Physiology & Behavior 153 (2016) 19–27

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Associations between temperament and gene polymorphisms in the brain dopaminergic system and the adrenal gland of sheep

Xiaoyan Qiu [a], Jason Ledger [a], Chen Zheng [a,b], Graeme B. Martin [a], Dominique Blache [a,*]

[a] UWA Institute of Agriculture and School of Animal Biology, The University of Western Australia, Crawley 6009, Australia
[b] College of Animal Science and Technology, Gansu Agricultural University, 730070, PR China

HIGHLIGHTS

- Sheep have SNPs for CYP17 (glucocorticoid synthesis) and DRD2 (dopamine receptor 2)
- A DRD2 variant is associated with a behavioural phenotype, temperament
- A CYP17 variant is associated with a physiological phenotype, the cortisol response
- Combinations of DRD2 SNP939 and CYP17 SNP628 could be used as markers for temperament

ARTICLE INFO

Article history:
Received 10 July 2015
Received in revised form 17 October 2015
Accepted 19 October 2015
Available online 20 October 2015

Keywords:
Dopamine receptor
Monoamine oxidase
Glucocorticoid
CYP17
Stress

ABSTRACT

Sheep of calm or nervous temperament differ in their physiological (cortisol secretion) and behavioural (motor activity) responses to stressors, perhaps due to variation in genes that regulate glucocorticoid synthesis or brain dopamine activity. Using ewes that had been selected over 20 generations for nervous (n = 58) or calm (n = 59) temperament, we confirmed the presence of a polymorphism in a gene specifically involved in cortisol production (CYP17), and identified polymorphisms in three genes specifically associated with personality and behavioural traits: dopamine receptors 2 and 4 (DRD2, DRD4), and monoamine oxidase A (MAOA). The calm and nervous lines differed in their frequencies of CYP17 SNP628 (single nucleotide A-G mutation at position 628) and DRD2 SNP939 (single nucleotide T–C mutation at position 939), but not for other SNPs detected in DRD2 or MAOA. In a second experiment, we then genotyped a large, non-selected flock of ewes for DRD2 SNP939 and CYP17 SNP628. Responses to the 'arena' and 'isolation box' challenges were associated with the DRD2 SNP939 genotype and the response to ACTH challenge was associated with the CYP17 SNP628 genotype. We conclude that, for sheep, a combination of the DRD2 SNP939 C allele and the CYP17 SNP628 A/A genotype could be used as a genetic marker for nervous temperament, and that a combination of DRD2 SNP939 T/T and CYP17 SNP628 G/G could be used as a genetic marker for calm temperament.

1. Introduction

Humans and animals exposed to a stimulus initially evaluate the situation at brain level on the basis of a variety of parameters such as suddenness, familiarity, pleasantness, controllability, predictability, expectations, and social norms [1]. Their subsequent emotional responses involve three components [2]: 1) a psychological response, such as positive and negative feelings, that cannot be directly assessed in animals; 2) a behavioural response; and 3) a physiological response that involves changes in the production of, for example, adrenaline, noradrenaline, serotonin, dopamine and glucocorticoids [2–4]. Individuals exposed to the same stimulus can show differences in all three components, and this variability is called temperament [5–9]. We have been investigating temperament in a flock of sheep that has been genetically selected for 20 generations on the basis of behavioural responses to isolation and human presence. There are two divergent lines, one that is hypo-responsive ('calm') and one that is hyper-responsive ('nervous'; [10,11]). This flock offers a unique model for studying the genetic basis of the relationships between temperament, behaviour and physiology. The temperament of an individual depends on genetic background [5,7,12–14] and life experience [12]. Genetic associations with personality, psychological disorders and behavioural traits are supported by an increasing body of evidence from a variety of animal models, including monkeys, birds, rodents, cattle, dogs and horses [15–21]. The genes involved could affect the perception or the process of evaluation of stressors at brain level, or modulate the physiological response at any level of the hypothalamic–pituitary–adrenal axis (HPA).

* Corresponding author at: School of Animal Biology M085, The University of Western Australia, 35 Stirling Highway, Crawley 6009, Australia.
E-mail address: dominique.blache@uwa.edu.au (D. Blache).

http://dx.doi.org/10.1016/j.physbeh.2015.10.022
0031-9384/

At brain level, human personality traits, including temperament, have been linked to genetic differences in neurotransmitter systems [22]. In humans and other primates, polymorphisms in genes encoding for tyrosine hydroxylase (TH), dopamine transporter (DAT), dopamine receptors 2, 3 and 4, tryptophan hydroxylase (TPH), monoamine oxidase A (MAOA, an enzyme responsible for the catabolism of 5-serotonin and dopamine), serotonin transporter (5-HTT) and serotonin receptors, have all been linked with personality, psychiatric disorders (neuroticism, schizophrenia), substance dependence and abuse, eating disorders, depression, anxiety, child abuse and suicidal behaviour [23]. Moreover, polymorphisms in dopamine receptors 2 and 4 (DRD2, DRD4) and in MAOA [24] have often been associated with impulsivity, aggression, fear and panic-related behaviours in humans [25–39]. These behavioural phenotypes are similar to those used to define temperament in our 'calm' and 'nervous' lines (fear, behavioural reactivity in response to the isolation [10,11]) so it is feasible that the same genes could be involved.

In the HPA axis, cortisol is the final effector and variation in the amplitude of the stressor-induced response in plasma cortisol concentrations has been associated with temperament in humans, monkeys, dogs, cattle, and sheep [40–46]. The full range of adrenal steroid hormones is synthesised in the adrenal cortex from the same precursor, and preference for cortisol over mineralocorticoids and androgens is controlled by several enzymes, with CYP17 (cytochrome P450 17α-hydroxylase/17,20-lyase) being a key branch-point [47]. Polymorphisms in the CYP17 gene are known to affect CYP17 enzyme activity and the cortisol response to a stressor in South African Angora goats and Merino sheep [48,49,50]. Moreover, the cortisol response to stressors differs between our 'calm' and 'nervous' sheep [46] so CYP17 is an obvious candidate gene.

Therefore, the present study aimed to identify polymorphisms associated with temperament for potential future use as markers to assist selection for behavioural reactivity. The physiological and behavioural differences between our 'calm' and 'nervous' lines of sheep have been well characterised, so we used an association study [51,52] to investigate whether the candidate genes were related to those differences. This approach is more appropriate than 'whole-genome association analysis' or 'linkage analysis', both of which are preferable when the underlying pathophysiological mechanisms are not known and false positive results are likely [52,53]. The present study thus began by testing whether polymorphisms of specific genes are distributed differently between the calm and nervous lines by quantifying the genes for CYP17, DRD4, DRD2 and MAOA. We then tested whether the allelic variants of the genes that we had identified are associated with differences in physiological and behavioural reactivity to isolation and human contact in sheep that had never been selected for their temperament.

2. Materials and methods

This experiment was carried out in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (8th Edition, 2013) and was approved by the Animal Ethics Committee of The University of Western Australia under RA/3/100/1252.

2.1. Part A: detection of gene polymorphisms associated with temperament

2.1.1. Animals

We used a flock of Merino sheep that had been selected for over 20 generations on criteria that reflect emotional reactivity. At about 16 weeks of age (2 weeks after weaning), the behavioural reactions of lambs to humans are measured in an 'arena test' and their reactions to social isolation are measured in an 'isolation box test' [10,11]. The two tests are described below (Section 2.2). All animals are assigned an overall selection score that combines these two tests, allowing them to be classified as 'nervous' or 'calm' on the basis of expression of high or low levels of movement or vocalisation. The 'nervous' line is more reactive to contact with humans and with isolation, whereas the 'calm' line is less reactive to humans and to isolation [11]. For the first part of this study, we used 58 ewes from the 'nervous' line and 59 ewes from the 'calm' line, all 16 weeks old and of similar live weight (20 ± 1.6 kg). The animals in the two lines were always managed together except at mating and lambing.

2.1.2. Genomic DNA isolation

Whole blood was sampled by jugular venepuncture into EDTA vacutainers (Greiner Bio-One, Australia). Genomic DNA was isolated using the DNeasy Blood & Tissue Kit (Qiagen, Germany) according to the instructions provided by the manufacturer. The isolation of genomic DNA was confirmed by gel electrophoresis and the concentration of genomic DNA was measured using a BioPhotometer Plus (Eppendorf, Hamburg, Germany).

2.1.3. Polymorphisms in CYP17, DRD4, DRD2 and MAOA

The primers used to amplify the single nucleotide polymorphism (SNP) located at position 628 (SNP628) in a fragment of CYP17 had previously been described for South African Merino sheep ([54]; Table 1). The other primers were designed using Primer Premier software (Version 5.0, PREMIER Biosoft, Palo Alto, CA, USA) to amplify a highly conserved fragment of DRD4, all 7 exons of DRD2, and 3 exons of MAOA, based on sequences obtained from Genebank (Table 1). All primers were synthetised by GeneWorks, Australia. Because of differences in fragment lengths, different PCR reaction conditions (annealing temperatures and elongation times) were used during the amplification of the different fragments (see description below).

Fragments of the CYP17 and DRD4 genes were amplified by PCR. Amplification reactions (10 μL) contained 2 μL of 5 × PCR buffer including 0.2 mM dNTPs (Fisher Biotec, Australia), 2.5 mM $MgCl_2$ (Fisher Biotec, Australia), 0.3 μM each of forward and reverse primers (GeneWorks, Australia), 1.1 U Taq DNA polymerase (5.5 U/μL; Fisher Biotec, Australia) and 20 ng genomic DNA. The fragments of CYP17 SNP628 and DRD4 were amplified at an initial denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 58 °C for 30 s, 72 °C for 15 s, and a final extension at 72 °C for 7 min.

Table 1
Oligonucleotide primers used in PCR amplification for cytochrome P450 17α-hydroxylase/17,20-lyase (CYP17), dopamine receptors 2 and 4 (DRD2, DRD4) and monoamine oxidase A (MAOA). Accession numbers from Genbank are NC_019479 for CYP17 mRNA, XM_004016032 for DRD2 mRNA, and XM_004022016 for MAOA mRNA. Note: CYP17 SNP628 has been reported previously [54].

Gene	Fragment	Gene oligonucleotide sequences (5′- to 3′-)
CYP17	SNP628	F: CCTGAAGGCCATACAAA
		R: GGATACTGTCAGGGTGTG
DRD4	Conserved fragment	F: TGCTCTGCTGGACGCCCT TCT TC
		R: GTGCGG AACTCGGCGTTGAAGAC
DRD2	Exon 1	F: TCCGCTGAACCTGTCCTGGTATGAT
		R: TGAGATGGCACGCTCTTGAGGGGT
	Exon 2	F: CCACCCATTATGTTGCTTTGTCT
		R: AAGGTGGTTGGTCTGGGTTG
	Exon 3	F: CAAAGTGGGGTGTCTCTGTGG
		R: CCAGGAGGACAGAGGCAGGACT
	Exon 4	F: GGCTTTCTTCCTCCTCCCCA
		R: CAGAGACATTGGGGGAGAGTGGT
	Exon 5	F: GTTTCTGTTCTCACCCGCCTC
		R: GTCCCTGACCTGAACACTTACCAC
	Exon 6	F: AGGAGATAAGGGAGCCTGAGTGAG
		R: CCTGTATTGCTGGGTCCGTCG
	Exon 7	F: CTCCCGCAGGTGTGTTCAT
		R: CAAGGACATGGCCGAGGCT
MAOA	Exon 1	F: GCTCAACCCCCGAATTCCCC
		R: CCCCCAAACGCCACTTTCAGA
	Exon 8	F: GCTTCCATCAGAGCGAAACCA
		R: TAAACACAGCCTACCCTTTTTCTTC
	Exon 15	F: CTCTGATGTCTTTGTAGATGCCACT
		R: TGAGCATGTCAACTTTAACTTCTTG