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Correlation analysis between single nucleotide polymorphism of *DRD1* gene and stereotyped behavior of blue fox

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ABSTRACT. This study was performed to investigate the correlation between stereotyped behavior of the blue fox and single nucleotide polymorphisms (SNPs) of the *DRD1* gene. We choose the *DRD1* gene as a major gene for investigating the correlation of gene polymorphism and self-biting disease by means of direct sequencing. Part of the *DRD1* gene exon of the blue fox was cloned; the length of the whole sequence was 864 bp. Four SNPs were detected and analyzed by the chi-square analysis; the results showed that the gene polymorphism of T206C in the *DRD1* gene had a significant correlation with self-biting ($P < 0.01$). Therefore, marker-assistant selection on self-biting of blue foxes using these SNPs can be applied to select healthy individuals.

Key words: Blue fox; Stereotyped behavior; SNP; *DRD1* gene

INTRODUCTION

Stereotypes are found in captive animals but are rare in nature (Mason, 1991). The caged environments of fur animals are sometimes considered poor living conditions. Although more attention has focused on environmental influences, the role of genetics in stereotypy cannot be overlooked. Stereotypy is heritable in bank voles (*Clethrionomys glareolus*) (Schoenecker and Heller, 2000) and African striped mice (*Rhabdomys pumilio*) (Schwaibold and Pillay, 2001), similarly, the importance of genetic transmission has also been noted in fur animals, such as mink (Hansen, 1993). Smith (1984) suggests that the occurrence of stereotypes in thoroughbred racehorses has a genetic origin. For example, stereotyping stallions produce stereotyping offspring. A similarly positive significant correlation between the occurrence of stereotypes in parents and their offspring has been indicated in other species (Kiley, 1977; Odberg, 1987; Hansen, 1993).

Dopamine is a neurotransmitter that plays a major role in a variety of brain functions, including motor control, cognition, motivation, reward, and endocrine regulation. Dopamine activity is regulated by a family of transmembrane G-protein-coupled receptors (i.e., D1, D2, D3, D4, and D5 receptors) that are encoded by 5 distinct genes (Jonsson et al., 2003). Activation of the D1 and D2 receptors mediates intracellular calcium levels via a single mechanism. The stimulation of phosphatidylinositol hydrolysis by phospholipase C results in the production of inositol 1,4,5-trisphosphate, which mobilizes intracellular calcium stores (Kötter, 1994; Missale et al., 1998; Lane et al., 2004). To date, calcium channels have received attention as mediators or potential therapeutic targets for stereotyped behavior (Jonsson et al., 2004). However, previous studies have only investigated a limited number of polymorphisms.

To investigate whether the *DRD1* gene could be susceptible to stereotyped behavior, the differences in genotype distribution and allele frequencies of this polymorphic region among stereotypic blue foxes and control subjects were analyzed.

MATERIAL AND METHODS

Study design and resource populations

The experiment was implemented at the Fur Animals Experiment Station of the Institute of Special Economic Animals and Plants of the Chinese Academy of Agricultural Sciences. Healthy (48) and stereotyped (54) blue foxes were raised in standard roofed sheds with open sides in individual cages (60 x 70 x 90 cm). Animals had free access to drinking water and were fed twice a day with a diet of similar ingredients and composition. Venous blood was collected from each fox, and coagulation was prevented with citrate in August and October of 2009 and August of 2010. Genomic DNA was extracted from thawed blood and stored at -20°C. Total genomic DNA was extracted from the blood samples using the phenol-chloroform procedure.

Genotyping of SNPs in *DRD1* genes

Primers were designed from homologous regions of dog DRD1 sequences (GenBank accession No. XM_546227). The primers were as follows: forward primer, 5'-AGACCATTC

ACTTTTCAGGCTTC-3'; and reverse primer, 5'-GCTGTCTGACTTGCTTCAATTTAAT-3'. Each PCR contained 100 ng genomic DNA, 0.35 µM of each primer, 0.1 mM dNTPs, 1X PCR buffer, 1.5 mM $MgCl_2$ and 1 U Taq polymerase in a total volume of 20 µL. PCR amplification was preformed using the Eppendorf AG (Gene Co., Ltd., Hamburg, Germany). The amplification conditions were 5 min at 94°C, 35 cycles of 30 s at 94°C, 30 s at 63°C, and 1 min at 72°C, and a 5-min final extension at 72°C. The PCR products were isolated and purified using the Agarose Gel DNA Extraction Kit and cloned into *Escherichia coli* strain JM109 via the PMD-18T vector. The sequences obtained were analyzed by the BLAST program for a similarity search.

Statistical analysis

A comparison of polymorphism frequency between cases and controls was performed using a chi-square test. The SAS8.0 software was used to analyze the data (SAS, 2004).

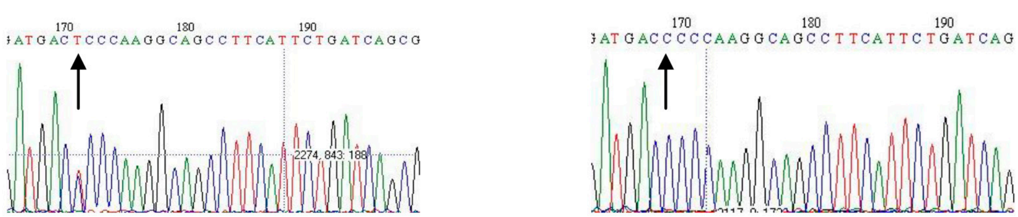
RESULTS

Sequencing and genomic organization of the *DRD1* gene

The *DRD1* gene was chosen as a candidate gene to study the correlation between gene polymorphisms and self-biting disease by direct sequencing. Part of the *DRD1* gene exon of the blue fox was cloned; the length of the whole sequences was 864 bp. The sequence was highly conservative overall for the dog and red fox *DRD1* gene nucleotide sequences (99%). Moreover, the predicted amino acid sequence of dogs was 95% conformity with human and murine proteins and in 96% conformity with porcine proteins.

Genotyping of SNPs within *DRD1* genes by DNA sequencing

PCR products from the 2 candidate genes were specifically amplified. After DNA sequencing analysis, 3 genotypes were clearly discerned at each polymorphic site in the studied population. Sequencing of individuals from the 2 groups showed a T/C SNP at bases 206 (Figure 1), 314 (Figure 2) and 688 (Figure 3) of the *DRD1* gene (i.e., homozygous AA and BB).



AA genotype BB genotype

Figure 1. Sequence results for the AA and BB genotypes at nucleotide T206C.

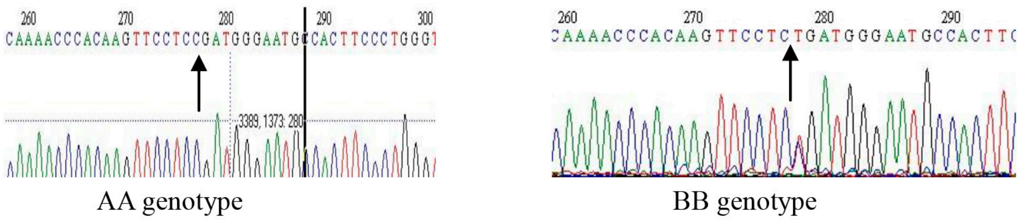


Figure 2. Sequence results for the AA and BB genotypes at nucleotide T314C.

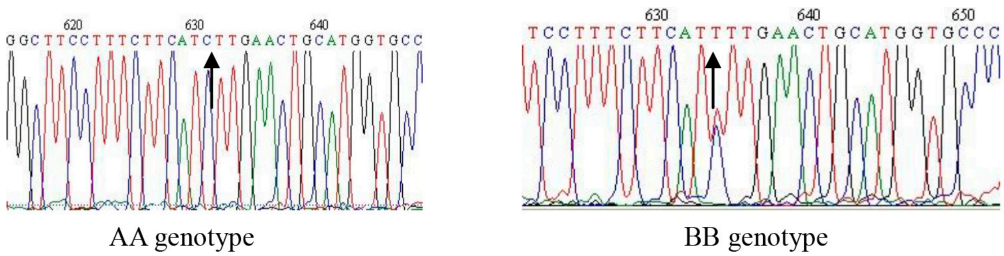


Figure 3. Sequence results for the AA and BB genotypes at nucleotide C688T.

Effects of the *DRD1* gene SNP

The *DRD1* gene polymorphism (C206T, C314T, C688T) was predominantly related to self-biting behavior in the 2 groups of blue foxes (Table 1). Three SNPs were detected in the research and analyzed by the least square analysis. The results showed that the gene polymorphism of T206C in the *DRD1* gene was significantly correlated with self-biting behavior ($P < 0.01$).

Table 1. Effects (P value) of *DRD1* gene polymorphism on 2 groups (i.e., healthy and self-biting) of the blue fox.

SNP	Group	Gene type			Reality ratio	Theoretical ratio	χ^2	P
		AA	AB	BB				
T206C	Health	0.4545	0.5000	0.0455	20:22:2	20.41:22.45:2.04	33.4915	0.0002
	Self-biting	0.5926	0.0370	0.3704	32:2:20	32.65:2.04:20.14		
C314T	Health	0.8182	0.1364	0.0450	36:6:2	36.43:6.12:2.04	3.4773	0.1758
	Self-biting	0.7778	0.2220	0.0000	42:12:0	42.86:12.24:0		
C688T	Health	0.8182	0.1364	0.0450	36:8:0	36.73:8.16:0	8.8668	0.0119
	Self-biting	0.7778	0.2220	0.0000	42:4:8	42.86:4.08:8.16		

DISSCUSSION

The stereotypic behavior or self-biting etiologies are usually multifarious, involving complex interactions among genetic, environmental, neurological, physiological, endocrinological and social factors (van der Kolk, 1994; De Bellis et al., 1999a,b; Siegel, 1999; Schore, 2002). In the wild, fur animals are considered relatively social animals. The caged environment of farmed fur animals is sometimes considered a poor living environment that can influence the emergence of stereotyped behaviors; however, this environment can possibly be enriched via reconstruction of the social system (Ahola et al., 2007). Studies of animal emotions are important approaches to ensuring animal welfare in applied ethology. Moe (2006) studied anticipatory behavior, which may be useful for the development of indicators of positive emotional states and, thus, positive welfare in farmed silver foxes. Sensitivity to the development of stereotypies has a genetic component (Hansen, 1993; Jeppesen et al., 2004); hence, different breeding strategies among farms could influence stereotyped behavior in a population. Research has shown that sensitivity to the development of self-biting behavior is affected by the genetic background of an individual (Li et al., 2008; Liu et al., 2011).

Correlations among behavioral problems and neurotransmitters, especially plasma and platelet concentrations of serotonin, dopamine and norepinephrine have also been found in some species such as rats, rabbits, humans, and dogs (Rogeness et al., 1992; Higley et al., 1992, 1996; Reisner et al., 1996; Reisner, 2002). There is additional evidence via the molecular methods used to manipulate the genes that control aggressive behavior and identify allelic variations, which may help to explain the differences in phenotype. Fewer data are available for impulsive/compulsive behaviors, partly because their studies are less unified across species. Nevertheless, clear genetic effects exist. For instance, strain affects barbering by mice (Garner et al., 2004) and tail biting by pigs (Breuer et al., 2003); it is also possible to breed high- and low-feather-pecking strains of laying hens (Reisner, 2002; Glatt et al., 2003). Lin and Bai (2008) showed that the polymorphisms of the 5-hydroxytryptamine receptor 1A gene and dopamine receptor D1 and D2 gene had distinguished tendencies to stereotype the behavior of mink. The current study was designed to investigate the associations of *DRD1* gene polymorphisms with self-biting disease. Results indicate that the gene polymorphism of T206C in the *DRD1* gene significantly correlated with self-biting behavior ($P < 0.01$). The results, therefore, point to *DRD1* as a major gene of quantitative trait loci that could be used to affect self-biting disease in the blue fox.

We should mention that the gene polymorphism of T206C in the *DRD1* gene was found in a single healthy individual. This might imply that there is a threshold in the blue fox central nervous system to exhibit self-biting behavior even though they carry the self-biting gene. Behavior is normally inherited in a polygenic, additive manner. Therefore, in the future, more genes should be investigated to estimate the contribution of each of the genes to the phenotypic variation of stereotypic behavior.

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