Wagner et al., 2007).

SAMPLES AND QUANTITATIVE PCR

Samples from 182 Rhodesian Ridgeback dogs and seven controls including four Bernese Mountain and three Deutsch Drahthaar dogs were used for quantification of the 133-kb duplication (Table S1). Rhodesian Ridgeback dogs under study were ridged without DS ($n = 77$), ridged with DS ($n = 101$), ridgeless without DS ($n = 2$), and ridgeless with DS ($n = 2$). Detection of 133-kb duplication was performed in triplicates using a SYBR-Green based quantitative PCR (qPCR) on a 7500 Real-Time PCR-System (ABI, Thermo Fisher Scientific) as previously reported (Waldo & Diaz, 2015). We calculated relative copy number (RCN) using each two different primer pairs for the 133-kb duplication and a region outside of the 133-kb duplication. For each qPCR run, a control sample of a dog without a ridge was included.

STATISTICAL ANALYSIS

Screening 178 ridged Rhodesian Ridgeback dogs showed RCN values of 1.5, 2, 2.5 and >2.5 in 29.8% ($n = 47$), 64.6% ($n = 96$), 18.5% ($n = 33$) and 1.1% ($n = 2$), respectively (Table 1). All four ridgeless Rhodesian Ridgebacks and seven dogs from other breeds had RCN of 1. Thus, we confirmed the reliability and effectiveness of the qPCR methodology for determining the RCN of the 133-kb duplication and its perfect association with the ridge locus (Salmon Hillbertz et al., 2007;

TABLE 1 Frequencies of relative copy number (RCN) variants and odds ratios (OR) among RCN variants and dermoid sinus (DS) with 95%-confidence intervals (95% CI) and *p*-values in 182 Rhodesian Ridgeback dogs

	Phenotype				Differences of RCN least squares means			
	Ridged and		Ridgeless and					
RCN	no DS ($n = 77$)	DS ($n = 101$)	no DS ($n = 2$)	DS ($n = 2$)	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
1 (WT)	0	0	2 (100%)	2 (100%)	1.00 (reference)			
1.5	33(42.86%)	14 (13.86%)	0	0	0.42 (0.05–3.32)	0.41	1.00 (reference)	
2.0	34 (44.16%)	62 (61.38%)	0	0	1.82 (0.25–13.53)	0.56	4.30 (2.03–9.12)	0.0001
2.5	10 (12.99%)	23 (22.77%)	0	0	2.50 (0.31–20.27)	0.39	5.89 (2.25–15.47)	0.0003
3.0	0	1 (0.99%)	0	0				
4.0	0	1 (0.99%)	0	0				

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Waldo & Diaz, 2015). The effect of the RCN variants was significant for DS frequency ($p < 0.001$). Significant odds ratios indicated a higher risk to DS for RCN of 2 or 2.5 than for RCN of 1.5 ($p < 0.001$). Odds ratios among Rhodesian Ridgebacks with RCN = 1 and RCN between 1.5 and 2.5 were at 0.42 to 2.5. Out of the 77 ridged Rhodesian Ridgebacks without a DS, 57.1% had RCN ≥2, refuting a perfect association among RCN and presence of DS. Our results did not confirm the hypothesis that DS is a Mendelian trait and that DS only develops in ridged Rhodesian Ridgeback dogs when they carry two copies of the 133-kb duplication on CFA18. In conclusion, we show that the 133-kb duplication responsible for the ridged phenotype is not identical with the hypothesized locus for DS.

ANIMALS AND DNA PREPARATION

Blood samples of 182 Rhodesian Ridgeback dogs and DNA samples of four Bernese Mountain and three Deutsch Drahthaar dogs were obtained from our biobank of the diagnostic lab. Monarch® Genomic DNA Purification Kit (New England Biolabs) was used for DNA preparation. For each extraction, DNA was quantified using Nanodrop (Thermo Fisher Scientific) and diluted in 0.1×TE buffer to 2 ng/µl.

ADVANCED SYBR GREEN-BASED RIDGE ALLELE RELATIVE COPY NUMBER (RCN) DIAGNOSIS

We followed the protocol reported by Waldo et al. (2015), but compared the average RCN values to the expected RCN values instead of the observed RCN values of the likely genotypes rr (homozygous for the wildtype allele r), Rr, and RR (homozygous for the ridge allele R). The target region was the region within the 133-kb ridge duplication (amplified by primer set 1 F/R or 2 F/R) and the reference region was outside of the 133-kb ridge duplication (amplified by primer sets 3 F/R or 4 F/R; Table S1). In each experiment, four values of RCN were calculated by comparing primer set 1 to 3 and 4 and primer set 2 to 3 and 4, and then averaged to generate an average RCN value. For each test dog, three average RCN values were determined in triplicate experiments, and compared to the expected values of RCN = 1, RCN = 1.5, RCN = 2, RCN = 2.5, RCN = 3, and RCN = 4. The genotype with the highest *p*-value was assigned to the test subject, as being statistically indistinguishable from the expected RCN.

DATA ANALYSIS

Association analysis and calculation of odds ratio among RCN variants and DS were performed using SAS v.9.4 (SAS Institute, 2021).

ACKNOWLEDGMENTS

We are grateful to the dog breeders for donating samples from their animals.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest.

DATA AVAILABILITY STATEMENT

None of the data were deposited in an official repository. The data supporting the findings of the present study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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