

## Supplementary Materials

### Cleft lip and palate in four full-sib puppies from a single litter of Staffordshire Bull Terrier dogs: An anatomical and genetic study

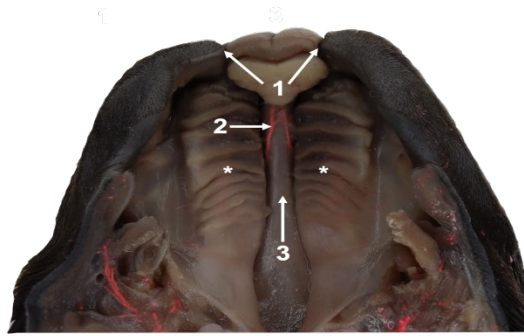
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**Table S1.** PCR primers used in the study of the *ADAMTS20*, *DLX6* and *MYH3* genes.

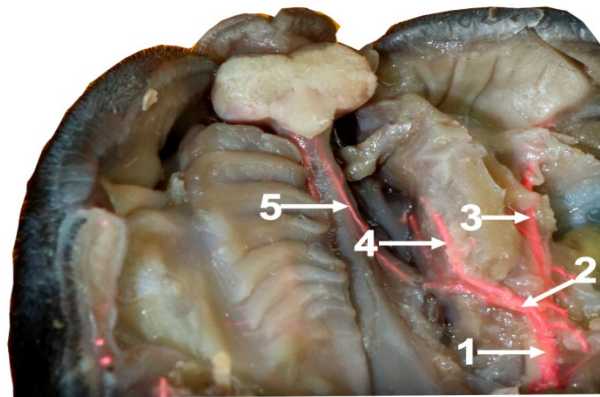
Gene	Analyzed variant	Primers sequence (genomic location of the studied regions according to CanFam3.1)	Annealing temperature	Amplicon length
<i>ADAMTS20</i>	2 bp deletion (c.1360_1361delAA)	F: 5' TGTTCACATGATGATAGTTTAAATG R: 5' CCCGGGTCTCTAGAATCACA (chr27:10553360 - 10553759)	56 °C	400 bp
<i>DLX6</i>	LINE 1 insertion in intron 2	F: 5' ACCATCGCTTTCAGCAAACCT R: 5' CTAGGCCCGAGAATTCCTCCT (chr14:22067918 - 22068089)	60 °C	172 bp
	control amplicon	F: 5' AAAACGGGGAAATCAGGTTC R: 5' ATCTGCAGGCAGGGTCAG (chr14:22067831 - 22068052)	60 °C	222 bp
<i>MYH3</i>	11-bp deletion and A>G substitution in exon 21	F: 5' TGAAAACCTTGTGGATGCAGA R: 5' TGATTCACAATGAGCACACG (chr5:34924947 - 34925232)	61 °C	286 bp



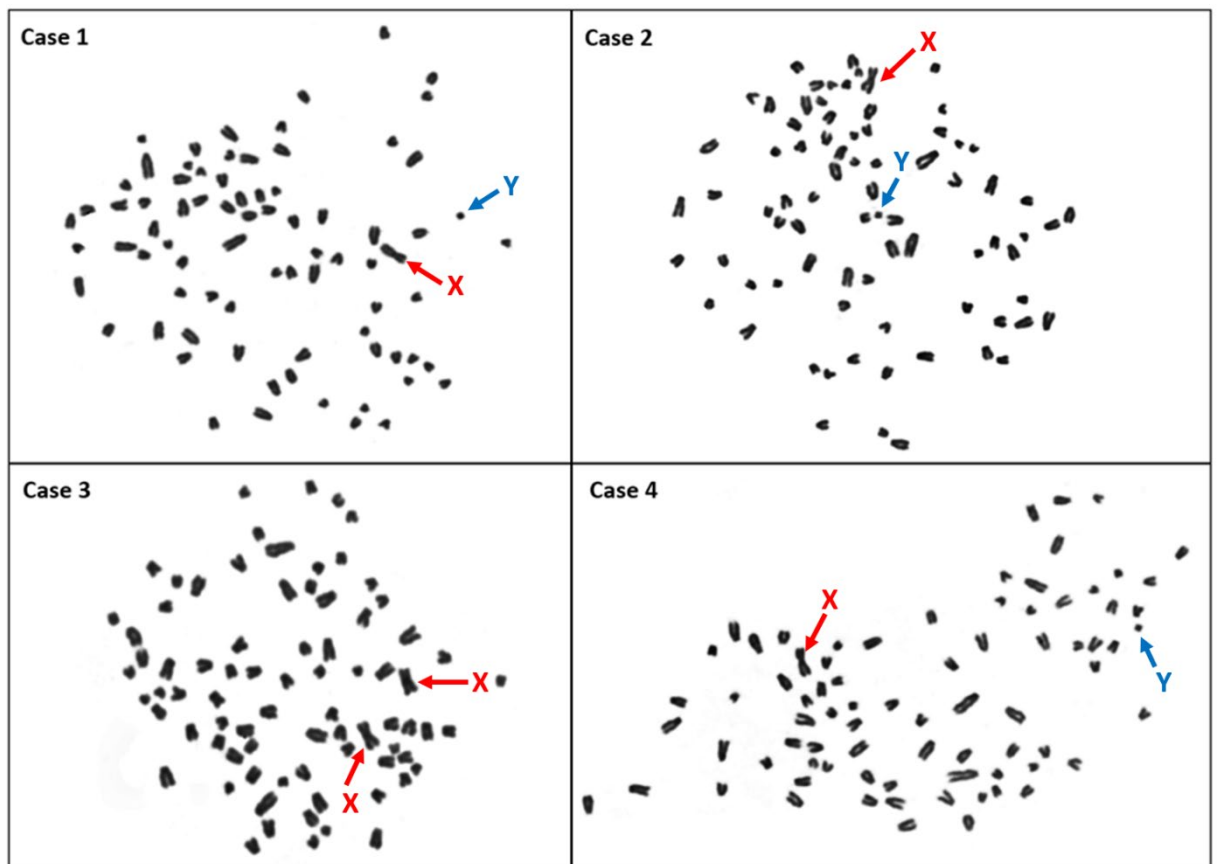
**Figure S1.** Bilateral cleft of the lip in Staffordshire Bull Terrier puppy (case 4). Black arrows indicate the clefts.



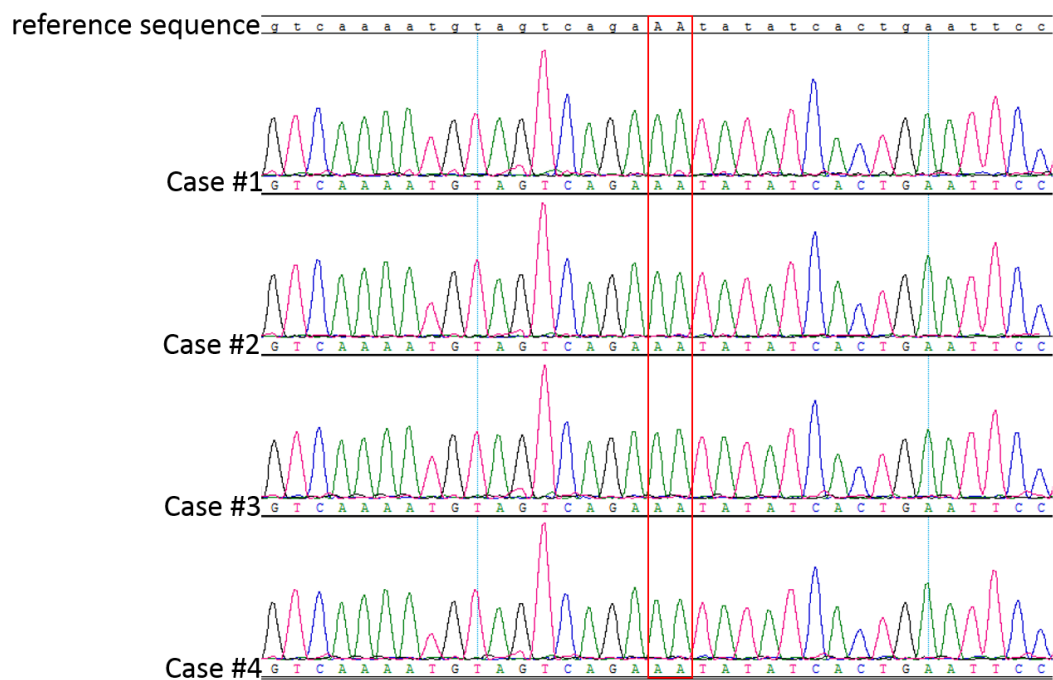
**Figure S2.** Anatomical preparation of the head of the Staffordshire Bull Terrier dog (case 4). The arterial vessels are visible due to the latex injection technique. 1: cleft of the lip; 2: septal nasal artery; 3: vomer; \*: disunited parts of the palate.



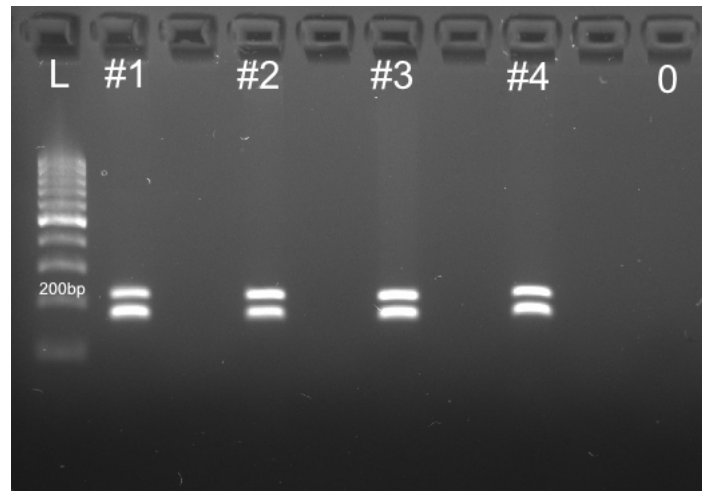
**Figure S3.** Latex preparation of the palatal region of the Staffordshire Bull Terrier puppy (case 1). 1: maxillary artery; 2: descending palatine artery; 3: infraorbital artery; 4: major palatine artery; 5: septal nasal artery.



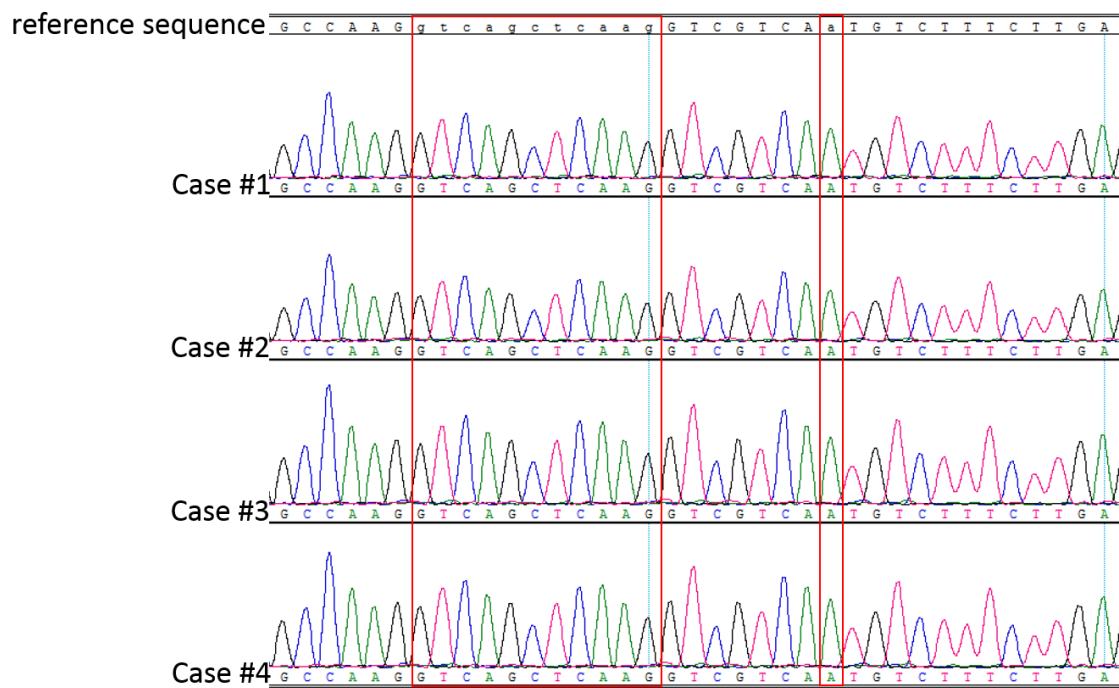
**Figure S4.** Representative metaphase spreads showing normal chromosome complement for male and female dogs in the puppies. Sex chromosomes are indicated by arrows (X: red, Y: blue).



**Figure S5.** Sequencing of the candidate fragment of the *ADAMTS20* gene in the dogs, showing the lack of 2 bp deletion (in the frame).



**Figure S6.** Agarose gel electrophoresis for the fragments in *DLX6* gene after PCR-duplex (222 bp: control amplicon; 172 bp fragment amplified if the LINE-1 insertion is not present in a homozygote status). L: DNA Ladder; 1–4: the affected puppies; 0: negative control (no DNA template).



**Figure S7.** Sequencing of the candidate region of the *MYH3* gene in the four dogs showing the lack of 11-bp deletion and A>G substitution (in frames).