The association of C789A polymorphism in the dopamine beta-hydroxylase gene (*DBH*) and aggressive behaviour in dogs

Daniel Polasik[1], Aneta Konieczna[2], Arkadiusz Terman[1], Andrzej Dybus[1]

West Pomeranian University of Technology in Szczecin, [1]Department of Genetics, [2]Faculty of Biotechnology and Animal Husbandry, Szczecin, Poland

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Abstract

The genetic basis of aggressive behaviour has been examined extensively, including analysis of genes encoding neurotransmitters, signalling molecules and regulatory enzymes, as well as their synthesis and degradation. Dopamine beta-hydroxylase, an enzyme catalysing the conversion of dopamine into norepinephrine in synaptic endings, significantly affects the modulation of emotional states and behaviour. The aim of this study was to determine the association of C789A polymorphism in the canine dopamine beta-hydroxylase gene (*DBH*) and aggressive behaviour in dogs. A total of 110 dogs of different breeds were analysed. All animals were classified according to their individual behavioural characteristics, defined by a veterinary interview and observation. Polymorphism was analysed using ACRS-PCR (amplification created restriction site-polymerase chain reaction) method. Significant differences in *DBH* genotypes and allele frequency between aggressive and non-aggressive dogs were observed ($\chi^2 = 16{,}232$, $P = 0.0003$). In aggressive dogs, the *CC* genotype (0.788) and *C* allele (0.815) were most frequent while in non-aggressive dogs, their frequencies were significantly lower (0.361 and 0.404, respectively). The obtained results indicate that *DBH* is a promising candidate gene for canine behavioural study.

Canis familiaris, aggression, genetics, SNP

Aggression is one of the most common behavioural problem in dogs. Results of studies on human populations indicate that genetic factors may contribute nearly 50% of the determination of behavioural traits, suggesting the multigenic (quantitative) nature of personality phenotypes (Bouchard et al. 1990). Neurotransmitters in the central nervous system are a very important factor in the modulation of emotional states and behavioural patterns in both humans and animals (Steimer 2002). Dopamine beta-hydroxylase (DβH) is an enzyme catalysing the conversion of dopamine into norepinephrine - one of the main neurotransmitters of the peripheral and central nervous system. Dopamine beta-hydroxylase is located almost entirely inside the synaptic vesicles of postganglionic sympathetic neurons (norepinephrinic) and the adrenal medulla (Gonzalez-Lopez and Vrana 2020).

Polymorphisms in genes encoding regulatory enzymes of neurotransmitters can be associated with various behaviours, including aggression. *DBH*, the structural locus encoding the DβH enzyme is the major quantitative trait locus influencing plasma DβH activity (Cubells and Zabetian 2004; Kopecková et al. 2006). An analysis showed that the C-1021T polymorphism, located 1,021 bp upstream of the transcriptional start site in the 5'-flanking region of the human *DBH* gene, is associated with 35–52% of the total variance in DBH plasma activity (Zabetian et al. 2001). Further studies took into consideration C-1021T single nucleotide polymorphism (SNP) in relation to affective disorders and personality styles. Results indicated that the *TT* genotype was correlated with personality features linked to impulsiveness and aggressive hostility. However, no relations were observed for affective disorders and *DBH* variants (Hess et al. 2009).

Address for correspondence:
Daniel Polasik
Department of Genetics
West Pomeranian University of Technology in Szczecin
Aleja Piastów 45, 70-311 Szczecin, Poland

Phone: +48 91 449 6782
E-mail: daniel.polasik@zut.edu.pl
http://actavet.vfu.cz/

In canines, the *DBH* gene is located on chromosome 9 (Cfa9) in the position 50,004,806-50, 024,130. It consists of 12 exons, whereas the transcript length is 2346 bp (Cunningham et al. 2019). Takeuchi et al. (2005) detected two SNPs in the canine *DBH* gene - C789A and A1819G. Of them, only C789A showed significant variation ($P < 0.01$) between five analysed breeds. A1819G polymorphism, however, was present only in one breed - Shiba. The C789A SNP (rs851198876) is located in exon 4 of the *DBH* gene and causes asparagine to lysine (Asn263Lys) substitution (Cunningham et al. 2019). A study performed later by Våge and Lingaas (2008) confirmed presence of the C789A variant as well as 5 additional nonsynonymous SNPs in the canine *DBH* gene. The authors also predicted a possible damaging effect of the amino acid substitutions caused by the C789A polymorphism as leading to positively charged/polar uncharged change. Because the C789A polymorphism in dogs was investigated only in relation to differences between breeds, we decided to evaluate its relation to different behaviours. The aim of this study was to determine the association of C789A polymorphism in the canine dopamine beta-hydroxylase gene (*DBH*) and aggression in dogs.

Materials and Methods

The study involved 110 individuals of different breeds of dogs (*Canis familiaris*) classified into 9 groups according to the Fédération Cynologique Internationale (FCI). The dogs (n =110) were assessed based on their individual behavioural characteristics defined by veterinary interview and observation, and then divided into those exhibiting aggressive behaviour (n = 27) and those not showing aggression (n = 83) (Table 1).

Table 1. Characteristics of investigated dogs.

Breed group[1]	n	Number of aggressive dogs within group
Sheepdogs and Cattledogs (except Swiss Cattledogs)	22	4
Pinscher and Schnauzer - Molossoid and Swiss Mountain and Cattledogs	35	10
Terriers	17	7
Dachshunds	4	-
Spitz and primitive types	6	-
Scent hounds and related breeds	1	-
Pointing Dogs	4	1
Retrievers - Flushing Dogs - Water Dogs	16	4
Companion and Toy Dogs	5	1

[1]Breed groups were assigned in accordance with the Fédération Cynologique Internationale (FCI)

Peripheral blood was collected by a veterinarian into tubes containing K_3EDTA. DNA isolation from the blood was performed using the MasterPure™ DNA Purification Kit for Blood II (Epicentre Biotechnologies, Illumina, Madison, Wisconsin, USA), according to the methodology given by the manufacturer. For polymorphism screening, the ACRS-PCR method was applied. In the close distance (12 bp) of the analysed *DBH* gene polymorphism is the second recognition site for the *Bsr*I enzyme, so we designed a forward primer to abolish this site, allowing for correct genotyping. The primers were as follows:

forward 5'-GGTCCACCACATAGAGATCTTACA-3',
reverse 5'-GAGGTGAGGGGACACAGGT-3' (underlined nucleotide introduces mismatch).

The PCR mixture contained: ~60 ng of genomic DNA, 10 pmol of each primer, 1 × PCR buffer, 1.5 mM $MgCl_2$, 200 µM dNTP, DMSO (5%), 0.4 units of *Taq* polymerase (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and deionized water filled up to 15 µl. The following thermal cycling was applied: denaturation at 94 °C/5 min, followed by 35 cycles of 95 °C/30 s, 64 °C/45 s, 72 °C/35 s and final synthesis at 72 °C/5 min. Obtained amplicons (210 bp) were digested with the *Bsr*I enzyme (Thermo Fisher Scientific, Waltham, Massachusetts, USA) at 65 °C/ 3 h. After incubation, restriction fragments were separated by horizontal electrophoresis in 3% agarose gels stained with ethidium bromide in 1 × TBE buffer. After separation, results were visualized in UV light using a transilluminator (Vilber Lourmat, Marne La Vallée, France).

Differences between the *DBH* genotype frequency in the analysed groups of dogs were estimated using the Chi-square test (χ^2) (Kwasiborski and Sobol 2011).

Results

In the analysed dogs, three genotypes of the *DBH* gene were identified: *CC* (176, 34 bp), *CA* (210, 176, 34 bp), and *AA* (210 bp). The *CC* genotype was most frequent in aggressive dogs (0.778), while in non-aggressive it reached significantly lower frequency (0.361). In case of the heterozygous genotype (*CA*), reverse tendency was observed. Frequency of the *AA* genotype was similar in both groups (Table 2). Analysing the numbers of aggressive dogs within groups, the highest were found in Terriers (41.2%) as well as Pinscher and Schnauzer - Molossoid and Swiss Mountain and Cattle dogs (28.6%).

Table 2. Genotype and allele frequencies for the effects of the *DBH* gene on dog behaviour characteristics.

Group	n	Genotype			Allele		χ^2 statistics
		CC	*CA*	*AA*	*C*	*A*	
Aggressive	27	0.778	0.074	0.148	0.815	0.185	
Non-aggressive	83	0.361	0.470	0.169	0.404	0.596	16,232 (P = 0.0003)
Total	110	0.464	0.373	0.164	0.650	0.350	

n – number of individuals; χ^2 – chi-square

Significant differences in *DBH* genotypes and allele frequency between aggressive and non-aggressive dogs were observed (χ^2 = 16,232, P = 0.0003). In aggressive dogs, the *CC* genotype (0.788) and *C* allele (0.815) were the most frequent whereas in non-aggressive dogs, their frequency was significantly lower (0.361 and 0.404, respectively).

Discussion

The difference between aggressive and non-aggressive dogs could be affected by genes that control the synthesis of the neurotransmitter and its reuptake, their receptors, enzymes that inactivate the neurotransmitters or genes which control expression of any of the above-mentioned genes (Houpt 2007; Proskura et al. 2013). Våge et al. (2010) studied the expression profiles in the brains of aggressive and non-aggressive dogs and found the increased expression of two genes (*UBE2V2* and *ZNF227*) strongly associated with the aggression phenotype. In another research, Wang et al. (2018) identified differentially expressed genes in brain tissues between tame and aggressive fox populations, with the top two significant candidate genes *PCDHGA1* and *DKKL1*. In the genome wide association (GWA) mapping of canine fear and aggression, Zapata et al. (2016) found that the *IGF-1* and *HMGA2* loci were associated with separation anxiety, touch sensitivity, owner-directed aggression and dog rivalry. The authors reported also two additional loci on the canine chromosome 18 and X (*GNAT3* and *CD36*, respectively) associated with several behavioural traits, including fear and aggression directed toward unfamiliar dogs and humans.

In this study, we analysed a gene encoding enzyme that catalyses the synthesis of norepinephrine from dopamine. The C789A polymorphism detected in the *DBH* gene was investigated earlier in 5 different breeds of dogs. Among them, the *AA* genotype and *A* allele were most frequent in Golden and Labrador Retrievers (Takeuchi et al. 2005). Golden Retrievers are characterized by low aggression toward other dogs, low snapping at children, low dominance over the owner and low territorial deference (Hart and Miller 1985). They are recognized as trainable and playful dogs but not aggressive (Prakash 2009). We did not find any differences regarding the *AA* genotype in both groups. Interestingly, when analysing the *CC* genotype, Takeuchi et al. (2005) found that it was

most frequent in the Shiba and Miniature Schnauzer breeds. They highlighted that Shiba is a less-domesticated dog breed and is genetically closer to wolves than other breeds under study. Miniature Schnauzer, however, belongs to the Pinscher and Schnauzer - Molossoid and Swiss Mountain and Cattledogs group, which was characterized in our study by one of the highest percentages of aggressive dogs.

The *DBH* gene was also investigated in relation to behavioural traits in other domestic animals. Ren et al. (2017) studied four candidate genes for temperament behaviour in the Mongolian horse. They identified polymorphism of the *DBH* gene in a similar position (G758A) to that found in our study (C789A). Different equine *DBH* variants were associated significantly with horse-friendly behaviour which is directly translated into other traits, e.g. comprehension ability, crossing the river, and swimming. Other studies were also conducted in pigs, where behavioural characteristics were analysed in relation to neurotransmitter-related genes in the Chinese indigenous Mi breed and Landrace × Large White crossbreeds. After backtesting and assessing aggressive behaviour, it was confirmed that Mi pigs are less active and less aggressive than European pigs. Genetic analysis confirmed that the most frequent haplotypes were significantly different between Mi and Landrace × Large White pigs for the *DBH* gene and few others (Chu et al. 2017). The last domestic animals include cows, where *TH* (tyrosine hydroxylase) and *DBH* genes polymorphisms were analysed in four different breeds. Significant *DBH* haplotype differences between Brahman cattle and the three *B. t. taurus* breeds, Charolais, Holstein, and Lidia, were found. The authors stated that detected polymorphism is interesting from a behavioural point of view because of the observed differences in temperament between these breeds (Lourenco-Jaramillo et al. 2012).

In conclusion, we found that *DBH* genotypes and allele frequencies differ significantly between aggressive and non-aggressive dogs. Thus, further studies should be conducted to confirm the usefulness of the *DBH* gene as a marker for behavioural traits in this species.

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