First genetically confirmed case of Lethal Acrodermatitis in a Bull Terrier in Türkiye

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ABSTRACT

Lethal acrodermatitis (LAD) is a rare disease affecting bull terriers and miniature bull terriers characterized by poor growth, progressive skin lesions, and immunodeficiency. A mutation in the Muskelin 1 (*MKLN1*) gene was determined as the causative mutation for LAD, and a genetic testing method for affected dogs has been established. A bull terrier representing symptoms similar to those of LAD was submitted to clinics, and a blood sample was taken for genetic testing. DNA was extracted, and direct mutation screening confirmed the causative mutation MKLN1:c.400+3A>C. Due to the severe progression of the disease and lack of available treatment, we have been informed that the patient was euthanised. This is the first reported case of LAD from Turkiye, and based on our findings, we strongly suggest that owners and breeders implement genetic testing before breeding to reduce and eventually eradicate this mutation from the population.

The Bull Terrier is a breed that originated from crossing Bulldogs and the now extinct White English Terrier back in 1835 by James Hinks in Britain. To develop breed characteristics and improve the overall quality of the breed, the Bull Terriers were outcrossed with Spanish Pointers, Dalmatians, and Greyhounds. The breed was initially bred for pit fights; thus, they have been famous for their agility, tenacity, and constitution (3). However, in time, probably due to changing demands and improvements in animal rights, breeders have added companionship to breed personality. Currently, the Bull Terriers are categorised according to their colour (white, coloured) and size (standard, miniature) (2). Even though there is a lot of information on Bull Terriers and their traits, there seems to be no publicly available information on their breeding records.

Lethal acrodermatitis (LAD) is a rare disease affecting approximately 12% of the bull terrier population as either carriers or affected patients (3). LAD is characterised by poor growth, skin lesions, and immune deficiency (1, 5-8). The disease was first recognised at the University of Pennsylvania genetics clinic veterinary school in 1982 (11). Because the disease was reported only in the Bull Terrier, a genetic background was suggested (5). In the following years, it was generally agreed that LAD must have a genetic background, no study was conducted to determine the causative mutation until 2018. A splicing defect on the canine MKLN1 gene, located on the 14th chromosome spanning across 5574374-5904923 (CanFam3.1), was associated with LAD in Bull Terriers (OMIA 002146-9615). The causative mutation is monogenic, transmitted in an autosomally recessive manner in bull terriers and miniature bull terriers (1).

The reported phenotypic representation of LAD is growth retardation, progressive skin lesions, paronychia, diarrhoea, abnormal behaviour, bronchopneumonia, and death by around one and a half years old. When affected puppies reach 8 weeks of age, they are noticeably smaller

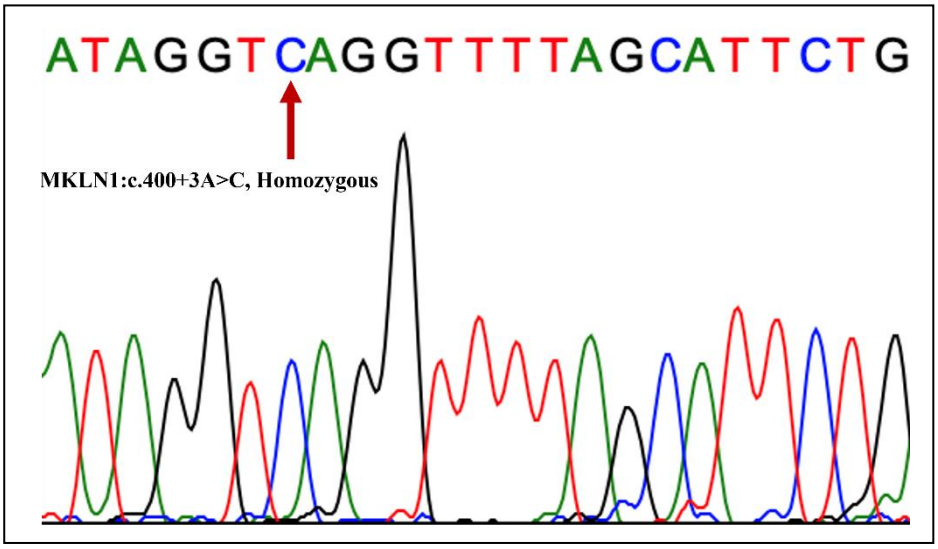

Figure 1. Sanger sequencing chromatography revealing the causative homozygous mutation MKLN1:c.400+3A>C (Arrow).

than their littermates. Some of the puppies affected by LAD show a lighter pigmentation that becomes pronounced with age compared to their normal/healthy littermates. Usually, characteristic skin lesions appear on the feet and face by the time the puppy reaches 6-8 weeks of age. Prominent symptoms begin in the extremities, consisting of splayed digits, erythema, interdigital pyoderma, and paronychia, followed by hyperkeratotic footpads. Affected dogs have difficulty eating solid foods and lodge the food into their abnormally arched hard palate. Many affected dogs also show symptoms such as diarrhoea, lethargy, and decreased responsiveness as the disease progresses. When affected puppies reach one year of age, they have half the body size and weight of a healthy littermate (5, 6, 8, 9). Due to their immunodeficient state and reduced IgA levels, they are susceptible to infections and suffer from skin (Malassezia, Candida) and respiratory tract infections. Bronchopneumonia is identified as a common cause of death (7, 9).

A blood sample from a bull terrier with LAD symptoms was submitted to Ankara University Faculty of Veterinary Medicine, Department of Genetics for mutation screening. The DNA was extracted from the whole blood with a commercial DNA extraction kit (GF-1 Blood DNA Extraction Kit, Vivantis, Malesia) following the manufacturer's instructions. DNA quality and quantity were measured spectrophotometrically (Nanodrop 2000, Thermofischer, USA), and DNA integrity was visualised via agarose gel electrophoresis (Kodak, Logic 200 imaging system, USA). Direct mutation screening for the candidate variant MKLN1:c.400+3A>C was performed by PCR and Sanger sequencing. To amplify the region harbouring the mutation, PCR was done using primers Dog: MKLN1F CCATGCACTGTAGCCACATC and Dog: MKLN1R TGGAAAAGGTTCCACTTGAAAT.

PCR was set up containing 80 ng DNA, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.5 µM of each primer, 1 X PCR Buffer, and 1 U/µl of Taq DNA polymerase (Fermentas, Thermo Fischer Scientific), and added ddH_2O to a final volume 25 µl. Thermal cycling was carried out using the Mastercycler thermocycler (Eppendorf, USA) with an initial denaturation at 94 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing at 60 °C for 30 sec extension at 72 °C for 30 sec and final extension at 72 °C for 5 minutes. PCR products were purified and sequenced in both directions. PCR products were visualised safely by dyed agarose gel electrophoresis and scanned (Gel Logic 200 Imaging System, Kodak). A 796 bp long PCR band was obtained, and then the PCR products were purified (GeneJet PCR purification Kit, ThermoScientific) following the manufacturer's instructions. Purified PCR products were used as templates in the BigDye Terminator v3.1 cycle sequencing reaction, bi-directionally, using the same primers. The sequencing reaction products were purified (DNA Sequence Purification Kit, Zymo, USA), and products were sequenced using an ABI310 automatic sequencer (Applied Biosystems, Foster City, CA, USA). Electroforegrams and chromatographs were analysed with Bioedit Sequence Alignment Editor (4), and aligned to the reference gene sequence (ENSCAFG00000001406), confirming the presence of the MKLN1:c.400+3A>C splicing defect mutation (Figure 1).

LAD has similarities to zinc-responsive dermatosis of canines, acrodermatitis enteropathica (AE) of humans, and lethal trait A46 of Black Pied Danish cattle. These diseases are associated with zinc absorption and/or metabolism. Although the skin lesions are similar in these diseases, oral or intravenous zinc supplementation does not show any curative signs in LAD patients (5, 8, 13). According to a study, LAD patients showed considerable

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improvements in skin lesions when zinc supplements was supported with vitamin complexes, omegas, and copper. Unfortunately, the outcome remained the same, which is either death caused by LAD or euthanasia (10). In our case, after the confirmation of the mutation, we have been informed, that with the owner's consent, the patient was euthanised.

Moreover, abnormal behaviour is considered one of the symptoms caused by LAD; in our case, abnormal behaviour was not reported. Also, in a study analysing behaviour differences in 28 dogs, none of the dogs showed any abnormal behaviour according to their owners (8). However, since behaviour is a subjective topic, it should not be considered a good indicator (12).

Although there are no publicly available records for Bull terrier breeding in Turkiye, it is public knowledge that it continues to this day. The breeders should acknowledge the LAD presence in the Bull terrier population. There is no known cure currently available for dogs affected by LAD. Early genetic diagnosis is crucial for eliminating this mutation from the population. To help the survival of the following generations of bull terriers, breeders should implement genetic testing when choosing the sires and dams to decrease and eventually eliminate LAD mutations from the population.

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Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

NB conceived and planned the experiments. FK carried out the experiments. FK took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical Statement

This study does not present any ethical concerns.

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