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Identification, Expression, and Pharmacology of a Cys_{23}–Ser_{23} Substitution in the Human 5-HT_{2C} Receptor Gene (HTR2C)

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The function of brain serotonin-2C (5-HT_{2C}) receptors, including behavioral and neurochemical responses to 5-HT_{2C} agonist challenge, has been suggested to be abnormal in individuals with neuropsychiatric disorders. Thus, it is important to identify polymorphisms and functional variants within this gene. Using SSCP analysis, we identified a Cys_{23}–Ser_{23} substitution (designated 5-HT_{2Ccys} and 5-HT_{2Cser}) in the first hydrophobic region of the human 5-HT_{2C} receptor. Allele frequencies in unrelated Caucasians were 0.13 and 0.87 for 5-HT_{2Cser} and 5-HT_{2Ccys}, respectively. DNAs from informative CEPH families were typed for this polymorphism and analyzed with respect to 20 linked markers on the X chromosome. Linkage analysis placed the 5-HT_{2C} receptor gene (HTR2C) on Xq24. To evaluate whether this amino acid substitution causes a variant function of this receptor, recombinant human 5-HT_{2Ccys} and 5-HT_{2Cser} receptors were expressed in *Xenopus* oocytes and tested for responses to 5-HT using electrophysiological techniques. Concentration–response curves for 5-HT were not significantly different in oocytes expressing either form of the receptor, suggesting that the 5-HT_{2Ccys} and 5-HT_{2Cser} receptor proteins may not differ in their responses to serotonin under baseline physiological conditions.

INTRODUCTION

Family, twin, and adoption studies indicate genetic vulnerability to several neuropsychiatric disorders, including alcoholism (Cloninger *et al.*, 1981), antisocial personality (Crowe, 1974), suicidal behavior (Roy *et al.*, 1991), panic disorder (Crowe, 1985), and anxiety (Woodman, 1993). Although psychiatric disorders are likely to arise from a complex combination of environmental, genetic, and biological factors, it may be possible to identify biological variables that predict these behaviors and thus facilitate implementation of preventive and therapeutic measures. Previously, the function of the brain serotonin (5-HT) system has been found to correlate with certain behaviors. For example, low levels of cerebrospinal fluid (CSF) 5-hydroxyindoleacetic acid (5-HIAA), the main metabolite of serotonin (5-HT), are found in impulsive individuals with alcoholism (Ballenger *et al.*, 1979), depression (Asberg *et al.*, 1976a), suicidal tendencies (Asberg *et al.*, 1976b), and aggression (Linnoila *et al.*, 1983; Virkkunen *et al.*, 1994b). Thus, it is important to identify polymorphisms including functional variants in genes that control serotonin function.

A candidate gene of special interest in neuropsychiatric disorders is the 5-HT_{2C} receptor (formerly 5-HT_{1C} receptor). The 5-HT_{2C} receptor gene (HTR2C) is widely expressed in the brain, where it is involved in the regulation of endocrine responses, including the production and secretion of adrenocorticotropic hormone (ACTH) (King *et al.*, 1989), oxytocin (Bagdy *et al.*, 1992), and prolactin (Aulakh *et al.*, 1992). Genes for rat (Julius *et al.*, 1988), mouse (Foguet *et al.*, 1992), and human (Saltzman *et al.*, 1991) 5-HT_{2C} receptors have been cloned. The functional state of 5-HT_{2C} receptors in normal controls and various patient groups has been studied *in vivo* by administering mCPP, a nonselective 5-HT_{2C} agonist, and measuring hormonal and psychological responses. In alcoholism (Benkelfat *et al.*, 1991), panic disorder (Charney *et al.*, 1987; Kahn *et al.*, 1988), seasonal affective disorder (Joseph-Vanderpool *et al.*, 1993), and obsessive–compulsive disorder (Hollander *et al.*, 1988; Zohar *et al.*, 1987), mCPP has been shown to induce different hormonal and psychological responses in patients and controls (Kahn and Wetzler, 1991).

In this article, we describe identification of a nonconservative amino acid substitution in HTR2C. The variant was found using single-strand conformational polymorphism (SSCP) analysis. By typing this polymorphism in CEPH families, the gene was genetically

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mapped to the long arm of the X chromosome. Since this polymorphism was not detectable as a conventional RFLP, we also developed a PCR–RFLP-based method that allows rapid genotyping in populations and families. In addition, both 5-HT_{2C} variants were expressed in *Xenopus laevis* oocytes and tested for electrophysiological responses to 5-HT.

MATERIALS AND METHODS

Subjects. DNA samples (total $n = 62$) for initial mutation screening by SSCP consisted of Finnish alcoholic violent offenders ($n = 16$), Finnish alcoholic arsonists ($n = 5$) with low CSF 5-HIAA concentrations, healthy Finnish controls free of mental disorders ($n = 15$), alcoholic American Indians (Jemez Pueblo $n = 8$, Cheyenne $n = 2$), and U.S. alcoholic male Caucasians ($n = 16$). A general characterization of these subjects and description of their psychiatric assessment have been published elsewhere (Brown *et al.*, 1993; Limson *et al.*, 1991; Virkkunen *et al.*, 1994a,b).

PCR of HTR2C. Amplification of genomic DNA isolated from Epstein–Barr immortalized lymphoblastoma cell lines was accomplished with four primer pairs: 5HT2C1 (5′ GTA TCA GTT CCT ATC CCT GTG 3′) and 5HT2C2 (5′ ATA GCC AAT CCA AAC AAA CAC 3′), 5HT2C3 (5′ GAA TGT GTT TGT TTG GAT TGG 3′) and 5HT2C4 (5′ AAA CAC TTT TTC AGA CAG CAA 3′), 5HT2C7 (5′ CAC CTA ATT GGC CTA TTG GTT 3′) and 5HT2C8 (5′ AAG GAT TGC CAG GAG AGA CAG 3′), and 5HT2C9 (5′ ATT ATG TCT GGC CAC TAC CTA 3′) and 5HT2C10 (5′ CCC AAA CAA TAG CAA TCT TCA 3′). These primer pairs amplify four nonoverlapping regions that are, respectively, 522, 442, 306, and 189 bp in length and that cover 98% of the HTR2C coding sequence. Since the human HTR2C intron sequences were unknown, primers were designed to be complementary to exon sequences adjacent to the exon–intron boundaries. For this reason, the proportion of the coding sequence that was not overlapped by primers was 90%. Positions of the intron–exon boundaries were assumed based on the published mouse sequence (Foguet *et al.*, 1992). Amplification was performed using 30 ng of DNA, 0.25 μM of each primer, 250 μM each of dATP, dCTP, dGTP, and dTTP, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, 10 mM Tris–HCl (pH 8.3), and 0.75 unit of AmpliTaq (Perkin Elmer Cetus). For single-strand conformational polymorphism analysis 1 μCi of [α-^{33}P]dCTP was included in the PCR reaction. Reactions were carried out in a volume of 7.5 μl. Samples were amplified for 30 cycles, each consisting of 1 min at 95°C, 2 min at 53°C (5HT2C1–2), 52°C (5HT2C3–4), 55°C (5HT2C7–8), or 51°C (5HT2C9–10), and 3 min at 72°C.

Single-strand conformation polymorphism analysis. To increase the sensitivity of the SSCP analysis, the amplified fragments were further digested with restriction enzymes to yield subfragments 79–253 bp in length. Fragments were digested with the following restriction enzymes: 5HT2C1–2 with *Nla*IV, 5HT2C3–4 with *Hin*cII, and 5HT2CB7–8 with *Rsa*I (all enzymes were obtained from New England Biolabs, Beverly, MA). The reaction mixtures consisted of 7.5 μl of PCR mixture, 5 units of enzyme, and the recommended buffer. After incubation, reaction mixture was diluted with 15 μl of 95% formamide, 10 mM NaOH, 0.05% xylene cylanol, and 0.05% bromphenol blue, incubated at 95°C for 3 min, and then placed on wet ice. Four microliters of the denatured DNA was loaded per lane in a mutation detection enhancement gel (AT Biochem, Malvern, PA). Electrophoresis was carried out at 4°C for 16 h at 6 W. The gels were dried and autoradiography was performed at −70°C.

Sequence determination. The PCR product was purified by agarose gel electrophoresis followed by extraction with glass beads (GeneClean, BIO 101, La Jolla, CA). DNA was directly sequenced by cycle sequencing (Life Technologies, Gaithersburg, MD).

PCR–RFLP. An artificial restriction site was created using a PCR primer that introduces a base substitution close to the codon of interest (Haliassos *et al.*, 1989). The PCR primers were REPA1 (5′ TTG GCC TAT TGG TTT GGG AAT 3′) and ARTIFACT2 (5′ GTC TGG GAA TTT GAA GCG TCC AC 3′). REPA1 introduces a C to G substitution 4 bp upstream from the polymorphic site, enabling the detection of this site as a *Hin*fI RFLP. Digestion with *Hin*fI yields two fragments (18 and 86 bp) in the case of 5-HT_{2Cser} or leaves the product undigested (104 bp) in the case of 5-HT_{2Ccys} (Fig. 2). Amplification was performed using 60 ng of DNA, 0.25 μM each primer, 250 μM each of dATP, dCTP, dGTP, and dTTP, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, 10 mM Tris–HCl (pH 8.3), and 0.75 unit of AmpliTaq (Perkin Elmer Cetus) in a total volume of 15 μl. Samples were amplified for 35 cycles, each consisting of 1 min at 95°C, 2 min at 50°C, and 3 min at 72°C. After amplification 10 units of *Hin*fI (New England Biolabs) and recommended buffer (1× concentration with respect to the final volume) were added directly to PCR tubes and incubated 10 h at 37°C. The DNA fragments were resolved by 10% polyacrylamide gel electrophoresis and stained with ethidium bromide. Typing 50 individuals either by PCR–RFLP or by SSCP analysis yielded identical results.

Linkage analysis. HTR2C genotype was determined in 10 informative CEPH families. Data were entered into programs provided by CEPH, and files with X chromosome markers were prepared. Two-point lod score analysis was performed using LINKAGE (Lathrop *et al.*, 1984) and MAPMAKER (Lander *et al.*, 1987), and the two-point values were utilized for multipoint analysis. Multipoint linkage analysis was performed using markers that showed the lowest recombination fraction. Also other highly informative markers were chosen from both the Généthon (Weissenbach *et al.*, 1992) and the NIH/CEPH consortium maps (NIH/CEPH Collaborative Mapping Group, 1992). HTR2C was placed in all positions of a map of markers ordered with greater than 1000:1 odds, using the TRY command of MAPMAKER.

Site-directed mutagenesis and synthesis of mRNA. The human HTR2C cDNA clone was provided by Dr. Alan Saltzman (Rhone-Poulenc Rorer Central Research, Collegeville, PA). The entire coding region of the human HTR2C cDNA (Saltzman *et al.*, 1991) was subcloned into a pSP72 vector at *Eco*RI and *Xba*I sites for mutagenesis. A single-base mutation that converts cysteine (TGT) to serine (TCT) was introduced into the human HTR2C cDNA at codon 23 by recombinant PCR (Vallette *et al.*, 1989). The authenticity of the single base mutation was confirmed by double-stranded DNA sequencing (United States Biochemical, Cleveland, OH). Complementary RNA was prepared by *in vitro* transcription using the mCAP kit from Stratagene (La Jolla, CA).

Oocyte preparation and electrophysiological recording. Oocytes were isolated from mature female *X. laevis*. The follicular layer of the oocytes was removed by treatment with 0.2% collagenase A for 2 h. Oocytes were then transferred into a modified Barth's saline (MBS) solution containing 88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.3 mM $Ca(NO_3)_2$, 0.9 mM $CaCl_2$, 0.8 mM $MgSO_4$, 10 mM Hepes (pH 7.5) to await cRNA injection. Approximately 16–20 ng cRNA was injected per oocyte using a microinjection pump (World Precision Instruments, New Haven, CT). Two or three days following cRNA microinjection, oocyte whole cell currents were measured in an MBS superfusion medium using the two-microelectrode voltage-clamp technique, with membrane potential held at −70 mV. Serotonin was superfused at the rate of ~3 ml/min for 30 s, with a period of 20 min between applications to allow for recovery from desensitization.

RESULTS

Identification of 5-HT_{2C} Variants

Amplification of genomic DNA with primers 5HT2C7 and 5HT2C8 followed by SSCP analysis revealed a mobility shift in some individuals. This DNA fragment corresponds to the mouse 5-HT_{2C} receptor gene exon 2 (Fig. 1). This polymorphism was observed in both alcoholics and controls. Sequencing of the polymorphic

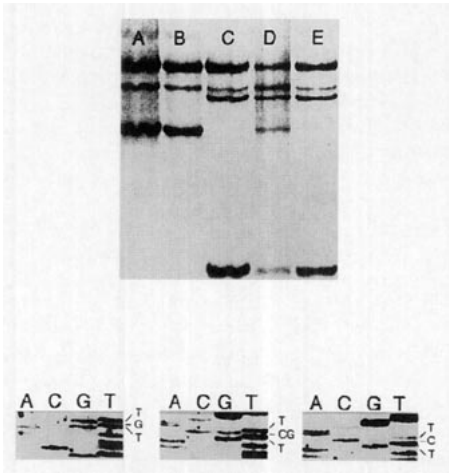

FIG. 1. (**Upper autoradiogram**) SSCP analysis of the second exon of HTR2C. Lanes **A** and **B** are males with 5-HT_{2Ccys} genotype. Lanes **C** and **E** are males with 5-HT_{2Cser} genotype. Lane **D** is a female who is heterozygous for 5-HT_{2Cser}/5-HT_{2Ccys} alleles. (**Lower autoradiogram**) Sequences of two males with 5-HT_{2Ccys} (left) and 5-HT_{2Cser} (right) genotypes and a female who is heterozygous for 5-HT_{2Ccys}/5-HT_{2Cser} (middle). G–C transversion causes cysteine to be replaced by serine at codon 23 (TGT or TCT).

DNA fragment revealed that the variant was caused by a G to C transversion at nucleotide 68 of the coding sequence substituting Cys_{23} for Ser_{23} (TGT for TCT) (Fig. 1). Hydrophilicity analysis (Mac Vector, International Biotechnologies Inc., CT) indicated that the human 5-HT_{2C} receptor contains eight hydrophobic regions, as previously suggested in mouse (Yu *et al.*, 1991). The site of the Cys_{23}–Ser_{23} substitution was placed within the first hydrophobic region of the human 5-HT_{2C} receptor (data not shown). A PCR method generating restriction site was developed to determine genotypes rapidly and also to confirm the existence of the substitution (Haliassos *et al.*, 1989) (Fig. 2). SSCP analysis after amplification of other regions of HTR2C did not reveal additional variants across the population sample.

Genetic Mapping of HTR2C

The HTR2C polymorphism was found to segregate in an X-linked codominant fashion in CEPH families

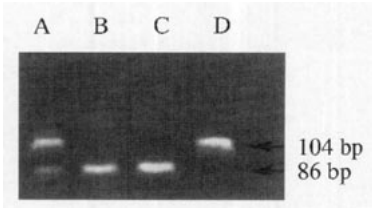

FIG. 2. PCR–RFLP genotyping of four individuals. Genomic DNA was amplified with primers REPA1 and ARTIFACT2. Digestion with *Hin*fI yields two fragments (86 and 18 bp) in the case of 5-HT_{2Cser} or leaves the product undigested (104 bp) in the case of 5-HT_{2Ccys}. Products were visualized in ethidium bromide-stained 10% polyacrylamide gel. Lane **A** is a heterozygous female, lanes **B** and **C** are males with the 5-HT_{2Cser} genotype, and lane **D** is a male with the 5-HT_{2Ccys} genotype.

TABLE 1

Markers Linked to HTR2C

Locus	Probe	Enzyme	Location	θ	Z_{max}
DXS447	RX-404	*Bgl*II	Xq21.1	13.8	3.38
DXS3	P19-2	*Msp*I	Xq21.3	9.5	3.15
DXS366	RX-329	*Taq*I	Xq21.1–q24	0.0	3.31
DXS456	XG30B	PCR	Xq21–q22	7.5	4.73
DXS350	RX-100M1	*Msp*I	Xq22.1–q24	0.0	4.51
DXS350	RX-100M2	*Msp*I	Xq22.1–q24	0.0	5.71
DXS362	RX-237	*Msp*I	Xq21.2–qter	2.7	8.24
DSX362	RX-237	*Taq*I	Xq21.2–qter	8.1	6.01
DXS328	QST-13	*Hin*dIII	Xq21.2–q22.1	12.2	5.14
DSX328	QST-47	*Hin*dIII	Xq21.2–q22.1	11.6	3.49
DSX327	QST-7	*Msp*I	Xq21.2–q22.1	5.0	3.69
DXS1001	248we5	$(AC)_n$	Xq24	8.6	3.67
DXS358	RX-187	*Hin*dIII	Xq24–qter	0.0	7.22
DXS358	RX-187A	*Taq*I	Xq24–qter	0.0	6.01
DXS358	RX-187B	*Taq*I	Xq24–qter	0.0	4.21
DXS329	KZO-7	*Hin*dIII	Xq24–q26	6.2	3.19
DXS734	RX-99	*Taq*I	Xq21.1–qter	0.0	10.22

Note. Loci are listed in order from pter to qter according to the data in the Genome Data Base (GDB, The Johns Hopkins University School of Medicine). The DXS markers are anonymous loci. Enzyme refers to the enzyme that detects the polymorphism; $(AC)_n$ represents CA dinucleotide repeat loci; location is the known physical location of the marker on the X chromosome. θ is the sex-averaged recombination frequency observed with *HTR2C*, and Z_{max} is the lod (log of odds) score at the value of the recombination frequency.

and was typed in 10 informative families. Allele frequencies in 47 unrelated Caucasians (CEPH parents) were 0.13 and 0.87 for 5-HT_{2Cser} and 5-HT_{2Ccys}, respectively. Table 1 shows the maximum two-point lod scores. Two-point analysis yielded a maximum lod score of 10.22 to DXS734, which has previously been assigned to Xq21.1–qter. There were also a number of other markers in this region that were linked to HTR2C. Multipoint analysis placed HTR2C on the long arm of the X chromosome and in the interval between DXS362 and DXS42 with odds greater than 1000:1. DXS362 and DXS42 have previously been mapped to Xq21.2–qter and Xq25, respectively. The marker DXS287, previously assigned to Xq23, appears to be centromeric to HTR2C, and the odds against the alternate order for the two loci were greater than 50:1. Sex-averaged intervals between the HTR2C locus and the markers are shown in Fig. 3.

Oocyte Expression and Pharmacological Characterization of 5-HT_{2Ccys} and 5-HT_{2Cser}

Xenopus oocytes have endogenous Ca^{2+}-activated chloride channels that serve as a functional indicator for receptor-mediated inositol triphosphate (IP3) formation. Taking advantage of this property, the oocyte expression system has been widely used to characterize 5-HT_{2C} function pharmacologically after microinjection of the synthetic receptor mRNA into the cells (Julius *et al.*, 1988). We compared the electrophysiological properties of 5-HT_{2Ccys} and 5-HT_{2Cser} under identical

FIG. 3. Genetic location of human HTR2C. Multipoint analysis in CEPH families placed the 5-HT_{2C} gene on the long arm of the X chromosome and in the interval between DXS362 and DXS42 with odds greater than 1000:1. Sex-averaged intervals between the HTR2C locus and the markers are shown.

conditions in oocytes expressing these receptors. 5-HT activated similar inward currents in oocytes expressing either 5-HT_{2Ccys} or 5-HT_{2Cser}. In agreement with others, we found that it was necessary to allow at least 20 min between 5-HT applications to permit recovery from desensitization (Lubbert *et al.*, 1987; Yu *et al.*, 1991). The currents activated by 10 n*M* 5-HT were antagonized in oocytes expressing either 5-HT_{2Ccys} or 5-HT_{2Cser} by 1 μM 1-(1-naphthyl)piperazine HCl (PPRZ), an antagonist of 5-HT_{2A} and 5-HT_{2C} receptors. The 5-HT-activated currents recovered 20 min after the beginning of washout of PPRZ in oocytes expressing either 5-HT_{2Ccys} or 5-HT_{2Cser}.

The amplitude of 5-HT-activated currents was concentration–dependent over a concentration range from 1 to 500 n*M*. Analysis of the concentration–response data was performed using the nonlinear curve-fitting program ALLFIT (DeLean *et al.*, 1978). Values reported from the concentration–response analysis are those obtained by fitting the data to the logistic equation $y = (E_{max} - E_{min})/[1 + (x/EC_{50})^{-n}] + E_{min}$, where x and y are concentration and response, respectively E_{min} is the minimal response, E_{max} is the maximal response, EC_{50} is the half-maximal response, and n is the slope factor (apparent Hill coefficient). The EC_{50} for 5-HT activated current was 6.2 ± 0.9 n*M* (mean ± SEM; n = 8 cells) and 6.5 ± 0.8 n*M* (n = 7 cells) for oocytes expressing 5-HT_{2Ccys} and 5-HT_{2Cser}, respectively (one-factor ANOVA test; $P > 0.05$). The apparent Hill coefficients for the 5-HT concentration response curves were 1.4 for 5-HT_{2Ccys} and 1.2 for 5-HT_{2Cser} (one-factor ANOVA test; $P > 0.05$). Similar maximal responses were obtained with 500 n*M* 5-HT in oocytes expressing either 5-HT_{2Ccys} or 5-HT_{2Cser}.

DISCUSSION

We identified a polymorphism in the human HTR2C gene by amplifying genomic DNA from 62 individuals and performing SSCP analysis, which is a rapid and efficacious method for detecting polymorphisms (Dean *et al.*, 1990; Glavac and Dean, 1993; Poduslo *et al.*, 1992). Although 90% of the HTR2C coding region was screened for sequence variants, no other variants were detected. However, SSCP is not 100% sensitive even when carefully optimized (Glavac and Dean, 1993), and approximately 10% of the HTR2C gene was not screened because intron sequences were not available for primer design. Thus, it is possible that other undetected 5-HT_{2C} variants exist in the sample population. The 5-HT_{2C} variant is a common (allele frequency 0.13) nonconservative amino acid substitution in which a G–C transversion causes a cysteine to be replaced by serine at codon 23. Since the polymorphism was not detectable as a conventional RFLP, a PCR primer that introduces a base substitution close to the codon of interest was used to create an artificial *Hin*fI restriction site with two allelic forms (Haliassos *et al.*, 1989). Digestion with *Hin*fI yields two fragments (18 and 86 bp) in the case of 5-HT_{2Cser} or leaves the product undigested (104 bp) in the case of 5-HT_{2Ccys}. Typing 50 individuals either by PCR–RFLP or by SSCP analysis yielded identical results, demonstrating reproducibility of this method. We have successfully utilized a similar approach in studies of two of the other serotonin receptors, 5-$HT_{1D\alpha}$ (Ozaki *et al.*, 1995) and 5-$HT_{1D\beta}$ (Lappalainen *et al.*, 1995). The conversion of the SSCP variant to the RFLP site facilitates the genotyping, makes the genotyping more portable to other laboratories, and also confirms the sequencing result. This is especially important when PCR products are directly sequenced using cycle sequencing.

A tight linkage was found between the HTR2C polymorphism and marker DXS734, which has previously been placed on Xq21.1–qter. Other markers that did not recombine with the HTR2C polymorphism were DXS358 (Xq24–qter), DXS366 (Xq21.2–q24), and DXS350 (Xq22.1–q24). Multipoint analysis placed HTR2C between DXS287 and DXS42, which have previously been mapped to Xq23 and Xq25, respectively. Thus, the location of HTR2C appears to be within Xq24, which is in agreement with the previous physical mapping result of Milatovich *et al.* (1992). The knowledge of the precise genetic location of HTR2C is important for future linkage studies with X-linked syndromes.

Since HTR2C has an important role in the regulation of several endocrinological responses, CSF production, and behavior, this locus becomes a potential candidate for any Xq24-linked syndromes that involve abnormal brain function.

In this study, a single Cys_{23}–Ser_{23} point mutation in the first hydrophobic domain of human HTR2C did not alter the 5-HT concentration–response curves in *Xenopus* oocytes expressing these receptors. This suggests that human 5-HT_{2C} variants may not be functionally different under baseline physiological conditions. It is possible, however, that the responses to pharmacological agents, neuropeptides, or hormones may be different. For example, Cys–Ser substitution may affect the receptor folding, hindering the formation of a normal hydrophobic pocket and subsequently the binding of bulky ligands. Theoretically, the presence of cysteine could alter protein folding due to its ability to form disulfide bonds (Strosberg, 1991). Dixon *et al.* (1987) have previously shown that substitution of Cys_{106} and Cys_{184} in hamster β_2-adrenergic receptor markedly diminishes agonist binding to the modified receptor. Thus, studies utilizing compounds of different molecular size might be required to demonstrate functional differences between 5-HT_{2Ccys} and 5-HT_{2Cser}.

As reviewed in the Introduction, several lines of evidence suggest abnormal function of 5-HT_{2C} receptors in certain neuropsychiatric disorders, including alcoholism (Benkelfat *et al.,* 1991), panic disorder (Charney *et al.,* 1987; Kahn *et al.,* 1988), seasonal affective disorder (Joseph-Vanderpool *et al.,* 1993), and obsessive–compulsive disorder (Hollander *et al.,* 1988; Zohar *et al.,* 1987). Thus, it may be valuable to search for a population association or linkage between 5-HT_{2Ccys} and 5-HT_{2Cser} to these disorders.

In conclusion, this is the first demonstration of a fairly common amino acid substitution in human HTR2C. This substitution was used for fine-scale linkage mapping of HTR2C which was placed on the long arm of the X chromosome. The described polymorphism should also provide a tool for genetic studies in populations and families. In electrophysiological studies, with the 5-HT_{2C} variants expressed in *Xenopus* oocytes, concentration–response curves for 5-HT were not significantly different. However, further electrophysiological studies utilizing different pharmacological compounds and also ligand binding experiments are required to rule out the possibility of functional divergence between 5-HT_{2Ccys} and 5-HT_{2Cser}.

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REFERENCES

Asberg, M., Thoren, P., Traskman, L., Bertilsson, L., and Ringberger, V. (1976a). Serotonin depression: A biochemical subgroup within the affective disorder. *Science* **191:** 478–480.

Asberg, M., Traskman, L., and Thoren, P. (1976b). 5-HIAA in the cerebrospinal fluid. *Arch. Gen. Psychiatry* **33:** 1193–1197.

Aulakh, C. S., Hill, J. L., and Murphy, D. (1992). Effects of various serotonin receptor subtype selective antagonists alone and on m-chlorophenylpiperazine-induced neuroendocrine changes in rats. *J. Pharm. Exp. Ther.* **263:** 588–595.

Bagdy, G., Kalogeras, K. T., and Szemeredi, K. (1992). Effect of 5-HT1C and 5-HT2 receptor stimulation on excessive grooming, penile erection and plasma oxytocin concentrations. *Eur. J. Pharmacol.* **229:** 9–14.

Ballenger, J. C., Goodwin, F. K., Major, L. F., and Brown, G. L. (1979). Alcohol and central serotonin in man. *Arch. Gen. Psychiatry* **36:** 224–227.

Benkelfat, C., Murphy, D. L., Hill, J. L., George, D. T., Nutt, D., and Linnoila, M. (1991). Ethanol-like properties of the serotonergic agonist m-chlorophenylpiperazine in chronic alcoholic patients. *Arch. Gen. Psychiatry* **48:** 383.

Brown, G. L., Albaugh, B., Robin, R., Goodson, S. G., Trunzo, M., Wynne, D., and Goldman, D. (1993). Alcoholism and substance abuse among selected Southern Cheyenne Indians. *Transcultural Psychiatry* **16:** 531–542.

Charney, D. S., Woods, S. W., Goodman, W. K., and Heninger, G. R. (1987). Serotonin function in anxiety. II. Effects of the serotonin agonist mCPP in panic disorder patients and healthy subjects. *Psychopharmacology* **92:** 14–24.

Cloninger, C. R., Bohman, M., and Sigvardsson, S. (1981). Inheritance of alcohol abuse: Cross-fostering analysis of adopted men. *Arch. Gen. Psychiatry* **38:** 861–868.

Crowe, R. R. (1974). An adoption study of antisocial personality. *Arch. Gen. Psychiatry* **31:** 785–791.

Crowe, R. R. (1985). The genetics of panic disorder and agoraphobia. *Psychiatr. Dev.* **3:** 171–185.

Dean, M., White, M. B., Amos, J., Gerrard, B. S. C., Khaw, K. T., and Leppert, M. (1990). Multiple mutations in highly conserved residues are found in mildly affected cystic fibrosis patients. *Cell* **61:** 863–870.

DeLean, A., Munson, P. J., and Rodbard, D. (1978). Simultaneous analysis of families of sigmoidal curves: Application to bioassays, radioligand assay and physiological dose–response curves. *Am. J. Physiol.* **235:** E97–E102.

Dixon, R. A. F., Sigal, I. S., Candelore, M. R., Register, R. B., Scattergood, W., Rands, E., and Strader, C. D. (1987). Structural features required for ligand binding to the β-adrenergic receptor. *EMBO J.* **6:** 3269–3275.

Foguet, M., Nguyen, H., Le, H., and Lubbert, H. (1992). Structure of the mouse 5-HT_{1C}, 5-HT_2 and stomach fundus serotonin receptor genes. *NeuroReport* **3:** 345–348.

Glavac, D., and Dean, M. (1993). Optimization of the single stranded conformation polymorphism (SSCP) technique for detection of point mutations. *Hum. Mutat.* **2:** 404–414.

Haliassos, A., Chomel, J. C., Tesson, L., Baudis, M., Kruh, J., Kaplan, J. C., and Kirtzis, A. (1989). Modification of enzymatically amplified DNA for the detection of point mutations. *Nucleic Acids Res.* **17:** 3606.

Hollander, E., Fay, M., Cohen, B., Campeas, R., Gorman, J. M., and Liebowitz, M. R. (1988). Serotonergic and noradrenergic sensitivity in obsessive–compulsive disorder: Behavioral findings. *Am. J. Psychiatry* **145:** 1015–1018.

Joseph-Vanderpool, J. R., Jacobsen, F. M., Murphy, D. L., Hill, J. L., and Rosenthal, N. E. (1993). Seasonal variation in behavioral responses to m-CPP in patients with seasonal affective disorder and controls. *Biol. Psychiatry* **33:** 496–504.

Julius, D., MacDermott, A. D., Axel, R., and Jessell, T. M. (1988). Molecular characterization of a functional cDNA encoding the serotonin 1c receptor. *Science* **241:** 558–564.

Kahn, R., and Wetzler, S. (1991). m-Chlorophenylpiperazine as a probe of serotonin function. *Biol. Psychiatry* **30:** 1139–1166.

Kahn, R. S., Asnis, G. M., Wetzler, S., and van Praag, H. M. (1988). Neuroendocrine evidence for serotonin receptor supersensitivity in patients with panic disorder. *Psychopharmacology* **96:** 360–364.

King, B. H., Brazell, C., Dourish, C. T., and Middlemiss, D. N. (1989).

MK-212 increases rat plasma ACTH concentration by activation of the 5-HT1C receptor subtype. *Neurosci. Lett.* **105:** 174–176.

Lander, E. S., Green, P., Abrahamson, J., Barlow, A., Daly, M. J., Lincoln, S. E., and Newburg, L. (1987). MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* **1:** 174–181.

Lappalainen, J., Dean, M., Charbonneau, L., Virkkunen, M., Linnoila, M., and Goldman, D. (1995). Mapping of the serotonin 5-HT1Dβ autoreceptor on chromosome 6 and direct analysis for sequence variants. *Neuropsychiatr. Genet.*, in press.

Lathrop, G. M., Lalouel, J. M., Julier, C., and Ott, J. (1984). Strategies for multilocus linkage analysis in humans. *Proc. Natl. Acad. Sci. USA* **81:** 3443–3446.

Limson, R., Goldman, D., Roy, A., Lamparski, D., Ravitz, B., Adinoff, B., and Linnoila, M. (1991). Personality and cerebrospinal fluid monoamines in alcoholics and controls. *Arch. Gen. Psychiatry* **48:** 437–441.

Linnoila, M., Virkkunen, M., Scheinin, M., Nuutila, M., Rimon, R., and Goodwin, F. K. (1983). Low cerebrospinal fluid 5-hydroxyindoleacetic acid concentration differentiates impulsive from nonimpulsive behavior. *Life Sci.* **33:** 2609–2614.

Lubbert, H., Hoffman, B. J., Snutch, T. P., van Dyke, T., Levine, R. J., Hartig, P. R., Lester, H. A., and Davidson, N. (1987). cDNA cloning of a serotonin 5-HT1c receptor by electrophysiological assays of mRNA-injected Xenopus oocytes. *Proc. Natl. Acad. Sci. USA* **84:** 4332–4336.

Milatovich, A., Hsieh, C.-H., Bonaminio, G., Tecott, L., Julius, D., and Francke, U. (1992). Serotonin receptor 1c assigned to X chromosome in human (band q24) and mouse (bands D-F4). *Hum. Mol. Genet.* **1:** 681–684.

NIH/CEPH Collaborative Mapping Group (1992). A comprehensive genetic linkage map of the human genome. *Science* **258:** 67–86.

Ozaki, N., Lappalainen, J., Dean, M., Virkkunen, M., Linnoila, M., and Goldman, D. (1995). Mapping of the serotonin 5-$HT_{1D\alpha}$ autoreceptor gene (HTR1D) on chromosome 1 using a coding region polymorphism. *Neuropsychiatric Genet.*, in press.

Poduslo, S., Dean, M., Kolch, U., and O'Brien, S. (1992). Detecting high resolution polymorphisms in human coding loci by combining PCR and single stranded conformation polymorphism (SSCP) analysis. *Am. J. Hum. Genet.* **49:** 106–111.

Roy, A., Segal, N. L., Centerwall, B. S., and Robinette, C. D. (1991). Suicide in twins. *Arch. Gen. Psychiatry* **48:** 29–32.

Saltzman, A. G., Morse, B., Whitman, M. M., Ivanshchenko, Y., Jaye, M., and Felder, S. (1991). Cloning of the human serotonin 5-HT2 and 5-HT1C receptor subtypes. *Biochem. Biophys. Res. Commun.* **181:** 1469–1478.

Strosberg, A. D. (1991). Structure/function relationship of proteins belonging to the family of receptors coupled to GTP-binding proteins. *Eur. J. Biochem.* **196:** 1–10.

Vallette, F. E., Mege, E., Reiss, A., and Adesnik, M. (1989). Construction of mutant and chimeric genes using the polymerase chain reaction. *Nucleic Acids Res.* **17:** 723–733.

Virkkunen, M., Kallio, E., Rawlings, R., Tokola, R., Poland, R., Guidotti, A., Nemeroff, C., Bissette, G., Kalogeras, K., Karonen, S.-L., and Linnoila, M. (1994a). Personality profiles and state aggressiveness in Finnish alcoholic, violent offenders, fire setters and healthy volunteers. *Arch. Gen. Psychiatry* **51:** 28–33.

Virkkunen, M., Rawlings, R., Tokola, R., Poland, R., Guidotti, A., Nemeroff, C., Bissette, G., Kalogeras, K., Karonen, S.-L., and Linnoila, M. (1994b). CSF biochemistries, glucose metabolism, and diurnal activity rhythms in alcoholic, violent offenders, fire setters, and healthy volunteers. *Arch. Gen. Psychiatry* **51:** 20–27.

Weissenbach, J., Gyapay, G., Dib, C., Vignal, A., Morissette, J., Millaseau, P., Vaysseix, G., and Lathrop, M. (1992). A second generation linkage map of the human genome. *Nature* **359:** 794–801.

Woodman, C. L. (1993). The genetics of panic disorder and generalized anxiety. *Ann. Clin. Psychiatry* **5:** 231–239.

Yu, L., Nguyen, H., Le, H., Bloem, L. L., Kozak, C. A., Hoffman, B. J., Snutch, T. P., Lester, H. A., Davidson, N., and Lubbert, H. (1991). The mouse 5-HT1c receptor contains eight hydrophobic domains and is X-linked. *Mol. Brain Res.* **11:** 143–149.

Zohar, J., Mueller, E. A., Insel, T. R., Zohar-Kadouch, R. C., and Murphy, D. L. (1987). Serotonergic responsivity in obsessive–compulsive disorder: Comparison of patients and controls. *Arch. Gen. Psychiatry* **44:** 946–951.