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Distribution of the C1473G polymorphism in tryptophan hydroxylase 2 gene in laboratory and wild mice

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The neurotransmitter serotonin is implicated in the regulation of various forms of behavior, including aggression, sexual behavior and stress response. The rate of brain serotonin synthesis is determined by the activity of neuronal-specific enzyme tryptophan hydroxylase 2. The missense C1473G substitution in mouse tryptophan hydroxylase 2 gene has been shown to lower the enzyme activity and brain serotonin level. Here, the C1473G polymorphism was investigated in 84 common laboratory inbred strains, 39 inbred and semi-inbred strains derived from wild ancestors (mostly from Eurasia) and in 75 wild mice trapped in different locations in Russia and Armenia. Among all the classical inbred strains studied, only substrains of BALB/c, A and DBA, as well as the IITES/Nga and NZW/NSlc strains were homozygous for the 1473G allele. In contrast to laboratory strains, the 1473G allele was not present in any of the samples from wild and wild-derived mice, although the wild mice varied substantially in the C1477T neutral substitution closely linked to the C1473G polymorphism. According to these results, the frequency of the 1473G allele in natural populations does not exceed 0.5%, and the C1473G polymorphism is in fact a rare mutation that is possibly eliminated by the forces of natural selection.

Keywords: C1473G polymorphism, inbred mouse strains, serotonin, tryptophan hydroxylase 2, wild-derived strains, wild mice

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The neuromediator serotonin (5-hydroxytryptamine, 5-HT) is involved in the regulation of various physiological processes and forms of behavior, such as aggression, sexual behavior, food intake, sleep and stress response (Chaouloff *et al*. 1999; Lucki 1998; Olivier 2004; Popova 2006). Impairments of the serotonergic system in humans have been associated with escalated aggression, pathological anxiety, depression and suicidality (Arango *et al*. 2003; Craig 2007; Lesch 2004; Mathew & Ho 2006). Tryptophan hydroxylase 2 (TPH2) that catalyses the hydroxylation of tryptophan to 5-HT is the key enzyme of brain 5-HT synthesis (Walther & Bader 2003), determining the activity of serotonergic synapses. It is assumed that mutations in human *TPH2* (*hTPH2*) gene may influence psychopathology risks (Harvey *et al*. 2004; Zhou *et al*. 2005b; Zill *et al*. 2004a,b).

To date, at least nine missense substitutions in *hTPH2* gene have been reported, and several of them have been shown to alter the enzyme activity, solubility or stability (Mckinney *et al*. 2009). There is evidence of association between functional mutations in *hTPH2* gene and psychiatric diseases: S41Y, P206S and bipolar affective disorder (Cichon *et al*. 2008; Lin *et al*. 2007), R441H and unipolar major depression (Zhang *et al*. 2005), and R303W and attention deficit/hyperactivity disorder (Mckinney *et al*. 2008).

One functional mutation has been found in mouse *tph2* gene – namely the C1473G polymorphism (rs33849125), which results in the substitution of Pro by Arg at the position 447 of the enzyme molecule (Zhang *et al*. 2004). This mutation leads to about 50% decrease in TPH2 activity when expressed in PC12 cells (Zhang *et al*. 2004) and *Escherichia coli* (Sakowski *et al*. 2006). It has been established that C1473G polymorphism is the main factor determining the hereditary variability of TPH2 activity in the mouse brain (Kulikov *et al*. 2005, 2007). It has been shown that the 1473G allele is linked to lowered intensity of intermale aggression (Kulikov *et al*. 2005; Osipova *et al*. 2009).

To date, the 1473G allele has been found only in a few inbred mouse strains (Crowley *et al*. 2005; Isles *et al*. 2005; Jacobsen *et al*. 2008; Kulikov *et al*. 2005; Zhang *et al*. 2004) and there were no data on its occurrence in nature.

According to the National Center for Biotechnology Information (NCBI) single nucleotide polymorphism (SNP) database (http://www.ncbi.nlm.nih.gov/snp), there is a polymorphic site close to the C1473G polymorphism – C1477T (rs45719309) that does not lead to an amino acid change. As this substitution is likely selectively neutral and closely linked (in 5 bp) to the C1473G polymorphism, its distribution might help to assess the heterogeneity of our sample.

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Table 1: Distribution of C and G alleles of the C1473G polymorphism in mice of classical inbred strains maintained at the RIKEN BioResource Center, Tsukuba, Japan

Strain	Genotype	*n*	Origin	Source
C57BL/10SnSlc	C	1	C57-related strains	2
C57BL/6CrSlc	C	1		2
C57BL/6J	C	1		5
C57BL/6JJcl	C	1		3
C57BL/6JJmsSlc	C	1		2
C57BL/6NCrlCrlj	C	1		4
C57BL/6NJcl	C	1		3
C57BL/6NTac	C	1		6
C57L/J	C	1		5
129+Ter/SvJcl	C	1	Castle's mice	3
129P3/J	C	1		5
129S2/SvPas	C	1		1 (RBRC01023)
129S6/SvTac	C	1		6
129X1/SvJJmsSlc	C	2		2
A/J	**G**	1		1 (RBRC01220)
A/JJmsSlc	**G**	1		2
A/KiKua	**G**	1		1 (RBRC02510)
Af/BiKiKua	**G**	1		1 (RBRC02511)
AKR/JMsStm	C	1		1 (RBRC00010)
AKR/NSlc	C	1		2
BALB/cAJcl	**G**	1		3
BALB/cAnNCrlCrlj	**G**	1		4
BALB/cAnNMs	**G**	1		1 (RBRC00641)
BALB/cAnNTac	**G**	1		6
BALB/cByJJcl	**G**	1		3
BALB/cCrSlc	**G**	1		2
BALB/cKiKua	**G**	1		1 (RBRC02513)
C3H/Bifb/KiKua	C	1		1 (RBRC02515)
C3H/HeJJcl	C	1		3
C3H/HeJYokSlc	C	1		2
C3H/HeNCrlCrlj	C	1		4
C3H/HeNJcl	C	1		3
C3H/HeNSlc	C	1		2
C3H/HeSlc	C	1		2
CBA/HStm	C	1		1 (RBRC00173)
CBA/J	C	1		5
CBA/JNCrlCrlj	C	1		4
CBA/NSlc	C	1		2
CBA/StKiKua	C	1		1 (RBRC02514)
CBA/StMs	C	1		1 (RBRC00179)
DBA/1JNCrlCrlj	**G**	1		4
DBA/1NSlc	**G**	1		2
DBA/2fKiKua	**G**	1		1 (RBRC02517)
DBA/2J	**G**	1		5
DBA/2JJcl	**G**	1		3
DBA/2KiKua	**G**	1		1 (RBRC02516)
DBA/2NCrlCrlj	**G**	1		4
DBA/2NJcl	**G**	1		3
FB/KiKua	C	1		1 (RBRC02518)
NZB/NSlc	C	1		2
NZW/NSlc	**G**	1		2
RFM/MsStm	C	1		1 (RBRC00216)
SM/J	C	1		1 (RBRC01221)
FGS/NgaJcl	C	1	Crossbred of CBA/Nga and RFM/Nga	1 (RBRC01909)
IITES/Nga	**G**	1	Crossbred of CS, DBA/2, NBC and ITES	1 (RBRC00197)
IXBL/Nga	C	1	Crossbred among Japanese and US stocks	1 (RBRC00795)
CF1/Sgn	C	1	Non-inbred CF1 from Carworth Farms	1 (RBRC01674)
DDD/Sgn	C	1	Strains derived from dd stock	1 (RBRC01670)
DDK/Nga	C	1		1 (RBRC00186)
DDN/Nga	C	1		1 (RBRC00792)
DDY/Stm	C	1		1 (RBRC00151)
DNI/Nga	C	1		1 (RBRC00793)
KR/CNga	C	1	Japanese dealers' stocks	1 (RBRC00794)
AA/Sgn	C	1		1 (RBRC01671)
KK/Sgn	C	1		1 (RBRC01677)
KK/TaJcl	C	1		3
KSN/Sgn	C	1		1 (RBRC01676)
KYF/Msldr	C	1		1 (RBRC01310)
RR/Sgn	C	3		1 (RBRC01672)
SS/Sgn	C	1		1 (RBRC01673)
NC/Nga	C	1	Japanese fancy mice	1 (RBRC01059)
NC/NgaSlc	C	1		2
NC/NgaTndCrlj	C	1		4
NC/Sgn	C	1		1 (RBRC01675)
RIII/ImrKiKua	C	1	European mammary tumor strain	1 (RBRC02519)
SL/Kh	C	1	Swiss mice	1 (RBRC00222)
SL/Ni	C	1		1 (RBRC00224)
FVB/NJcl	C	1		3
GRS/A	C	1		1 (RBRC00191)
NFS/N	C	1		1 (RBRC00888)
NOD/ShiJic	C	1		3
NSY/Hos	C	1		2
SHN	C	1		1 (RBRC00521)
SJL/JOrlCrlCrlj	C	1		4

n, the number of individuals studied.
Sources: 1, RIKEN BRC, RBRC catalogue numbers are marked in parentheses; 2, Japan SLC, Inc. (Hamamatsu, Japan); 3, CLEA Japan, Inc. (Tokyo, Japan); 4, Charles River Laboratories Japan, Inc. (Yokohama, Japan); 5, The Jackson Laboratory (Bar Harbor, ME, USA) and 6, Taconic Farms, Inc. (Hudson, NY, USA).

The present work was aimed to investigate the distribution of C1473G and C1477T polymorphisms in laboratory and wild mice. Specifically, the study addressed (1) C1473G alleles distribution in (a) common laboratory strains, (b) new inbred strains derived from wild ancestors and (c) in natural populations of the house mouse; and (2) C1477T alleles distribution in laboratory strains and in natural populations of the house mouse.

Materials and methods

The C1473G polymorphism was investigated in mice of 84 classical inbred strains (Table 1) and 39 strains derived from wild ancestors – mostly *Mus musculus* and several specimens of *Mus spretus* and *Mus spicilegus* – from different regions of Eurasia, one sample from Tunisia and one sample from Canada (Table 2). All these 123 strains were obtained from commercial breeders and the RIKEN BRC as listed in Tables 1 and 2. Furthermore, the C1473G and C1477T polymorphisms were analyzed in 75 wild mice of the

Table 2: Distribution of C and G alleles of the C1473G polymorphism in mice of new strains derived from wild mice and maintained at the RIKEN BioResource Center, Tsukuba, Japan

Strain	Genotype	Origin	*n*	Source*
HMI/Ms	C	*M. m. castaneus* (Hemei, Taiwan)	1	RBRC00657
MYS/Mz	C	*M. m. castaneus* (Mysore, India)	1	RBRC01196
WMP/Pas	C	*M. m. domesticus* (Monastir, Tunisia)	1	RBRC01167
BFM/2Ms	C	*M. m. domesticus* (Montpellier, France)	1	RBRC00659
PGN2/Ms	C	*M. m. domesticus* (Pegion, Canada)	1	RBRC00667
MOM/Nga	C	*M. m. molossinus* (Aichi, Japan)	1	RBRC01837
AIZ/Stm	C	*M. m. molossinus* (Fukushima, Japan)	1	RBRC00430
KOR1/Stm	C	*M. m. molossinus* (Fukushima, Japan)	1	RBRC00427
KOR5/Stm	C	*M. m. molossinus* (Fukushima, Japan)	1	RBRC00428
KOR7/Stm	C	*M. m. molossinus* (Fukushima, Japan)	1	RBRC00429
MAE/Stm	C	*M. m. molossinus* (Iwate, Japan)	1	RBRC00431
JF1/Ms	C	*M. m. molossinus* (Japan)	1	RBRC00639
STM1/Stm	C	*M. m. molossinus* (Saitama, Japan)	1	RBRC00265
STM2/Stm	C	*M. m. molossinus* (Saitama, Japan)	1	RBRC00266
MSM/Ms	C	*M. m. molossinus* (Shizuoka, Japan)	1	RBRC00209
AKT/MzTua	C	*M. m. musculus* (Aktubinsk, Kazakhstan)	3	RBRC01238
KAZ/Tua	C	*M. m. musculus* (Almaty, Kazakhstan)	1	RBRC01237
AST/MzTua	C	*M. m. musculus* (Astrakhan, Russia)	3	RBRC01249
MBT/Pas	C	*M. m. musculus* (General-Toshevo, Bulgaria)	1	RBRC01164
GOR/MzTua	C	*M. m. musculus* (Gorno-Altaisk, Russia)	3	RBRC01242
IRK/MzTua	C	*M. m. musculus* (Irkutsk, Russia)	2	RBRC01241
LUZ/MzTua	C	*M. m. musculus* (Khabarovsk, Russia)	3	RBRC01244
NJL/Ms	C	*M. m. musculus* (Northern Jutland, Denmark)	1	RBRC00207
NOV/Tua	C	*M. m. musculus* (Novo-Kachalinsk, Russia)	1	RBRC01246
OKH/Tua	C	*M. m. musculus* (Okha, Russia)	1	RBRC01245
PWK/Rp	C	*M. m. musculus* (Prague, Czech Republic)	1	RBRC00213
TOM/Tua	C	*M. m. musculus* (Tomsk, Russia)	1	RBRC01243
BLG2/Ms	C	*M. m. musculus* (Toshevo, Bulgaria)	1	RBRC00653
CHD/Ms	C	*M. musculus* spp (Chengdu, China)	1	RBRC00662
JIL/Oda	C	*M. musculus* spp (Jilin, China)	3	RBRC00841
KJR/Ms	C	*M. musculus* spp (Kojuri, Korea)	1	RBRC00655
SWN/Ms	C	*M. musculus* spp (Suweon, Korea)	1	RBRC00654
ZBN/Ms	C	*M. spicilegus* (Kranero, Bulgaria)	1	RBRC00661
SPI/Tua	C	*M. spicilegus* (Mt. Caocasus, Bulgaria)	2	RBRC01250
SPRET/EiJ	C	*M. spretus* (Cadiz, Spain)	1	The Jackson Laboratory
SEG/Pas	C	*M. spretus* (Granada, Spain)	1	RBRC01165
SPR2/Rbrc	C	*M. spretus* (Spain)	1	RBRC00208
WLA/Pas	C	*M. spretus* (Toulouse, France)	1	RBRC01168
STF/Pas	C	*M. spretus* (Tunisia)	1	RBRC01166

n, the number of individuals studied.
*All strains, except for SPRET/EiJ, were obtained from RIKEN BRC; RBRC catalogue numbers are provided. The SPRET/EiJ strain was purchased from The Jackson Laboratory.

Mus musculus species trapped in different regions of Russia and Armenia (Fig. 1; Table 3).

For the samples listed in Tables 1 and 2, genomic DNA was extracted from tail biopsies (1–1.5 cm) with Dneasy Blood & Tissue Kit (Qiagen K.K., Tokyo, Japan). For the samples listed in Table 3, tail biopsies were treated with proteinase K (18 h, 50°C), and genomic DNA was purified by NaCl precipitation of proteins (Miller *et al*. 1988). The C1473G genotype was determined by allele-specific polymerase chain reaction (PCR) (Zhang *et al*. 2004), using outer primers for positive control (F: 5′-tttgacccaaagacgacctgct tgca and R: 5′-tgcatgcttactagccaaccatgaca) and allele-specific primers (C-specific 5′-cagaatttcaatgctctgcgtgtggg and G-specific 5′-cagaatttc aatgctctgcgtgtggc). Genotyping for C1477T was performed using the same positive control primers and the following allele-specific primers: 5′-tctttcagaatttcaatgctctgcttg (C-specific) and 5′-tctttcagaatttcaatgctctgctta (T-specific).

Results

Among 84 inbred strains maintained at the RIKEN BRC, only 21 were homozygous for the 1473G allele. These were substrains of BALB/c, A and DBA, as well as the IITES/Nga and NZW/NSlc strains (Table 1). All the investigated animals from 39 inbred and partially inbred strains derived from

Figure 1: Location of wild mouse populations genotyped in this study. Geographic sources of inbred and semi-inbred wild-derived strains maintained at the RIKEN BRC are marked in black; sources of wild mice trapped in nature are indicated in gray. Location of one sample from Canada is not marked here.

wild ancestors, as well as 75 mice trapped in nature, were homozygous for the 1473C allele (Tables 2 and 3).

Genotyping of 11 inbred mouse strains maintained at the Institute of Cytology and Genetics (Novosibirsk, Russia) for the C1477T polymorphism showed that the strains C3H/He, PT/Y, DD/He, A/He, DBA/2, BALB/c, ICR, CBA/Lac, AKR/J, C57BL/6J and CC57BR/Mv all carried the 1477C allele. At the same time, there was a substantial diversity by this polymorphism in 75 wild mice trapped in nature, with the frequency of 1477T allele ranging from 0% to 71% (Table 3).

Discussion

Most of the common inbred strains under study carried the 1473C allele, and only substrains of BALB/c, A and DBA, as well as the IITES/Nga and NZW/NSlc mice were homozygous for the 1473G allele (Table 1).

This result agrees with the data obtained in other laboratories. The 1473C allele has been previously shown in 129X1/SvJ, 129/SvHsd, C57BL/6J, NMRI, C3H/HeJ, AKR/J, FVB/NJ, NOD/LtJ, BTBR T+ tf/J, KK/HlJ and NZW/LacJ, CBA/Lac, DD/He, PT/Y and YT/Y mice, whereas the 1473G allele has been found in DBA/2J, BALB/cJ, A/J, A/He, CBA/Ca and CC57BR/Mv substrains (Crowley *et al*. 2005; Isles *et al*. 2005; Jacobsen *et al*. 2008; Kulikov *et al*. 2005; Zhang *et al*. 2004; dbSNP build 131). Genealogy analysis shows that the substrains of BALB/c, A and DBA along with CBA/Ca all have originated from the stocks of W.E. Castle and his student C.C. Little, who were among the founders of modern mouse genetics (Beck *et al*. 2000). W.E. Castle received most of the mice for his studies from Abbie Lathrop, a mouse fancier who bred and sold animals in Granby, MA, USA. She obtained her mice from dealers, European fanciers, and those captured in the wild (Davisson & Linder 2004). In Europe, mouse breeding culture developed mainly in England in the 19th century and was taken over from China and Japan, where fanciers collected mice with unusual phenotypes from neighboring territories (Keeler 1931). Europeans imported some of these lines and bred them to local mice to create new varieties (Silver 1995).

The IITES strain has originally been developed from the multiple cross of CS, DBA/2, NBC and ITES strains by Dr Kyoji Kondo at Nagoya University, School of Agriculture, and presumably has received the 1473G allele from DBA/2. The CC57BR/Mv strain generated by Dr N. Medvedev from the cross between BALB/cN and C57BL/N mice (Medvedev 1969) has evidently received the 1473G allele from BALB/c. The New Zealand White (NZW) mice were created by W.H. Hall at the Otago University from a random bred stock brought in 1930 to New Zealand from the Imperial Cancer Research Fund Laboratories at Mill Hill, London (Bielschowsky & Goodall 1970; Hall & Simpson 1975). Although there is no data available on the origin of the UK stock, it is possibly related to the stock that gave rise to the classical inbred strains. Interestingly, the substrains NZW/NSlc and NZW/LacJ as well as CBA/Ca and CBA/Lac differ by C1473G genotype. It points to heterogeneity of the stocks that were used to generate these strains.

It is worth noting that all the classical inbred strains established before 1922 (e.g. substrains of DBA, BALB/c, A, C3H, C57BL, SWR, 129), as well as many newer strains (e.g. substrains DD/He, NMRI/Lac, NZB/Ibm and NZW/Ola), have the same mtDNA type belonging to the Western European house mouse – *M. m. domesticus* (Bayona-Bafaluy *et al*. 2003; Ferris *et al*. 1983). At the same time, the level of mtDNA variation among wild representatives of *M. m. domesticus* subspecies is much greater, with the 'old inbred' type being

Table 3: Distribution of alleles of the C1473G and C1477T polymorphisms in wild mice of *Mus musculus* species captured in different regions of Eurasia

		1473			1477				
Region	*n*	C/C	C/G	G/G	C/C	C/T	T/T	C (%)	T (%)
Novosibirsk, Russia	33	33	0	0	14	18	1	70	30
Kalmykia, Russia	19	19	0	0	0	11	8	29	71
Armenia	11	11	0	0	10	0	0	100	0
Moscow, Russia	10	10	0	0	1	5	4	35	65
Yamalo-Nenets Autonomous Okrug, Russia	2	2	0	0	1	1	0	75	25
All	75	75	0	0	27	35	13	59	41

n, the number of individuals studied.

present at only 4% frequency (Ferris *et al*. 1983). This implies that all the classical laboratory strains were derived from the same stock of some pet dealers, most probably in northern Europe. The abundance of the 'old inbred' mtDNA type may result from the genetic drift either in the common pet mouse stock or in the wild population from which that stock had been selected (Ferris *et al*. 1983). Thus, one may assume that all the classical inbred strains are derived by maternal line from a very small population of wild mice.

All the investigated animals from 39 inbred and partially inbred strains derived from wild ancestors were homozygous for the 1473C allele (Table 2). The 1473G allele was also not detected in any of the 75 DNA samples obtained from wild mice trapped in nature (Table 3). On the whole, among 126 wild and wild-derived mice mostly from different regions of Eurasia (Fig. 1), 118 of which belong to *Mus musculus* species, there were no 1473G allele carriers. Therefore, one may suggest that the frequency of the 1473G allele in natural populations of house mouse does not exceed 0.5%. Thus, 1473C is the 'wild-type' allele, and 1473G is a rare mutation.

It must be noted that because of their origins in mouse fancy trade, the classical laboratory strains represent a genetic mix of four different subspecies (Silver 1995; Wade *et al*. 2002): *M. m. domesticus* (Western Europe), *M. m. musculus* (Eastern Europe, Russia, Northern China), *M. m. molossinus* (Japan) and *M. m. castaneus* (Western Asia, Southeastern Asia, Southern China). A variation map of the genomes of laboratory strains has been generated using 8.27 million SNPs that allows one to determine the ancestry for a selected genome region (Frazer *et al*. 2007). According to these data (http://mouse.perlegen.com/mouse/mousehap.html), the part of the chromosome 10 containing *tph2* gene derives from *M. m. domesticus* both in the strains bearing the 1473G allele (DBA/2, A and BALB/c) and in those carrying the 1473C allele (e.g. C57BL/6, C3H and AKR). As our sample includes only three wild-derived representatives of this subspecies, we still cannot rule out the possibility that the 1473G allele is confined to *M. m. domesticus* and that it could be found with noticeable frequency in natural house mouse populations of Western Europe.

To assess the diversity of our sample, we have studied the distribution of the selectively neutral C1477T polymorphism in wild mice and in several classical inbred mouse strains. Previously, the 1477T allele has been found only in strains derived from wild representatives of *M. m. domesticus* (WSB/EiJ), *M. m. musculus* (PWD/PhJ), *M. m. castaneus* (CAST/EiJ) and *M. m. molossinus* (MOLF/EiJ), and not in classical laboratory strains (NCBI SNP database, http://www.ncbi.nlm.nih.gov/snp). Consistent with these data, we also detected the 1477C allele in the 11 laboratory strains studied both with 1473C/C and 1473G/G genotypes. At the same time, a substantial diversity by the C1477T polymorphism was observed in our sample of wild mice (presumably *M. m. musculus*), with the frequency of the 1477T allele ranging from 0% to 71% (Table 3). Therefore, the populations studied, although heterogeneous for the neutral C1477T change, all bear the conserved 1473C allele that proves to be the ancestral and dominating type in the investigated regions.

A question arises whether the 1473G allele is just not typical for the analyzed subspecies or its rareness results from a negative selection in all natural populations of domestic mice. Although our data do not allow us to answer this question, the second hypothesis seems more probable.

As the C1473G polymorphism alters brain 5-HT level (Zhang *et al*. 2004), one may expect that it would also lead to changes in serotonin-related functions, such as sexual behavior, food intake, sleep, stress response, anxiety or aggression. Previously, we have shown an association between the C1473G polymorphism and intermale aggressiveness in inbred mouse strains: the number of attacks in mice homozygous for the 1473G allele was substantially lower compared with those homozygous for the 1473C allele (Kulikov *et al*. 2005). The linkage has been confirmed by transferring the 1473G allele onto the C57BL/6J genome (Osipova *et al*. 2009). As intermale aggression and consequent territorial dominance are the main factors of differential reproduction in domestic mice (Osadchuk & Naumenko 1981), it can be assumed that there is an intensive natural selection against the 1473G allele. Mice of the 1473G/G genotype are characterized by a lowered aggression and, therefore, a decreased probability to leave progeny, as compared with mice homozygous for the 1473C allele. It should be noted that even minor difference in competitive ability and, consequently, in reproductive success, although insignificant under laboratory conditions, may become essential in natural environment (Carroll & Potts 2006). At the same time, the conserved 1473G allele in several related laboratory mouse strains seems to have been fixed as a result of artificial selection aimed at other traits.

Similar to the mouse C1473G polymorphism is the G1463A substitution in *hTPH2* gene. The 1463A allele, which lowers the enzyme activity, has been originally detected only in 12 of the 306 subjects studied (Zhang *et al*. 2005). It has been shown that the frequency of the 1463A allele is higher in patients with unipolar depression (13.8%) compared with healthy controls (1.8%) (Zhang *et al*. 2005). The mutant allele is so rare that other investigators have failed to show the 1463A allele in different populations (Garriock *et al*. 2005; Glatt *et al*. 2005; Van Den Bogaert *et al*. 2005; Zhou *et al*. 2005a).

Thus, the distribution of the C1473G polymorphism in common mouse inbred strains, those derived from wild ancestors and in natural populations of domestic mice points to an extreme rarity of the 1473G allele. And this fact, in turn, might be explained by a great importance of normal TPH2 activity for survival or reproductive success of mice in natural conditions, and by elimination of mutant alleles leading to a decreased activity through natural selection.

References

Arango, V., Huang, Y.Y., Underwood, M.D. & Mann, J.J. (2003) Genetics of the serotonergic system in suicidal behavior. *J Psychiatr Res* **37**, 375–386.

Bayona-Bafaluy, M.P., Acin-Perez, R., Mullikin, J.C., Park, J.S., Moreno-Loshuertos, R., Hu, P., Perez-Martos, A., Fernandez-Silva, P., Bai, Y. & Enriquez, J.A. (2003) Revisiting the mouse mitochondrial DNA sequence. *Nucleic Acids Res* **31**, 5349–5355.

Beck, J.A., Lloyd, S., Hafezparast, M., Lennon-Pierce, M., Eppig, J.T., Festing, M.F. & Fisher, E.M. (2000) Genealogies of mouse inbred strains. *Nat Genet* **24**, 23–25.

Bielschowsky, M. & Goodall, C.M. (1970) Origin of inbred NZ mouse strains. *Cancer Res* **30**, 834–836.

Carroll, L.S. & Potts, W.K. (2006) Functional genomics requires ecology. In Brockmann, H.J., Slater, P.J.B., Snowdon, C.T., Roper, T.J., Naguib, M. & Wynne-Edwards, K.E. (eds), *Advances in the Study of Behavior*. Vol. 36. Academic Press, San Diego, pp. 173–215.

Chaouloff, F., Berton, O. & Mormede, P. (1999) Serotonin and stress. *Neuropsychopharmacology* **21**, 28S–32S.

Cichon, S., Winge, I., Mattheisen, M., *et al*. (2008) Brain-specific tryptophan hydroxylase 2 (TPH2): a functional Pro206Ser substitution and variation in the 5′-region are associated with bipolar affective disorder. *Hum Mol Genet* **17**, 87–97.

Craig, I.W. (2007) The importance of stress and genetic variation in human aggression. *Bioessays* **29**, 227–236.

Crowley, J.J., Blendy, J.A. & Lucki, I. (2005) Strain-dependent antidepressant-like effects of citalopram in the mouse tail suspension test. *Psychopharmacology (Berl)* **183**, 257–264.

Davisson, M.T. & Linder, C.C. (2004) Historical foundations. In Hedrich, H.J. (ed), *The Laboratory Mouse*. Academic Press, San Diego, pp. 15–24.

Ferris, S.D., Sage, R.D., Prager, E.M., Ritte, U. & Wilson, A.C. (1983) Mitochondrial DNA evolution in mice. *Genetics* **105**, 681–721.

Frazer, K.A., Eskin, E., Kang, H.M., Bogue, M.A., Hinds, D.A., Beilharz, E.J., Gupta, R.V., Montgomery, J., Morenzoni, M.M., Nilsen, G.B., Pethiyagoda, C.L., Stuve, L.L., Johnson, F.M., Daly, M.J., Wade, C.M. & Cox, D.R. (2007) A sequence-based variation map of 8.27 million SNPs in inbred mouse strains. *Nature* **448**, 1050–1053.

Garriock, H.A., Allen, J.J., Delgado, P., Nahaz, Z., Kling, M.A., Carpenter, L., Burke, M., Burke, W., Schwartz, T., Marangell, L.B., Husain, M., Erickson, R.P. & Moreno, F.A. (2005) Lack of association of TPH2 exon XI polymorphisms with major depression and treatment resistance. *Mol Psychiatry* **10**, 976–977.

Glatt, C.E., Carlson, E., Taylor, T.R., Risch, N., Reus, V.I. & Schaefer, C.A. (2005) Response to Zhang *et al*. (2005): loss-of-function mutation in tryptophan hydroxylase-2 identified in unipolar major depression. *Neuron* **45**, 11–16. *Neuron* **48**, 704–705; author reply 705–706.

Hall, W.H. & Simpson, L.O. (1975) The origins of some hitherto undescribed inbred mouse strains. *Lab Anim* **9**, 139–142.

Harvey, M., Shink, E., Tremblay, M., Gagne, B., Raymond, C., Labbe, M., Walther, D.J., Bader, M. & Barden, N. (2004) Support for the involvement of TPH2 gene in affective disorders. *Mol Psychiatry* **9**, 980–981.

Isles, A.R., Hathway, G.J., Humby, T., de la Riva, C., Kendrick, K.M. & Wilkinson, L.S. (2005) An mTph2 SNP gives rise to alterations in extracellular 5-HT levels, but not in performance on a delayed-reinforcement task. *Eur J Neurosci* **22**, 997–1000.

Jacobsen, J.P., Nielsen, E.O., Hummel, R., Redrobe, J.P., Mirza, N. & Weikop, P. (2008) Insensitivity of NMRI mice to selective serotonin reuptake inhibitors in the tail suspension test can be reversed by co-treatment with 5-hydroxytryptophan. *Psychopharmacology (Berl)* **199**, 137–150.

Keeler, C.E. (1931) *The Laboratory Mouse; its Origin, Heredity, and Culture*. Harvard University Press, Cambridge.

Kulikov, A.V., Osipova, D.V., Naumenko, V.S. & Popova, N.K. (2005) Association between Tph2 gene polymorphism, brain tryptophan hydroxylase activity and aggressiveness in mouse strains. *Genes Brain Behav* **4**, 482–485.

Kulikov, A.V., Osipova, D.V. & Popova, N.K. (2007) The C1473G polymorphism in gene tph2 is the main factor mediating the genetically defined variability of tryptophan hydroxylase-2 activity in the mouse brain. *Russ J Genet* **43**, 1408–1412.

Lesch, K.P. (2004) Gene-environment interaction and the genetics of depression. *J Psychiatry Neurosci* **29**, 174–184.

Lin, Y.M., Chao, S.C., Chen, T.M., Lai, T.J., Chen, J.S. & Sun, H.S. (2007) Association of functional polymorphisms of the human tryptophan hydroxylase 2 gene with risk for bipolar disorder in Han Chinese. *Arch Gen Psychiatry* **64**, 1015–1024.

Lucki, I. (1998) The spectrum of behaviors influenced by serotonin. *Biol Psychiatry* **44**, 151–162.

Mathew, S.J. & Ho, S. (2006) Etiology and neurobiology of social anxiety disorder. *J Clin Psychiatry* **67** (Suppl. 12), 9–13.

McKinney, J., Johansson, S., Halmoy, A., Dramsdahl, M., Winge, I., Knappskog, P.M. & Haavik, J. (2008) A loss-of-function mutation in tryptophan hydroxylase 2 segregating with attention-deficit/hyperactivity disorder. *Mol Psychiatry* **13**, 365–367.

McKinney, J.A., Turel, B., Winge, I., Knappskog, P.M. & Haavik, J. (2009) Functional properties of missense variants of human tryptophan hydroxylase 2. *Hum Mutat* **30**, 787–794.

Medvedev, N.N. (1969) Inbreeding, fertility and viability. *Sov Genet* **5**, 376–387.

Miller, S.A., Dykes, D.D. & Polesky, H.F. (1988) A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* **16**, 1215.

Olivier, B. (2004) Serotonin and aggression. *Ann N Y Acad Sci* **1036**, 382–392.

Osadchuk, A.V. & Naumenko, E.V. (1981) Ethological genetic mechanisms of differential reproduction in male laboratory mice. *Dokl Akad Nauk SSSR* **261**, 1238–1241.

Osipova, D.V., Kulikov, A.V. & Popova, N.K. (2009) C1473G polymorphism in mouse tph2 gene is linked to tryptophan hydroxylase-2 activity in the brain, intermale aggression, and depressive-like behavior in the forced swim test. *J Neurosci Res* **87**, 1168–1174.

Popova, N.K. (2006) From genes to aggressive behavior: the role of serotonergic system. *Bioessays* **28**, 495–503.

Sakowski, S.A., Geddes, T.J. & Kuhn, D.M. (2006) Mouse tryptophan hydroxylase isoform 2 and the role of proline 447 in enzyme function. *J Neurochem* **96**, 758–765.

Silver, L.M. (1995) *Mouse Genetics: Concepts and Applications*. Oxford University Press, New York/Oxford.

Van Den Bogaert, A., De Zutter, S., Heyrman, L., Mendlewicz, J., Adolfsson, R., Van Broeckhoven, C. & Del-Favero, J. (2005) Response to Zhang *et al*. (2005): loss-of-function mutation in tryptophan hydroxylase-2 identified in unipolar major depression. *Neuron* **45**, 11–16. *Neuron* **48**, 704; author reply 705–706.

Wade, C.M., Kulbokas, E.J. III, Kirby, A.W., Zody, M.C., Mullikin, J.C., Lander, E.S., Lindblad-Toh, K. & Daly, M.J. (2002) The mosaic structure of variation in the laboratory mouse genome. *Nature* **420**, 574–578.

Walther, D.J. & Bader, M. (2003) A unique central tryptophan hydroxylase isoform. *Biochem Pharmacol* **66**, 1673–1680.

Zhang, X., Beaulieu, J.M., Sotnikova, T.D., Gainetdinov, R.R. & Caron, M.G. (2004) Tryptophan hydroxylase-2 controls brain serotonin synthesis. *Science* **305,** 217.

Zhang, X., Gainetdinov, R.R., Beaulieu, J.M., Sotnikova, T.D., Burch, L.H., Williams, R.B., Schwartz, D.A., Krishnan, K.R. & Caron, M.G. (2005) Loss-of-function mutation in tryptophan hydroxylase-2 identified in unipolar major depression. *Neuron* **45**, 11–16.

Zhou, Z., Peters, E.J., Hamilton, S.P., McMahon, F., Thomas, C., McGrath, P.J., Rush, J., Trivedi, M.H., Charney, D.S., Roy, A., Wisniewski, S., Lipsky, R. & Goldman, D. (2005a) Response to Zhang *et al*. (2005): loss-of-function mutation in tryptophan hydroxylase-2 identified in unipolar major depression. *Neuron* **45**, 11–16. *Neuron* **48**, 702–703; author reply 705–706.

Zhou, Z., Roy, A., Lipsky, R., Kuchipudi, K., Zhu, G., Taubman, J., Enoch, M.A., Virkkunen, M. & Goldman, D. (2005b) Haplotype-based linkage of tryptophan hydroxylase 2 to suicide attempt, major depression, and cerebrospinal fluid 5-hydroxyindoleacetic acid in 4 populations. *Arch Gen Psychiatry* **62**, 1109–1118.

Zill, P., Baghai, T.C., Zwanzger, P., Schule, C., Eser, D., Rupprecht, R., Moller, H.J., Bondy, B. & Ackenheil, M. (2004a) SNP and haplotype analysis of a novel tryptophan hydroxylase isoform (TPH2) gene

provide evidence for association with major depression. *Mol Psychiatry* **9**, 1030–1036.
Zill, P., Buttner, A., Eisenmenger, W., Moller, H.J., Bondy, B. & Ackenheil, M. (2004b) Single nucleotide polymorphism and haplotype analysis of a novel tryptophan hydroxylase isoform (TPH2) gene in suicide victims. *Biol Psychiatry* **56**, 581–586.

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