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Genetic Diversity of the Human *Serotonin Receptor 1B* (*HTR1B*) Gene

Alan R. Sanders,*,† Qiuhe Cao,† Jennifer Taylor,†,‡ Tamara E. Levin,* Judith A. Badner,*,† Anibal Cravchik,†,[1] Josep M. Comeron,§ Saitou Naruya,¶ Amado Del Rosario,* Debra A. Salvi,* Katherine A. Walczyk,* Bryan J. Mowry,‡ Douglas F. Levinson,‖ Raymond R. Crowe,** Jeremy M. Silverman,†† and Pablo V. Gejman*,†,[2]

**Schizophrenia Genetics Research Program, Department of Psychiatry, The University of Chicago, Chicago, Illinois 60637; †Unit on Molecular Clinical Investigation, Clinical Neurogenetics Branch, National Institute of Mental Health, Bethesda, Maryland 20892; ‡Department of Psychiatry, University of Queensland, and Queensland Centre for Schizophrenia Research, Wolston Park Hospital, Brisbane, Queensland, Australia; §Department of Ecology and Evolution, The University of Chicago, Chicago, Illinois 60637; ¶Laboratory of Evolutionary Genetics, National Institute of Genetics, Mishima, Japan; ‖Department of Psychiatry, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104; **Mental Health Clinical Research Center and Department of Psychiatry, The University of Iowa College of Medicine, Iowa City, Iowa 52242; and ††Department of Psychiatry, Mount Sinai School of Medicine, New York, New York 10029*

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We systematically and comprehensively investigated polymorphisms of the *HTR1B* gene as well as their linkage disequilibrium and ancestral relationships. We have detected the following polymorphisms in our sample via denaturing gradient gel electrophoresis, database comparisons, and/or previously published assays: G-511T, T-261G, –182INS/DEL-181, A-161T, C129T, T371G, T655C, C705T, G861C, A1099G, G1120A, and A1180G. The results of the intermarker analyses showed strong linkage disequilibrium between the C129T and the G861C polymorphisms and revealed four common haplotypes: ancestral (via chimpanzee comparisons), 129T/861C, -161T, and -182DEL-181. The results of association tests with schizophrenia were negative, although A-161T had a nominal $P = 0.04$ via ASPEX/sib_tdt. The expressed missense substitutions, Phe124Cys, Phe219Leu, Ile367Val, and Glu374Lys, could potentially affect ligand binding or interaction with G proteins and thus modify drug response in carriers of these variants. On average, the human cSNPs and differences among other primates clustered in the more thermodynamically unstable regions of the mRNA, which suggests that the evolutionary survival of nucleotide sequence variation may be influenced by the mRNA structure of this gene.

All database accession numbers are registered with the dbSNP (Single-Nucleotide Polymorphism Database), curated by the National Center for Biotechnology Information (NCBI at http://www.ncbi.nlm.nih.gov/SNP). Below are our group's recommended SNP names for these polymorphisms, followed by the NCBI Assay ID numbers, accessed by http://www.ncbi.nlm.nih.gov/SNP/snp_retrieve.cgi?subsnp_id=#, with the pertinent number replacing the pound sign at the end of the URL. *HTR1B*_G-511T, *HTR1B*_−182INS/DEL-181, *HTR1B*_A-161T, *HTR1B*_C129T, *HTR1B*_G276A, *HTR1B*_T371G, *HTR1B*_T655C, *HTR1B*_C705T, *HTR1B*_G861C, *HTR1B*_A1099G, *HTR1B*_G1120A, NCBI Assay ID 137614; NCBI Assay ID 137615; NCBI Assay ID 137616; NCBI Assay ID 137617; NCBI Assay ID 137618; NCBI Assay ID 137619; NCBI Assay ID 137620; NCBI Assay ID 137621; NCBI Assay ID 137622; NCBI Assay ID 137623; and NCBI Assay ID 137624.

[1] Current affiliation: Medical Affairs Department, Celera Genomics, Rockville, MD 20850.

[2] To whom correspondence and reprint requests should be addressed at Jules F. Knapp Research Center, The University of Chicago, 924 East 57th Street, Room R-010, Chicago, IL 60637. Telephone: (773) 834-3060. Fax: (773) 834-2970. E-mail: pgejman@uchicago.edu.

INTRODUCTION

Serotonin (HT)[3] is a neurotransmitter with a critical role in various brain functions including feeding, sleep, pain, mood, aggression, thermoregulation, locomotion, and learning and in the pharmacology of depression and psychosis. HT elicits its effects through a superfamily of receptors, whose common properties are that they are integral membrane proteins, have seven hydrophobic regions, and bind the receptors with nanomolar affinity. The HTR1 subfamily of receptors contains the subtypes *HTR1A–F*, and upon activation at low concentrations of serotonin they inhibit adenylyl cyclase (Barnes and Sharp, 1999). The intronless human *HTR1B* gene, which is located at 6q14.3–q16.3 (GDB 132312), encodes a 390-amino-acid polypeptide (Jin *et al.*, 1992). *HTR1B* mRNA is predominantly present in the caudate-putamen and cortex, but it is also detected in the hippocampus, cerebellum, and ce-

[3] Abbreviations used: HT, serotonin, also known as 5-hydroxytryptamine; *HTR*, serotonin receptor; *HTR1B*, serotonin receptor 1B, also known as serotonin receptor 1Dβ, 5HT1Dβ, and HTR1Dβ; SNP, single-nucleotide polymorphism; UTR, untranslated region; DGGE, denaturing gradient gel electrophoresis; ASA, allele-specific amplification; RFLP, restriction fragment length polymorphism; SSCP, single-strand conformational polymorphism; TDT, transmission test for linkage disequilibrium; PCR, polymerase chain reaction; INS/DEL, insertion/deletion; DHPLC, denaturing high-performance liquid chromatography; VDA, variant detector arrays.

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TABLE 1

Subjects

Data set	Number of individuals	Number of families	Number of full trios[a]	Complete data set: European-American	Complete data set: African-American	Complete data set: Other	Screening sample set: Number of unrelateds used	Screening sample set: European-American	Screening sample set: African-American	Screening sample set: Other
National Institute of Mental Health (NIMH) Intramural Collection[b]	371	82	45	75%	2%	23%	67	73%	3%	24%
NIMH Schizophrenia Genetics Initiative Sample[c]	340	75	21	61%	36%	3%	27	33%	63%	4%
United States/ Australian Sample[d]	119	36	28	75%	19%	6%	0	NA	NA	NA
Total	830	193	94	70%	18%	12%	94	62%	20%	18%

[a] Full trios are composed of an affected offspring and both parents, and a maximum of one full trio per family is tabulated here.
[b] Cao *et al.* (1997).
[c] Cloninger *et al.* (1998).
[d] Levinson *et al.* (1998).

rebral arteries (Bruinvels *et al.*, 1994). The *HTR1B* protein has both autoreceptor (presynaptic) and heteroreceptor (postsynaptic) function in various brain areas and is probably involved with arterial contractile response mechanisms and with behavioral traits such as locomotion, feeding, and thermoregulation (Barnes and Sharp, 1999); it is thought to mediate the therapeutic effect of the agonist sumatriptan for migraine headache (Ferrari and Saxena, 1993) and is a candidate as a molecular target for lithium action (Massot *et al.*, 1999). Mice that lack the *HTR1B* gene show a variety of interesting behavioral changes, namely, increased aggression (Saudou *et al.*, 1994; Zhuang *et al.*, 1999), increased exploratory and decreased anxiety behavior (Zhuang *et al.*, 1999), increased motor impulsivity (Brunner and Hen, 1997), facilitated learning abilities (Malleret *et al.*, 1999), increased vulnerability to cocaine reinforcement (Rocha *et al.*, 1998), elevated alcohol consumption (Crabbe *et al.*, 1996), attenuated locomotor response to 3,4-methylenedioxymethamphetamine (Scearce-Levie *et al.*, 1999), disturbances in paradoxical (rapid eye movement) sleep (Boutrel *et al.*, 1999), and differences in sensorimotor gating such as prepulse inhibition (Dulawa *et al.*, 1997). In humans, the *HTR1B* gene has been reported as associated with antisocial alcoholism (Lappalainen *et al.*, 1998). However, a definitive test of association with a human disease phenotype remains elusive. Most association studies (Ohara *et al.*, 1996; Lappalainen *et al.*, 1998; MaassenVanDenBrink *et al.*, 1998; Hinney *et al.*, 1999; Huang *et al.*, 1999; Vincent *et al.*, 1999) typically have examined only one *HTR1B* gene polymorphism and have not performed haplotype analyses or taken into account the allelic distribution in various ethnic groups. A disease locus for schizophrenia probably exists in 6q13–q26 (OMIM 603175), the linkage region encompassing approximately 60 cM via the initial report (Cao *et al.*, 1997) and narrowed to approximately 20 cM within 6q14–q21 with an increased sample size from pooling multiple family sets recently (Martinez *et al.*, 1999; Levinson *et al.*, 2000). The latter large replication effort consisting of 1937 affected individuals in 734 families (824 independent affected sibling pairs (ASPs), 1003 ASPs counting all possible pairs) provided strong support (empirical $P = 0.0004$ without inclusion of the original data set) for the 6q13–q26 linkage findings (Levinson *et al.*, 2000). This region also contains the *HTR1B* gene (mapped to 6q14.3–q16.3). We have screened for variants of the *HTR1B* gene in the same data sets (Cao *et al.*, 1997; Cloninger *et al.*, 1998; Levinson *et al.*, 1998) used for the original linkage study (Cao *et al.*, 1997) plus an additional data set (Martinez *et al.*, 1999).

To study the genetic diversity of the *HTR1B* gene, we have employed denaturing gradient gel electrophoresis (DGGE) analysis, database comparisons, and previously published assays of the coding sequences of the *HTR1B* gene. Our goals were (1) to detect and study polymorphisms by multiple complementary approaches, (2) to analyze their ancestral and haplotypic relationships, and (3) to perform association tests in our available schizophrenia pedigree samples.

MATERIALS AND METHODS

Subjects. We screened 94 unrelated individuals, consisting of 62% European Americans, 20% African Americans, and 18% other groups from schizophrenia pedigree collections, for polymorphisms. Analytically, the screening of 94 subjects has a probability of 0.85 of detecting at least one variant with a frequency of 0.01 and a probability of 1.00 for a variant with a frequency of 0.05, given the ability to detect any variations actually present. When a DNA variant was detected, the whole pedigree collection, i.e., 830 individuals from 193 families with 267 unrelated members including families from three data sets, was genotyped (Cao *et al.*, 1997; Cloninger *et al.*, 1998; Levinson *et al.*, 1998), as described in Table 1. Since these data sets are well described in the literature (Cao *et al.*, 1997; Cloninger *et al.*,

TABLE 2

Ethnic Diversity

Group	Chr[a]	Power[b] to detect a variant more common than 0.01	Power[b] to detect a variant more common than 0.05	G-511T	T-261G	−179INS/ DEL-178	-182INS/ DEL-181	A-161T	C129T	G276A	T371G	T655C	C705T	G861C	A1099G	G1120A	A1180G
North American[c]	16	0.28	0.81	0	NT	0	0	0.13	0.81	0	0	0	0	0.81	0	0	NT
South American[d]	16	0.28	0.81	0	NT	0	0	0.25	0.63	0	0	0	0	0.63	0	0	NT
Northeast Asian[e]	16	0.28	0.81	0	NT	0	0	0.06	0.63	0	0	0	0	0.63	0	0	NT
Southeast Asian (China)[f]	16	0.28	0.81	0	NT	0	0	0	0.69	0	0	0	0	0.69	0	0	NT
Southeast Asian (Taiwan)[g]	16	0.28	0.81	0	NT	0	0	0	0.13	0	0	0	0	0.13	0	0	NT
Caucasoid (Indo-Pakistani)[h]	16	0.28	0.81	0	NT	0	0.13	0.13	0.31	0	0	0	0	0.31	0	0	NT
Caucasoid (Middle Eastern)[i]	16	0.28	0.81	0	NT	0	0	0.31	0.38	0	0	0	0	0.38	0	0	NT
Caucasoid (north of Sahara African)[j]	14	0.25	0.76	0	NT	0	0.07	0.21	0.21	0	0	0	0	0.21	0	0	NT
African (Sub-Saharan African)[k]	20	0.33	0.87	0	NT	0	0.10	0.10	0.05	0	0	0	0	0.05	0	0	NT
Pacific Island[l]	14	0.25	0.76	0	NT	0	0	0.07	0.29	0	0	0	0	0.29	0	0	NT
Total	160	0.80	1.00														

Note. Polymorphisms listed were tested except T-261G and A1180G. C129T and G861C were in complete linkage disequilibrium.

[a] Number of chromosomes examined.

[b] Power was calculated for a given number of chromosomes scanned (N), to find a minimum or higher minor allele frequency (m), assuming the variant detection method would detect any polymorphisms actually present: Power = $1 - N\hat{}(1 - m)$.

[c] Pima, Jemez; Coriell Cell Repository (CCR) Nos. NA14308, NA14309, NA14310, NA14311, NA12060, NA12061, NA12062, NA12063.

[d] Karitiana; CCR Nos. NA10965, NA10966, NA10967, NA10968, NA10969, NA10970, NA10971, NA10972.

[e] Japanese; CCR Nos. NA02345, NA04535, NA05289, NA10810, NA11587, NA11589, NA11590, NA14819.

[f] Chinese, South Chinese; CCR Nos. NA06090, NA07426, NA09820, NA11322, NA11323, NA11324, NA11325, NA11321A.

[g] Taiwan aboriginal: Atayal; CCR Nos. NA13597, NA13598, NA13599, NA13600, NA13601, NA13602, NA13603, NA13604.

[h] Indo-Pakistani, CCR Nos. NA01032A, NA01225, NA04300, NA10176, NA10667, NA07895, NA11860, NA14611.

[i] Druze, Lebanese, Persian, Iranian; CCR Nos. NA11521, NA11522, NA11523, NA11524, NA11525, NA08925, NA06417, NA02781B.

[j] Egyptian, Moroccan, Algerian; CCR Nos. GM02994, GM02998, NA06231, GM06331, GM07711, GM08100, GM08687.

[k] Mbuti, Biaka; CCR Nos. NA10492, NA10493, NA10494, NA10495A, NA10496A, NA10469A, NA10470, NA10471, NA10472A, NA10473A.

[l] Hawaiian, Maori, Nasioi; CCR Nos. NA10539, NA10540, NA10541A, NA10542A, NA10543A, GM02545, GM04932.

TABLE 3

PCR Primers and DGGE Conditions for the *HTR1B* Gene

	Primer sequence 5′ to 3′	Position[a] 5′	Position[a] 3′	Length (bp)	Position of GC clamp[b]	Annealing temperature (°C)	Gradient range (%)
−1 SS	**AGCGCTGCTCCTAGACTTCACC**	**−595**	**−574**	234	3′	57	0–80
AS	**TGACCAAATGGGATCTCTTACC**	**−362**	**−383**				
−2 SS	**GCTCAGCCTCAAGCAACTG**	**−403**	**−385**	255	3′	57	0–90
AS	**AACTAGAGGTCATGGGTGCG**	**−149**	**−168**				
−3 SS	**AGGAACAACCACAGACGC**	**−243**	**−226**	252	3′	57	0–80
AS	TTCCTCCAT**GGCTCTCCTC**	9	**−10**				
1 SS	**CTCCATGCCCAAGAGCTG**	**−45**	**−28**	234	5′	57	0–80
AS	GGTGGCCAAGGTGATGAG	189	172				
2 SS	TCTGCTCCCTCCCAAAACTGC	79	99	323	3′	67	0–90
AS	GTGCAACAAGTGATGTCCGACGAC	401	378				
3 SS	TGTACACTGTCACCGGCCGC	323	342	257	3′	60	0–80
AS	TTCGGCCTTAGCCTGACGCCA	579	559				
4 SS	ATCGCGCTGGTGTGGGTCTTC	508	528	252	3′	70	0–80
AS	TCGGGTCAAGCGCTTGCCGGTC	759	738				
5 SS	CGCATCTACGTAGAAGCCCGCT	688	709	300	5′	64	0–80
AS	GGGTAGCCAACACACAATAAAG	987	966				
6 SS	ATGGCCGCTAGGGAGCGCAAAG	913	934	289	3′	65	0–90
AS	*CTTAGGCGACCCCACTGCAAA*T	*1201*	*1180*				

Note. Character codes for bases: 5′ noncoding region is in boldface type; coding region is in normal text; 3′ noncoding region is in italics.
[a] A of the ATG start codon is position +1.
[b] GC-clamp sequence is 5′-GCGGCCGCCCGTCCCGGCGCCCCCGCCCCGCCGCGGCCGC-3′.

1998; Levinson *et al.,* 1998), a brief summary follows. The Cao *et al.* (1997) families contained sibships with two (~91%) or more (~9%) siblings diagnosed by DSM-III-R (American Psychiatric Association, 1987) as having schizophrenia or schizoaffective disorder (depressive or manic types). Families were recruited opportunistically through advertisements, an advocacy group (the National Alliance for the Mentally Ill (NAMI)), and clinical collaborators, and there were no known instances of both parents of an affected individual being affected themselves. The information collected to make final best estimate diagnoses consisted of a semistructured diagnostic interview—the Schedule for Affective Disorders and Schizophrenia–Lifetime Version (Endicott and Spitzer, 1978), systematic review of medical records, and a family history interview (Gershon *et al.,* 1988). The Cloninger *et al.* (1998) families used in this study contained sibships with two (~78%) or more (~22%) siblings diagnosed by DSM-III-R (American Psychiatric Association, 1987) as having schizophrenia or schizoaffective disorder (depressive type only), with at least one of the siblings being diagnosed as having schizophrenia. Families were recruited by a combination of mostly systematic ascertainment through screening of patients in psychiatric hospitals and clinics and some opportunistic ascertainment from other sources such as clinician referrals and NAMI, and families were excluded if both parents were schizophrenic. The information collected to make final best estimate diagnoses consisted of a structured interview—the Diagnostic Interview for Genetic Studies (Nurnberger *et al.,* 1994), medical records review, and a family history interview—the Family Instrument for Genetic Studies. The Levinson *et al.* (1998) families used in this study contained sibships with two (~68%) or more (~32%) siblings diagnosed by DSM-III-R (American Psychiatric Association, 1987) as having schizophrenia, schizoaffective disorder, or nonaffective psychoses (atypical nonaffective psychosis, delusional disorder, and schizophreniform disorder), although we used only trios from such families in the analyses (one trio per family). Families were recruited from a combination of clinical and research settings, and families were excluded if there was suspicion or demonstration of bilinearity within two degrees of the proband. The information collected to make final best estimate diagnoses consisted of a modified Schedule for Affective Disorders and Schizophrenia (Endicott and Spitzer, 1978) or the Comprehensive Assessment of Symptoms and History (Andreasen *et al.,* 1992) diagnostic interviews, and medical record review. As can be seen from the above review, the data sets were very similar with respect to source, methods used, diagnostic systems, and general exclusion and inclusion criteria. To address ethnicity differences in polymorphism detection and allele frequencies, we screened with DGGE a sample of 10 different ethnic groups of 7 to 10 unrelated individuals each and genotyped this ethnic diversity panel, described in Table 2, for any polymorphisms detected in the original, largely European American sample. This ethnic diversity panel allowed us a reasonable chance (power ranging from 0.76 to 0.87) of detecting at least one variant with a frequency of 0.05 in each of the 10 different ethnic groups of 7 to 10 unrelated individuals each. If a polymorphism was evenly distributed across all of the groups, this power was 0.80 for detecting variants with minor allele frequencies down to 0.01.

DNA isolation and amplification. A 35-μl total volume contained 50 ng genomic DNA isolated from whole blood, 12 pmol of each primer, 1.2 U *Taq* Gold DNA polymerase (Perkin–Elmer), 50 mM KCl, 2.5 mM $MgCl_2$, 10 mM Tris–HCl (pH 8.3), 0.01% gelatin, and 0.25 mM dNTPs. Samples were processed in a GeneAmp 9600 PCR (polymerase chain reaction) System (Perkin–Elmer). Annealing temperature varied from 57 to 70°C. Experiments included a sample with no DNA for control of contamination.

Variant analysis. We have employed DGGE, which is based on two principles: (1) thermal DNA denaturation is remarkably sensitive to DNA sequence and pairing and (2) partial denaturation of a DNA duplex retards its electrophoretic mobility in polyacrylamide gels. A computer algorithm (Melt87) that simulates melting contour probabilities of a DNA duplex at increasing temperatures was employed (Lerman and Silverstein, 1987). Selected PCR primers used are described in Table 3. DGGE and DNA sequencing were performed as previously described (Cao *et al.,* 1997; Gejman *et al.,* 1998); the latter was performed on an ABI Prism 377 DNA Sequencer (Perkin–Elmer) to characterize and confirm putative polymorphisms. Previous variant detection attempts have employed the methods of single-strand conformational polymorphism (SSCP) analysis (Nöthen *et al.,* 1994; Lappalainen *et al.,* 1995; Huang *et al.,* 1999), dideoxy sequencing (Ohara *et al.,* 1996), and denaturing high-performance liquid chromatography (DHPLC) and variant detector

TABLE 4

Variant Scans of the *HTR1B* Gene

Base change[a]	Minor allele frequency, if detected in scans below					
	Nöthen *et al.* (1994)[b]	Lappalainen *et al.* (1995)[c]	Ohara *et al.* (1996)[d]	Cargill *et al.* (1999)[e]	Huang *et al.* (1999)[f]	Current paper[g]
G-511T	1.09%	Not scanned	Not scanned	—	Not scanned	1.07%
T-261G	48.91%	Not scanned	Not scanned	—	Not scanned	NT
−179INS/DEL-178	1.09%	Not scanned	Not scanned	—	Not scanned	0.00%
−182INS/DEL-181	—	Not scanned	Not scanned	—	Not scanned	3.60%
A-161T	—	Not scanned	Not scanned	—	Not scanned	32.78%
C129T	—	—	—	16 to 50%	16.85%	25.86%
G276A	—	—	≥2.50%	—	—	0.00%
T371G	1.09%	—	—	—	—	1.17%
T655C	—	—	—	—	—	0.15%
C705T	—	—	—	—	—	0.15%
G861C	—	27.94%	—	16 to 50%	16.85%	27.39%
A1099G	—	—	—	—	—	0.18%
G1120A	—	—	—	—	—	0.15%
A1180G	—	—	Not scanned	5 to 15%	—	NT

[a] A of the ATG start codon is position +1. The coding sequence is intronless and 1173 bp long with the stop codon.
[b] Nöthen *et al.* (1994) used SSCP to scan 1800 bp (−573 to 1227) of 92 chromosomes in Germans.
[c] Lappalainen *et al.* (1995) used SSCP to scan 1286 bp (−40 to 1246) of 136 chromosomes (*c*) in Finnish ($c = 84$), American Indians ($c = 20$), and European Americans ($c = 32$).
[d] Ohara *et al.* (1996) used sequencing to scan 1171 bp (−4 to 1167) of 80 chromosomes in Japanese.
[e] Cargill *et al.* (1999) used DHPLC and hybridization to VDAs to scan 1258 bp (1173 coding and 85 noncoding bp) of an average of 114 chromosomes in Europeans, African Americans, African Pygmies, and Asians.
[f] Huang *et al.* (1999) used SSCP to scan 1231 bp (22 to 1252) of 356 chromosomes (*c*) in European Americans ($c = 212$), African Americans ($c = 86$), and American "other" ($c = 58$), and they noted C129T and G861C to be in complete linkage disequilibrium.
[g] We used DGGE to scan 1746 bp (−566 to 1180) of 1658 chromosomes in European Americans and African Americans.

arrays (VDA) (Cargill *et al.*, 1999) to scan the coding sequence of the *HTR1B* gene in approximately 778 chromosomes of mostly European descent (see Table 4) and have had variable success.

Database comparisons. To find putative single-nucleotide polymorphisms (SNPs), we utilized database comparisons within the coding region and nearby noncoding region of the *HTR1B* gene to generate more markers for extended haplotype analyses and linkage disequilibrium approaches. We obtained nine sequences containing complete coding sequence from GenBank (M81590, D10995, L09732, M75128, M83180, M89478, M84986, AB041370, and NT_001959) and performed alignment comparisons by means of the MacVector version 6.0.1 program (Oxford Molecular) to investigate putative new polymorphisms. Database sequence was also used to infer the ancestral human allele for the identified polymorphic sites, specifically, the chimpanzee (*Pan troglodytes*) and the gorilla (*Gorilla gorilla*) *HTR1B* gene sequences, GenBank Accession Nos. AB041371 and AB041372, respectively, from the Silver Project (http://sayer.lab.nig.ac.jp/~silver/).

Genotyping methods. Several polymorphisms were assayed by standard restriction fragment length polymorphism (RFLP) (Kan and Dozy, 1978) analysis of PCR-amplified DNA segments without (G-511T, A-161T, C705T, and G1120A) or with (T371G) mutagenic primers (Vallette *et al.*, 1989), using digestion conditions according to the manufacturer's recommendations (New England BioLabs). Allele-specific assays (ASA) (Glisic and Alavantic, 1996) were used for several other polymorphisms. The insertion/deletion polymorphism was assayed by detection of fluorescently labeled PCR products on an ABI Prism 377 DNA Sequencer (Perkin–Elmer). All polymorphisms and their genotyping assays are registered with the dbSNP (Single-Nucleotide Polymorphism Database), curated by the National Center for Biotechnology Information ((NCBI) http://www.ncbi.nlm.nih.gov/SNP), under the following identifications (all prefixed with "*HTR1B_*"): G-511T; −182INS/DEL-181; A-161T; C129T; G276A; T371G; T655C; C705T; G861C; A1099G; and G1120A.

Statistical analysis. For the estimation of the allele frequencies in each marker, the haplotype frequency, and the testing of linkage disequilibrium between polymorphisms, we used the EH program (Xie and Ott, 1993; Terwilliger and Ott, 1994). For individuals with a missing genotype in the schizophrenia families (24%), we assigned the major allele at that site to estimate the full haplotype. Genotyping was 100% complete on the ethnic diversity panel. We have tested association with disease by the transmission test for linkage disequilibrium (TDT) (Spielman *et al.*, 1993). TDT considers parents who are heterozygous for a locus and evaluates the frequency with which an allele or its alternate is transmitted to affected offspring. Nontransmitted alleles provide an internal control because they are of the same ancestry as the "case" chromosomes. TDT will detect linkage between the marker locus and the disease locus only if association (due to linkage disequilibrium) is also present. To control for the effects of known linkage in this region in the families tested, we conditioned on the observed identity by descent (IBD) sharing via the ASPEX program (Hinds and Risch, 1998), sib_tdt option, as reported in Table 5. To determine the haplotype(s) contributing the most to any increased number of transmissions of an allele to affected offspring, we utilized the 4-locus TDT (for the most common polymorphisms) as estimated by GENEHUNTER (Kruglyak *et al.*, 1996; Kruglyak and Lander, 1998), which was also used to generate haplotypes from the linkage files. Fisher's Exact Test (Fisher, 1935) was utilized to estimate the probability of linkage disequilibrium among markers that had at least 10 individuals possessing the minor allele. In addition, Hardy–Weinberg disequilibrium was assessed by means of an exact test using a Markov chain.

RNA folding analysis. The coding region of 1173 bp for the human *HTR1B* mRNA was analyzed by mfold version 3.0 (Mathews *et al.*, 1999; Zuker *et al.*, 1999). The *HTR1B* mRNA appears to be ~4.5 kb via Northern analysis (Hamel *et al.*, 1993), which exceeds the capacity of the mfold server, currently 3000 bp. We selected the most stable folding (lowest final dG value) and examined the position of the polymorphic sites for pairing predictions. We also performed the same analyses as described above for 3000 bp (the limit of the mfold server) of the *HTR1B* mRNA sequence, which consisted of part of the

TABLE 5

TDT for the *HTR1B* Gene and Schizophrenia

Mutation	Minor allele frequency	Allele most frequently transmitted to affected offspring	T[a]	NT[b]	*P* level not conditioned on IBD[c]	*P* level conditioned on IBD[d]
G-511T	1.07%	NA	0	0	1.0	1.0
−182INS/DEL-181	3.60%	Major allele	7	5	0.56	0.80
A-161T	32.78%	Major allele	108	78	0.028	0.041
C129T	25.86%	Major allele	80	73	0.57	0.63
T371G	1.17%	Major allele	8	5	0.41	0.63
T655C	0.15%	NA	0	0	1.0	1.0
C705T	0.15%	Minor allele	1	0	1.0	1.0
G861C	27.39%	Major allele	80	74	0.63	0.70
A1099G	0.18%	NA	0	0	1.0	1.0
G1120A	0.15%	NA	0	0	1.0	1.0
Any minor variant	NA	Minor allele	222	170	0.0086	0.044

[a] T is the number of transmissions to affected offspring from heterozygous parents for the more commonly transmitted allele.
[b] NT is the number of nontransmissions to affected offspring from heterozygous parents for the more commonly transmitted allele.
[c] *P* level refers to the nominal probability of the observation being due to chance, not conditioned by observed IBD sharing.
[d] *P* level refers to the nominal probability of the observation being due to chance, conditional on the observed IBD sharing, i.e., controlling for the effects of linkage. This was performed by means of the ASPEX program, sib_tdt option.

5′-untranslated region (5′-UTR), the entire coding region, and the entire 3′-UTR, except for the poly(A) tail.

RESULTS

DGGE

Multiple DNA segments of the *HTR1B* gene revealed DGGE patterns consistent with DNA variations in a heteroduplex form. See Tables 3 and 4 for a summary of the DGGE fragments studied and the polymorphisms detected. Subsequent sequence analyses demonstrated the presence of three new missense substitutions in the *HTR1B* gene (Table 6), located in transmembrane domain 5 (TMD5) Phe219Leu, TMD7 (Ile367Val), and in the cytoplasmic part of the receptor beyond TMD7 (Glu374Lys). Additionally, we detected another missense substitution, Phe124Cys (Nöthen *et al.*, 1994). The newly detected synonymous variant was C705T (Ala235Ala), and we also found C129T (Ser43Ser) (Cargill *et al.*, 1999) and G861C (Val287Val) (Lappalainen *et al.*, 1995). We found no evidence of G276A (Ala92Ala) (Ohara *et al.*, 1996), found thus far in Japanese populations only, in our sample. In the 5′ noncoding region, we detected G-511T and T-261G (Nöthen *et al.*, 1994). We have detected the new A-161T, which had interfered with attempts at our assessment of −179INS/DEL-178 through the annealing step of the PCR using the originally reported primers (Nöthen *et al.*, 1994). We found

TABLE 6

DNA Variants in the *HTR1B* Gene

Base change[a]	Amino acid	Type of change	Transition vs transversion[b]	Position[c]	Minor allele frequency[d]	First described[e]
G-511T	Noncoding	Noncoding	Transversion	—	1.07%	A
T-261G	Noncoding	Noncoding	Transversion	—	NT	A
−179INS/DEL-178	Noncoding	Noncoding	Not applicable	—	0.00%	A
−182INS/DEL-181	Noncoding	Noncoding	Not applicable	—	3.60%	Here
A-161T	Noncoding	Noncoding	Transversion	—	32.78%	Here
C129T	Ser-Ser (43)	Synonymous	Transition	—	25.86%	B
G276A	Ala-Ala (92)	Synonymous	Transition	—	0.00%	C
T371G	Phe-Cys (124)	Nonconservative	Transversion	TMD 2	1.17%	A
T655C	Phe-Leu (219)	Conservative	Transition	TMD 5	0.15%	Here
C705T	Ala-Ala (235)	Synonymous	Transition	—	0.15%	Here
G861C	Val-Val (287)	Synonymous	Transversion	—	27.39%	D
A1099G	Ile-Val (367)	Conservative	Transition	TMD 7	0.18%	Here
G1120A	Glu-Lys (374)	Nonconservative	Transition	Cytoplasmic	0.15%	Here
A1180G	Noncoding	Noncoding	Transition	—	NT	B

[a] A of the ATG start codon is position +1. The coding sequence is intronless and 1173 bp long.
[b] Transitions are purines (A or G) for purines or pyrimidines (C or T) for pyrimidines; transversions are the other kind of change.
[c] Position notes potential localization in protein for nonsynonymous changes.
[d] Minor allele frequency refers to our sample of families. We detected neither −179DEL/INS-178 nor G276A in our families. NT, not tested.
[e] First descriptions: A, Nöthen *et al.* (1994); B, Cargill *et al.* (1999); C, Ohara *et al.* (1996); D, Lappalainen *et al.* (1995).

TABLE 7

***HTR1B* Nucleotide and Amino Acid Sequence Variation in Human, Chimpanzee, and Gorilla**

Base position[a]	*Homo sapiens*[b]	*Pan troglodytes*[c]	*Gorilla gorilla*[d]	Amino acid	Type of change
133	T	T	G	Ser vs Ala	Nonconservative
288	G	T	G	Leu	Synonymous
489	C	T	C	Pro	Synonymous
546	G	G	A	Leu	Synonymous
570	T	C	C	Ala	Synonymous
618	C	T	C	Ile	Synonymous
846	A	A	G	Gly	Synonymous

[a] A of the ATG start codon is position +1. The coding sequence is intronless and 1173 bp long.
[b] *H. sapiens* sequence from NM_000863 (human consensus sequence verified in this investigation).
[c] *P. troglodytes* sequence from AB041371 (one sequence).
[d] *G. gorilla* sequence from AB041372 (one sequence).

no evidence of −179INS/DEL-178 (Nöthen *et al.*, 1994) in our samples with an improved set of PCR primers, however, we did detect the presence of −182INS/DEL-181 as a 2-bp insertion/deletion with approximately the same minor allele frequency. This probably is the same polymorphism as described by Nöthen et al. (1994), but with a difference in the nucleotide sequence determination. We sequenced one heterozygous member of each family segregating this size polymorphism and found that all of them were accounted for by −182INS/DEL-181 and none by the previously reported −179INS/DEL-178 (Nöthen *et al.*, 1994). In the 3′ noncoding region, we found A1180G (Cargill *et al.*, 1999).

Database Analysis

Via our sequence comparisons of nine sequences (seven of which were deposited in 1992) and containing complete coding sequence (M81590, D10995, L09732, M75128, M83180, M89478, M84986, AB041370, and NT_001959), we detected four SNPs. Three were coding region polymorphisms: C129T (Ser43Ser) (Cargill *et al.*, 1999), G276A (Ala92Ala) (Ohara *et al.*, 1996), and G861C (Val287Val) (Lappalainen *et al.*, 1995), and the other was the 3′ noncoding region A1180G (Cargill *et al.*, 1999). In addition, the possible presence of several other SNPs and/or insertion/deletions was indicated in the 3′ and 5′ noncoding regions by the same means. We therefore investigated whether any of these additional putative 3′ or 5′ noncoding region polymorphisms were present in our data set by means of ASA, RFLP analysis of PCR-amplified fragments, and size determination upon the screening of 94 unrelated individuals. None of the database sequence differences in the coding region, each with a minor allele found in one of the nine available database sequences, represented false positives. Uncommon (≤1% minor allele) SNPs, detected by DGGE, were not seen in the database sequences, as expected. In the 3′ noncoding region, the true positive A1180G was found in two of eight available database sequences. There were three false positives, represented by one (two cases) or two (one case) sequences. For database sequence differences in the 5′ noncoding region, there was one true positive, −182INS/DEL-181, and six false positives in two to four available sequences, with a decreasing amount of available database sequence as the distance from the coding region increased. Hence, 100% (3/3) of the cDNA database differences were true positives, and 18% (2/11) of the database differences outside of the cDNA were true positives. We have confirmed the consensus *HTR1B* gene sequence (NM_000863, derived from D10995) and the Sanger Centre sequence for this area (NT_001959) as containing the major allele at each polymorphic site we examined. In addition, the ancestral human allele for each of the identified polymorphic sites appears to be that found in the consensus *HTR1B* gene sequence (NM_000863) from comparisons to the chimpanzee (AB041371) and gorilla (AB041372) sequences, which both have 99.7% (1169/1173) identity with the human cDNA sequence. The nonhuman primate sequence does not include enough of the 5′ noncoding sequence to determine this for G-511T, −182INS/DEL-181, and A-161T sites. Also of note is the finding that, of the seven differences (7/1173 = 0.6%) between the human and the nonhuman primates, six are synonymous and only one (Ser45Ala) is a missense change; see Table 7.

Association Tests among Markers

Fisher's Exact Test revealed several intermarker linkage disequilibria in the schizophrenia families; most notable was that between C129T and G861C ($D' = 0.922$; Fisher's $P < 0.001$, still significant after Bonferroni correction). A-161T was in linkage disequilibrium with both C129T ($D' = -0.283$; Fisher's $P = 0.009$) and G861C ($D' = -0.232$; Fisher's $P = 0.034$), and T371G was in linkage disequilibrium with G861C ($D' = 0.668$; Fisher's $P = 0.004$, still significant after Bonferroni correction). Although a much reduced sample size in each group resulted in decreased power to detect several intermarker linkage disequilibria, similar findings were present in the ethnic diversity groups: $P < 0.05$ linkage disequilibrium between C129T and G861C (Atayal, Indo-Pakistani, Japanese,

Karitiana, and Southern Chinese), A-161T and C129T (Karitiana), and A-161T and G861C (Karitiana). The only marker found to be in Hardy–Weinberg disequilibrium was A-161T ($P = 0.0024$), which was found to originate from the European American schizophrenia families that had an excess of heterozygotes ($P = 0.002$), whereas the African American schizophrenia families appeared to be in Hardy–Weinberg equilibrium.

Additional Ethnic Groups

We found no evidence for additional polymorphisms in the coding region, besides those already discovered in the original, largely European American sample. We found −182INS/DEL-181, A-161T, C129T, and G861C in our sample of 10 different ethnic groups of 7 to 10 unrelated individuals each; see Table 2. As previously noted (Huang *et al.*, 1999), C129T and G861C appeared to be in total linkage disequilibrium in these ethnic groups, i.e., only two haplotypes appeared to exist: C129/G861 and 129T/861C. The frequency of the 129T/861C haplotype varied widely, from 5% in the Sub-Saharan African (Mbutu and Biaka) group to 81% in the North American (Pima and Jemez) group. The −182INS/DEL-181 polymorphism was detected in only three groups: Caucasoid Indo-Pakistani, Caucasoid North of Sahara African (Egyptian, Moroccan, and Algerian), and Sub-Saharan African (Mbutu and Biaka) groups at 13, 7, and 10% minor allele frequency, respectively. Each time DEL-181 was detected (five times), it was always on the C129/G861 haplotype, as evidenced by being seen only in C129/G861 homozygotes. A-161T was not detected in the Southeast Asian Chinese (Chinese and South Chinese) or the Southeast Asian Taiwanese (Atayal) groups, but was found in other groups at a minor allele frequency ranging from 6 to 31%. Each time -161T was found (20 times), it was in either a C129/G861 homozygote (11 times) or a heterozygote (C129/G861 129T/861C) (9 times); -161T was never found in the 129T/861C homozygotes. This is consistent with the possibility of T-161 existing only on the C129/G861 haplotype, however, with only unrelated individuals in our ethnic diversity sample, this was not demonstrated definitively. Similarly, each time DEL-181 was detected (5 times), it was in either an A-161 homozygote (4 times) or an A-161T heterozygote (1 time); DEL-181 was never found in the -161T homozygotes. This is consistent with the possibility of DEL-181 existing only on haplotypes containing -161T; however, again with only unrelated individuals in our ethnic diversity sample, this was not demonstrated definitively. None of the rare (≤1%) polymorphisms found in the larger, predominantly European American sample was detected in this smaller ethnic diversity sample, as expected (see Tables 2 and 8), although we had reasonable (>80%) power to detect a ≥1% minor allele frequency polymorphism, assuming it was evenly distributed among the groups. In the collection of schizophrenia families, the rare (≤1%) variants were found in either exclusively European Americans (-511T in one family, 371G in eight of eight families, 1099G in one family, and 1120A in one family) or exclusively African Americans (655C in one family and 705T in one family).

Association Tests for Schizophrenia

The results of association tests with schizophrenia in the linked data sets were negative. No polymorphism was detected primarily in affected individuals, suggesting that these polymorphisms do not have a large effect on disease. We analyzed the transmission of the biallelic polymorphisms located in the *HTR1B* gene, for which A-161T, C129T, and G861C had by far the most informative parent–offspring combinations. The observed number of transmissions to affected offspring was not significantly different from what would be expected by chance after correcting for multiple testing, although A-161T had 108 transmissions of the A-161 allele to affected offspring versus 78 nontransmissions (nominal $P = 0.028$ unconditioned by observed IBD sharing and $P = 0.041$ controlling for the effects of linkage via ASPEX/sib_tdt) (Table 5). Since most polymorphisms were rare (≤1%), we also examined for association of any variant allele with schizophrenia, which interestingly revealed 222 transmissions to affected offspring versus 170 nontransmissions (nominal $P = 0.0086$ unconditioned by observed IBD sharing and $P = 0.044$ controlling for the effects of linkage via ASPEX/sib_tdt) (Table 5). Via GENEHUNTER 4 locus TDT (−182INS/DEL-181_A-161T_C129T_G861C), we determined the haplotypes contributing the most to the larger number of transmissions of the A-161 allele to affected offspring: there were 19 transmissions of −182INS-181_A-161_129T_861C versus 5 nontransmissions (nominal $P = 0.0043$), while there were 2 transmissions of −182INS-181_-161T_129T_861C versus 8 nontransmissions (nominal $P = 0.058$). Hence, the possible transmission distortion of the A-161T polymorphism appears to act primarily upon the −182INS-181_ 129T_861C haplotype.

RNA Folding Analysis

Mfold analysis revealed that in the resultant structure of the coding region mRNA of the *HTR1B* gene, the polymorphic sites appeared to be preferentially located in the periphery of the molecule (see Fig. 1). This was also the case for the sites that differ between the human and the chimpanzee or gorilla *HTR1B* gene coding sequence (see Fig. 1). Mfold returned 41 competing RNA secondary structures for the coding region mRNA of the *HTR1B* gene, ranging in final dG from −373.94 (Fig. 1) to −353.16 (least stable). The pattern described above held for the majority of these foldings, especially the more stable foldings (data not shown). Similar patterns were found when the entire 3000-bp capacity of the mfold server was utilized in the exam-

TABLE 8

Haplotypes of the *HTR1B* Gene

Ancestral Group[1]											Polymorphic Site[2]														
North American	South American	Northeast Asian	Southeast Asian (China)	Southeast Asian (Taiwan)	Caucasoid (Indo-Pakistani)	Caucasoid (Middle Eastern)	Caucasoid (North of Sahara African)	African (Sub-Saharan African)	Pacific Island	European American SZ marry-in parents[3]	G-511T	-182INS/DEL-181	A-161T	C129T	G276A	T371G	T655C	C705T	G861C	A1099G	G1120A	Haplotype (1 = major and 2 = minor allele)	TOTAL Diversity Panel count	Diversity Panel %	TOTAL Schizophrenia Unrelateds count	Schizophrenia Unrelateds %
1	2	9	5	14	7	5	7	15	9	6												11111111111	80	45.5%	366	49.5%
13	10	6	11	2	5	6	3	1	4	6												11121111211	67	38.1%	137	18.5%
2	4	1	0	0	2	5	3	2	1	4												11211111111	24	13.6%	148	20.0%
0	0	0	0	0	2	0	1	2	0	0												12111111111	5	2.8%	12	1.6%
0	0	0	0	0	0	0	0	0	0	0	?	?	?	?		?	?	?	?	?	?	any other	0	0.0%	76	10.3%
16	16	16	16	16	16	16	14	20	14	16													176		739	

[1] Ancestral groups are as defined in Table 2.
[2] A of the ATG start codon is position +1.
[3] SZ (schizophrenia or schizoaffective disorder) marry-in parents have no known family history of the illness themselves.

ination of 3000-bp comprising part of the 5′-UTR, the entire coding region, and the entire 3′-UTR of the *HTR1B* mRNA (data not shown).

DISCUSSION

Nucleotide Diversity

We have performed an intensive DNA variation analysis by DGGE of genomic sequences of the *HTR1B* gene and have detected 12 polymorphisms, 10 of which we genotyped. The cSNPs had a mean minor allele frequency of 8%, as opposed to those detected in noncoding regions (12%). Also of note is that 6 of the 8 currently known polymorphic sites in the coding region represent transitions, rather than transversions (see Table 6), consistent with known patterns of mammalian genome evolution (Epstein *et al.*, 2000). The ratios of transitions to transversions in the cDNA (6:2) versus the noncoding regions (1:3) of this gene were not statistically different (Fisher's Exact Test, $P = 0.14$), quite possibly due to the relatively small number of observations. We found an average of one SNP per 159 bp, on a par with previous reports of larger surveys (Halushka *et al.*, 1999), and calculated the nucleotide diversity (per basepair) in the scanned sequence of the human *HTR1B* gene as 7.1×10^{-4}, with the cDNAvalue being 6.8×10^{-4} and the noncoding region being 7.8×10^{-4}. These values are consistent with previously reported observations from larger surveys (Halushka *et al.*, 1999; Cargill *et al.*, 1999). The actual diversity in the noncoding regions surrounding the *HTR1B* cDNA is likely to be higher than this value, as we did not genotype two noncoding region SNPs, T-261G in the 5′-UTR and A1180G in the 3′-UTR. Previous estimates of SNP frequency (for SNPs with ≤1% minor allele frequency) have been one per 721 bp and slightly less common than this in coding regions, i.e., for cSNPs (Wang *et al.*, 1998). We have found six such SNPs in 1746 bp scanned for a ≤1% minor allele SNP frequency of one per 291 bp, and for such cSNPs we have found three in 1173 bp scanned for a cSNP frequency of one per 391 bp. The large sample size screened for variations allowed a large number of potentially functional changes to be identified. Furthermore, it is improbable that a great deal more diversity in the coding region of this gene exists. These polymorphisms and their haplotypes can be used to reach ro-

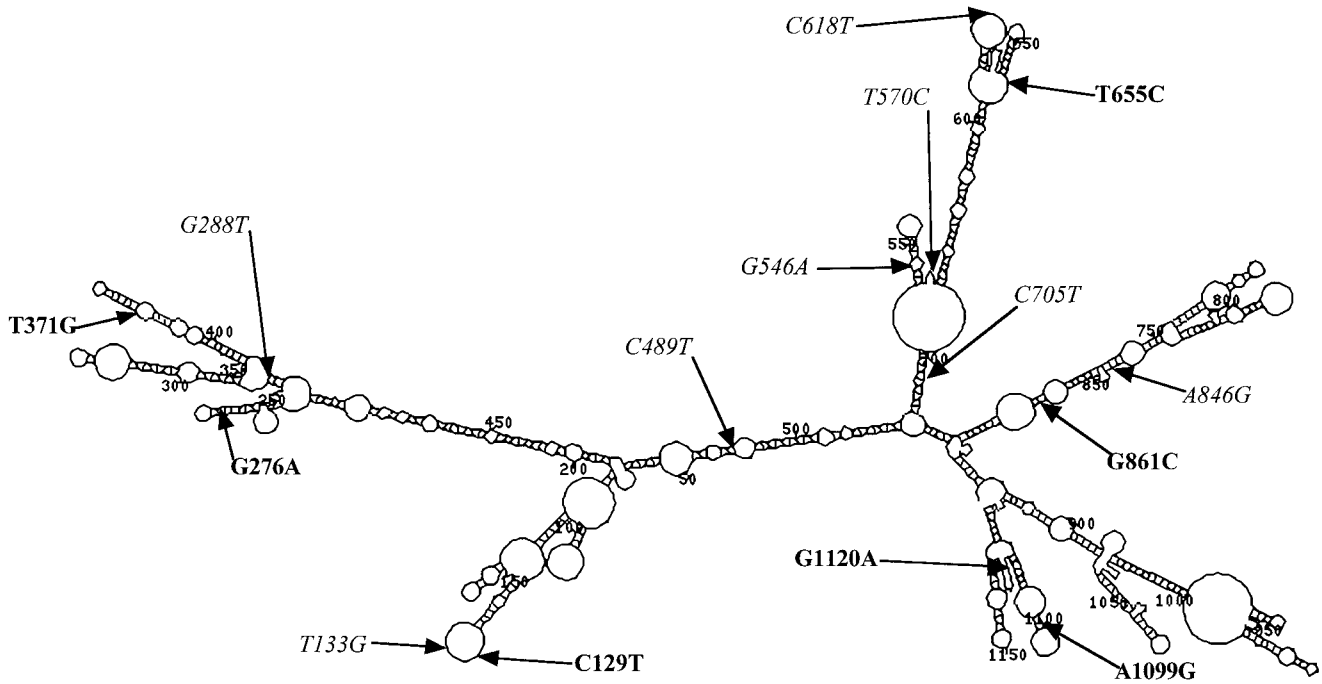

FIG. 1. Mfold version 3.0 analysis of human *HTR1B* gene with polymorphic sites. This image represents the result of submitting the CDS of the human *HTR1B* gene (NM_000863) to mfold version 3.0 (Mathews *et al.*, 1999; Zuker *et al.*, 1999) and selecting the most stable folding (lowest final dG value). The known polymorphic sites among humans are indicated in boldface type and appear to cluster in the periphery, as do the sites that vary in human versus chimpanzee and/or gorilla sequence, which are indicated in italics. For the known human polymorphisms, the first base is the major allele. For the human versus chimpanzee and/or gorilla differences, the first base is the human nucleotide, and the second base is the variant chimpanzee and/or gorilla base.

bust conclusions regarding the relationship between several human disease phenotypes and the *HTR1B* gene.

Missense Polymorphisms

The missense SNPs were population dependent, found exclusively in either European American families (T371G, A1099G, and G1120A) or African American families (T655C), and rarer (≤1% minor allele frequency), as previously described (Halushka *et al.*, 1999). DGGE experiments detected three new missense substitutions, one located in TMD5, T655C (Phe219Leu); another in TMD7, A1099G (Ile367Val); and one in the cytoplasmic part of the receptor beyond TMD7, G1120A (Glu374Lys); in addition to T371G (Phe124Cys) located in TMD3 (Nöthen *et al.*, 1994) (refer to Fig. 2 for the illustration of these positions in the receptor). We discovered one new synonymous variant, C705T (Ala235Ala), and also detected C129T (Ser43Ser) (Cargill *et al.*, 1999) and G861C (Val287Val) (Lappalainen *et al.*, 1995). Two of the missense changes are chemically nonconservative: Phe124Cys (aromatic hydrophobic to a sulfur hydrophilic neutral residue) and Glu374Lys (basic to acidic), and another two are chemically conservative: Phe219Leu (both hydrophobic and neutral) and Ile367Val (both hydrophobic). It should also be noted that Phe124Cys potentially interferes with the normal formation of a disulfide bridge between the cysteine residues at positions 122 and 199, due to its close proximity to position 122.

Evolutionary Context

Phe124, Ile367, and Glu374 in the human are largely conserved when compared to other available mammal sequences (opossum U04311, mouse M85151, rat M89954, hamster X86458, mole rat AF027184, guinea pig U82175, rabbit U60826, gorilla AB041372, and chimpanzee AB041371) at the analogous amino acid position, with the exceptions of Leu124 in the rabbit and Asp374 in the opossum. However, this is not the case with Phe219 at which residue all primates and the opossum have Phe219 and all rodents and the rabbit have Leu219, which is the variant human amino acid at this position. Among other human HT receptors, the analogous residue to *HTR1B*-Ile367 varies among other hydrophobic and neutral residues, e.g., isoleucine (*HTR1B*, *1D*, and *6*), valine (*HTR1A*), leucine (*HTR1E*, *1F*, *2A*, *2B*, *2C*, and *5A*), and phenylalanine (*HTR7*), providing a further suggestion that the Ile367Val change may be tolerated. The residue analogous to *HTR1B*-Glu374 varies among other hydrophilic residues, sometimes basic and other times acidic, e.g., glutamic acid (*HTR1B*, *1D*, *1E*, and *1F*), arginine (*HTR6* and *7*), and lysine (*HTR1A*, *2A*, *2B*, *2C*, and *5A*), suggesting that acidic to basic changes here may be tolerated. In contrast, the residue analogous to *HTR1B*-Phe124 varies only among other hydrophobic and

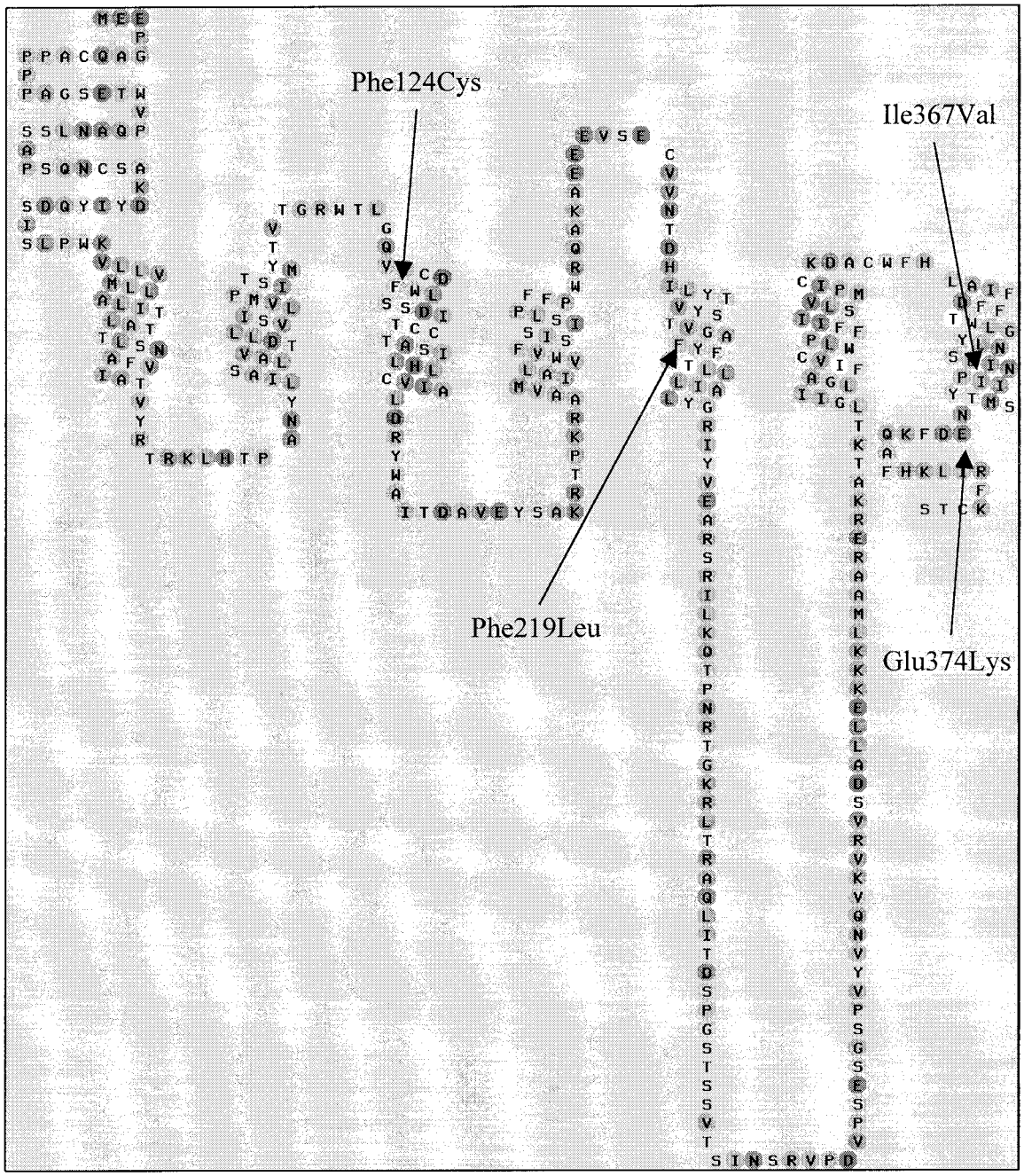

FIG. 2. Snake-like diagram of the *HTR1B* receptor including the identified missense substitutions. The diagram was obtained from the Viseur based snake-like diagrams html page (Campagne *et al.*, 1999) (http://www.gpcr.org/7tm/seq/vis/5H1B_HUMAN/5H1B_HUMAN.html).

neutral residues, e.g., phenylalanine (*HTR1B*), leucine (*HTR1A*, *5A*, and *6*), isoleucine (*HTR1D* and *1F*), valine (*HTR1E*, *2A*, *2C*, and *7*), and alanine (*HTR2B*), suggesting that the Phe124Cys might affect the function of the receptor. Finally, the residue analogous to *HTR1B*-Phe219 varies among other hydrophobic and neutral residues, e.g., phenylalanine (*HTR1B*), leucine (*HTR5A*, and *6*), and isoleucine (*HTR1D*, *1E*, *1F*, *2A*, *2C*, and *7*) except for the hydrophilic and neutral threonine (*HTR2B*), suggesting that the change to leucine, a neutral hydrophobic residue, is likely a functionally tolerable change.

Location of Polymorphisms in the Receptor

It is known that amino acid changes that modify the tertiary structure of the receptors may result in functional variations. Some amino acid changes in TMD6 and TMD7 are particularly important for the specificity of the *HTR1B* protein. The human *HTR1B* protein binds β-adrenergic antagonists with much less affinity than the rodent versions (Adham *et al.*, 1994). This property is conferred by the amino acid residues of TMD7, and residue 355 appears to be the key position via site-directed mutagenesis experiments (Thr355Asn) in which the human version has been shown to become identical to

the rodent version in this regard (Oksenberg *et al.*, 1992). None of the site-directed mutagenesis experiments yet reported with the human *HTR1B* gene (Oksenberg *et al.*, 1992; Granas *et al.*, 1998a, b; Granas and Larhammar, 1999; Pauwels *et al.*, 1999) has targeted the residues with missense polymorphisms (Phe124Cys, Phe219Leu, Ile367Val, and Glu374Lys) for which we have detected heterozygous individuals. This would be an area meriting further exploration as has been achieved for missense variants in other genes, e.g., the human *dopamine D2 receptor* and *dopamine D5 receptor* genes (Cravchik *et al.*, 1996, 1999; Cravchik and Gejman, 1999). Another related avenue of investigation has arisen for this receptor with the development of homozygous transgenic mice that express only the human, and not the murine, *HTR1B* gene (Bonaventure *et al.*, 1999).

DGGE Utility

Among the polymorphisms noted in our survey, many were not previously reported by other groups. This is not unexpected with those having uncommon minor alleles (T655C, C705T, A1099G, and G1120A) given our comparatively large sample size (see Table 4), but was somewhat surprising for the more common A-161T and −182INS/DEL-181. No previous single study has found more than four different variants, but more than one group has noted the presence of the more common polymorphisms (T-261G, C129T, T371G, G861C, and A1180G) (Table 4). The results of the present study support the usefulness of DGGE screening of genes previously studied by other variant analysis methods, and we report previously undetected missense, synonymous, and 5′ noncoding region polymorphisms in the *HTR1B* gene.

Database Comparisons

The utility of database comparisons was highest in (e.g., C129T, G276A, and G861C) or immediately adjacent to (e.g., A1180G) the possibly higher quality sequence of the coding region for common (e.g., C129T, G861C, and A1180G) SNPs, found more than once (e.g., A1180G). In summary, five polymorphisms (one 5′ noncoding region, three coding regions, and one 3′ noncoding region) were confirmed out of 13 candidates (six 5′ noncoding region, three coding region, and four 3′ noncoding region) for a 38% confirmation rate. This is less than some previous database screens (Picoult-Newberg *et al.*, 1999) for SNPs that have achieved >60% confirmation rate after multiple filtering procedures, which we have not performed.

Linkage Disequilibrium among Markers

We have confirmed the observation of almost complete linkage disequilibrium between C129T and G861C (Huang *et al.*, 1999), found evidence for linkage disequilibrium between T371G and G861C, and have also observed linkage disequilibrium to a lesser extent (no longer significant after Bonferroni corrections) between A-161T and both C129T and G861C. The finding in our European American schizophrenic families of Hardy–Weinberg disequilibrium suggests further caution in interpreting the nominally significant association with schizophrenia at that site (A-161T). Namely, one or more of the assumptions of Hardy–Weinberg equilibrium seem to have been violated: no natural selection, no immigration, no emigration, no mutation, random mating, and/or a large population.

Comparisons among Ethnic Groups

The C129T and G861C polymorphisms appear to be in total linkage disequilibrium with each other in multiple ethnic groups, but at widely varying "minor" allele frequencies. −182DEL/INS-181 was detected only in Caucasoid and African groups, such as comprise most of our larger schizophrenia sample. Hence, we would have likely missed this polymorphism if we relied on other ethnic groups for variation scanning, and, of course, if a nearby 2-bp polymorphism had not been previously reported (Nöthen *et al.*, 1994) to draw our attention to this area. A-161T was found in 8 of the 10 groups and would have been detectable in DGGE variant scanning efforts from many various ethnic groups. We attempted to construct a cladogram for our data by parsimony and bootstrap methods, but were unable to obtain significant support for one tree versus another, except that, unsurprisingly, the chimpanzee and gorilla sequences were always positioned as outgroups versus all of the human haplotypes (data not shown).

Linkage Disequilibrium with Schizophrenia

We have not found evidence of association of *HTR1B* gene polymorphisms with schizophrenia that would remain statistically significant after correcting for the multiple tests performed. However, it is quite possible that we do not have the power to detect linkage disequilibrium because the informative sample size is relatively small. Furthermore, we cannot rule out the possibility that DNA variations in regulatory sequences (not yet examined by us or by others), not necessarily in linkage disequilibrium with the known polymorphisms, confer susceptibility to schizophrenia in a subset of patients. Nevertheless, our data and data of the previous studies tend to exclude common (and large effect) variations in the *HTR1B* gene in schizophrenia, with the possible exception of excess transmission of the A-161 allele to affected offspring, acting upon the −182INS-181_ 129T_861C haplotype.

RNA Folding Analysis

HTR1B gene polymorphic sites appeared to be clustered in the periphery of the folded mRNA molecule, as did those sites that differed between human and chimpanzee or gorilla *HTR1B* coding sequence (see Fig. 1). This appeared to be the case both for the coding region

only and for the more complete mRNA (part of the 5′-UTR, the entire coding region, and the entire 3′-UTR, except for the poly(A) tail), up to the limits of the mfold program (Mathews *et al.*, 1999; Zuker *et al.*, 1999). These observations are intriguing, as they suggest the possibility of another route by which synonymous variants might, at the mRNA level (e.g., through altered stability of the mRNA, or key portions thereof), lead to differences in expression of the protein. This hypothesis remains to be tested.

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