Allelic Variation of the Serotonin Transporter Gene Polymorphic Region in Apes

MIHO INOUE-MURAYAMA, YOUKO NIIMI, *Gifu University*
OSAMU TAKENAKA, *Kyoto University*
KYOKO OKADA, ICHIYO MATSUZAKI, *University of Tsukuba*
SHIN'ICHI ITO, *Gifu University*
and YUICHI MURAYAMA, *National Institute of Animal Health*

ABSTRACT. To assess the change of serotonin transporter (5-HTT) gene-linked polymorphic region that has occurred during the process of hominization, we examined the allelic variation of 5-HTT gene-linked polymorphic region (5-HTTLPR) in anthropoid apes such as chimpanzees, gorillas, orang-utans, and gibbons, and determined the DNA sequences of the alleles in each species. All chimpanzees examined shared only the 17.5 repeat allele, while polymorphism was observed in the other apes and the 16 and 20 repeat alleles were most frequent in gorillas and orang-utans, respectively. 5-HTTLPR was highly polymorphic in gibbons and the 17 and 23 repeat alleles were most common among 5 alleles. Alleles with extra-long repeated (22 and 23) sequences were found in orang-utans and gibbons, and the alleles of these Asian apes were similar to the rhesus monkey allele.

Key Words: Serotonin transporter; Promoter sequence; Allelic variation; Hominization.

INTRODUCTION

The receptors for neurotransmitters are distributed in specific areas of the brain and are expressed in neural cells having effects upon various neurological and psychological functions. Genetic polymorphism has been reported in several genes encoding the above cell surface receptors. For example, human serotonin transporter (5-HTT) gene is polymorphic in terms of the repeat numbers of the GC-rich 20–23 bp sequence located in the 5-HTT gene-linked polymorphic region (5-HTTLPR) proximal to the transcription initiation site (HEILS et al., 1996) and 14–20 repeat alleles have been reported in humans (HEILS et al., 1996; DELBRÜCK et al., 1997; MICHAELOVSKY et al., 1999), with the 14 and 16 repeat alleles being most common in human populations. The promoter activity of the 14 repeat allele was found to be reduced as compared to that of the 16 repeat allele *in vitro* (HEILS et al., 1996), resulting in a decreased 5-HTT expression and serotonin (5-HT) re-uptake (LESCH et al., 1996; GREENBERG et al., 1999).

Since 5-HTT modulates the amount of secreted serotonin in the synapses, 5-HTT is involved in the regulation of various functions such as mood, cognition, food intake, and sleep. An association between genetic variants of the 5-HTT gene and several neuropsychiatric disorders has been proposed (OLIVEIRA et al., 1998; ROSENTHAL et al., 1998). In addition, allelic variation of the 5-HTT gene has been shown to be possibly related to human personality traits (LESCH et al., 1996; MAZZANTI et al., 1998; KATSURAGI et al., 1999). That is, individuals with 14 repeats in the 5-HTT gene promoter displayed higher scores in a personality test for anxiety/neuroticism than did those with 16 repeats. However, other reports (GELERNTER et al., 1998; FLORY et al., 1999; KUMAKIRI et al., 1999) showed no significant association between 5-HTT genotypes and the above personality traits. It remains unclear whether 5-HTT gene locus is itself affecting anxiety/neuroticism or is linked to another unknown functional variant.

5-HTTLPR has been identified in humans, apes, Old World monkeys, and New World monkeys but not in prosimians (LESCH et al., 1997). It has been suggested therefore that a progenitor 5-HTTLPR sequence derived from a viral genome or a transposable element may have been introduced into the genome of the ancestral simian primate. DNA sequences of 5-HTTLPR have been described in the chimpanzee, orang-utan, and rhesus monkey (LESCH et al., 1997). These non-human primates have relatively longer 5-HTTLPR sequences with an increased number of tandem repeats as compared to human sequences. However, only a few individuals of each species were examined in the above apes, and the DNA sequences and variations of 5-HTTLPR in other apes such as the gorilla and gibbon remain obscure. Such data are indispensable for assessing the change of 5-HTTLPR that occurred during the process of hominization. The present study describes the allelic variation of 5-HTTLPR observed in 54 individuals from 4 ape species and the DNA sequences of typical alleles of each species.

MATERIALS AND METHODS

PRIMATES

Blood or tissue samples were collected from the following species: chimpanzees (*Pan troglodytes*, n=16), gorillas (*Gorilla gorilla*, n=14), orang-utans (*Pongo pygmaeus*, n=9), and MÜLLER's gibbons (*Hylobates muelleri*, n=15). In addition, we determined polymorphism in humans (healthy Japanese subjects, n=102) and the DNA sequences of three alleles (the 14, 16, and 20 repeat alleles) of 5-HTTLPR. Most of the non-human primates were kept at the Primate Research Institute of Kyoto University, Japan Monkey Centre, Sanwa Kagaku Kenkyusho CO., LTD., and Tama Zoo in Japan. Fourteen blood samples from MÜLLER's gibbons were obtained from pet animals bred on Borneo Island, Indonesia. As for the all samples, the genealogy is not known.

POLYMERASE CHAIN REACTION (PCR) AND DNA SEQUENCE

Genomic DNA was extracted by the conventional phenol-chloroform method or with a QIAamp blood kit (QIAGEN, Chatsworth, CA). The 5-HTTLPR containing the repeated sequence was amplified by the PCR according to HEILS et al. (1996). The primers were synthesized based on the human 5-HTT 5'-regulatory region. The primer sequences employed were 5'-GGCGTTGCCGCTCTGAATGC-3' and 5'-GAGGGACTGAGCTGGACAACCAC-3', corresponding to the nucleotide positions of -1416 to -1397 and -910 to -888, respectively. The predicted size of the PCR product is 484 bp in the case of the 14 repeat allele and 528 bp in the 16 repeat allele. The DNA (20 – 100 ng) was exposed to 20 μl of PCR buffer containing 0.5 μM each of primer, 0.5 U of ExTaq polymerase (Takara, Kyoto, Japan), 125 μM of dATP, dTTP, and dCTP, and 62.5 μM of dGTP and 7-deaza-dGTP (Boehringer Mannheim, Germany). The resultant solution was subjected to 30 cycles of incubation, with each cycle consisting of denaturation for 30 sec at 95°C, annealing for 30 sec at 65°C, and extension for 1 min at 72°C. Each sample was separated on 2% agarose gel. The genotype was estimated from the product size and confirmed from its nucleotide sequence.

The PCR products were extracted from the gel and then directly sequenced by the dye termination method using a Perkin Elmer 377 sequencer. The nucleotide sequence was determined in both strands at least twice. The sequences of the repeated units contained in 5-HTTLPR were aligned using CLUSTALW (THOMPSON et al., 1994).

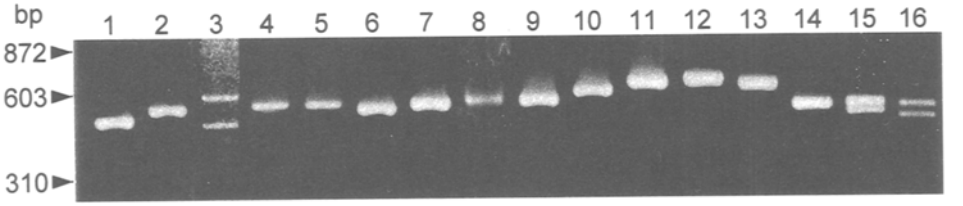

Fig. 1. Example of PCR-based detection of 5-HTTLPR in apes. The human alleles (lane 1: 14/14; lane 2: 16/16; lane 3: 14/20) are also given for comparison with the ape alleles. The species included in the figure are: chimpanzees (lanes 4 and 5: 17.5/17.5), gorillas (lane 6: 16/16; lane 7: 17/17; lane 8:18/18), orang-utans (lane 9: 18/18; lane 10: 20/20; lane 11: 22/22), and gibbons (lane 12: 23/23; lane 13: 22/22; lane 14: 17/17; lane 15: 16/17; lane 16: 15/17). The numbers of tandem repeats were confirmed by sequence analysis.

RESULTS AND DISCUSSION

Allelic Variation of 5-HTTLPR in Apes and Humans

The number of repeated units of 5-HTTLPR was estimated from the size of the PCR product (Fig. 1). As shown in Table 1, the 14 repeat allele has been found only in humans and this allele was most frequent in the Japanese in contrast to Caucasians as reported previously (Ishiguro et al., 1997; Delbrück et al., 1997). We also identified the 20 repeat allele in the Japanese, which has been reported to be unique in African individuals (Delbrück et al., 1997).

Among the apes, all 16 chimpanzees examined shared only one genotype with the homozygote of the 17.5 repeat allele, which was estimated from the size of the PCR product. Although the 18 repeat allele has been reported in chimpanzees (Lesch et al., 1997), we failed to identify this allele by both electrophoresis and sequence analysis: only the 17.5-repeat allele was found in our specimens. Common chimpanzees can be classified into four subspecies (Morin et al., 1994; Gonder et al., 1997), and the use of different subspecies in the experiments (*P. t. verus* was examined in the present study while subspecies examined by Lesch et al. was unknown) could thus be reflected in the above disparity. On the other hand, polymorphism was noted in the other apes, with the 16 and 20 repeat alleles being most frequent in the gorillas and orang-utans, respectively. 5-HTTLPR was highly polymorphic in the gibbons, and 17 repeat allele was most frequent among five alleles. Twenty and more repeat alleles were rare or absent in the humans, chimpanzees, and gorillas, whereas these alleles were common in the orang-utans and gibbons.

Table 1. Allele frequency of the serotonin transporter gene-linked polymorphic region.

Allele*	Human n=102	Chimpanzee n=16	Gorilla n=14	Orang-utan n=9	Gibbon n=15
14	0.775				
15					0.067
16	0.221		0.536		0.033
17			0.143		0.500
17.5		1.000			
18			0.321	0.111	
20	0.004			0.778	
22				0.111	0.067
23					0.333

*Allele with the given number of tandem repeats in the promoter sequence.

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                 1                     2                     3      Region A       4                     5
J14    CCCTACTGCAGCC-TCCCAGCAT --------------------- --------------------- --------------------- ---------------------  22
J16    CCCTACTGCAGCC-TCCCAGCAT --------------------- --------------------- --------------------- ---------------------  22
J20    CCCTACTGCAGCC-TCCCAGCAT --------------------- --------------------- --------------------- ---------------------  22
C17.5  CCCTACTGCAGCCCTCCCAGCAT --------------------- --------------------- --------------------- ---------------------  23
Go16   CCTAACTGCAGCCATCCCAGCAT --------------------- --------------------- --------------------- ---------------------  23
Go17   CCTAACTGCAGCCATCCCAGCAT --------------------- --------------------- --------------------- ---------------------  23
Go18   CCTAACTGCAGCCATCCCAGCAT --------------------- --------------------- --------------------- ---------------------  23
O18    CCCTGCTGCAGCCCTCCCAGCTT --------------------- --------------------- --------------------- ---------------------  23
O20    CCCTGCTGCAGCCCTCCCAGCTT --------------------- --------------------- --------------------- ---------------------  23
O22    CCCTGCTGCAGCCCTCCCAGCAT CTCTCTGTACTCCTCCTAGGAT CTCCCCTGCACCCCCCATTAT CCTCCCTGCACCCCTTGCGGCAT CCCCCCTGCACCC--CCAGCAT  109
Gi15   CCCTGCTGCAGCCCTCTCAGCAT --------------------- --------------------- --------------------- ---------------------  23
Gi16   CCCTGCTGCAGCCCTCTCAGCAT --------------------- --------------------- --------------------- ---------------------  23
Gi17   CCCTGCTGCAGCCCTCTCAGCAT --------------------- --------------------- --------------------- ---------------------  23
Gi22   CCCTGCTGCAGCCCTCCCAGCAT CTCTCTGTACTCCTCCTAGGAT CTCCCCTGCACCCCCCATTAT CCTCCCTGCACCCCTTGCGGCAT CCCCCCTGCACCC--CCAGCAT  109
Gi23   CCCTGCTGCAGCCCTCCCAGCAT CTCCCTGTACCCCTCCTAGGAT CTCCCCTGCACCCCCCATTAT CCTCCCTACACCCC--CCAGCAT CCCCCCTGCAGCCTCCCAGCAT  109
Rh23   CCCTGCTGCAGCCCTCCCAGCAT CTCCCTGCACCCCTCCTAGGAT CTCCCCTGCACCCCCCATTAT CCTCCCTACACCCC--CCAGCAT CCCCCCTGCAGCCTCCCAGCAT  109

                  6                      7                       8                       9                      10
J14    --------------------- ---------------------- CCCCCCTGCAACCTCCCAGCAA -CTCCCTGTACCCCTCCTAGGAT CGCTCCTGCATCCCCCATTAT  87
J16    --------------------- ---------------------- CCCCCCTGCAACCTCCCAGCAA -CTCCCTGTACCCCTCCTAGGAT CGCTCCTGCATCCCCCATTAT  87
JL20   --------------------- ---------------------- CCCCCCTGCAACCTCCCAGCAA -CTCCCTGTACCCCTCCTAGGAT CGCTCCTGCATCCCCCATTAT  87
C17.5  --------------------- ---------------------- CCCCCCTGCAACCTCCCAGCAA -CTCCCTGTACCCCTCCTAGGAT CGCTCCTGCATCCCCCATTAT  88
Go16   --------------------- ---------------------- CCCCCCTGCAACCTCCCAGCAA -CTCCCTGTACCCCTCCTAGGAT CGCTCCTGCATCCCCCATTAT  88
Go17   --------------------- ---------------------- CCCCCCTGCAACCTCCCAGCAA -CTCCCTGTACCCCTCCTAGGAT CGCTCCTGCATCCCCCATTAT  88
Go18   --------------------- ---------------------- CCCCCCTGCAACCTCCCAGCAA -CTCCCTGTACCCCTCCTAGGAT CGCTCCTGCATCCCCCATTAT  88
O18    --------------------- ---------------------- CCCCCCTGCACCCTCCCAGCAA -CTCCCTGTACCCCTCCTAGGAT CGCCTCTGCATCCCCCATCAT  88
O20    --------------------- ---------------------- CCCCCCTGCACCCTCCCAGCAA -CTCCCTGTACCCCTCCTAGGAT CGCCTCTGCATCCCCCATCAT  88
O22    --------------------- CCCCCCTGCAGCCCTTTCAGCGT ---------------------- -CCCCCTGCACCCCTCCCAGGAT CTCCCTTGCA-CCCCCATTAT  174
Gi15   --------------------- ---------------------- CCCCCCTGCACCCTCCCAGCAT -CTCCCTGTACCCCTCCTAGGAT CGCCCCTGCATCCCCCATTAT  88
Gi16   --------------------- ---------------------- CCCCCCTGCACCCTCCCAGCAT -CTCCCTGTACCCCTCCTAGGAT CGCCCCTGCATCCCCCATTAT  88
Gi17   --------------------- ---------------------- CCCCCCTGCACCCTCCCAGCAT -CTCCCTGTACCCCTCCTAGGAT CGCCCCTGCATCCCCCATTAT  88
Gi22   --------------------- CCCCCCTGCAGCCCTTTCAGCGT ---------------------- -CCCCCTGCACCCCTCCCAGGAT CTCCCTTGCA-CCCCCATTAT  174
Gi23   CTCCCCTGCA-CCCCCAGCAT CCCCCCTGCAGCCCTTTCAGCAT ---------------------- -CCCCCTGCACCCCTCCCAGGAT CTCCCTTGCAT-CCCCATTAT  194
Rh23   CTCCCCTGCACCCCCCAGCAT -CCCCCTGCAG-CCTTTCAGCAT ---------------------- CCCCCCTGCACCCCTCCCAGGAT CTCCCTTGCAT-CCCCATTAT  195

                  11                     12                      13                   14      Region B     15
J14    CCCCCCCTTCACTCCTCGCGGCAT CCCCCCTGCACCCCCCAGCAT ---------------------- ---------------------- ----------------------  132
J16    CCCCCCCTTCACTCCTCGCGGCAT CCCCCCTGCACCCCCCAGCAT ---------------------- ---------------------- ----------------------  132
J20    CCCCCCCTTCACTCCTCGCGGCAT CCCCCCTGCACCCCCCAGCAT CCCCCCTGCAGCCCTTCCAGCAT C-CCCCTGCA-CCCCCAGCAT CCCCCCTGCAGCCCTTCCAGCAT  197
C17.5  CCCCCCCTTCACCCCTCGCGGCAT CCCCC-----------ATTAT C--------------------- ---------------------- CCCCCCCTTCACCCCTCGCGGCAT  146
Go16   CCCCCCCTTCACCCCTCGCGGCAT CCCCCCTGCACCCCCCAGCAT ---------------------- ---------------------- ----------------------  133
Go17   CCCCCCCTTCACCCCTCGCGGCAT CCCCCCTGCACCCCCCAGCAT ---------------------- ---------------------- ----------------------  133
Go18   CCCCCCCTTCACCCCTCGCGGCAT CCCCCCTGCACCCCCCAGCAT ---------------------- ---------------------- GCCCCCTGCACCCCTCGCGGCAT  156
O18    -CCCCCCTGCACCCCTCGCGGCAT CCCCCCTGCACCCCCCAGCAT ---------------------- ---------------------- CCCCCCTGCAGCCC-CCCAGCAT  154
O20    -CCCCCCTGCACCCCTCGCGGCAT CCCCCCTGCACCCCCCAGCAT CCCCCCTGCAGCCC-CCCAGCAT CTCCCCTGCACCCCCCAGCAT -CCCCCTGCAGCCC-CCCAGCAT  196
O22    -CTCCCCTGCACCCCTCGCAGCAT CCCCCC-GCA-CCTCCATTAT ---------------------- ---------------------- -CCCCCTGCACCCCTTGCGGCAT  238
Gi15   -CCCCCCTGCACCCCTCGCGGCAT CCCCC-TGCACCCCCCAGCAT ---------------------- ---------------------- ----------------------  131
Gi16   -CCCCCCTGCACCCCTCGCGGCAT CCCCCCTGCACCCCCCAGCAT ---------------------- ---------------------- -GCCCCTGCACCCCTCGCGGCAT  154
Gi17   -CCCCCCTGCACCCCTCGCGGCAT CCCCCCTGCACCCCCCAGCAT ---------------------- ---------------------- -GCCCCTGCACCCCTCGCGGCAT  154
Gi22   -CTCCCCTGCACCCCTCGCAGCAT CCCCCC-GCA-CCTCCATTAT ---------------------- ---------------------- -CCCCCTGCACCCCTTGCGGCAT  238
Gi23   -CTCCCCTGCACCCCTCTCAGTAT CCCCCC-GCA-CCTCCATTAT ---------------------- ---------------------- CCCCCCTGCACCCCTTGCGGAAT  259
Rh23   -CTCCCCTGCACCCCTCGCAGTAT CCCCCC-GCA-CCTCCATTAT ---------------------- ---------------------- CCCCCCTGCACCCCTTGCGGCAT  260
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(continued)

(continued)

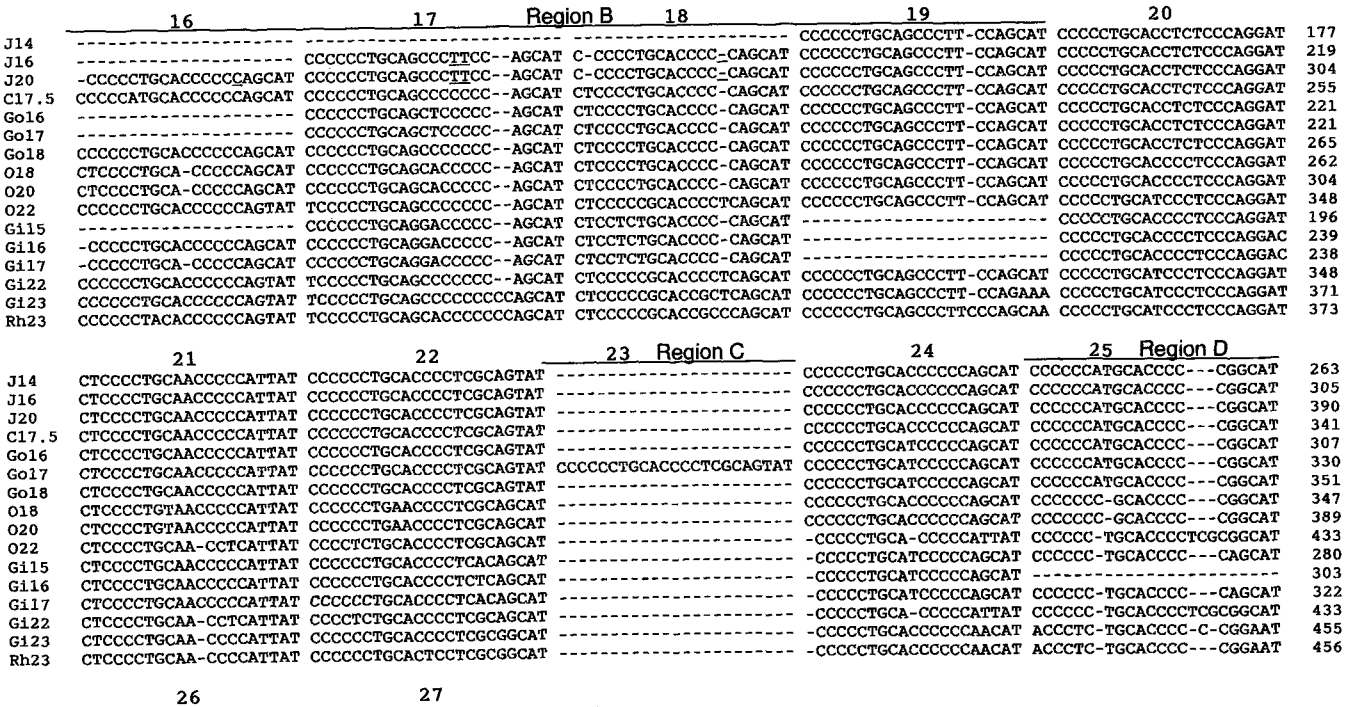

Fig. 2. Multiple alignment of 5-HTTLPR sequences from humans (Japanese), apes and rhesus monkeys. PCR-derived nucleotide sequences from humans and apes were aligned with the previously reported rhesus monkey sequence (LESCH et al., 1997). The repeat units are numbered serially. Dashes represent gaps introduced to optimize the alignment. Underlines in the Japanese sequences indicate nucleotide substitutions differing from those of Caucasians. J: Japanese; C: chimpanzee; Go: gorilla; O: orang-utan; Gi: gibbon; Rh: rhesus monkey.

COMPARISON AMONG DNA SEQUENCES OF 5-HTTLPR

We determined the nucleotide sequences of all 5-HTTLPR allelic variants. Figure 2 presents the DNA sequences of the ape 5-HTTLPR. The human and rhesus monkey (*Macaca mulatta*, LESCH et al., 1997) sequences are also included in this figure. In orang-utans, we determined the sequences of the 20 and 22 repeat alleles, in addition to the 18 repeat allele which has been reported in a previous paper (LESCH et al., 1997). The sequences of 5-HTTLPR in gorillas and gibbons were newly determined in the present study.

Insertion or deletion of unit(s) was recognized at four sites of the sequences in the apes. Region A located between the second and the seventh units (serial numbers of repeated units) was specific to orang-utans and gibbons. In these apes, extra-long repeat alleles were found and alleles such as O22, Gi22, and Gi23 had additional sequences in Region A, which were highly homologous with the rhesus monkey sequence, Rh23. Region B corresponding to the 13th – 19th units was a common polymorphic site present in humans, gorillas, orang-utans, and gibbons. In the vicinity of Region B, the 12th unit of the chimpanzee sequence was incomplete with the deletion of 11 nucleotides. Region C in the 23rd unit and Region D in the 25th unit were specific to gorillas and gibbons, respectively. The polymorphic sites of 5-HTTLPR thus differed between each species of ape.

The polymorphism observed in orang-utans and gibbons has strong implications for the origin of 5-HTTLPR in apes. In these Asian apes, extra-long repeat (22 and 23) alleles were recognized and their sequences were similar to the Rh23 sequence. The Gi23 allele was estimated to be maintained at a relatively high frequency of 0.333 in MÜLLER's gibbons. These findings suggest that the ancestral ape might have had such extra-long repeat alleles.

It is inferred that dynamic changes of 5-HTTLPR in its length had occurred at the stage of orang-utans and gibbons. These apes are arboreal primates and display a relatively small social structure consisting of solo or family members, when compared with those of the chimpanzee and gorilla. The development of various alleles and an increased frequency of shorter repeat alleles during the process of hominization could reflect the changes in habitats and social structures. In addition to molecular genetic studies of 5-HTTLPR, biochemical and behavioral scientific analysis is required if we are to succeed in elucidating the detailed significance of 5-HTTLPR polymorphism in ape societies.

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REFERENCES

DELBRÜCK, S. J.; WENDEL, B.; GRUNEWALD, I.; SANDER, T.; MORRIS-ROSENDAHL, D.; CROCQ, M. A.; BERRETTINI, W. H.; HOEHE, M. R. 1997. A novel allelic variant of the human serotonin transporter gene regulatory polymorphism. *Cytogenet. Cell Genet.*, 79: 214–220.

FLORY, J. D.; MANUCK, S. B.; FERRELL, R. E.; DENT, K. M.; PETERS, D. G.; MULDOON, M. F. 1999. Neuroticism is not associated with the serotonin transporter (5-HTTLPR) polymorphism. *Mol. Psychiatry*, 4: 93–96.

GELERNTER, J.; KRANZLER, H.; COCCARO, E. F.; SIEVER, L. J.; NEW, A. S. 1998. Serotonin transporter protein gene polymorphism and personality measures in African American and European American subjects. *Amer. J. Psychiatry*, 155: 1332–1338.

GONDER, M. K.; OATES, J. F.; DISOTELL, T. R.; FORSTNER, M. R.; MORALES, J. C.; MELNICK, D. J. 1997. A new west African chimpanzee subspecies? *Nature*, 388: 337.

GREENBERG, B. D.; TOLLIVER, T. J.; HUANG, S. J.; LI, Q.; BENGEL, D.; MURPHY, D. L. 1999. Genetic variation in the serotonin transporter promoter region affects serotonin uptake in human blood platelets. *Amer. J. Med. Genet.*, 88: 83–87.

HEILS, A.; TEUFEL, A.; PETRI, S.; STÖBER, G.; RIEDERER, P.; BENGEL, D.; LESCH, K. P. 1996. Allelic variation of human serotonin transporter gene expression. *J. Neurochem.*, 66: 2621–2624.

ISHIGURO, H.; ARINAMI, T.; YAMADA, K.; OTSUKA, Y.; TORU, M.; SHIBUYA, H. 1997. An association study between a transcriptional polymorphism in the serotonin transporter gene and panic disorder in a Japanese population. *Psychiatry Clin. Neurosci.*, 51: 333–335.

KATSURAGI, S.; KUNUGI, H.; SANO, A.; TSUTSUMI, T.; ISOGAWA, K.; NANKO, S.; AKIYOSHI, J. 1999. Association between serotonin transporter gene polymorphism and anxiety-related traits. *Biol. Psychiatry*, 45: 368–370.

KUMAKIRI, C.; KODAMA, K.; SHIMIZU, E.; YAMANOUCHI, N.; OKADA, S.; NODA, S.; OKAMOTO, H.; SATO, T.; SHIRASAWA, H. 1999. Study of the association between the serotonin transporter gene regulatory region polymorphism and personality traits in a Japanese population. *Neurosci. Lett.*, 263: 205–207.

LESCH, K. P.; BENGEL, D.; HELIS, A.; SABOL, S. Z.; GREENBERG, B. D.; PETRI, S.; BENJAMIN, J.; MÜLLER, C. R.; HAMER, D. H.; MURPHY, D. L. 1996. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science*, 274: 1527–1531.

LESCH, K. P.; MEYER, J.; GLATZ, K.; FLÜGGE, G.; HINNEY, A.; HEBEBRAND, J.; KLAUCK, S. M.; POUSTKA, A.; POUSTKA, F.; BENGEL, D.; MÖSSNER, R.; RIEDERER, P.; HEILS, A. 1997. The 5-HT transporter gene-linked polymorphic region (5-HTTLPR) in evolutionary perspective: alternative biallelic variation in rhesus monkeys. *J. Neural. Transm.*, 104: 1259–1266.

MAZZANTI, C. M.; LAPPALAINEN, J.; LONG, J. C.; BENGEL, D.; NAUKKARINEN, H.; EGGERT, M.; VIRKKUNEN, M.; LINNOILA, M.; GOLDMAN, D. 1998. Role of the serotonin transporter promoter polymorphism in anxiety-related traits. *Arch. Gen. Psychiatry*, 55: 936–940.

MICHAELOVSKY, E.; FRISCH, A.; ROCKAH, R.; PELEG, L.; MAGAL, N.; SHOHAT, M.; WEIZMAN, R. 1999. A novel allele in the promoter region of the human serotonin transporter gene. *Mol. Psychiatry*, 4: 97-99.

MORIN, P. A.; MOORE, J. J.; CHAKRABORTY, R.; JIN, L.; GOODALL, J.; WOODRUFF, D. S. 1994. Kin selection, social structure, gene flow, and the evolution of chimpanzees. *Science*, 265: 1193–1201.

OLIVEIRA, J. R.; GALLINDO, R. M.; MAIA, L. G.; BRITO-MARQUES, P. R.; OTTO, P. A.; PASSOS-BUENO, M. R.; MORAIS, M. A. JR.; ZATZ, M. 1998. The short variant of the polymorphism within the promoter region of the serotonin transporter gene is a risk factor for late onset Alzheimer's disease. *Mol. Psychiatry*, 3: 438–441.

ROSENTHAL, N. E.; MAZZANTI, C. M.; BARNETT, R. L.; HARDIN, T. A.; TURNER, E. H.; LAM, G. K.; OZAKI, N.; GOLDMAN, D. 1998. Role of serotonin transporter promoter repeat length polymorphism (5-HTTLPR) in seasonality and seasonal affective disorder. *Mol. Psychiatry*, 3: 175–177.

THOMPSON, J. D.; HIGGINS, D. G.; GIBSON, T. J. 1994. CLUSTALW. *Nucleic Acids Res.*, 22: 4673–4680.

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Authors' Names and Addresses: MIHO INOUE-MURAYAMA and YOUKO NIIMI, *Department of Biological Diversity and Resources, Faculty of Agriculture, Gifu University, Gifu, Japan*; OSAMU TAKENAKA, *Primate Research Institute, Kyoto University, Inuyama, Aichi, Japan*; KYOKO OKADA, *Institute of Environmental Science, University of Tsukuba, Ibaraki, Japan*; ICHIYO MATSUZAKI, *Institute of Community Medicine, Univeristy of Tsukuba, Ibaraki, Japan*; SHIN'ICHI ITO, *Department of Biological Diversity and Resources, Faculty of Agriculture, Gifu University, Gifu, Japan*; YUICHI MURAYAMA, *National Institute of Animal Health, Kannondai, Tsukuba, Ibaraki 305-0856, Japan*. e-mail: mrspock@bc.mbn.or.jp