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ANIMAL GENETICS

Cytochromes P450 2F and Genes of Behavioral Traits: Covariations of Expression in the Human Brain and Polymorphism of the Orthologs in Domestic Goats

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Abstract—Human cytochrome P450 2F1, as well as its ortholog 2F3 in domestic goat, is considered to be a rather unusual enzyme. The only type of reaction it catalyzes has been described: the conversion of skatole, a product of anaerobic tryptophan metabolism, into a pulmonary toxin. Endogenous substrates of CYP2F are unknown, and although more than 30 years have passed since the discovery of the enzyme, its biological role remains unclear. We hypothesized that the physiological functions of CYP2F can be specifically implemented in the brain, remaining previously unnoticed owing to the high compartmentalization of the organ. Using open data, we studied the covariation of the expression of *CYP2F1* and genes for behavioral traits: in the human brain, as well as the polymorphism of their orthologs and *CYP2F3* in 180 populations of domestic goats (*Capra hircus*). Two SNPs were found in the *CYP2F3* gene, one of which had pronounced traces of selection, and the frequency of homozygotes increased with geographic distance from the center of domestication. Expression of *CYP2F1* mRNA in the human brain also had regional specificity. In both species, factor analysis revealed the relationship between CYP2F1/3 and a number of genes regulating behavior: the serotonin transporter *SLC6A4* and its receptor *HTR2A3*, the *ABCB1* transporter, the purine receptor *P2RX7*, the GABA receptor *GABRA4*, the circadian rhythm regulator *PER3*, and T-cadherin *CDH13*. Thus, analysis of the genomic data of the domestic goat and human transcriptomic data revealed the evolutionary and functional relationships of CYP2F cytochromes and neurochemical systems for regulating behavior. This evidence of the cerebral function of the enzyme is indirect, since it is based on correlation analysis, but indicates the promise of further search in this direction.

Keywords: cytochrome, brain, personality, behavior, domestication, serotonin, goat

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INTRODUCTION

Cytochromes P450 catalyze the transfer of oxygen atoms to small organic molecules, allowing them to obtain energy and also carry out the interconversion of such molecules. Thanks to cytochromes, aerobic organisms arose. The evolution and functioning of the nervous and endocrine systems are closely related to cytochromes. However, these enzymes have the received most attention for their ability to oxidize xenobiotics, in particular, toxins and drugs. The physiological role of many representatives of the superfamily has been studied much less fully. One of the most mysterious representatives belongs to the 2F family and is described in humans (CYP2F1), mice (CYP2F2), and goats (CYP2F3) as a lung-specific enzyme for which only one type of catalyzed reaction is known—the partial oxidation of 3-methylindole (skatole), leading to selective pneumotoxicity of this initially harmless substance [1]. The endogenous substrates and physiological function of CYP2F have not been described.

When analyzing the goat genome in our previous work [2], it was noticed that the distribution of alleles of *CYP2F3* in ancient and modern breeds differs from random. This observation stimulated the search for a possible physiological role of the enzyme. The connection of gene *CYP2F3* with domestication may indicate its role in cerebral processes, since behavioral change underlies domestication. A logical question as to why it has not yet been discovered can be addressed to classical methods of studying a new enzyme. Among them, one of the main ones is the search for cells and tissues with enhanced expression of the mRNA of the compound being studied. Studies are

usually conducted on rodents, using relatively large anatomical structures as samples. However, it should be taken into account that, in comparison with other organs, the brain is highly compartmentalized, that is, divided into many microanatomical structures. For example, in humans, the relative volume of the hypothalamus is about 1.5% of the total brain volume, and the hypothalamus itself consists of more than 50 pairs of nuclei. It can be calculated that a conditional 100-fold increase in the level of enzyme expression in one of the nuclei will increase the overall expression level by less than 1%, which is less than the measurement error [3]. There are other reasons for the possible cerebral function of CYP2F. Thus, some of the most ancient signaling molecules in the brain, serotonin, melatonin, and kynurenine, as well as CYP2F substrates, are derivatives of tryptophan [1]. This suggests that the physiological role of CYP2F may be related to neurotransmitter systems.

In this work, to study the possible involvement CYP2F1/3 in neurochemical processes, two sets of open data were used that successfully corresponded to the task. The first set contains genome-wide SNP genotypes of more than 5000 domestic goats (*Capra hircus*) from 188 breeds and populations located throughout the globe. This unified data set is one of the largest in terms of number of genotypes and geographic distribution of any mammal. With its help, the population-geographical and evolutionary relationships of polymorphism of gene *CYP2F3* and genes of neurochemical systems were studied. The second set contains microarray data on the mRNA levels of hundreds of thousands of genes in the human brain at the microstructural level. It made it possible to study functional anatomical features and regional expression profiles of *CYP2F1* and other genes. The advantages of these datasets relate to the population-world scale of the domestic goat genomic data and the high anatomical detail of the human transcriptomic data, and the fact that the genetic sequence and catalytic activity of the enzyme are most similar in these species [1].

Selecting genes associated with neurochemical processes in humans was not a difficult task, since mRNA expression levels were determined in the brain itself. In the case of the domestic goat, there was evidence of single nucleotide substitutions in genomic DNA linked to chromosomal coordinates. It was necessary to identify genes with functionally significant polymorphism for brain function caused by one of the presented substitutions with a high frequency of occurrence in the population. Thus, the phenotypic traits corresponding to them must have population variation, have high heritability, and also be measurable so that their variation can be compared with the genotypic one.

One of the direct products of brain activity is behavior. Many behavioral traits are characterized by individual differences in their expression, which are stable throughout life and in different contexts. Such differences constitute the established formal academic definition of personality traits.

It is worth noting that personality, in most understandings, is more than just a collection of personality traits; the latter, however, constitute a part of it that is potentially measurable and thus can be studied by mathematical methods. The presence of personality traits, as defined here, has been demonstrated in humans and many other animals with developed brains and social structures, and has been particularly well studied in domesticated species, in particular, the domestic goat.

The severity of personality traits is variable, with high heritability, and they are based on neurochemical processes. On the basis of this, as genes associated with neurochemical processes, in this work, genes were selected whose polymorphism is associated with the severity of personality traits [4], and in the case of goats, orthologs of these genes. The presence and prevalence of polymorphic variants of these genes make it possible to confirm (or refute) the assumption that their polymorphism may be nonrandomly associated with polymorphism of gene *CYP2F1/3*.

MATERIALS AND METHODS

Working Domestic Goat Whole Genome Database

A dataset was used from our previous work [2], including (1) genotypes of animals from the AdaptMap project, described in the article by L. Colli et al. [5]; (2) data from the study by T.E. Deniskova et al. [6], presenting the genotypes of seven goat breeds from Russia; (3) genotypes of six populations from five regions of China described by H. Berihulay et al. [7]; (4) data from five Mongolian goat populations genotyped in our study. Genotyping of all populations was performed using a Goat 50K BeadChip (Illumina Inc., San Diego, CA, USA). The final combined dataset contained 38 276 SNPs from 5176 animals in 188 populations [2].

Search for DNA Polymorphisms in Goats

Using PLINK 1.9, we searched for SNPs in the gene *CYP2F3* and orthologs of 18 genes (Table 1) associated with human personality, also recently identified in the horse genome [7]. Chromosomal coordinates were obtained from the goat genome annotated in Ensembl (Table 1). The ARS1 genome assembly (ASM170441v1) was used. Gene *CYP3F3* was located on chromosome 18 and had two SNP markers (snp21618-scaffold2118-550706, snp21617-scaffold2118-498278). Minor allele frequencies (MAF) were calculated for the identified SNP markers in the world population, as well as for each animal population consisting of at least ten individuals. The number of such populations was 158, with an average number

Table 1. SNPs in goat genes—orthologs of human personality trait genes

Gene	Protein	Position on chromosome	SNP ID on chip
FAAH	Fatty acid amide hydrolase	3: 21026588–21047140	snp17501-scaffold181-440831 snp17502-scaffold181-510286
PER3	Period circadian regulator 3	16: 44082685–44149046	snp44080-scaffold597-1100913
CDH13	Cadherin 13	18: 10713157–11547248	snp6116-scaffold1216-1475632
NPY	Neuropeptide Y	4: 48587984–48618665	snp27655-scaffold295-4443665
LEP	Leptin	4: 27665917–27682282	snp24940-scaffold2551-297044 snp7099-scaffold1264-42116
HSD11B1	Hydroxysteroid 11-beta dehydrogenase 1	16: 72192303–72232521	snp23279-scaffold2329-257953 snp23276-scaffold2329-78243
ANKK1	Ankyrin repeat and kinase domain containing 1	15: 58404829–58418257	snp57901-scaffold937-1037722
DRD2	Dopamine receptor D2	15: 58322506–58393525	snp57902-scaffold937-1074027
BDNF	Brain derived neurotrophic factor	15: 24503417–24569735	snp54722-scaffold837-1900767
COMT	Catechol-O-methyltransferase	1: 547660–553624	snp2811-scaffold1082-402075 snp2810-scaffold1082-348665
P2RX7	Purinergic receptor P2X 7	17: 17203049–17255098	snp29995-scaffold327-1964517 snp30001-scaffold327-2285684
APOE	Apolipoprotein E	18: 58979223–58984704	snp46071-scaffold632-588199 snp46070-scaffold632-517251
CNR1	Cannabinoid receptor 1	9: 49082848–49084266	snp47005-scaffold657-454462 np47003-scaffold657-348206
SLC6A4	Solute carrier family 6 member 4	19: 21107560–21127442	snp6455-scaffold1229-2653423
GABRA6	Gamma-aminobutyric acid type A receptor subunit alpha 6	7: 37186194–37207432	snp41489-scaffold54-1527889 snp41488-scaffold54-1473617
HTR2A	5-Hydroxytryptamine receptor 2A	12: 69719711–69785162	snp24155-scaffold246-2237548
DGKH	Dacylglycerol kinase eta	12: 74178634–74373984	snp52847-scaffold792-1018579 snp52845-scaffold792-943854

of animals of 50 ± 2.2 (M ± SEM). For all populations, the same allele was always considered minor on the basis of its frequency in the world sample, even if it was predominant in a particular population.

Distribution of DNA Variants in Goats

The statistical distribution of SNP allele frequencies was visualized by histograms with MAF on the *X* axis and the number of goat populations along the *Y* axis. The mean (M), standard deviation (SD), skewness, and kurtosis were calculated to provide numerical data on allele distribution.

The geographic distribution of allele frequencies was visualized by means of ArcGis 10.8 using marker color intensity as a representation of the MAF for each population.

Analysis of mRNA Expression in the Human Brain

Data on mRNA expression in the brains of six donors were provided by the Allen Human Brain Atlas [8]. Tissue samples for mRNA isolation were obtained by sequential dissection. At the first stage, brain sections 0.5–1 cm thick were obtained, which were divided into blocks and stored at –80°C. Next, sections 25 μm thick were obtained in cryostats. Sections (one at a time) were used for histological staining or further processing. Manual macrodissection was used for relatively large and easily identifiable brain structures, and laser microdissection was used for smaller

Table 2. Parameters of statistical distribution of frequencies of minor alleles of the *CYP2F3* gene in goat populations

Parameter	SNP1	SNP2
M	0.23	0.48
SD	0.17	0.26
Asymmetry	1.12	0.35
Excess	1.62	–0.83
Minimum	0.00	0.03
Maximum	0.90	1.00

M—mean, SD—standard deviation.

and irregularly shaped structures that required microscopic visualization. Areas sampled by macrodissection included the cerebral cortex and cerebellum, as well as large, regularly shaped subcortical nuclei such as the caudate, putamen, and globus pallidus. Other subcortical nuclei, in particular, the amygdala, thalamus and hypothalamus, and cerebellar nuclei, were sampled using laser microdissection. Neuroanatomical structures were identified on the basis of histological Nissl or silver staining of the remaining sections. During macrodissection, 50 to 200 mg of tissue was excised, depending on the region. The average weight of cortical samples was 100 mg. Laser microdissection extracted samples with an average volume of 3.6 mm^3. In total, there were about 500 samples for each cerebral hemisphere. More details about these procedures, the methods for isolating mRNA, the use of microarrays, normalization of expression data, and also donor data are available on the project's website (Allen Human Brain Atlas, Documentation section). The ontology and nomenclature of microstructures were compiled in accordance with several sources and are described on the project's website in the subsection "Ontology and Nomenclature."

To obtain numerical data, on the main page of the brain atlas, select the "Human brain" option and then "Microarray," enter the names of the genes of interest in the search window and select the type of expression intensity color scale with microstructure resolution for the search results, and then select the "Download data" option. In the first step, values of *CYP2F1* expression were extracted in the main parts and microstructures of the brain. Next, genes associated with personality traits were selected, and data associated with gene expression were downloaded. The values of expression are presented as normalized values.

Factor analysis was performed in SPSS Statistics v26. In the matrices used, the rows ("observations") corresponded to brain microstructures, and the columns ("variables") corresponded to genes (their transcripts). The factor extraction method was the maximum likelihood method with promax rotation with Kaiser normalization. The number of rotation iterations was limited to 15. The Kaiser–Meyer–Olkin (KMO) test was used to test sampling adequacy. The eigenvalue plot was used to determine the number of factors to be extracted.

RESULTS

Analysis of CYP2F3 Polymorphism in Goats

Using PLINK 1.9 within the chromosomal coordinates of the gene *CYP2F3*, two SNPs were discovered with minor allele frequencies (MAF) of 0.224 and 0.491 (hereinafter referred to as SNP1 and SNP2) (Table 2). MAFs were also calculated for each animal population.

As can be seen from the histogram (Fig. 1), the distribution of alleles is somewhat different from normal. SNP2 has a tendency to fix extreme variants, homozygotes for the minor and major alleles, while SNP1 demonstrates a distribution shift toward the minor allele.

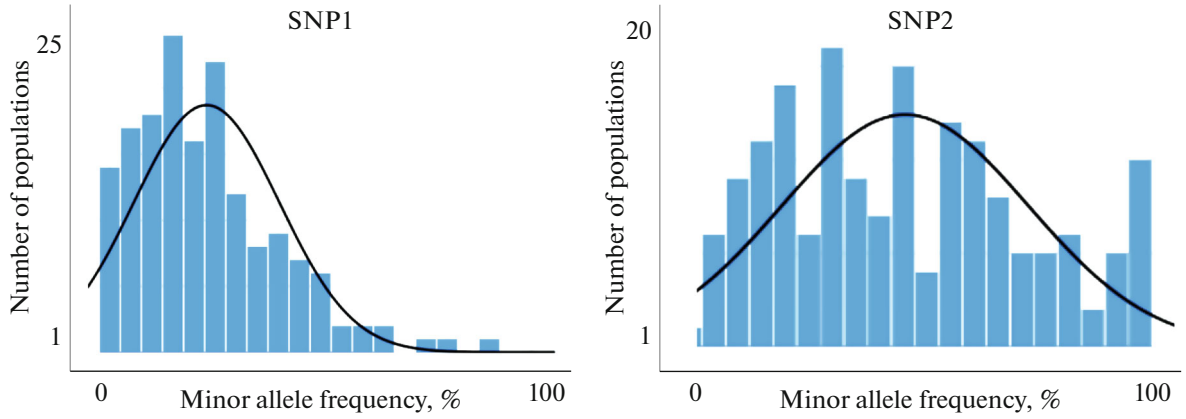

Fig. 1. Distribution of minor allele frequencies (MAF) of SNP1 and SNP2 of gene *CYP2F3* in goat populations.

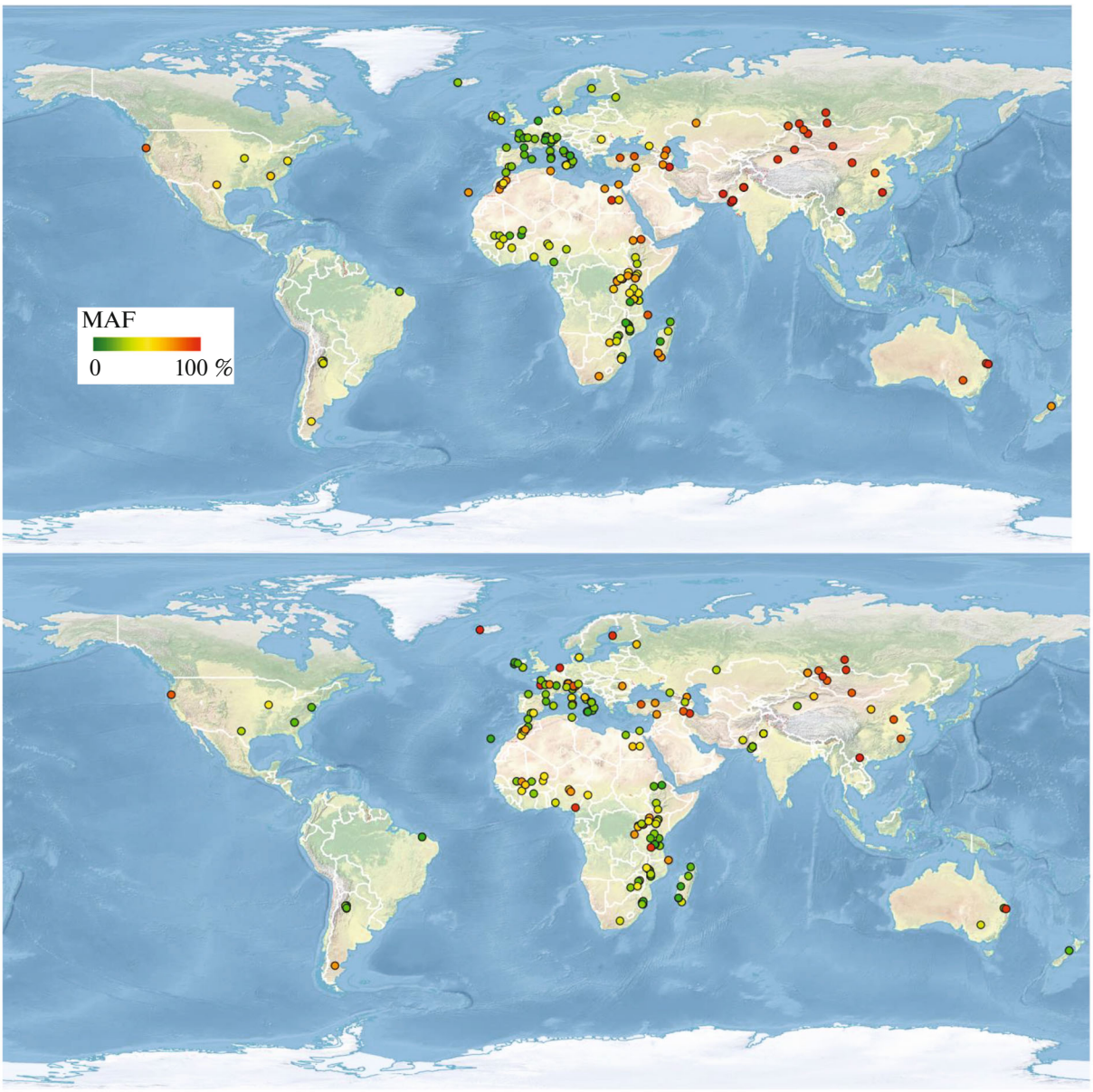

Fig. 2. Distribution of minor allele frequencies of gene *CYP2F3* for SNP1 (top) and SNP2 (bottom) in domestic goat populations.

Geographical Distribution of CYP2F3 Alleles

The world map (Fig. 2) shows the distribution of allele frequencies of *CYP2F3* by SNP1 and SNP2. For each population, the proportion of the minor allele is displayed using a color gradient. Here, the allele was also considered minor on the basis of the frequency in the entire world sample. The frequencies of minor alleles SNP1 and SNP2 in the wild ancestor (bezoar goat) were 1 and 0.64, respectively.

The frequency of occurrence of SNP1 in animal populations shows a pronounced geographic dependence. In European populations, a major allele is actually recorded; in Asian populations, a minor one is recorded; and in the wild ancestor (bezoar goat), only a minor allele is found. SNP2 alleles do not have such a pronounced geographic distribution pattern; however, it is noticeable that, with distance from the source of primary domestication in ancient Anatolia (on the territory of modern Pakistan), a "sharpening" of genetic variation occurs: that is, the emergence of populations with an increasing predominance of one of the alleles.

Next, we studied the relationship between alleles of gene *CYP2F3* and alleles of key genes associated with behavior. The bulk of the SNPs of behavioral genes used were identified by searching within chromosomal coordinates for genes orthologous to genes associated with the expression of personality traits in humans

Table 3. Factor analysis of frequencies of minor alleles of genes of behavior and *CYP2F3* in goat populations (factor loadings are given)

Allele	I	II	III	IV	V	VI	VII	VIII
HTR2A	**0.89**	0.26	0.27	–0.11	0.10	0.09	–0.31	0.18
CDH13_6	**–0.86**	0.18	–0.09	0.03	0.08	0.03	–0.36	–0.15
CYP2F3_SNP2	**0.69**	–0.25	0.08	0.04	0.03	0.36	0.15	–0.06
PER3	**0.65**	0.14	–0.18	–0.11	–0.07	0.10	0.08	0.18
LEP_2	**0.60**	–0.25	0.00	0.21	–0.12	–0.10	0.05	0.01
BDNF	**0.59**	0.15	0.00	–0.21	–0.06	–0.02	–0.03	0.00
ANKK1	**0.52**	0.24	–0.38	–0.06	–0.12	–0.03	–0.26	–0.13
GABRA6_1	**0.40**	–0.38	0.01	0.02	0.21	0.06	–0.24	0.09
CNR1_1	0.27	**0.78**	0.07	0.01	–0.35	–0.09	0.06	0.18
DGKH_2	0.03	**0.67**	–0.13	0.07	–0.21	–0.04	–0.01	–0.13
P2RX7_1	–0.16	**0.67**	0.02	–0.04	–0.03	0.05	0.18	0.24
LEP_1	–0.02	**–0.57**	–0.01	–0.18	–0.20	–0.26	–0.13	0.10
CNR1_2	–0.05	**0.56**	0.24	0.06	0.13	0.15	–0.10	0.12
HSD11B1_1	0.00	**–0.53**	0.13	0.07	–0.13	0.19	–0.01	–0.08
FAAH_2	0.20	**0.52**	0.19	–0.07	0.03	–0.30	0.08	–0.11
APOE_1	0.30	**–0.34**	–0.17	0.10	–0.01	0.19	–0.08	0.25
HSD11B1_2	–0.11	0.04	**0.63**	–0.19	0.10	–0.07	0.14	0.16
ABC_B1_SNP1	–0.28	0.04	**0.62**	–0.13	–0.15	0.42	0.05	–0.27
FAAH_1	–0.14	–0.05	**0.62**	0.07	0.34	–0.18	–0.19	0.43
DRD2_1	–0.11	–0.08	**–0.57**	–0.02	–0.04	–0.07	0.03	–0.01
APOE_2	0.09	0.14	**–0.46**	0.00	**0.43**	0.18	0.14	0.14
COMT_2	0.05	0.25	0.06	**1.01**	–0.18	0.00	0.11	0.21
COMT_1	–0.30	–0.12	–0.10	**0.86**	0.07	0.08	0.02	0.11
SLC6A4	–0.14	–0.11	0.13	–0.03	**0.85**	–0.09	0.21	0.09
CYP2F3_SNP1	0.14	0.05	–0.23	–0.11	**0.53**	–0.05	**0.52**	–0.15
ABC_B1_SNP2	0.15	0.14	0.23	0.19	**0.38**	–0.24	0.05	–0.24
NPY	–0.08	0.13	–0.06	–0.05	0.12	**–0.65**	–0.04	0.08
P2RX7_2	–0.07	0.16	–0.04	0.10	0.25	0.07	**0.77**	–0.10
DRD2_2	–0.23	–0.10	–0.10	–0.20	–0.07	0.07	0.09	**–0.46**

according to the Big Five model, as in the work of T. Yokomori et al. [7] (Table 1). In this model, personality is defined as a set of stable individual behavioral characteristics. Thus, genes associated with personality traits can be considered as regulators of behavior. Seven SNPs with the highest MAF-to-MAF correlation modulus values of *CYP2F3* were identified in genes *GABRA6*, *CDH13*, *HSD11B1*, *HTR2A*, and *LEP*. Then a factor analysis was carried out in which the frequencies of minor alleles in individual populations were used as variables (Table 3). The minor allele frequency of *CYP2F3* turned out to be one of the "strongest" variables, being included in the first factor, explaining 26% of the total variance. This is quite an interesting result, given that this gene has not previously been seen in connection with neurochemical processes. In addition to it, the first factor included *HTR2A*, *LEP*, *ABC_B1*, *ANKK1*, *CDH13*, *PER3*, and *BDNF*. The second allele of the gene *CYP2F3* was included in factors V and VII, jointly explaining 6% of the total variance, together with *SLC6A4* and *ABCB1*—genes encoding one of the main serotonin transporters [9], and *P2RX7*—a purine receptor that serves as a modulator of serotonergic transmission [9].

Analysis of CYP2F1 Expression in the Human Brain according to the Expression Atlas of the Allen Institute

Analysis of *CYP2F1* mRNA expression was conducted on the basis of open data from the Allen Institute (Seattle, USA). The mRNA levels in this study were determined using a microarray in previously iso-

Table 4. Transcripts of *CYP2F1* in the human brain

	Transcript 1	Transcript 2
NCBI no.	NM_000774.3	NM_000774.3
Length, bp	60	60
Subsequence	TCTTTTTGTACCCACAGAGCTTGTTCTATGG-CACGCCCTTTTCTGGGCTTTTTGTATCAT	AGAGGAGAAGGAGGACCCACTGAGC-CACTTCCACATGGATACCCTGCTGATGAC-CACACA
GC, %	43	55

Table 5. Factor analysis of mRNA expression level of genes of behavior and *CYP2F1* in the microstructures of the human brain (factor loadings are given)

Gene	I	II	III	IV	V
HSD11B1	**0.77**	0.46	0.17	–0.52	–0.01
P2RX7	**0.76**	0.18	–0.13	–0.34	0.2
SLC6A4	**–0.74**	–0.42	–0.46	–0.01	0.02
CYP2F1_2	**–0.7**	–0.33	–0.28	0.14	0.12
LEP	**–0.66**	–0.27	–0.33	–0.17	0.01
PER3	**0.62**	0.85	0.31	–0.37	–0.03
ABCB1	**0.51**	**0.84**	0.46	–0.19	0.05
COMT	0.31	**0.76**	0.22	–0.19	0.13
FAAH2	**0.54**	**0.75**	0.46	–0.08	–0.09
BDNF	0.17	**0.60**	0.46	0.09	–0.04
DGKH	0.06	**–0.56**	0.29	0.21	0.16
GABRA4	**0.52**	0.63	**0.77**	0.04	–0.15
CNR1	0.35	0.31	**0.74**	0.21	–0.24
CYP2F1_1	–0.09	–0.19	**–0.72**	0.15	–0.1
HTR2A3	**0.56**	0.31	**0.67**	0.31	–0.08
DRD2	–0.2	–0.17	0.17	**0.85**	0.03
DRD2_2	–0.11	–0.27	–0.24	**0.59**	–0.09
NPY	0.01	–0.07	–0.18	–0.05	**0.91**
CDH13	0.07	0.29	**0.54**	–0.02	**0.62**

lated microanatomical structures of the brain representing the main cortical and nuclear elements of all brain regions. Expression levels of *CYP2F1* were identified in the brains of six donors aged 25 to 57 years, five of whom were men. The number of microstructures was 169 per brain; the total number of samples was 1014. Two transcripts of gene *CYP2F1* were identified, general information about which is contained in Table 4. Regional transcript expression profiles of *CYP2F1* were not identical: the correlation between the average values of the mRNA levels of two transcripts in 145 brain structures was $r = 0.17$. Next, genes associated with personality traits were selected, the orthologs of which were studied in goats (see above). Factor analysis was conducted using the same parameters for extracting latent variables (factors).

As can be seen from Table 5, transcripts of *CYP2F1* were included in factors I and III, along with genes *ABCB*, *ANK1*, *BDNF*, *CDH13*, *CNR1*, *FAAH2*, *GABRA4*, *HSD11B1*, *HTR2A3*, *LEP*, *P2RX7*, *PER3*, and *SLC6A4*. Genes *SLC6A4* and *HTR2A3*, a transporter and receptor of serotonin, had a load on both factors. Moreover, one of the transcripts had a positive and the second had a negative relationship to expression of both *SLC6A4* and *HTR2A3*.

Table 6 compares genes associated with *CYP2F* according to factor analysis of their polymorphism and expression in goats and humans, respectively. Of the 13 such genes, eight were common to the two species, two were associated only with *CYP2F1* of humans, and three were associated only with *CYP2F3* of goats.

Table 6. Genes associated with *CYP2F1* of humans and *CYP2F3* of goats according to factor analysis of their polymorphism and expression

	Human (mRNA)	Goat (SNPs)
ABCB1	+	+
ANK1		+
BDNF		+
CDH13	+	+
CNR1	+	
FAAH2	+	
GABRA4	+	+
HSD11B1	+	
HTR2A3	+	+
LEP	+	+
P2RX7	+	+
PER3	+	+
SLC6A4	+	+

The symbol "+" indicates that the gene is part of the same latent variable with *CYP2F1/3*.

Comparative Analysis of the Expression Level of CYP2F1 Transcripts in Human Brain Microstructures

The highest levels of expression of the first transcript of *CYP2F1* were identified in the superior frontal gyrus, in Cornu Ammonis area IV, in the paravermis, in the amygdala-hippocampal transition zone, in the paracentral lobule, in the cingulate gyrus, and in the precentral gyrus; those of the second transcript were identified in the motor nucleus of the trigeminal nerve, in the mastoid body, in the nuclei of the cochlea, in the motor nucleus of the facial nerve, in the substantia nigra, in the reticular formation, in the hypoglossal nucleus, and in the interstitial nucleus of Cajal (Table 7). Since, according to factor analysis, the expression of the second transcript is negatively associated with the expression of the serotonin receptor, areas of minimal expression of this transcript were also determined. They were found in Brodmann area VIIIA, in the medial supramammillary nucleus, in the inferior dorsomedial nucleus of the hypothalamus, and in some other parts of the limbic system in the neocortex. High expression of the serotonin receptor of *HTR2A3* was observed in these same structures.

DISCUSSION

The statistical and geographical distribution of alleles of *CYP2F3* in domestic goat populations turned out to be nonrandom and had pronounced patterns that indicate that the gene has functions associated with domestication. The gradual change in allele frequencies of the gene with distance from the source of domestication along ancient migration routes, as well as the fixation of homozygous variants in modern populations, apparently indicates that the functions of the gene *CYP2F3* not only played an important role in the early stages of domestication but also are significant at the present time for adaptation to human-created conditions.

A pronounced statistical relationship was found in the analysis of correlations and joint variance of *CYP2F3* and genes homologous to human genes, which, according to genome-wide analysis [8], are associated to the greatest degree with the severity of personality traits, i.e., individual differences in behavior.

Data of expression of gene *CYP2F1* in the human brain revealed regional specificity of the expression of two transcripts with the highest levels of expression of the first transcript in the nuclei of the limbic system, which mediate emotional reactions [9, 10], and in the main serotonergic structures—the raphe nucleus and the red nucleus, which have multiple effects on the higher parts. High levels of expression were also found in integrative areas of the neocortex associated with awareness, goal-directed behavior, and working memory capacity [11]. High levels of the second transcript of *CYP2F* were also found in nuclei and cortical structures associated with the regulation of eating behavior, digestion, and circadian rhythms. The latter pattern could hypothetically be associated with the fact that intestinal bacteria produce tryptophan derivatives [12], which could act as signaling compounds and also be substrates of *CYP2F*. Moreover, since we are unaware of metabolic pathways that directly link serotonergic transmission to substrate specificity of *CYP2F* in relation to indole compounds, there is reason to assume that the activity of *CYP2F* in the brain may be associated with the existence of an alternative (co)transmitter system of indole compounds, which could hypothetically mediate the effect of the intestinal microbiota on feeding behavior. Correlation analysis of expression levels of *CYP2F1* mRNA in the human brain also showed the involvement of the gene in functional networks regulating behavior. As in goats, the strongest associations were found with genes of the serotonergic system. Interestingly, according to factor analysis and comparison of regional mRNA levels, one of the transcripts of *CYP2F1* and serotonin receptor of *HTR2A3* were expressed in antiphase. If one assumes that one of the precursors or metabolites of serotonin may undergo bioactivation into a toxin under the influence of *CYP2F1*, then a decrease in the level of expression of the latter can be considered as a mechanism of protection against toxic effects in brain structures rich in serotonin.

New and quite convincing data have been obtained that indicate the fate of *CYP2F1/3* in the neurochemical processes of regulation of behavior. To our knowledge, this is the first evidence of a physiological role for the cytochrome family *CYP2F*.

The obtained evidence of physiological function of *CYP2F* is quite diverse, but at the same time, it is indi-

Table 7. Expression levels of transcripts of *CYP2F1* in the human brain (normalized values)

Structure	Number of samples	Transcript 1		Transcript 2	
		M	SEM	M	SEM
Ventral thalamus	67	2.67	0.58	3.40	0.58
Midbrain	51	2.45	0.66	2.89	0.66
Medulla	48	2.42	0.67	2.72	0.67
Cerebellar nuclei	337	2.39	0.27	2.78	0.27
Operculum	31	2.38	0.82	2.83	0.82
Basal pontine ganglia	148	2.37	0.40	2.69	0.40
Amygdala	47	2.31	0.68	3.04	0.68
Dorsal thalamus	175	2.26	0.37	2.85	0.37
Globus pallidus	15	2.26	1.12	2.77	1.12
Claustrum	537	2.24	0.21	3.23	0.21
Hippocampus	39	2.08	0.74	3.23	0.74
Epithalamus	188	2.05	0.35	2.60	0.35
Hypothalamus	102	2.02	0.47	2.50	0.47
Striatum	182	1.95	0.36	2.78	0.36
Basal forebrain	279	1.92	0.29	2.73	0.29
Parahippocampal gyrus	212	1.91	0.33	2.67	0.33
Frontal lobe	58	1.84	0.62	3.14	0.62
Parietal lobe	290	1.77	0.29	2.96	0.29
Temporal lobe	170	1.75	0.37	3.05	0.37
Cerebellar cortex	14	1.75	1.15	3.09	1.15
Cingulate gyrus	5	1.72	2.00	2.72	2.00
Occipital lobe	470	1.71	0.23	3.00	0.23
White matter	34	1.69	0.79	2.53	0.79
Insular cortex	15	1.66	1.12	2.93	1.12

M—mean, SEM—standard error.

rect, since it is based on variance-correlation analysis. In the future, direct evidence and clarification of the role of *CYP2F* in the brain can be obtained, for example, using tools to measure and inhibit enzyme activity. However, it is necessary to take into account that, if the enzyme function is not realized constitutively, but is associated with certain functional states, such experiments may turn out to be uninformative. Therefore, it probably makes sense to delve further into correlation analysis, as well as use modeling of enzyme activity of *CYP2F* depending on the polymorphism of the gene and its transcripts.

Overall, conclusions about the physiological role of *CYP2F* should be made with caution, since cytochromes, owing to their ancient origin, can serve as an interaction point for many signaling pathways. The observed processes may be generally more complex and multidimensional than the concepts with which a person is able to operate.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was reviewed and approved at the meeting of the Committee of the Bioethics Commission of the Vavilov Institute of General Genetics, Russian Academy of Sciences, protocol no. 47, dated September 14, 2020.

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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