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SHORT COMMUNICATION

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A new Finnish flavor of feline coat coloration, "salmiak," is associated with a 95-kb deletion downstream of the *KIT* gene

Abstract

Cats with a distinctive white hair pattern of unknown molecular cause have been discovered in the Finnish domestic cat population. Based on the unique appearance of these cats, we have named this phenotype salmiak ("salty licorice"). The use of a commercially available panel test to genotype four salmiak-colored cats revealed the absence of all known variants associated with white-haired phenotypic loci: full White (*W*), Spotting (W^S) and the Birman white Gloves associated (w^g) allele of the *KIT proto-oncogene* (*KIT*) gene. Whole-genome sequencing on two salmiak-colored cats was conducted to search for candidate causal variants in the *KIT* gene. Despite a lack of coding variants, visual inspection of the short read alignments revealed a large ~95 kb deletion located ~65 kb downstream of the *KIT* gene in the salmiak cats. Additional PCR genotyping of 180 domestic cats and three salmiak-colored cats confirmed the homozygous derived variant genotype fully concordant with the salmiak phenotype. We suggest the newly identified variant be designated as w^{sal} for "w salmiak".

Variation in the *KIT proto-oncogene* (*KIT*) gene associated with an absence of melanocytes in the skin and hair follicles is a common cause of white coat appearance, especially in various domestic animal species (Brooks et al., 2007; Henkel et al., 2019; Küttel et al., 2019). Across cat breeds, breed types and populations, the appearance of white patterning or full white is explained by a feline endogenous retrovirus (FERV-1) insertion in intron one of the *KIT* gene (Anderson et al., 2022; David et al., 2014; Frischknecht et al., 2015). An insertion of a full-length 7125 bp FERV-1 element is responsible for the white spotting (W^S) pattern widely distributed in the cat population, while a partial 632 bp fragment of FERV-1 at the same position is associated with the full white (*W*) coat coloration (David et al., 2014). The presence of white paws is also associated with W^S heterozygosity (Anderson et al., 2022). Additionally, a 2 bp deletion insertion variant in the *KIT* gene (w^g allele) has been associated with the Birman white paws (gloves) phenotype (Montague et al., 2014). The sparse hair with roaning (the mixing of hairs with full and white color) phenotype observed in the Lykoi cat breed is attributed to variants of the *hairless* (*HR*) gene (Buckley et al., 2020a, 2020b).

A unique white-haired coat color pattern was first noted in 2007 in one cat population in Petäjävesi, central Finland (Figure S1). The color patterning phenotype resembles a normal "tuxedo" pattern of ventral white but with additional color gradation within hairs showing full coloration at the base and no color (white) at the tips throughout the dorsal regions. The decrease in color is less prominent near the head and in some individuals a band of more intense coloring is seen in the scapular region. However, the patterning shows individual variation with some individuals manifesting only few white hairs in the colored areas. Colored spots are also noticeable in the white areas of the front legs and chest (Figure 1). The end of the cat's tail is white or nearly white. All known cats have yellow and green eyes, which are also the most common eye colors in domestic cats. We have named this phenotype salmiak (salty licorice). This salmiak patterning of white hairs is most noticeable in solid black cats, but it can occur with different color backgrounds, including, for example, brown tabby, tortoiseshell and dilution (blue) and in both male and female cats (Figure 1). The unique coloring is present at birth and seems to stay highly similar throughout life (Figure S2). Previously, salmiak-colored cats were considered possibly infertile solely owing to the maintained low prevalence of this coat color in the cat population, but a recent observation of a salmiak-colored cat giving birth to four kittens refuted this belief (cat shown with kittens in Figure S2A). To our knowledge, salmiak cats have not been suspected of exhibiting hearing impairments. However, to definitively exclude any potential auditory issues, it would be necessary to conduct brainstem auditory evoked response testing.

DNA analyses were conducted to unravel the genetic background of the salmiak-colored cats. The study sample consisted of domestic cat blood samples collected by the owners' written consent in the Helsinki University biobank

under the permit ESAVI/25696/2020 by the Animal Ethics Committee of State Provincial Office of Southern Finland, Hämeenlinna, Finland. In total we received DNA samples from five salmiak-colored cats. DNA samples from four of the five salmiak-colored cats, two males and two females, were genotyped for known coat color variants using the MyCatDNA™ panel test based on a custom developed Illumina Infinium XT platform (Wisdom Panel/Mars Petcare Science & Diagnostics) (Table S1). In this panel test, the genotyping assay for the *W* and W^S alleles detected the presence of either variant without distinguishing between them, while the w^g allele was detected separately. All four salmiak cats were genotyped as wild type (*w*, full color) with no white-associated *W* alleles present. The solid black or blue parts of the coat were explained by a non-agouti (*aa*, solid color) genotype of the *agouti-signaling protein* (*ASIP*) gene in all four cats (Figure 1a,b,e) (Eizirik et al., 2003). Additionally, the blue cat had two copies of the d allele of the *melanophilin* (*MLPH*), resulting in a diluted (*dd*, blue) coat color (Figure 1b) (Ishida et al., 2006). Further, genome-wide low-resolution mapping (418 kb average distance) was performed using the neutral SNP data obtained as a part of MyCatDNA™/Optimal Selection Feline tests for four salmiak-colored cats and 21 random-bred cats as controls using the PLINK association test. We found no significant region of homozygosity between the

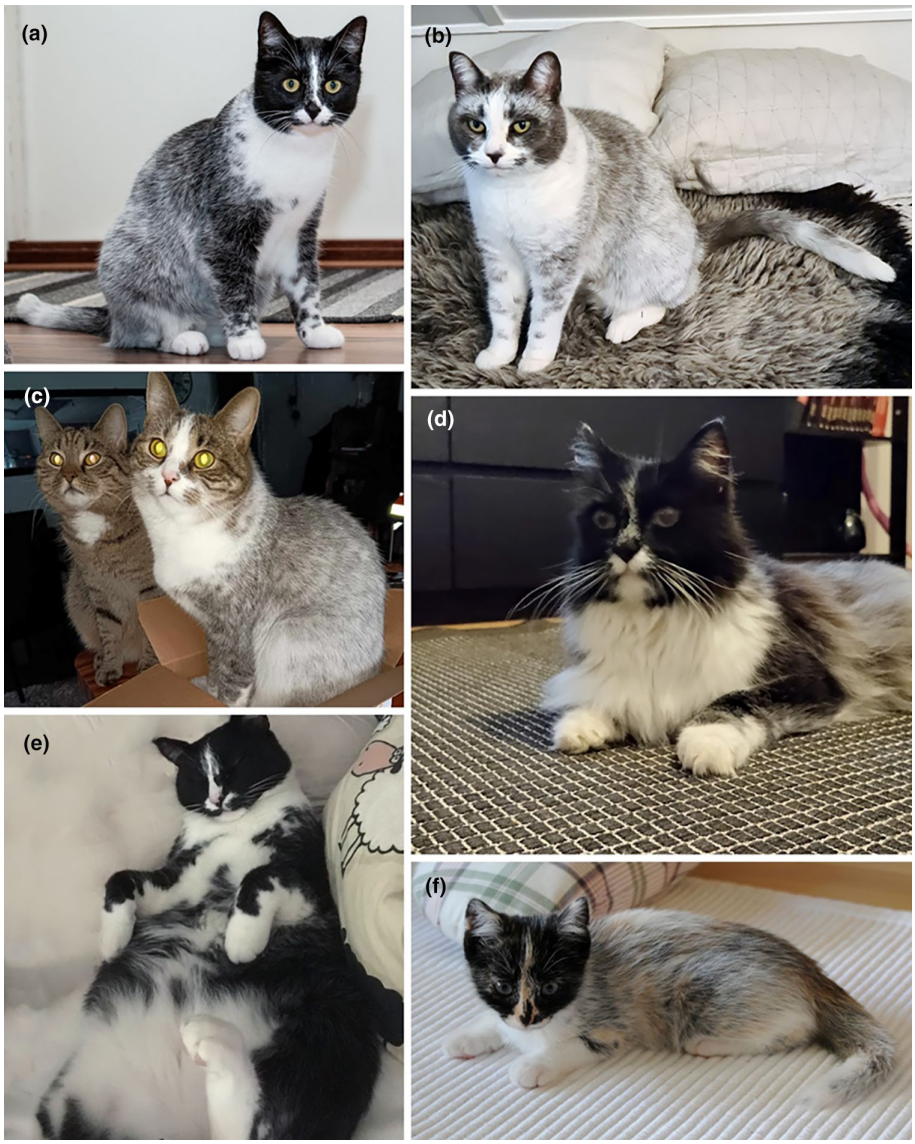

FIGURE 1 Salmiak coloring in cats. Prominent features of the coloring are: "tuxedo" (a.k.a. bicolor) white spotting in the absence of white spotting alleles (W^s, *g*), and additional gradation of the pigment within hairs of primary color toward no pigmentation at the tips in the body, legs and tail. Additionally, there is primary colored spotting in the white areas of the front legs and chest, more intense coloring in the scapular region, and a very pale tip of the tail. (a) Salmiak solid black cat ($aa/w^{sal}w^{sal}$), (b) salmiak solid blue cat (diluted black, $aa/dd/w^{sal}w^{sal}$), (c) salmiak brown mackerel tabby ($w^{sal}w^{sal}$) (right) and his normal-colored brother heterozygous for salmiak ($w^{sal}w$), (d) salmiak phenotype on a long-haired solid black cat (not genotyped), (e) salmiak solid black cat ($aa/w^{sal}w^{sal}$) and (f) salmiak phenotype on a tortoiseshell cat (not genotyped). Cat a was sequenced, and cats b, c and e were genotyped for salmiak. Photo credits: (a) Ari Kankainen and (b–e) courtesy of the cat owners.

four unrelated salmiak cats (Data S1). This is likely to be explained by low extent of linkage disequilibrium (~17 kb) in random-bred domestic cats (Alhaddad et al., 2013).

We conducted whole-genome sequencing for two salmiak-colored cats (which were also genotyped with MyCatDNA™ panel) at Novogene (Novogen (HK) Company Limited, China) using the Illumina HiSeqX platform with high coverage (read depth ≈ 30×).The raw data are deposited in the Sequence Read Archive under the Bioproject ID PRJNA1075502 and accession numbers SRR27953328 and SRR27953327. The DRAGEN pipeline from the Illumina DRAGEN Complete Suite v3.9.5 was used to map and align the raw data (FASTQ files) to the Felis_catus_9.0 genome assembly as a reference sequence (Buckley et al., 2020a, 2020b) and for joint genotyping of this data to create a multi-VCF. SNPEFF v5.1 was used to annotate and predict the functional effects of the variants (Cingolani et al., 2012). SNPSIFT v5.1 was used to predict the significance of the variants and filter the most significant for further analysis (Cingolani et al., 2012). We did not identify unique *KIT* variants segregating with the salmiak phenotype compared with other unrelated feline full genomes sequences or VCF from the 99 Lives Genome Sequencing Initiative (http://felinegenetics.missouri.edu/99lives). A visual inspection of short read alignments using INTEGRATIVE GENOMICS VIEWER (Robinson et al., 2011) revealed a novel 94 991 bp deletion in the 65 875 bp downstream region of the *KIT* gene in salmiak-colored cats (Figure 2). The deletion coordinates are chrB1:163792100-163 887 091, and the *KIT* coding sequence coordinates are on the opposite strand at chrB1:163953936-164 039 268 (NM_001009837.3) in the Felis_catus_9.0 genome assembly.

We performed PCR and gel electrophoresis-based genotyping together with Sanger sequencing to genotype the newly identified variant of 180 DNA samples of Finnish domestic cats available in the feline biobank at the University of Helsinki and manifesting salmiak and non-salmiak phenotypes. Two set of primers, 5′GGGAGGAGGGACACAATCAA and 3′ACAA CCCAAATACCCATCAATGA (product size 386 bp), and 5′TTCAAAGGCAGCCACATTCC 3′CCCCAG CTCCCTACTTCATC (product size 550 bp), were used to confirm the variant and genotype the ends of deletion breakpoints (deletion present assays), and primer sets 5′GGGAGGAGGGACACAATCAA and 3′ACCCCGAAGCTCAGATGATG (product size 380 bp) and 5′ACCCGTAACCGTGAACAGAT and 3′ATTGA ACCCCAGCTCCCTAC (product size 361 bp) were used for genotyping the wild-type allele. The primer designs used the Felis_catus_9.0 as a reference sequence. The newly identified variant, that we refer to as w^{sal} for "w salmiak" for clarity hereafter, was fully concordant with the salmiak phenotype. Three salmiak-colored cats that were not whole-genome sequenced were also confirmed as homozygous for the w^{sal} allele, bringing the total to five. Three of the non-salmiak cats were heterozygous for the w^{sal} allele, and 175 were homozygous for the reference (wild-type) allele. All heterozygotes had a non-salmiak phenotype, which is consistent with an autosomal recessive mode of inheritance (Figure 1c). Anecdotally, heterozygous cats may have some white within their coat, although it is currently unclear whether this is due to the w^{sal} allele or has another genetic cause, such as an unknown allele for white lockets or white belly spots. The distribution of w^{sal} allele between observed salmiak and non-salmiak phenotypes was evaluated statistically significant ($p < 0.001$) with the Freeman–Halton extension of Fisher's Exact Probability test for a 2 × 3 table (Freeman & Halton, 1951) (Table 1). We also genotyped one domestic cat from Romania and one from the UK, which manifested another type of unusual white patterning referred to as karpati of yet unknown molecular

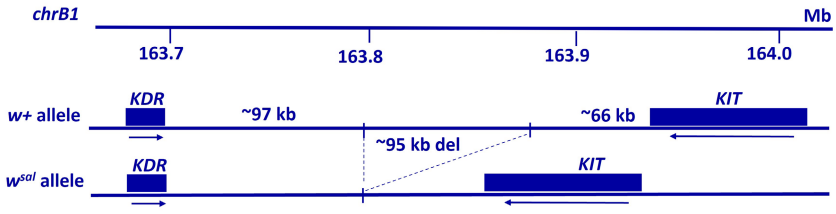

FIGURE 2 Schematic presentation of the newly identified w^{sal} allele of the *KIT* locus. A 94991 bp deletion is present in the 65 875 bp 3′flanking region of the *KIT* gene (*note*: *KIT* gene read direction is from the opposite strand).

TABLE 1 Finnish domestic cats genotyped for the novel w^{sal} allele and its association with the salmiak phenotype ($p < 0.001$).

Phenotype	Breed	Two copies of w^{sal}	One copy of w^{sal}	No copies of w^{sal}	Total
Salmiak	Domestic cat (Finland)	5	0	0	5
Non-salmiak	Domestic cat (Finland)	0	3	175	178
Totals		5	3	175	183

cause. We confirmed that the w^{sal} allele was absent in both of the karpati-colored cats.

Here, we show a large deletion in the 3′ flanking region of the *KIT* gene for the first time associated with the uncommon white-haired phenotype in cats. Other structural variants downstream of the *KIT* gene have been previously associated with coat color phenotypes in cattle, goats and horses (Brooks et al., 2007; Henkel et al., 2019; Küttel et al., 2019). In two Pakistani goat breeds, of which one is completely white and another one is white with colored patches, there is a copy number variation starting ~63 kb downstream of *KIT* and spanning a ~100 kb region that has a disrupted variant in a genomic region most similar to the salmiak variant (Henkel et al., 2019). In summary, comparative data from other species and genotype segregation analysis support the newly discovered *KIT* region deletion as potentially being a cause of salmiak coat color in cats.

KEYWORDS

coat color, domestic cat, feline, Finland, *KIT*, phenotype, *W* locus

AUTHOR CONTRIBUTIONS

Heidi Anderson: Conceptualization; data curation; formal analysis; investigation; methodology; project administration; resources; visualization; writing – original draft. **Milla Salonen:** Conceptualization; investigation; project administration; resources; visualization; writing – original draft. **Sari Toivola:** Conceptualization; data curation; investigation; resources; visualization; writing – review and editing. **Matthew Blades:** Data curation; formal analysis; methodology; resources; writing – review and editing. **Leslie A. Lyons:** Resources; writing – review and editing. **Oliver P. Forman:** Investigation; supervision; writing – review and editing. **Marjo K. Hytönen:** Investigation; methodology; resources; validation; writing – review and editing. **Hannes Lohi:** Conceptualization; funding acquisition; project administration; supervision; writing – original draft; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

HA and OPF are Wisdom Panel, Mars Petcare Science & Diagnostics employees that offer canine and feline DNA testing as a commercial service. MB was an employee of the Wisdom Panel at the time of this study.

DATA AVAILABILITY STATEMENT

The data that support the finding of this study are openly available in the Sequence Read Archive under Bioproject ID PRJNA1075502 and accession numbers SRR27953328 and SRR27953327.

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