Short Communication

A second *KRT71* allele in curly coated dogs

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Running title: second canine *KRT71* allele

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Summary

Major characteristics of coat variation in dogs can be explained by variants in only a few genes. Until now, only one missense variant in the *KRT71* gene, p.Arg151Trp, has been reported to cause curly hair in dogs. However, this variant does not explain the curly coat in all breeds as the mutant ^{151}Trp allele is for example absent in Curly Coated Retrievers. We sequenced the genome of a Curly Coated Retriever at 22x coverage and searched for variants in the *KRT71* gene. Only one protein-changing variant was present in a homozygous state in the Curly Coated Retriever and absent or present in a heterozygous state in 221 control dogs from different dog breeds. This variant, NM_001197029.1:c.1266_1273delinsACA, was an indel variant in exon 7 that caused a frameshift and an altered and probably extended C-terminus of the KRT71 protein NP_001183958.1:p.(Ser422ArgfsTer?). Using Sanger sequencing, we found that the variant was fixed in a cohort of 125 Curly Coated Retrievers, and segregating in 5 of 14 additionally tested breeds with a curly or wavy coat. *KRT71* variants cause curly hair in humans, mice, rats, cats, and dogs. Specific *KRT71* variants were further shown to cause alopecia. Based on this knowledge from other species and the predicted molecular consequence of the newly identified canine *KRT71* variant, it is a compelling candidate causing a second curly hair allele in dogs. It might cause a slightly different coat phenotype than the previously published p.Arg151Trp variant and could potentially be associated with follicular dysplasia in dogs.

Keywords: canis lupus familiaris, hair, hair follicle, dermatology, morphology, keratin, whole genome sequencing, animal model

In 2009, Cadieu and colleagues reported that major characteristics of coat variation in dogs are influenced by variants in three genes. A missense variant in *FGF5* explained long hair, a 167 bp insertion into the 3'-UTR of *RSPO2* was reported to cause wiry hair and furnishings (increased hair growth on face and legs), and a *KRT71* missense variant (c.451C>T, p.Arg151Trp) was found in dogs with a curly or wavy coat (Cadieu *et al.* 2009). Specific combinations of alleles with these variants lead to the desired breed standards, and genetic testing enables breeders to avoid unwanted coat phenotypes to a certain degree. However, in some cases genetic testing for the known variants fails to explain the coat texture. According to dog owners, the commercially testable *KRT71*:c.451C>T variant does not explain the curly hair in Curly Coated Retrievers. The standard coat of a Curly Coated Retriever is of black or liver colour with tight, crisp curls on the body and smooth, straight and very short hair on face and legs. Curly Coated Retrievers often have a sparsely haired tail ("rat tail"). Follicular dysplasia, characterized by symmetrical, non-pruritic hair loss, often with a waxing and waning course, is a relatively common coat disorder in this breed (Bond et al. 2016). During breed formation, Curly Coated Retrievers most likely got their curly coat from Irish Water Spaniels, another breed that is predisposed to follicular dysplasia (Cerundolo et al. 1999; Cerundolo et al. 2000; Bond et al. 2016). A genetic background for this disorder is suspected. In the present study, we aimed to identify the causative variant for the curly coat in Curly Coated Retrievers. Given that the known *KRT71*:c.451C>T variant was not present in several tested Curly Coated Retrievers, we hypothesized that another genetic variant is likely to cause their curls and probably fixed in the breed. We therefore sequenced the genome from a Curly Coated Retriever at 22x coverage (PRJNE448733, SAMEA104091556) and compared the sequencing data to 221 dog genomes from different breeds (Table S1). We called SNVs and short indels with respect to the canine reference genome assembly CanFam 3.1 as described earlier (Bauer *et al.* 2017). Applying a candidate gene approach, we extracted variants in the *KRT71* gene that were present in any of the 222 canine whole genome sequences (Table S2). Focusing on protein-changing variants, we found a single *KRT71* indel variant present in a homozygous state in the Curly Coated Retriever that was absent or heterozygous in all other

dogs. The variant can be formally described as NM_001197029.1:c.1266_1273delinsACA or NP_001183958.1:p.(Ser422ArgfsTer?). The identified novel *KRT71* variant is predicted to cause a frameshift with an extended mutant reading frame compared to the wildtype transcript, KRT71:p.(Ser422ArgfsTer212). Compared to the wildtype 525 aa KRT71, the predicted mutant protein has a length of 632 aa (Figure S1). RNA-seq data from skin biopsies demonstrated expression of the expected transcript without any splicing alterations from the allele with the indel variant (Figure 1).

To investigate the *KRT71* allele and genotype distribution in different dog breeds with curly or wavy coat, we genotyped 1286 dogs from 15 breeds for the previously described c.451C>T missense variant and the c.1266_1273delinsACA variant using Sanger sequencing (Table 1). For ease of reading we will from now on refer to the mutant alleles at these two variants as c^1 and c^2, respectively.

All 125 tested Curly Coated Retrievers were homozygous wildtype at c.451C>T and homozygous mutant c^2/c^2 at c.1266_1273delinsACA. While the c^1 allele overall was more common than the c^2 allele, there were many curly coated breeds, in which both alleles segregated. We also observed two dogs carrying both variants on one haplotype suggesting a past recombination or gene conversion event. This rare haplotype should be considered during future genetic testing applications.

KRT71 encodes a type II keratin specifically expressed in the inner root sheath of the hair follicle (Aoki *et al.* 2001; Langbein *et al.* 2002). Other than in dogs, *KRT71* variants have been identified and studied at a molecular level in humans, mice, rats and cats.

In humans, a *KRT71* missense variant affecting the helix initiation motif was discovered in a Japanese family with autosomal dominant woolly hair and hypotrichosis (Fujimoto *et al.* 2012). In mice, different dominant *Krt71* variants were described in spontaneous or induced mutants with curly or wavy hair such as the caracul or the reduced coat 12 and 13 mutants (Kikkawa *et al.* 2003; Runkel *et al.* 2006). Furthermore, a recessive 10 bp deletion variant in exon 1 of *Krt71* was reported to cause progressive alopecia in the reduced coat 3 mouse mutant (Peters *et al.* 2003). In rats, a *Krt71* splice defect was identified as the semidominant *Rex* allele that

causes curly hair in heterozygous animals and hair loss in homozygous mutant rats (Kuramoto *et al.* 2010). In rexoid cats, *KRT71* splice defects were found in the Devon Rex breed and in curly coated Selkirk Rex cats (Gandolfi *et al.* 2010; Gandolfi *et al.* 2013). Finally, in another cat breed, the hairless Sphinx, a splice site variant in *KRT71* leading to a frameshift and a premature stop codon was found, mostly in a homozygous state or compound heterozygous with the Devon Rex allele (Gandolfi *et al.* 2010).

The canine c.1266_1273delinsACA variant identified in the present study affects the sequence coding for the helix termination motif, altering approximately three quarters of its sequence as well as the whole C-terminal tail. In view of the numerous examples of *KRT71* variants causing curly hair, the inner root sheath restricted expression of KRT71 in the hair follicle, and the predicted effect of the c.1266_1273delinsACA variant on the protein, we think that it is a compelling candidate causative variant for the curly coat in Curly Coated Retrievers and other dog breeds. As specific *KRT71* variants can lead to hair loss in addition to curls in humans, mice, rats and cats, we further hypothesized that the canine c^2 allele might represent a genetic risk factor for some forms of follicular dysplasia in dogs. As Curly Coated Retrievers are fixed for the c^2 allele, we could not test for an association with follicular dysplasia in this breed. However, it is noteworthy that the Curly Coated Retriever breed standard calls for a smooth and straight coat on the forehead, face, front of forelegs, and feet (http://images.akc.org/pdf/breeds/standards/CurlyCoatedRetriever.pdf). This is quite distinct from breeds such as e.g. the Poodle, which has curls on the entire coat. Poodles are very nearly fixed for the c^1 allele. We thus speculate that the functional effect of the c^2 allele may be slightly different from that of the c^1 allele and that this hypothetical functional difference may be involved in the expression of the characteristic coat phenotype of Curly Coated Retrievers. Our cohort contained three curly coated dogs affected with follicular dysplasia that had been diagnosed by veterinary pathologists (1 Chesapeake Bay Retriever, 1 Lagotto Romagnolo, 1 Perro de Agua Español). All three follicular dysplasia cases were homozygous c^2/c^2. Our cohort also contained five Lagotto Romagnolos from Sweden diagnosed with adult onset

spontaneous, symmetrical, non-inflammatory alopecia affecting the trunk that were homozygous c^1/c^1. They were otherwise healthy.

In the Lagotto Romagnolo breed, c^1 and c^2 alleles were observed in all possible genotype combinations. Initially, we did not have phenotype information on the coat quality of most sampled Lagotto Romagnolo dogs in our biobank. Upon follow-up of the c^2/c^2 dogs, one of the owners reported severe alopecia on the back (Figure S2). Other c^2/c^2 Lagotto Romagnolo dogs showed a normal coat but several of their owners reported that the hair of these dogs could very easily be plucked.

Given the low frequency of the c^2 allele in Poodles, it is very striking that the only identified homozygous c^2/c^2 Poodle was alopecic and the potential differentials included follicular dysplasia. Similarly, the only observed homozygous c^2/c^2 Chesapeake Bay Retriever was diagnosed with follicular dysplasia. On the other hand, across breeds only a relatively small proportion of homozygous c^2/c^2 dogs in our study showed hair loss. It is therefore conceivable that the c^2 allele possibly represents a predisposing genetic risk factor for follicular dysplasia. However, even if this hypothesis is correct, other genetic and/or environmental factors will be required for the manifestation of follicular dysplasia.

In conclusion, we identified the *KRT71*:c.1266_1273delinsACA indel variant as the most likely causal variant underlying a second curly hair allele in dogs. This allele possibly causes a slightly different coat phenotype as the previously described curly allele with the *KRT71*:c.451C>T missense variant. Further studies are required to confirm whether the new curly allele modulates the risk for follicular dysplasia.

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Table 1. *KRT71* diplotypes in 1286 dogs from 15 different breeds with curly or wavy hair. We refer to the mutant alleles at the previously described c.451C>T missense variant as c^1 and the c.1266_1273delinsACA variant as c^2. In five of the tested breeds, both alleles were segregating.

Breed	***KRT71* diplotype**							**N**
	wt/wt	c^1/wt	c^1/c^1	c^2/wt	c^1/c^2	c^2/c^2	c^1/c^1c^2	
Airedale Terrier			5					5
Barbet	44	2						46
Bergamasco Shepherd dog	8	4						12
Bolonka Zwetna	8	8	8					24
Cão de Serra de Aires	15							15
Chesapeake Bay Retriever			3		9	1[1]		13
Curly Coated Retriever						125		125
Lagotto Romagnolo		14	559	3	172	12[1]		760
Mudi			11		18	3	2	34
Perro de Agua Español			73		20	2[1]		95
Poodle			89		6	1[2]		96
Portugese Water dog		3						3
Puli			1					1
Schapendoes	44	4						48
Soft Coated Wheaten Terrier	7	2						9
								1286

[1]The cohort contained 3 dogs, which had been diagnosed with follicular dysplasia. They all had the diplotype c^2/c^2.
[2]This poodle had pronounced alopecia on the body and an almost completely hairless tail. Unfortunately no histopathological examination of a skin biopsy was performed to confirm the suspected follicular dysplasia.

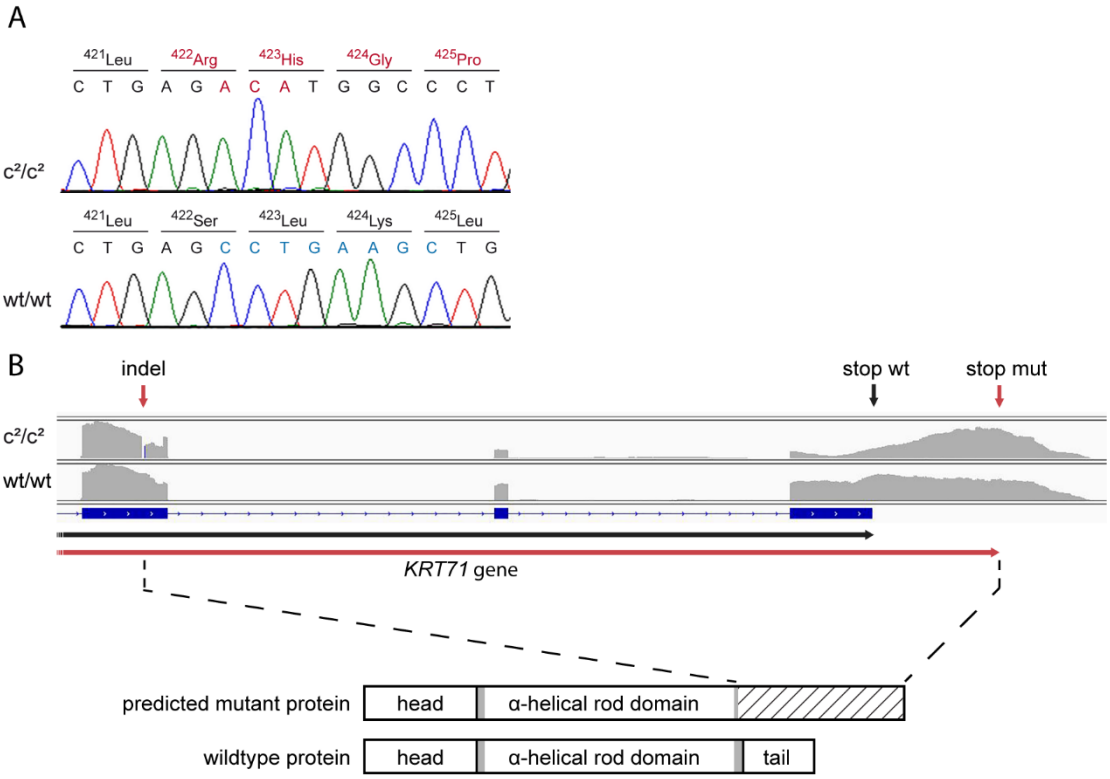

Figure1. Illustration of the *KRT71*:c.1266_1273delinsACA variant and its predicted consequence on the KRT71 protein.
A) Sanger electropherograms of dogs with c^2/c^2 and wt/wt genotypes. Bases shown in red are inserted, bases in blue are deleted in the mutant c^2 allele. The variant leads to a shift in the reading frame and thus an altered amino acid sequence in the mutant protein (amino acids written in red letters). (B) RNA-seq data from a Curly Coated Retriever with c^2/c^2 genotype and a Greyhound with *wt/wt* genotype showing the read coverage mapped to the last three exons of the *KRT71* gene. As a result of the indel variant in exon 7 (red arrow), the mutant open reading frame is extended and ends at a later termination codon (stop mut, red arrow) compared to the wildtype open reading frame (ending at stop wt). A scheme of the predicted mutant and wildtype protein is shown below the RNA-seq data. Grey boxes indicate helix initiation and helix termination motifs. The frameshift is predicted to affect part of the helix termination motif and the whole C-Terminal tail of the protein (hatched area).

Supplementary Material

Table S1. Sample designations and breed information on 222 dogs with genome sequences.

Table S2. Genotypes of 222 dogs at *KRT71* variants where the sequenced Curly Coated Retriever was homozygous for the mutant allele.

Table S3. Sample designations and breed information on dogs with RNA-seq data.

Figure S1. Alignments of wildtype and mutant canine *KRT71* transcripts and KRT71 proteins

Figure S2. Coat phenotypes in dogs with different *KRT71* genotypes.