

## Analysis of Single Nucleotide Polymorphism (SNP) for Dopamine Receptor D4 (DRD4) Gene in Horse

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**Abstract:** The objective of the present study was to detect the Single Nucleotide Polymorphism (SNP) of Dopamine Receptor D4 (DRD4) gene from 143 horses. We observed C of 147th base→T substitution and A of 292th base→G substitution SNP in 15 individuals from 143 horses. In C→T substitution case, SNP was observed from 7 individuals, among them 5 individuals of 7 horses were evaluated to be wild and fierce in temper generally by horse management supervisor and veterinarian and 2 individuals from 8 horses observed A→G substitution were evaluated to have wild and fierce character in common. The SNP of DRD4 gene was observed by 23.3% (7 individuals from 30 horses) from wild and fierce character ones and 7.1% (8 individuals from 113 horses) from mild temper ones which result in little SNP detection among most mild temper ones.

**Key words:** DRD4, fierce, horse, SNP, wild, detection

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### INTRODUCTION

Benjamin *et al.* (1996) published a research that the variation of dopamine receptor is associated with novelty-seeking which has been researched by a lot of people, later. Novelty-seeking is one of the items of the testing tool to measure a person's character and it is known that, if this disposition that one has is higher, one responds to an impulse, more actively, gets more exploratory and reacts more quickly to a new stimulus and has a character lacking a stable behavior (Benjamin *et al.*, 1996; Ebstein *et al.*, 1996).

In recent studies, in a gene, it has been known that genetic polymorphism has a correlation with a neurotransmitter or hormone that affects individual difference in character (Inoue-Murayama *et al.*, 2002). Dopamine Receptor D4 (*DRD4*) gene is one of the candidate genes that encodes one of the sub-types of the dopamine receptor and implies the basic individual difference in the characters of a lot of species including human. It was reported that these have a correlation with novelty-seeking (Benjamin *et al.*, 1996; Ebstein *et al.*, 1996).

Concerning the existence of DRD4 polymorphism, studies of the correlation between unique disposition or character and DRD4 gene are conducted continuously in nonhuman primates such as monkey and chimpanzee, etc.

and animals like dog based on research on men (Ito *et al.*, 2004). For example, it was reported that DRD4 sequence of a chimpanzee, a nonhuman primate was more closely associated with human sequence than that of gorilla (Livak *et al.*, 1995) and it was reported that the exon III region of DRD4, VNTR has a correlation with aggressiveness, activeness and impulsiveness in the dog (Hejjas *et al.*, 2007).

In addition with Equus that includes a zebra and a donkey, Hasegawa *et al.* (2002) analyzed a nucleotide sequence in part of the *DRD4* gene, included 18 bp repetition unit in the region and revealed that there was an intraspecies or interspecies difference in the number of repetitions.

In particular, a horse is the animal in which most importance is attached to disposition in terms of utilization as compared to other animals which is one of the stock species, for which comparative research with individual genetic trait and disposition is most necessary. However, there are insufficient studies of the mutual relationship between a horse's disposition and the region of *DRD4* gene and diversity. In a horse, DRD4 is located in Chromosome 12 (Momozawa *et al.*, 2007) which consists of 18 bp (6-amino acid) of VNTR and some Single Nucleotide Polymorphism (SNP) in exon III region (Hasegawa *et al.*, 2002). It is reported that, one of the SNP, G292A is associated with the point in the measurement of

a horse's disposition. It has been reported that, in G292, an allele A has correlations with low curiosity and high wariness in a thoroughbred (Momozawa *et al.*, 2005).

Therefore, it is judged that, for the discovery of specific genetic characteristics of horses which are assumed to allow them to have appropriate dispositions as saddle horses through an analysis of the *DRD4* gene, an analysis of SNP through a sequence analysis of the region of *DRD4* gene will be useful.

Under such a background, this study conducted research, aiming to explore the specific genetic characteristics of the region of *DRD4* gene related to the horse's disposition with 113 heads of horse with docile disposition and 30 heads of horse with fierce disposition, raised in South Korea to understand individual genetic traits and select saddle horses with docile disposition which have specific genetic characteristics.

## MATERIALS AND METHODS

**Testing materials:** Of a total of 143 heads of horse raised in Jangsu Stud Farm and Training Center of Korea Racing Authority, Busan-Gyeongnam Race Park and private equestrian fields with 30 heads of fierce horse and 113 heads of tractable horse, blood collected from the jugular vein was centrifuged, using Heparin Tube (Becton Dickinson, USA) and then, using the buffy coat as a testing material, Single Nucleotide Polymorphism (SNP) of *DRD4* gene was analyzed.

**Genomic DNA isolation:** Extraction of Genomic DNA was carried out, using Toyobo kit (Osaka, Japan) according to the method of Tozaki *et al.* (2001). First, lysis solution 750  $\mu$ L and magnetic bead 40  $\mu$ L were mixed with a buffy coat 200  $\mu$ L; vortexing was conducted for 5 min; it was fixed to a trapper and the rest of the solution was thrown out, excluding the bead. Washing solution 900  $\mu$ L was added to the fixed bead; vortexing was conducted for 1 min; it was fixed to the trapper and the rest of the solution was thrown out, excluding the bead. This cleaning process was carried out one more time. The 70% ethanol 900  $\mu$ L was added to the fixed bead; vortexing was conducted for 1 min; the bead was fixed to the trapper; the rest of the solution was thrown out and the above process was carried out one more time. Then, at room temperature, ethanol was completely evaporated; and then, it was dissolved in sterile distilled water 100  $\mu$ L.

For the quantification of the separated DNA, absorbance was measured at wavelengths of 260 and 280 nm using Nanodrop™ 8000 Spectrophotometer (Thermo, USA) and DNA extracted based on the

absorbance of 260 nm as 1.0 (Path length = 10.0 mm) was diluted and the concentration was adjusted till it became 50 ng/ $\mu$ L. In addition, it was judged that samples with excessively higher or lower than the A260/A280 ratio of 1.8 had low purity, so, DNA was extracted again from blood to use it in an experiment. Then, in order to check the DNA extracted and quantified by naked eye, using Mupid-2 Plus Electrophoresis Cell (Takara, Japan), electro-phoresis was carried out in 2.5% agarose gel at 100 V for 30 min.

**Single sequencing analysis of *DRD4* gene:** DNA concentration was adjusted equally using a spectrophotometer with template DNA extracted from blood and a PCR was conducted as follows.

The PCR was conducted, adding Template DNA 1  $\mu$ L and the primer proposed by Momozawa *et al.* (2005) to AccuPower HL PCR Premix (Bioneer®, Korea) and adding DNA free distilled water 17  $\mu$ L in T-Gradient Thermoblock (Biometra, Germany) and the conditions of reaction were as follows: Predenaturation at 95°C for 3 min; denaturation at 95°C for 30 sec; annealing at 56-59°C for 30 sec and extension at 72°C for 40 sec were repeated 30 times; the final extension at 72°C for 10 min; after the final reaction, 1 h electro-phoresis at a speed of 6 v/cm by dropping the amplified fragment 5  $\mu$ L each on 1.5% agarose gel (Bioneer, Korea) to which EtBr was added was added and purification of the amplified fragment using DNA Purification kit (Qiagen, USA) to use it in a sequence analysis (Momozawa *et al.*, 2005).

In order to analyze the *DRD4* gene sequence, purified amplified fragment 1  $\mu$ L (3-10 ng/ $\mu$ L), BigDye v3.1 Sequencing Mixture (Applied Biosystem, USA) 1  $\mu$ L, 5×Solution Buffer (Applied Biosystem, USA) 2  $\mu$ L and primer (3 pmole/ $\mu$ L) 1  $\mu$ L were mixed, sterile distilled water 5  $\mu$ L was added to adjust the amount to 10  $\mu$ L, finally and according to the sequencing cycling primer, in the beginning, denaturation was induced at 96°C for 1 min. and this process was repeated 25 times at 96°C for 10 sec, at 50°C for 5 sec and at 72°C for 4 min. After the final reaction, the sequence-cycling product was purified with Bigdye X Terminator Purification kit (Applied Biosystem, USA) once again and then, analyzed with ABI 3130XL DNA Analyser (Applied Biosystem, USA).

## RESULTS AND DISCUSSION

**Analysis of Single Nucleotide Polymorphism (SNP) in *DRD4* gene:** As a result of an analysis of SNP in *DRD4* gene, of the 143 heads of horse, in 15 heads, -147 base C→T substitution SNP and -292 base A→G substitution SNP were observed (Table 1). In C→T substitution, the

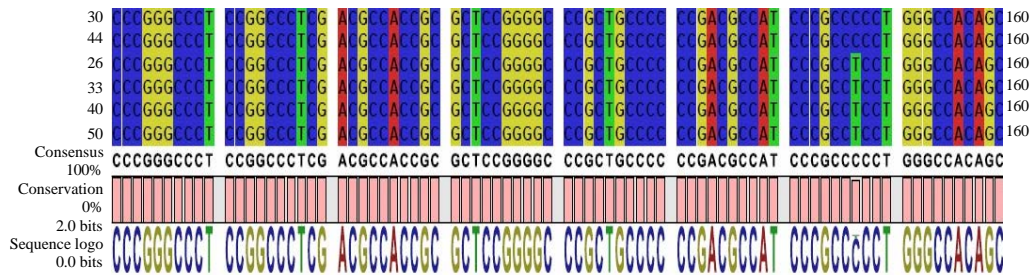


Fig. 1: Multiple alignment of the equine *DRD4* gene. The identified SNP on -147 genomic position (C→T substitution)

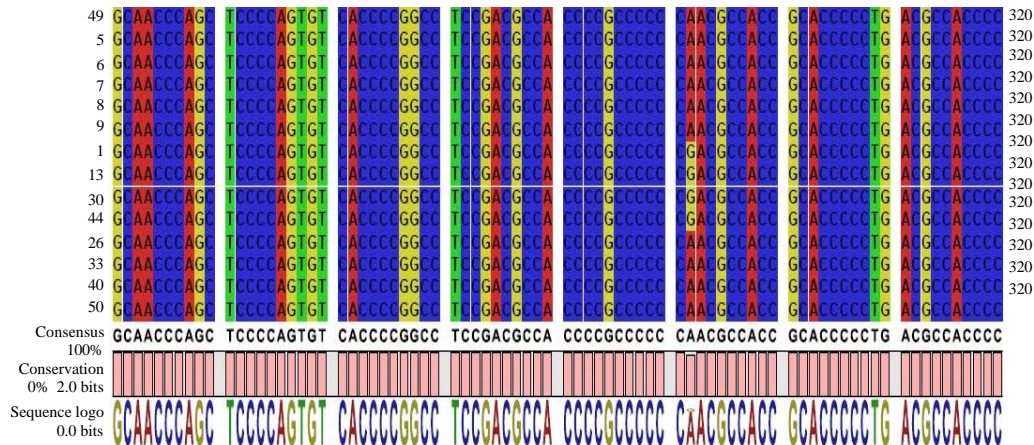


Fig. 2: Multiple alignment of the equine *DRD4* gene. The identified SNP on -292 genomic position (A→G substitution)

Table 1: Detection of SNPs of *DRD4* gene in 143 horses

SNPs (substitution)	No. of detected horse (%)
C→T	7(0.05)
A→G	8(0.06)

SNP was observed in seven heads of horse and of the seven heads of horse, five heads were evaluated to have a fierce disposition generally by the horse manager and veterinarian etc. and of the eight heads of horse in which A→G substitution was observed, two heads of horse were evaluated to have fierce disposition, generally (Fig. 1 and 2). However, there were no individuals in which two SNPs where C→T and A→G substitutions were observed simultaneously.

According to the result of Momozawa *et al.* (2005) that reported on the correlation between the SNP in *DRD4* gene and the horse's disposition, it was reported that, in C→T substitution, no significant correlation with the horse's disposition was observed; however, in A→G substitution, relatively higher curiosity was observed in individuals in which SNP was observed than in those in which no SNP was observed and it was reported that a rather relatively low degree of wariness was observed (Momozawa *et al.*, 2005).

Since, this study did not conduct a stereoscopic investigation on the horse's disposition, a simple comparison to the existing studies is difficult but as a result of an analysis, an individual in which SNP was observed, generally has a wild disposition, so a difference from the result of Momozawa *et al.* (2005) was observed. Yet, Momozawa *et al.* (2007) reported that it a simple evaluation of the horse's disposition according to SNP in *DRD4* gene would be difficult (Momozawa *et al.*, 2007), but as a result of an analysis of the correlation between the SNP in *DRD4* and the horse's disposition in the previous studies, in thoroughbred horses, high excitability, fearfulness and obstinacy were observed in the horses in which SNP trait was observed while low grades of mildness, patience and competitive spirit were observed. In Jeju horses, there is a difference in the item of trained ability according to sex and a higher trained ability was observed in female horses than in the control group and high memory, obstinacy and mildness were observed. In addition, in Paint species, a high trained ability, memory, mildness and intimacy with other horses were observed while low curiosity was observed.

In the results of this study, the SNP was observed in some individuals; however, in most individuals with docile disposition, no SNP was observed.

### CONCLUSION

This results will be useful in wild and fierce character exclusion in the domestic riding horse fertilization scheme by analyzing and comparing between SNP of DRD4 gene and horse temper correlation through prefertilization analysis in future.

### RECOMMENDATION

In the future, the finding would be useful as a means to exclude wild horses through an analysis before breeding in setting up a plan for the production of domestic saddle horses by a comparative analysis of the correlation between the SNP in *DRD4* gene and the horse's disposition.

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