Polymorphisms in Human Muscarinic Receptor Subtype Genes

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Abstract A wide range of polymorphisms have been reported in muscarinic receptor subtype genes, mostly in M_1 and M_2 and, to a lesser extent, M_3 receptors. Most studies linking such genetic variability to phenotype have been performed for brain functions, but a more limited amount of information is also available for cardiac and airway function. Unfortunately, for none of the phenotypes under investigation a robust association with genotype has emerged. Moreover, it remains mostly unclear whether a reported association indicates a causative role of the polymorphism under investigation or merely a role as indicator of other polymorphisms affecting expression and/or function of the receptor. Also, most data on genotype–phenotype associations of muscarinic receptor subtypes are based on cross-sectional samples. Mechanistic studies linking polymorphisms to molecular, cellular, and tissue functions are largely missing. Finally, studies on a possible impact of muscarinic receptor polymorphisms on drug responsiveness are also largely missing. Thus, the field of genomics of muscarinic receptor subtypes is still in an early stage and a considerably greater number of studies will be required to judge the role of muscarinic receptor gene variability in physiology, pathophysiology, and drug treatment.

Keywords Intelligence • Muscarinic receptor • Single nucleotide polymorphism • Tandem repeat polymorphism

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1 Introduction

Most genes exhibit sequence variability in the general population. Such gene variants are of interest as they can change human physiology and/or be associated with disease. They may also affect how other molecules bind to a given protein and hence may change drug responsiveness. The latter type of gene variant is the subject of pharmacogenomics. In an ideal world, the functional consequences of a gene variant are known at the molecular, cellular, tissue, organism, and population level. However, in most cases only information about association of gene variants with phenotypes at the population level is available, which leaves considerable uncertainty for mechanistic interpretation. Thus, for most gene variants, it remains unclear whether reported associations with physiology, disease states or drug responses are causal or merely represent indicators.

Gene variants can be rare and then mostly are referred to as mutations. Such mutations may affect the function of the gene product in a profound way in an individual subject, but typically have only little impact at the population level due to their low prevalence. In contrast, more frequent gene variants, e.g., those occurring in at least 1% of the population, are referred to as polymorphisms and have the potential to be functionally relevant at the population level. Therefore, this chapter will primarily focus on the more frequent polymorphisms.

Various types of gene variants exist. The most frequently observed type is single nucleotide polymorphisms (SNPs), i.e., variants in which a single nucleotide is exchanged. When occurring within the coding region of a gene, this can affect the structure of its product if it results in an amino acid change. Other types of gene variants include the deletion or addition of nucleotides. Within the coding region this can lead to nonsense or truncated proteins. A variation of this theme is seen in variants in which the number of repeats, e.g., of CA nucleotide doublets, varies between subjects. These are mostly found in non-coding gene parts and can affect the activity of the gene promoter and/or mRNA stability. Moreover, any of these gene variants can affect gene splicing. Of note, early detection of gene polymorphisms was based upon differential susceptibility of DNA to various digestion enzymes (Detera-Wadleigh et al. 1989). While such work was important to get the field started, it has provided limited direct information on the underlying molecular gene variant.

Gene variants can affect the function of the corresponding protein in multiple ways. Variants within the coding region resulting in an amino acid change can structurally alter the protein and hence may affect its function and/or stability. Variants outside the coding region may affect the promoter activity, gene splicing, and/or mRNA stability. This may result in an altered expression of structurally unchanged protein.

This chapter will review variants in the five muscarinic receptor subtype genes and their associations with physiology, disease states, and/or drug responses. It is based on a search in the NCBI database of gene variants and a systematic Medline search completed in May 2011 using the key word combination “muscarinic

receptor" and "polymorphism." Additional references were taken from the retrieved articles as well as from congress abstracts. Studies on polymorphisms in non-human muscarinic receptors (Klett and Printz 1995; Du et al. 1996) were not considered.

There are two types of gene variants which can affect muscarinic physiology and/or drug response that will not be dealt with in this chapter. Firstly, variants of genes encoding proteins which interact with muscarinic receptors, e.g., G-proteins or other signal transduction molecules (Rosskopf et al. 2003), may affect the function of the corresponding protein and hence secondarily the function of muscarinic receptors and drug responsiveness. Secondly, specific for the pharmacogenomics of muscarinic receptors, it needs to be considered that gene variants may relate not to the receptors or their signal transduction but rather to the pharmacokinetic properties of the muscarinic receptor ligands. For example, some muscarinic receptor antagonists are metabolized by cytochrome P450 2D6, and the genotype for this enzyme may affect the exposure to such drugs (Brynne et al. 1998; Müller et al. 1993) which can have consequences for their efficacy and/or tolerability (Brynne et al. 1998; Diefenbach et al. 2008).

2 M_1 Receptors

The human M_1 receptor gene (CHRM1) is located on chromosome 11q13 and consists of a single exon containing the entire coding region. The NCBI database contains 82 SNPs for this gene. Several of them are in linkage disequilibrium, indicating the presence of haplotypes (Lucas et al. 2001; Maeda et al. 2006; Lou et al. 2006). While sequencing the exon of the M_1 receptor gene of 245 subjects of various ethnic groups, 15 SNPs were identified of which 9 were in the coding region; only 1 of them was non-synonymous (T1249C, Cys417Arg[1]) but found in only a single subject (Lucas et al. 2001). While most other detected variants were also rare, 43% of subjects exhibited at least one variation. Another study did not detect any non-synonymous SNP of the CHRM1 among 74 subjects, including 48 with dementia (Weiner et al. 2004). Moreover, this study found based on phenotypic screening that the potency of the muscarinic agonist carbachol was similar to that in wild-type cells in all cases. Thus, non-synonymous polymorphisms appear rare within the coding region of the M_1 receptor.

The vast majority of studies on CHRM1 polymorphisms have explored associations with brain functions, but some studies on airway and ocular function have also been reported. With regard to dementia, a small study with receptor sequencing from samples of brains from nine patients with Alzheimer's, six with vascular dementia and three controls, a synonymous SNP was found in two patients

[1] This manuscript refers to specific nucleotides by the single and specific amino acids by the three letter code. Where possible, we provide the rs identification number.

(Ohara et al. 1994). More importantly, a study investigating a single synonymous SNP (rs2067477) in 232 Alzheimer patients and 169 controls found no difference in allele frequency or age of onset of the disease between genotypes (Liu et al. 2005).

Comparing brain samples from 20 schizophrenia patients and controls each, a reduction in M_1 receptor mRNA by about 28% was found, but this could not be linked to the prevalence of four SNPs in the coding region of the receptor gene (Mancama et al. 2003). In another study, 243 schizophrenic patients were not compared to controls but rather patients were assessed according to genotype (rs2067477) (Liao et al. 2003). Genotypes differed in responses in the Wisconsin Card Sorting Test but not in other parameters including age of onset, chlorpromazine equivalents, and Brief Psychiatric Rating Scale.

In 290 patients with acute sciatica, the relationship between 121 SNPs in 14 genes including that for the CHRM1 and pain-related reduction in movement was explored (Mishra et al. 2007). For the CHRM1, this was done for four SNPs (rs17157628, rs544978, rs542269, rs2075748), but after Bonferroni correction for multiple comparisons, none of the SNPs significantly correlated with phenotype (Roland-Morris Disability Questionnaire). A possible relationship between CHRM1 polymorphisms and nicotine dependence has explored a sample of 2,037 European and African American subjects from 602 families (Lou et al. 2006). A haplotype based on 6 SNPs (rs2507821, rs4963323, rs544978, rs542269, rs2075748, rs1938677) was found to be protective against nicotine dependence within the African but not with the European American part of the sample.

Two studies have explored a link between CHRM1 polymorphisms and airway disease. A case-control study of 325 asthma patients and 333 healthy subjects looked at nine SNPs in the M_1 receptor gene (Maeda et al. 2006). Among these, the homozygous T-allele of one SNP upstream of the coding sequence (rs2075748) was associated with a lower risk for asthma (odds ratio 0.29) whereas the homozygous G-allele of another upstream SNP (rs1942499) was associated with a higher risk for asthma (odds ratio 1.86). Taking a third SNP also into consideration, such associations were even stronger at the haplotype level. In vitro experiments using a reporter assay demonstrated that the TA haplotype was associated with lower luciferase activity as compared to the CG haplotype, indicating that these SNPs are not only markers but may actually contribute to the regulation of CHRM1 transcription (Maeda et al. 2006). On the other hand, the odds ratio for responding to the muscarinic antagonist tiotropium was not affected by two other SNPs (rs10897304, rs2067480) in the CHRM1 in a cohort of 138 asthma patients (Park et al. 2009).

A single study of 194 patients with high myopia and 109 control subjects explored four SNPs within the CHRM1, of which two (rs44978 and rs542269) were associated with myopia (Lin et al. 2009) but these results have been questioned on technical grounds (Guggenheim et al. 2010). However, these data are difficult to interpret because homozygosity for the minor allele decreased the odds ratio for myopia, whereas heterozygosity increased it relative to homozygosity for the major allele.

Taken together there are only few if any frequently occurring non-synonymous SNPs within the coding region of the CHRM1. While no robust pattern of genotype/

phenotype relationships is emerging, it is noteworthy that two SNPs upstream of the coding sequence are not only associated with asthma but also with a change in promoter activity. However, this needs to be confirmed in an independent sample.

3 M_2 Receptors

The human M_2 receptor gene (CHRM2) is located on chromosome 7q31-35 and consists of six exons, the last of which contains the entire coding region, as well as a large 5′UTR encoded by multiple exons that can be alternatively spliced (Wang et al. 2004). A splice site is present 46 bp upstream from the start codon (Fenech et al. 2004). The gene has multiple transcription start sites. While an early study with brains from 15 dementia and control patients did not detect gene variants of the CHRM2 (Ohara et al. 1994), later studies including one based on sequencing of 46 control subjects and 46 asthma patients identified SNPs in the coding region and 3′UTR, but the coding SNPs (T1197C and A976C) were synonymous (Fenech et al. 2001). At present the NCBI database contains >500 SNPs for this gene, and some of them are in linkage disequilibrium (Wang et al. 2004; Jones et al. 2004; Hautala et al. 2006; Cohen-Woods et al. 2009). Moreover, a multi-allelic CA tandem repeat polymorphism has been identified 96 bp downstream of the most 5′ transcription start site of the gene (Fenech et al. 2004). This CA repeat, at least in human airway smooth muscle and BEAS-2B cells, can influence transcription of the gene implying functional importance. Most studies on CHRM2 polymorphisms have focused on CNS functions, but several have also explored associations with airway, cardiovascular, and urogenital function.

Most studies regarding brain functions have explored associations with cognition and intelligence. An initial study reported an association of the A1890T SNP in the 3′UTR (rs8191992) with intelligence (Wechsler Adult Intelligence Scale-Revised) based on 828 subjects (Comings et al. 2003). The association exhibited a “gene dose–response” with homozygous T allele carriers having the highest IQ (about four points more than homozygous A allele carriers and explaining about 1% in total variation). This was detected in the overall cohort and in the male and female subgroups. A different group of investigators looking at six different SNPs found that one SNP from intron 5 (rs324650) yielded a similarly strong correlation with IQ in a study of 177 twin pairs as well as 793 members of 316 extended twin families (Gosso et al. 2006). In a follow-up study, these investigators confirmed the relevance of this locus to intelligence using a denser SNP mapping but also reported that genotype at this site did not correlate with CHRM2 mRNA expression in the brain (Gosso et al. 2007). An association of multiple SNPs in the CHRM2 with intelligence was confirmed based on the COGA (Collaborative study of the Genetics of Alcoholism) study of 2,158 individuals from 200 families involving multiple ethnic groups (Dick et al. 2007). In line with these association studies, it has been reported based on 1,337 individuals from 253 families, also derived from the COGA study, that some SNPs in the CHRM2 (rs2350786, rs8191992) associate

with theta band visual-evoked brain oscillations (Jones et al. 2004), which are considered to reflect higher cognitive processing in humans. On the other hand, a study with three cohorts from Australia (1,537 subjects from 730 families), England (758 subjects) and Scotland (2,091 subjects) looking at a range of SNPs in the M_2 receptor gene did not detect an association with cognitive function tests (Lind et al. 2009). Differences between the latter and the previous studies have been attributed to potential bias in subject selection.

An initial study based on 760 subjects reported an association of a A1890T SNP (rs8191992) in the 3′UTR of the CHRM2 with major depression in women but not men (Comings et al. 2002). An association of intronic SNPs from the CHRM2 has also been reported with alcoholism and major depressive syndrome based on the COGA study (2,310 subjects from 262 families) (Wang et al. 2004). While one of these SNPs (rs1824024) was associated with both conditions, several others were associated with one but not the other. However, a later case-control study with 1,420 cases and 1,624 controls failed to confirm an association with depression, including the rs1824024 SNP (Cohen-Woods et al. 2009). Similarly, a study with 474 subjects from 152 families did not detect an association with bipolar disorder with any of 13 SNPs including rs1824024 and rs8191992 (Shi et al. 2007). Within a single study of 406 subjects, rs8191992 did not associate with visual attention or working memory, but significantly modulated the association with an SNP in one of the nicotinic receptor subtypes (Greenwood et al. 2009). Finally, the T-allele of the rs324650 SNP was associated with bipolar but not monopolar depression and accompanied by a significantly increased expression of the receptor at the protein level in at least some brain areas as assessed in PET studies (Cannon et al. 2011). The rs324650SNP was also associated with smoking behavior/nicotine addiction in a study of 5,500 subjects (Mobascher et al. 2009) but only very weakly with alcoholism in a study of 155 subjects (Jung et al. 2011). Within the latter study, however, another SNP, rs1824024, was associated much stronger with alcoholism. Other investigators reported the rs1455858 SNP to be associated adolescent substance abuse (Hendershot et al. 2011).

A smaller number of studies have explored associations between CHRM2 polymorphisms and cardiovascular function. A study of 95 sedentary men reported that heart rate recovery following exercise, known to be under control of M_2 receptors, was associated with rs324640 and exhibited a gene dose–response curve in this regard (Hautala et al. 2006). A similar association was found with rs8191992, as expected based upon the strong linkage between the two SNPs. These authors extended their findings by demonstrating an association between cardiac mortality and these SNPs in a sample of 491 patients with a former myocardial infarction (Hautala et al. 2009).

Although M_3 receptors are the key determinant of airway tone, CHRM2 can play a role in the regulation of airway function by indirectly acting on smooth muscle tone and/or by altering ganglionic transmission. However, studies comparing present and "outgrown" asthma patients (Yamamoto et al. 2002) or asthmatic and control children (Szczepankiewicz et al. 2009) did not detect associations of CHRM2 SNPs with airway disease. Similarly, none of the four SNPs in the M_2

receptor was associated with responder status to the muscarinic antagonist tiotropium (Park et al. 2009), but another study reported a minor association with the degree of response to another muscarinic antagonist, ipratropium (Szczepankiewicz et al. 2009). While mechanistic studies had demonstrated a role for a CA repeat polymorphism in the promoter of the CHRM2 for promoter activity in human airways smooth muscle cells (Fenech et al. 2004), unfortunately no phenotypic in vivo studies have been reported for this polymorphism with regard to airway disease. However, due to a similar role of M_2 receptors in airways and urinary bladder (Michel and Parra 2008), an association between a CA repeat polymorphism in the CHRM2 promoter and lower urinary tract function has been studied (Michel et al. 2009). Within a sample of 1,015 men with voiding dysfunction attributed to benign prostatic hyperplasia, this polymorphism was not associated with any of more than 30 parameters of lower urinary tract function. Finally, an SNP (rs1993068) located close CHRM2 gene demonstrated linkage with body mass index in a genome-wide association study in both linked families and a case-control sample (Laramie et al. 2009).

In conclusion, the non-coding rs324640 from intron 5 and rs8191992 from the 3′UTR are in strong linkage (Hautala et al. 2006) and show the greatest potential to be associated with phenotypes of M_2 receptor function in the CNS and peripheral tissues. The functional role of the CA repeat polymorphism in the CHRM2 promotor also is promising. However, most of the reported associations remain controversial and/or unconfirmed. Moreover, as the two SNPs exist in distinct parts of the gene the question arises which, if any, of them is functionally relevant and which is mainly a genetic marker.

4 M_3 Receptors

The human M_3 receptor gene (CHRM3) is located on chromosome 1q43 and consists of a single exon. The NCBI database contains >1,100 SNPs in this gene, making this the apparently most polymorphic gene among the muscarinic receptors. Moreover, short tandem repeat polymorphisms were detected in the M_3 gene promoter (Donfack et al. 2003).

While screening 46 asthma patients and 46 controls for polymorphisms, no gene variants were detected in the coding region or flanking region of the CHRM3 (Fenech et al. 2001). Similarly, only one rare CHRM3 mutation was found during the sequencing of 102 asthma patients and 70 controls (Yamamoto et al. 2002). Screening 60 Caucasian and African American subjects with and without asthma, four SNPs and two short tandem repeat polymorphisms were detected in the CHRM3 promoter, but none of them was more frequent in a group of 76 asthma patients as compared to 81 controls (Donfack et al. 2003). Another study in 138 asthmatic subjects did not detect associations of five different SNPs in the CHRM3 with responder status to the muscarinic antagonist tiotropium (Park et al. 2009). One study reported that an SNP (rs2165870) located upstream of the promoter of

the CHRM3 gene was associated with the susceptibility for post-operative nausea and vomiting (Janicky et al. 2011). Thus, despite the prominent function of CHRM3 in human physiology, particularly in the airways and the bladder (Michel and Parra 2008) and the large number of polymorphisms reported for this receptor subtype, no consistent evidence for a pathophysiological relevance of these variations has been reported.

5 M_4 Receptors

The human M_4 receptor gene (CHRM4) is located on chromosome 11p12-p11.2 and consists of a single exon. The NCBI database contains only few SNPs of this gene. However, most of those within the coding region are synonymous and for none of them data have been reported linking genetic variability to phenotype.

6 M_5 Receptors

The human M_5 receptor gene (CHRM5) is located on chromosome 15q26 and consists of a single coding exon and three alternatively spliced 5′-UTRs (Anney et al. 2007). The NCBI database contains >200 SNPs of this gene. We have identified only a single study linking a polymorphism of the M_5 gene to phenotype (Anney et al. 2007). In that study, a non-coding C > T SNP (rs7162140) was reported to have a prevalence of 19% and to be associated with the number of cigarettes smoked and cannabis dependence but not with nicotine or alcohol dependence in a sample of 815 Australians of European descent.

7 Conclusions

Major progress has been made in exploring associations between muscarinic receptor subtype gene polymorphisms and physiological and pathophysiological variables. However, in most cases proposed associations remain to be confirmed in independent studies or have proven to be inconsistent. Even in cases where a considerable body of evidence supports an association between genotype and phenotype, in most cases it remains uncertain which specific chain of events connects the gene variant to a given phenotype. Muscarinic receptor polymorphisms with relevance for drug responsiveness have not been elucidated on a large scale. Thus, this research field has promise but apparently is still in its infancy.

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