Short Communication

A single base deletion in the *SLC45A2* gene in a Bullmastiff with oculocutaneous albinism

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Running title: Canine *SLC45A2* single base deletion

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Summary

Oculocutaneous albinism type 4 (OCA4) in humans and similar phenotypes in many animal species are caused by variants in the *SLC45A2* gene, encoding a putative sugar transporter. In dogs two independent *SLC45A2* variants are known that cause oculocutaneous albinism in Doberman Pinschers and several small dog breeds, respectively. For the present study, we investigated a Bullmastiff with oculocutaneous albinism. The affected dog was highly inbred and resulted from the mating of a sire to its own grandmother. We obtained whole genome sequence data from the affected dog and specifically searched for variants in candidate genes known to cause albinism. We detected a single base deletion in exon 6 of the *SLC45A2* gene (NM_001037947.1:c.1287delC) that has not been reported so far. This deletion is predicted to result in an early premature stop codon. It was confirmed by Sanger sequencing and perfectly co-segregated with the phenotype in the available family members. We genotyped 174 unrelated dogs from diverse breeds, which were all homozygous wildtype. We therefore suggest that *SLC45A2:*c.1287delC causes the observed oculocutaneous albinism in the affected Bullmastiff.

Keywords: Canis lupus familiaris; dog; melanocyte; pigmentation; cream; coat colour; *SLC45A2*; oculocutaneous albinism

Coat colour in mammals is dependent on the synthesis of two different pigments in melanocytes, the black/brown eumelanin and the yellow/red phaeomelanin (Barsh, 1996). Oculocutaneous albinism (OCA) in humans summarizes several autosomal recessive disorders where this pigment synthesis is distorted, which results in a reduction of pigmentation in skin, hair, and eyes. To date, seven different types, OCA 1-7, are known and causative variants have been identified in six different genes, namely *TYR*, *OCA2*, *TYRP1*, *SLC45A2*, *SLC24A5,* and *C10orf11* (Grønskov *et al.* 2007, Montoliu *et al.* 2014). Variants in *SLC45A2* encoding solute carrier family 45 member 2, formerly also called *AIM-1* or *MATP*, have been associated with OCA4 in humans (OMIM #606574; Newton *et al.* 2001). *Underwhite* mice, cream coloured horses, a single albinistic Western Lowland Gorilla, white tigers, silver chicken and orange-red coloured medaka b mutants were reported to be caused by variants in the *SLC45A2* gene (Newton *et al.* 2001; Mariat *et al.* 2003; Prado-Martinez *et al.* 2013; Xu *et al.* 2013; Gunnarsson *et al.* 2007; Fukamachi *et al.* 2001). *SLC45A2* has been investigated early on as a candidate gene in dog colour genetics, but initially no consistent variant explaining all cases of cream coloured dogs could be identified (Schmutz & Berryere, 2007). Subsequently, white Doberman Pinschers were shown to be homozygous for a large deletion of 4.1 kb including the last exon of *SLC45A2*. White Dobermans are characterized by OCA including health problems such as photophobia and an increased risk of cutaneous melanocytic neoplasms (Winkler *et al.* 2014). Several small, long haired albino dogs, including a Lhasa Apso, a Pekinese, two Pomeranians and a mixed breed dog were shown to be homozygous for a missense variant (p.Gly493Asp) in exon 7 of *SLC45A2*, leading to OCA (Wijesena & Schmutz, 2015). Based on these findings, we hypothesized that a variant in *SLC45A2* could be responsible for the phenotype of the OCA affected Bullmastiff in our study.

We received samples from an almost white male Bullmastiff puppy and several of its relatives. The affected dog had only minimal pale pigmentation of the skin and hair and blue eyes (Figure 1). The owner did not report any additional health problems such as eye squinting or photophobia. Pedigree analysis of the affected Bullmastiff revealed an inbreeding loop involving the mating of a male sire to his own grandmother. The OCA affected dog was born in a litter of 10, from which 8 puppies were euthanized after a few days due to atresia ani, a congenital anomaly of the rectum and anus (Figure 2A). One of the two surviving puppies showed oculocutaneous albinism, both parents and the other surviving puppy had a fawn phenotype, which is typical for Bullmastiffs.

The complete genome of the OCA affected Bullmastiff was sequenced at 14x genome coverage using 2 x 150 bp reads on an Illumina HiSeq 3000 instrument (ENA project accession PRJEB16012, sample accession SAMEA103949042). Private variants were identified by comparing the obtained sequence to 373 control dog genomes generated for previous projects and the canine reference genome assembly CanFam 3.1 as described (Bauer *et al.* 2017). We

detected 13 private homozygous protein-changing variants including a frame-shifting single base deletion in exon 6 of the *SLC45A2* gene (CanFam3.1:chr4:73,864,860delC; NM_001037947.1:c.1287delC; Table S1). This frameshift is predicted to result in a premature stop codon in the open reading frame (NP_001033036.1:p.(Met430CysfsTer4), which truncates the last 101 amino acids of the wildtype SLC45A2 protein.

We confirmed the variant in the affected Bullmastiff by Sanger sequencing (Figure 2B). Primers SLC45A2_F1, CTGGTCCTTCCTTGTGGAAA, and SLC45A2_R1, GAGCTGCAGAGGAAAGAGGA, were used to amplify exon 6 of the *SLC45A2* gene together with flanking sequences from the affected dog, five closely related family members as well as 174 unrelated dogs of various breeds with normal pigmentation (Table S2). Primer SLC45A2_F2, CACCGCCAGCTGTAATTTCT, was used as sequencing primer to obtain sequences on an ABI3730 capillary sequencer. The Sanger sequencing confirmed perfect co-segregation of the recessive deletion allele with the phenotype in the investigated family. None of the 174 unrelated dogs from various breeds carried the mutant allele.

An interesting feature of *SLC45A2* variants is the genotype-phenotype correlation considering the impact of the variant and its effect on the production of eumelanin versus phaeomelanin. The *SLC45A2* missense variant p.Ala477Val with recessive inheritance in tigers and the two chicken missense variants p.Tyr277Cys and p.Leu347Met with dominant inheritance probably have the least impact and retain some functional activity (hypomorphic alleles). These alleles affect mostly phaeomelanin but not eumelanin production (Xu *et al*. 2013, Gunnarsson *et al*. 2007). In horses, the missense variant p.Asp153Asn prevents phaeomelanin synthesis in heterozygotes, whereas in homozygotes both phaeomelanin and eumelanin synthesis are inhibited (Mariat *et al*. 2003). Sex-linked imperfect albinism in Japanese quail, *underwhite* in mice, and oculocutaneous albinism in Doberman Pinschers are caused by recessive null alleles involving frame-shift or deletion variants, where both eumelanin and phaeomelanin synthesis is blocked (Gunnarsson *et al*. 2007, Newton *et al*. 2001, Winkler *et al.* 2014). While most *SLC45A2* missense variants in domestic animals primarily have an effect on phaeomelanin, p.Gly493Asp found in small dog breeds apparently has a more profound effect and also blocks phaeomelanin and eumelanin production (Wijesena & Schmutz, 2015).

In our study, we identified a new *SLC45A2* variant, which most likely causes oculocutaneous albinism in a Bullmastiff. The effect of this frame-shift variant is in agreement with previous results as it leads to a nearly complete loss of both phaeomelanin and eumelanin production.

Acknowledgements

The authors would like to thank the dog breeder for donating samples and pictures and for sharing pedigree information of her dogs. The authors also wish to thank Nathalie Besuchet, Muriel Fragnière, and Sabrina Schenk for expert technical assistance. The Next Generation

Sequencing Platform and the Interfaculty Bioinformatics Unit of the University of Bern are acknowledged for performing the whole genome re-sequencing experiments and providing high performance computing infrastructure. This study was supported by grants from the Swiss National Science Foundation (31003A_149313) and the Albert-Heim Foundation (no. 105).

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Figure 1 Bullmastiff with oculocutaneous albinism.

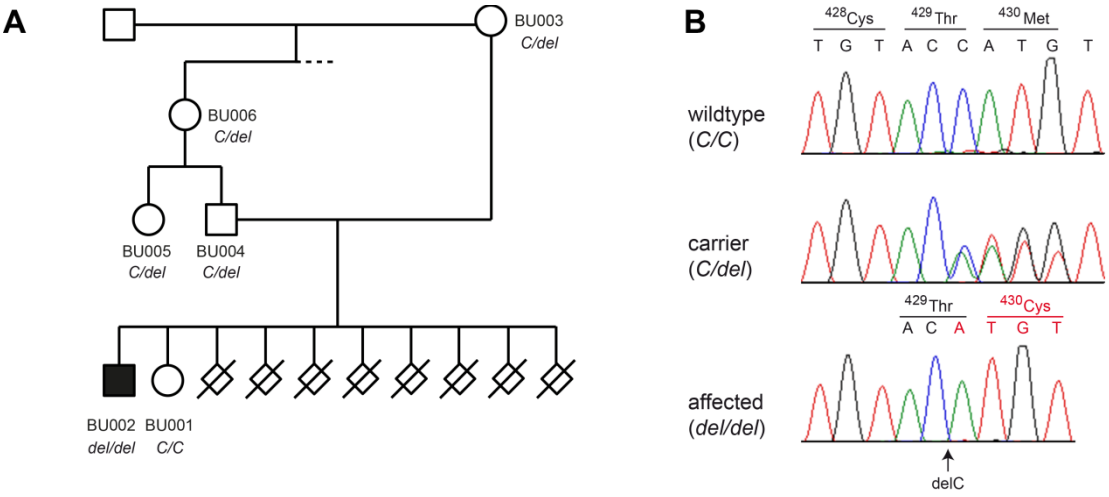

Figure 2 Genotypes of the affected Bullmastiff and its relatives. **A** The pedigree of the Bullmastiff family reveals an inbreeding loop. Genotypes of the sampled dogs are indicated. **B** Sanger sequencing chromatograms illustrate sequences of a homozygous wildtype dog, a heterozygous carrier and the homozygous affected dog. A homozygous deletion of a single cytosine is visible in the dog with oculocutaneous albinism (*SLC45A2:*c.1287delC).

Supplementary Material

Table S1. Private homozygous protein-changing variants in the affected Bullmastiff.

Table S2. Control dogs.