

Single Nucleotide Polymorphisms of the Human M1 Muscarinic Acetylcholine Receptor Gene

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ABSTRACT The gene encoding the human muscarinic receptor, type 1 (*CHRM1*), was genotyped from 245 samples of the Coriell Collection (Coriell Institute for Medical Research, Camden, NJ). Fifteen single nucleotide polymorphisms (SNPs) were discovered, 9 of which are located in the coding region of the receptor. Of these, 8 represent synonymous SNPs, indicating that *CHRM1* is highly conserved in humans. Only a single allele was found to contain a nonsynonymous SNP, which encodes an amino acid change of Cys to Arg at position 417. This may have functional consequences because a C417S point mutation in rat M1 was previously shown to affect receptor binding and coupling. Furthermore, 0 of 4 SNPs within *CHRM1* previously deduced from sequencing of the human genome were found in this study despite a prediction that a majority of such inferred SNPs are accurate. The consensus sequence of *CHRM1* obtained in our study differs from the deposited reference sequence (AC NM_000738) in 2 adjacent nucleotides, leading to a V173M change, suggesting a sequencing error in the reference sequence. The extraordinary sequence conservation of the *CHRM1* gene-coding region was unexpected as M1-knockout mice show only minimal functional impairments.

KEYWORDS: pharmacogenetics, muscarinic acetylcholine, receptor, single nucleotide polymorphism, G protein coupled receptor, *CHRM1*

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INTRODUCTION

The muscarinic receptor family, of which there are 5 subtypes (M1-M5), plays an important functional role throughout the body. Muscarinic receptors are involved in a variety of cell-type specific signaling pathways. These include the regulation of cyclic adenosine monophosphate concentration in the cell, activation of tyrosine and mitogen-activated kinases, and the regulation of ion channel function in the cell¹.

The muscarinic receptors have a broad and overlapping distribution in the body. The M1, M2, and M4 receptors are the most abundant. The M1 receptor is found in the hippocampal and cortical regions of the brain as well as in the parasympathetic ganglia^{2,3,4}. The M1 receptor is involved in many processes, including the initiation of seizures, learning and memory, and regulation of the force and rate of heart contractions^{5,6}. Because of its involvement in these processes, the M1 receptor is a compelling drug target for Alzheimer's disease and other neurological and psychiatric disorders⁶. Indeed, for the past 20 years, the cholinergic hypothesis has proposed that loss of cholinergic function is responsible for the cognitive symptoms of Alzheimer's⁷. It is thought that stimulation of the M1 receptor would alleviate some of the symptoms of Alzheimer's by several pathways, including increased secretion of the nontoxic α -amyloid peptide and decreased secretion of the toxic β -amyloids generated from the amyloid precursor protein^{8,9}.

The M1 receptor is a member of the G-protein coupled receptor (GPCR) superfamily. Many GPCRs have been found to contain single nucleotide polymorphisms (SNPs) that are involved in disease susceptibility or drug response (for a

review, see reference¹⁰). The R16G allele in the β_2 adrenergic receptor, for example, is associated with nocturnal asthma. Several of the serotonergic receptors have alleles associated with psychotic symptoms in Alzheimer's disease, whereas certain haplotypes of the μ opioid receptor have been associated with substance abuse¹⁰. Although the muscarinic receptors are thought to contribute to certain neurological and psychiatric disorders, no sequence variations or alleles have been found that affect susceptibility to these diseases or response to muscarinic agonists. In this study we have genotyped the *CHRM1* gene-encoding human M1 receptor in 245 individuals of the Coriell Collection.

MATERIALS AND METHODS

Genotyping of *CHRM1* was done as described in Wang et al¹¹. Briefly, the genomic DNA from 245 individuals was amplified by polymerase chain reaction (PCR) (Applied Biosystems Gene Amp PCR System 9700, Foster City, CA) using the following primers:

F1 5'GAGGAAGCCCTGTAGCG;
R3 5'GATCACCCTTCGGAGCC;
F2 5'AGCTCTGATGATCGGCCT;
R1 5'CCAAGGAATACTTAATGTTAAGCCT.

They were then sequenced on an Applied Biosystems 3700 DNA analyzer with a 3700 POP-6 polymer

matrix. The resulting sequence tracings were then analyzed using Sequencher 4.0 (Gene Code Corp., Ann Arbor, MI). Haplotype analysis was done using an expectation-maximum algorithm¹². Sequence alignments were done using the sequence alignment program Clustal W, which is freely available.

RESULTS AND DISCUSSION

The hM1 receptor gene (*CHRM1*) resides on chromosome 11. The *CHRM1* gene consists of 1 large exon containing the entire coding region of the hM1 receptor. A promoter region and 3 noncoding exons for the hM1 gene based on similarity to the rat gene have been reported approximately 14 kilobases (kb) upstream of the hM1 coding region¹³. However, a Basic Local Alignment Search Tool (BLAST) search of these sequences against the human genome sequence showed these DNA segments to be 213 kb upstream of *CHRM1*. The promoter region and noncoding exons were not sequenced in this study.

The human M1 muscarinic receptor gene, *CHRM1*, was sequenced from 245 samples of the Coriell Collection. The samples used are ethnically diverse as follows: 100 Caucasians, 100 African Americans, 30 Asians, 10 Hispanics, and 7 Pacific Islanders. Fifteen SNPs were discovered, 9 of which are located in the coding region of the receptor (Table 1,

Table 1. Single Nucleotide Polymorphisms (SNPs) of Human Muscarinic Receptor, Type 1

SNP	Base Position	Codon	Amino Acid	Total Frequency (n = 490)	CA (n = 200)	AA (n = 198)	AS (n = 58)	HIS (n = 20)	PA (n = 14)
1	96	ACG→ACA	T32	0.002	0.005	0	0	0	0
2	162	ACG→ACA	T54	0.002	0	0.005	0	0	0
3	267	GGC→GGA	G89	0.053	0.050	0.020	0.121	0.050	0.286
4	783	CGC→CGT	R261	0.004	0.005	0.005	0	0	0
5	1044	CAG→CAA	Q348	0.080	0.045	0.131	0.017	0.050	0.143
6	1140	CCG→CCA	P380	0.002	0	0.005	0	0	0
7	1221	TGC→TGT	C407	0.120	0.165	0.116	0	0.150	0
8	1249	TGC→CGC	C417R	0.002	0	0.005	0	0	0
9	1353	TCC→TCT	S451	0.055	0.055	0.02	0.121	0.050	0.286
10	Exon+2	A→G		0.039	0.01	0.086	0	0	0
11	Exon+13	C→A		0.035	0.005	0.081	0	0	0
12	Exon+171	C→T		0.002	0.005	0	0	0	0
13	Exon+221	C→A		0.002	0.005	0	0	0	0
14	Exon+222	T→G		0.002	0.005	0	0	0	0
15	Exon+299	T→C		0.002	0	0.005	0	0	0

CA indicates Caucasian; AA, African American; AS, Asian; HIS, Hispanic; PA, Pacific Islander.

Figure 1). Eight of these are synonymous (ie, leading to no change in the protein sequence). The 1 nonsynonymous SNP encodes an amino acid change of Cys to Arg at position 417, which is located in transmembrane domain VII (Figure 1). This SNP was found in only 1 heterozygous individual. Despite the high amino acid sequence conservation, 43% of individuals had at least 1 variation in their DNA sequence. The results indicate that the hM1 receptor is highly conserved in humans.

The *CHRM1* -hM1 sequence conservation extends to other mammalian species as well. The DNA sequences of rhesus monkey, rat, and mouse (AC: AF026262, S7397.1, NM_007698.1, respectively)

were 98.4%, 91.6%, and 91.4% identical as compared to the consensus sequence obtained from the *CHRM1* genotyping. The protein sequences (AC: P56489, P08482, NP_031724.1, respectively) were 99.6%, 98.7%, and 98.0% identical. Most of the variations in the DNA sequence were in the "wobble" site of the codon, resulting in conservation of the amino acid residue. This indicates that there is evolutionary pressure for the protein sequences to remain conserved. Furthermore, sequence alignments with the human sequences of the other muscarinic receptors and several animal species indicate that the entire family is conserved across species, but the M1 receptor is the most highly conserved.

Figure 1. Hm1 receptor SNPs

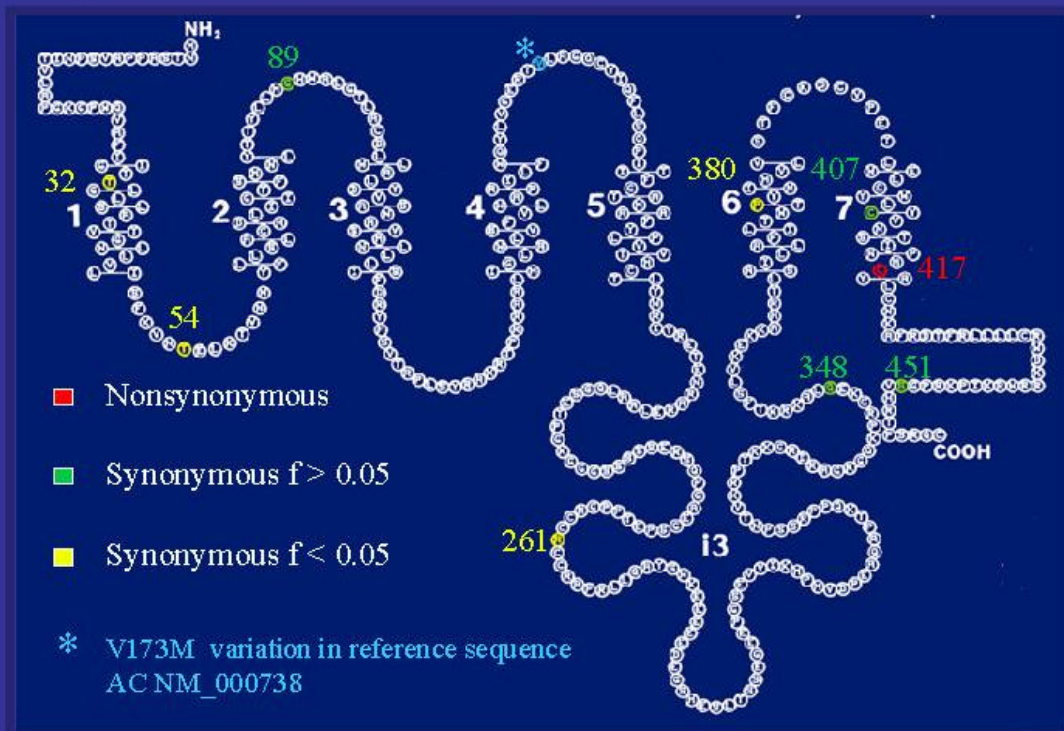


Figure 1. Schematic showing location of single nucleotide polymorphisms (SNPs) on the hM1 receptor. Red = nonsynonymous SNP; green = synonymous SNP with $f > .05$; yellow = synonymous SNP with $f < .05$

Although the high conservation of the M1 gene in humans and mammals emphasizes its importance, the M1 gene is not essential for survival. A knockout of the M1 receptor in mice is not lethal; the M1-deficient mouse strain displays no significant differences in body weight, longevity, fertility, or overt behavior compared with wild-type mice⁶.

There is suggestive evidence that the 1 nonsynonymous variation, C417R, may exhibit a change in M1 receptor function. Cys 417, which is in transmembrane helix VII, is highly conserved; it is present in all 5 muscarinic subtypes in humans and all other species analyzed, including rhesus monkey, rat, mouse, chimpanzee, pig, and chicken. Cys 417 has been shown to influence receptor-ligand interactions. Previous mutagenesis studies with rat M1 have demonstrated that mutating Cys 417 to Ser resulted in increased receptor affinity to carbachol, a muscarinic agonist, and to several antagonists. The C417S mutation also caused a shift to the left in the carbachol dose response curve for phosphoinositol hydrolysis, resulting in an EC₅₀ value 13-fold lower than wild type¹⁴. Cys 417 therefore has a critical role in M1 receptor function. Because a Cys to Ser change is also more conservative than the Cys to Arg change encoded by a human allele, it is likely that this SNP will affect M1 receptor function and may warrant further study, despite its low frequency (1:490 alleles).

A haplotype analysis of *CHRM1* SNPs was also done using an expectation-maximum algorithm. Haplotype analysis serves to characterize linkage disequilibrium in various populations^{15,16} and has the potential to identify disease-predisposing alleles in a population¹². Only hM1 SNPs with a frequency greater than 1% were analyzed. Of the 15 SNPs found in the hM1 receptor, 6 fit this category, resulting in 8 distinct haplotypes (Table 2). Of these, by far the predominant one was wild type; 4 contained 1 SNP, and 3 involved 2 SNPs. This result could serve as a basis for studying possible disease association and may also involve SNPs adjacent to the *CHRM1* gene.

The recent publication of the human genome sequence has brought to light many SNPs. There are 8 SNPs reported in the genomic sequence of *CHRM1* (AC XM_006058). Of these, 4 SNPs were found as a result of the genome sequencing project and 4 SNPs were recently added to the accession sequence on the NCBI (National Center for Biotechnology Information) website. These were obtained from samples of 100 individuals, without frequency distribution provided, as a result of a separate genotyping of *CHRM1* (unpublished). None of the 4 SNPs found as a result of sequencing for the human genome project were found in either study involving multiple samples, whereas all 4 of the SNPs identified in the alternate *CHRM1* sequencing study were found. Failure to reproduce

Table 2. Haplotypes of Human Muscarinic Receptor, Type 1

Haplotype*	Base Position	Overall Frequency	Chi-Square	Approximate p-value
AAAAAA	w.t	0.725	253.2	$p < .000001$
ABAAAA	1044	0.042	29.7	$p < .000001$
AABAAA	1221	0.105	79.2	$p < .000001$
AAAABA	Exon+2	0.006	4.7	$p < .05$
AAAAAB	Exon+13	0.028	19.0	$p < .0001$
BAABAA	267 1353	0.044	31.3	$p < .000001$
ABAABA	1044 Exon+2	0.028	19.3	$p < 0.0001$
AABAAB	1221 Exon+13	0.006	4.0	$p < .05$

*Haplotype analysis was done using an expectation maximum algorithm. Only single nucleotide polymorphisms with a frequency greater than 1% were analyzed.

the 4 genomic SNPs is surprising because it has been previously reported that 85% of SNPs inferred from the human genome project are correct¹⁷. The consensus sequence obtained from this study also differed from the reference sequence for hM1 (AC NM_000738). The purpose of the NCBI reference sequences is to provide sequence standards for chromosomes, mRNA and proteins for use in mutation analysis, gene expression discovery, and polymorphism discovery and therefore should reflect the wild-type sequence of a particular gene. At base pairs 516 and 517, a GA in our consensus sequence was an AG in the reference sequence. This resulted in a Val to Met amino acid change at position 173. The V173M variation was not observed in this study, so it is either a very rare allele or (more likely) a sequencing error.

CONCLUSION

The results of genotyping the coding region of *CHRM1* reveal that it is highly conserved in humans. Only 1 nonsynonymous SNP, C417R, was found in 1 heterozygote with potential consequences for receptor function. Even though the allele is rare, it may contribute to pathophysiology in some ethnic populations. The promoter region and other regulatory elements were not sequenced in this study. It is therefore possible that additional polymorphisms may be found in the regulatory elements of the *CHRM1* gene. Polymorphisms in this region could influence receptor expression, thereby affecting drug response or disease susceptibility.

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