Examination of Single Nucleotide Polymorphisms in Acetylcholine Receptors in Chronic Fatigue Syndrome Patients

Sonya Marshall-Gradisnik[1,2], Peter Smith[2], Bernd Nilius[3] and Donald R. Staines[2]

[1]School of Medical Science, Griffith University, Gold Coast, Australia. [2]The National Centre for Neuroimmunology and Emerging Diseases, Menzies Health Institute, Griffith University, Gold Coast, Australia. [3]Department of Cellular and Molecular Medicine, Laboratory of Ion Channel Research, KU Leuven, Belgium.

ABSTRACT

OBJECTIVE: Chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME) is a disorder characterized by debilitating fatigue accompanied by pain and impairments in memory, cognition, and concentration. Acetylcholine (ACh) has a plethora of roles in neuronal and neuromuscular transmission. There are two types of ACh receptors, muscarinic and nicotinic, comprising 17 different subunits of the nicotinic ACh receptor (nAChR) and five different subtypes of the muscarinic receptor (mAChR) that have been identified in humans. The purpose of this study was to determine the role of ACh receptor (nAChRs and mAChRs) single nucleotide polymorphisms (SNPs) in CFS/ME patients.

METHODS: One-hundred and fifteen CFS/ME patients (age = 48.68 ± 1.06 years) and 90 nonfatigued controls (age = 46.48 ± 1.22 years) participated in this study, where CFS/ME patients were defined according to the 1994 Center for Disease Prevention and Control (CDC) criteria. A total of 464 SNPs for nine mammalian ACh receptor genes (*M1, M2, M3, M4, M5, alpha 2, 5, 7,* and *10*) were examined via the Agena Biosciences iPLEX Gold assay. Statistical analysis was performed using the PLINK analysis software.

RESULTS: Seventeen SNPs were significantly associated with CFS/ME patients compared with the controls. Nine of these SNPs were associated with *mAChRM3* (rs4463655; $P = 0.00281$, rs589962; $P = 0.00348$, rs1072320; $P = 0.00371$, rs7543259; $P = 0.00513$, rs6661621; $P = 0.00536$ rs7520974; $P = 0.0167$, rs726169; $P = 0.02349$, rsrs6669810; $P = 0.02361$, rsrs6429157; $P = 0.0375$), while the remainder were associated with *nAChR alpha 10* (rs2672211; $P = 0.0107$, rs2672214; $P = 0.0108$, rs2741868; $P = 0.01185$, rs2741870; $P = 0.01281$, rs2741862; $P = 0.03043$), *alpha 5* (rs951266; $P = 0.01153$; rs7180002, $P = 0.03682$), and *alpha 2* (rs2565048; $P = 0.01403$).

CONCLUSION: The data from this pilot study suggest an association between ACh receptors, predominantly M3 and CFS. ACh receptor SNPs may contribute to the pathomechanism of CFS/ME.

KEYWORDS: chronic fatigue syndrome, acetylcholine receptor, single nucleotide polymorphisms

CITATION: Marshall-Gradisnik et al. Examination of Single Nucleotide Polymorphisms in Acetylcholine Receptors in Chronic Fatigue Syndrome Patients. *Immunology and Immunogenetics Insights* 2015:7 7–20 doi:10.4137/III.S25105.

RECEIVED: February 18, 2015. **RESUBMITTED:** May 6, 2015. **ACCEPTED FOR PUBLICATION:** May 6, 2015.

ACADEMIC EDITOR: Ignacio J. Dávila, editorial board member

TYPE: Original Research

FUNDING: This study was supported by funding from the Alison Hunter Memorial Foundation and the Mason Foundation. The authors confirm that the funder had no influence over the study design, content of the article, or selection of this journal.

COMPETING INTERESTS: SM-G, DRS and PS have a provisional patent pending on diagnostic methods. BN discloses no competing interests.

CORRESPONDENCE: s.marshall-gradisnik@griffith.edu.au

Paper subject to independent expert blind peer review by minimum of two reviewers. All editorial decisions made by independent academic editor. Upon submission manuscript was subject to anti-plagiarism scanning. Prior to publication all authors have given signed confirmation of agreement to article publication and compliance with all applicable ethical and legal requirements, including the accuracy of author and contributor information, disclosure of competing interests and funding sources, compliance with ethical requirements relating to human and animal study participants, and compliance with any copyright requirements of third parties. This journal is a member of the Committee on Publication Ethics (COPE).

Published by Libertas Academica. Learn more about this journal.

Introduction

The physiological functions of acetylcholine (ACh) are mediated by two membrane proteins, namely, the muscarinic (mAChR) and nicotinic (nAChR) receptors. Both receptor types have several subtypes and are located in the central and peripheral nervous systems, including the autonomic nervous system. ACh also performs functions in smooth muscle, sweat glands, β pancreatic cells, glial cells, lymphocytes, ocular lens cells, brain vascular endothelium,[1] and the central nervous system (CNS).[2–6] The degradation of ACh into choline and acetate is catalyzed by the enzyme acetylcholinesterase (AChE).[7,8]

There are five main mAChR subtypes: M1, M2, M3, M4, and M5, where M2 and M4 are inhibitory receptors, and M1, M2, and M3 are stimulatory receptors.[7,8] Once activated, each subtype has distinctive functions. Activation of M1, M3, and M5 receptors results in increased intracellular calcium signaling. In contrast, activated M2 and M4 receptors inhibit adenylate cyclase activity and mediate the function of nonselective cation channels, transient receptor potential channels, and potassium channels.[7–10]

nAChRs are fast ionotropic cationic nicotinic receptor channels that allow the influx of cations such as potassium, calcium, and sodium ions into the cell. Depending upon the combinational subunit binding, AChRs can form either heteromers or homomers.[11]

Chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME) is a disorder characterized by debilitating fatigue accompanied by impairments in memory, cognition, and concentration as well as pain and dysregulation of the gastrointestinal, cardiovascular, and immune systems.[12–29]

Importantly, peripheral cholinergic function is noted to be abnormal in CFS/ME patients exposed to ACh challenge,

whereby blood flow peaks take a longer time to return to normal. Increased sensitivity to ACh is noted in peripheral vascular endothelium.[30,31] ACh influences immune cell function[32] and is manufactured and secreted by a wide range of immune cells including lymphocytes.[32–35] We, along with others, have reported changes in immune cell phenotype and function as well as noting cardiac and neurological effects in disease.[14–16,18–20,22,24,26,27,29] Additionally, we have reported single nucleotide polymorphisms (SNPs) in transient receptor potential (TRP) ion channels in CFS/ME patients.[36] SNPs occur in the coding sequences, noncoding regions, or intergenic regions of genes, where these SNPs, depending upon their location and the influence of additional splicing mechanisms, may lead to human disease.[10,11,14–16,18–20,22,24,26,27,29] Given these collective reports and the role of TRPs and AChRs in cellular functions, the aim of the current study was to examine SNPs in AChRs in the same cohort of CFS/ME patients in whom we had previously reported TRP SNP anomalies.

Methodology

Subjects. CFS patients were defined in accordance with the 1994 Center for Disease Prevention and Control (CDC) criteria for CFS.[37] A total of 115 CFS patients and 90 nonfatigued controls were recruited for this study, and a volume of 10 mL of whole blood was collected from all participants in EDTA tubes. Written consent was obtained from all participants prior to the collection of samples and this research was approved by Griffith University Human Research Ethics Committee (MSC/18/13/HREC) in accordance with the National Human Research Ethical Standards and in accordance with the Declaration of Helsinki.

DNA extraction. Genomic DNA was extracted from all whole-blood samples using the Qiagen DNA blood mini-kit as per manufacturer's instructions (Qiagen). Quality and quantity of the DNA extracted were determined by the The Nanodrop (Nanodrop), where approximately 2 μg of genomic DNA was used to perform the SNP assay.

SNP genotyping studies. Geneworks completed the SNP analysis as previously defined (MassARRAY iPLEX Gold Assay).[38] Customized assays were developed for 464 SNPs across the nine mammalian acetylcholine receptor genes (*M1, M2, M3, M4, M5, alpha 2, 5, 7,* and *10*), which were selected because of the locations of these receptors in the central and peripheral nervous system, endocrine system, smooth muscle cells, β pancreatic cells, glial cells, lymphocytes, ocular lens cells, and brain vascular endothelium.[1–6] Primers and extension primers were created for each of the SNPs using the Assay Designer[38] according to the manufacturer's instructions. The amplification of the DNA was done as previously described.[39] Briefly, DNA was amplified via polymerase chain reaction (PCR) under the following conditions: 94°C for 2 minutes, 94°C for 30 seconds, 56°C for 30 seconds, and 72°C for 1 minute. Amplification products were then treated with shrimp alkaline phosphatase (SAP) at 37°C for 40 minutes, 85°C for 5 minutes reaction, and a final incubation at 4°C. Extension primers were optimized to control the signal-to-noise ratio where unextended primers (UEPs) were examined on the spectroCHIP and evaluated in Typer 4.0 to enable the division into low-mass, medium-mass, and high-mass UEPs. To perform the iPLEX extension reaction, a mixture containing iPLEX Gold reaction was prepared using iPLEX Gold Buffer Plus, iPLEX termination mix, iPLEX enzyme, and primer mix. The iPLEX reaction was cycled at an initial denaturation of 94°C for 30 seconds, annealing at 52°C for 5 minutes, extension at 80°C for 5 minutes (5 cycles of annealing and extension were performed, but the whole reaction was performed in 40 cycles), and extension again at 72°C for 3 minutes. Resin beads were used to rinse all iPLEX Gold reaction products. Following iPLEX Gold reaction, MassARRAY was performed using the MassARRAY mass spectrometer, and the data generated were analyzed using the TyperAnalyzer software.

Statistical analysis. The PLINK v1.07[40] whole-genome analysis toolset was implemented to determine the association between the CFS patients and the nonfatigued control group. A two-column χ^2 test was used, where the alpha level of significance was set at a P-value of <0.05.

Results

Participants. A total of 115 CFS patients (age = 48.68 ± 1.06 years) comprising 84 (73.04%) women and 31 (26.96%) men were recruited. The 90 nonfatigued controls (age = 46.48 ± 1.22 years) comprised 59 (65.56%) women and 31 (34.44%) men. All participants in both groups were of European descent. All participants were aged between 25 and 65 years and some of them were excluded following any symptoms and their replies in the affirmative to a medical questionnaire comprising whether they were taking any medications; showing signs of a comorbid chronic illness such as cardiovascular disease, diabetes, or autoimmune diseases; had been previously diagnosed with psychosis or epilepsy; or were pregnant, breastfeeding, or smokers. Furthermore, the nonfatigued controls were excluded if they reported symptoms of fatigue after completing the medical and symptom questionnaires.[15,17] All were residents of Australia at the time of blood collection.

SNP association studies. Of the 464 SNPs examined in the present study, 393 were successfully identified in both participants groups. Of the 393, seventeen were observed to be significantly associated with CFS (Table 1). Nine of these SNPs were associated with *mAChRM3* (rs4463655; $P = 0.00281$, rs589962; $P = 0.00348$, rs1072320; $P = 0.00371$, rs7543259; $P = 0.00513$, rs6661621; $P = 0.00536$, rs7520974; $P = 0.0167$, rs726169; $P = 0.02349$, rsrs6669810; $P = 0.02361$, rsrs6429157; $P = 0.0375$), while the remainder were associated with *nAChR alpha 10* (rs2672211; $P = 0.0107$, rs2672214; $P = 0.0108$, rs2741868; $P = 0.01185$, rs2741870; $P = 0.01281$, rs2741862; $P = 0.03043$), *alpha 5* (rs951266; $P = 0.01153$; rs7180002, $P = 0.03682$), and *alpha 2* (rs2565048; $P = 0.01403$). A summary of the SNPs that were not significant is given in Supplementary Table 1.

Table 1. Analysis of the frequency distribution and significance of acetylcholine receptor (AChR) single nucleotide polymorphisms (SNPs) in CFS patients and nonfatigued controls in rank order of significance.

GENE	CHR	RefSNPID	BP	A1	FREQUENCY_A	FREQUENCY_U	A2	χ^2	*P*-VALUE
mAchM3	1	rs4463655	239820994	T	0.3077	0.4671	C	8.932	0.00281*
mAchM3	1	rs589962	239826664	C	0.2416	0.3919	T	8.539	0.00348*
mAchM3	1	rs1072320	239819076	G	0.3242	0.1842	A	8.423	0.00371*
mAchM3	1	rs7543259	239815886	A	0.3187	0.1842	G	7.834	0.00513*
mAchM3	1	rs6661621	239821503	C	0.3022	0.1711	G	7.755	0.00536*
nAchα10	11	rs2672211	3669048	C	0.3736	0.2434	T	6.515	0.0107*
nAchα10	11	rs2672214	3670282	C	0.3708	0.24	T	6.498	0.0108*
nAchα5	15	rs951266	78586199	T	0.3944	0.2632	C	6.382	0.01153*
nAchα10	11	rs2741868	3668953	T	0.3693	0.24	A	6.333	0.01185*
nAchα10	11	rs2741870	3668879	G	0.3708	0.2434	C	6.195	0.01281*
nAchα2	8	rs2565048	27472615	C	0.0989	0.1933	T	6.034	0.01403*
mAchM3	1	rs7520974	239903960	G	0.4205	0.5533	A	5.727	0.0167*
mAchM3	1	rs726169	239630977	G	0.2833	0.4013	A	5.132	0.02349*
mAchM3	1	rs6669810	239905329	G	0.4213	0.5467	C	5.123	0.02361*
nAchα10	11	rs2741862	3666755	C	0.2857	0.1842	T	4.685	0.03043*
nAchα5	15	rs7180002	78581651	T	0.3846	0.2763	A	4.359	0.03682*
mAchM3	1	rs6429157	239818343	G	0.522	0.4079	A	4.327	0.0375*
nAchα2	8	rs55828312	42734459	G	0.2386	0.1513	A	3.914	0.04789
nAchα5	15	rs2175886	32063744	C	0.4944	0.3867	T	3.847	0.04982
mAchM3	1	rs12036141	239902696	A	0.4121	0.3092	G	3.781	0.05184
mAchM3	1	rs6429147	239631494	C	0.4444	0.34	G	3.728	0.05349
mAchM3	1	rs1594513	239848453	G	0.2198	0.3133	T	3.722	0.05371
nAchα2	8	rs16891561	42724596	T	0.2472	0.1597	C	3.696	0.05454

Notes: SNP, single nucleotide polymorphism with 115 CFS/ME patients and 90 controls. Data presented is included for $P \leq 0.05$. Data presented for gene, chromosome location for muscarinic M3, nicotinic alpha 2, 5, and 10, (CHR), reference SNP identification (RefSNPID), base pair (BP) location of SNP, alleles (A1 and A2), Allelic Frequency A (Frequency_A) of this allele in CFS cases, Frequency U (Frequency_U) of this allele in controls, Chi-square (χ^2) for basic allelic test (1df), and *P*-value for this test set at a significance of $P < 0.05$.

Discussion

This pilot study revealed a number of AChR SNP variations in CFS/ME patients. Specifically, within the coding sequences of nine AChR genes out of 464 SNPs examined, 17 significant alleles were found associated with CFS/ME patients compared to the nonfatigued controls. Moreover, these alleles were located in the gene sequence of one of the muscarinic acetylcholine receptors (mAChRM3) and three nicotinic acetylcholine alpha receptors (nAChRα2, nAChRα5, and nAChRα10). Interestingly, in our previous study, we identified a number of SNPs in the TRP family, namely, TRPM3. The importance of SNPs in mAChRM3 and TRPM3 is that the latter couples with mAChRM3 and can be activated by ACh.[41–43]

There is limited information available on the role of these AChR SNPs. However, alternate splicing in the noncoding sequences may have significant, unexpected outcomes on the splicing mechanism of the gene transcripts.[44,45] Splicing genetic variants that are intronic variants play a role in alternative splicing mechanisms, resulting in diverse protein isoforms. Incidentally, the human gene has the largest average number of mRNA isoforms per gene,[46] with an average of seven isoforms.[47,48] Additionally, enhancers and silencers that are located in the introns are integral to the recognition of the correct exon sequence[49] for correct protein coding. Also, introns have been documented to generate active spliceosomes, giving rise to alternative splicing events.[50,51] Importantly, our data show that the proportion of intron variants in the SNP has been previously reported to be associated with smooth muscle and respiratory function (rs4463655, rs589962, rs1072320, rs7543259, and rs6661621), neurocognitive function (rs951266), neuropathic pain (rs2741868), and gastric function (rs7520974).[52,53] Additionally, in the current study, we report SNP rs6669810 in CFS/ME patients, which is responsible for the gene *ART1*, an ADP-ribosyltransferase that catalyzes the ADP-ribosylation of arginine residues in proteins. Mono-ADP-ribosylation is a posttranslational modification of proteins affected by a variety of bacterial toxins, including cholera, pertussis, and heat-labile enterotoxins of *Escherichia coli*. Interestingly, previous researchers have reported increases in viral loads, respiratory-associated

illnesses, neurocognitive changes, and neuropathic pain from CFS/ME patients.[54–57]

SNPs were found in mAChR receptors, and are responsible for initiating smooth muscle contraction, such as in the gastrointestinal and genitourinary tracts, as well as effects in immune cells, epithelial, ovarian, and ocular skin cells, and respiratory and secretory glands.[5,32–35,58–67] Additionally, nAChRs are reported on T and B lymphocytes.[68,69] Human T lymphocytes express the α3, α4, α7, β2, and β4 receptor subunits,[70] while in the mouse and human thymus mAChR expression has been found to play a role in T-lymphocyte development and proliferation.[68,71–73] The α4 or α7 subunits have also been reported on B lymphocytes and found to stimulate proliferation while decreasing antibody production.[74] Such findings provide possible insight regarding the SNPs found in the current paper, noting that previous investigations have reported compromise to immune function in CFS/ME patients. Importantly, changes in numbers and function of lymphocytes, such as natural killer (NK) lymphocytes and T and B lymphocytes, in these studies suggest increased influx of Ca^{2+}.

The mAChRM3 receptors are located in the gastrointestinal tract and are controlled in part by the parasympathetic nervous system through the vagus nerve.[75] Moreover, clinical data report that nAChRs are involved in inflammatory bowel disease.[76] CFS/ME patients often exhibit gastrointestinal-associated issues, such as irritable bowel syndrome and constipation.[12,28]

Dysregulation of mAChRM3 receptors may affect metabolic and cardiac responses. In normal pancreas, mAChRM3 receptors play a role in regulating insulin and glucagon secretion.[77,78] Muscarinic ACh receptors expressed by pancreatic β cells have been reported to play a significant role in maintaining proper insulin release and whole-body glucose homeostasis.[77] Changes in Ca^{2+}-mediated channels may result in adverse glucose metabolic outcomes as implied in CFS/ME patients.[79] Pancreatic β cells rely on transient changes in Ca^{2+} to initiate the complex sequence of events resulting in insulin secretion following glucose exposure. Thus, it may be argued that aberrant intracellular Ca^{2+} concentrations through permissive AChR activity will impede the usual and necessary sequence of events required to initiate insulin response to glucose in CFS patients.

Cardiac mAChRM3 receptors perform an array of pathological and physiological functions. mAChM2 is not the only muscarinic receptor involved in cardiac function. Rather, mAChRM3 parasympathetic control of cardiac function is well established.[48] A report by van Borren et al[80] has shown the effect of muscarinic AChR stimulation on Ca^{2+} transients, cAMP production, and pacemaker frequency in sinoatrial (SA) nodes of the rabbit. These findings imply that muscarinic agonism will have an effect on SA node function, exacerbating disturbances of proper cardioregulatory mechanisms, particularly in an environment where Ca^{2+} intracellular concentrations are likely to be altered due to the direct effects of receptor activity. Clinical consequences such as altered orthostatic cardiovascular responses could be predicted and could align with symptom presentation in CFS/ME.[13,21,25,27,29]

In the vascular system, the endothelium contains nAChRs, including α3, α5, α7, α10, β2, β3, and β4.[63,81,82] Depending upon the type of smooth muscle, a specific subtype of nAChR is present: α3 and α5 are found in arteries, while α7 is widespread although not present in the renal circulatory system. nAChR α5, α7, β2, and β3 have been found in brain endothelial cells[83] and are an important component of the blood–brain barrier. This study identified SNPs in the α5 and α3 nAChR subunits, implying anomalies of signal transduction in our patient cohort. nAChRs are reported to be involved in arousal, sleep, and fatigue as well as those functions that are responsible for processing of pain, memory, and cognition.[84–86]

Conclusion

In conclusion, we report for the first time the presence of SNPs in receptors for ACh in patients with CFS/ME. Many detrimental consequences for physiological homeostasis are possible through aberrant AChR function in these patients. These scenarios require further investigation to establish whether the AChR SNPs identified in this study result in changes of function and conceivably may be associated with CFS/ME pathomechanisms and symptomatology.

Acknowledgments

The authors would like to thank Dr Lavinia Gordon, Australian Genome Research Facility, Melbourne, Australia, for completing the bioinformatics SNP analysis.

Author Contribution

Designed and developed experiments: SMG, PS, BN, DRS. Completed the analysis, revisions, and final preparation of the manuscript: SMG, DRS. All authors reviewed and approved of the final manuscript.

REFERENCES

1. Beckmann J, Lips KS. The non-neuronal cholinergic system in health and disease. *Pharmacology*. 2013;92(5–6):286–302.
2. Elhusseiny A, Hamel E. Muscarinic—but not nicotinic—acetylcholine receptors mediate a nitric oxide-dependent dilation in brain cortical arterioles: a possible role for the M5 receptor subtype. *J Cereb Blood Flow Metab*. 2000;20(2):298–305.
3. Felder CC, Bymaster FP, Ward J, DeLapp N. Therapeutic opportunities for muscarinic receptors in the central nervous system. *J Med Chem*. 2000;43(23):4333–4353.
4. Oki T, Takagi Y, Inagaki S, et al. Quantitative analysis of binding parameters of [3H]N-methylscopolamine in central nervous system of muscarinic acetylcholine receptor knockout mice. *Brain Res Mol Brain Res*. 2005;133(1):6–11.
5. Sharma G, Vijayaraghavan S. Nicotinic cholinergic signaling in hippocampal astrocytes involves calcium-induced calcium release from intracellular stores. *Proc Natl Acad Sci U S A*. 2001;98(7):4148–4153.
6. Wess J, Duttaroy A, Zhang W, et al. M1–M5 muscarinic receptor knockout mice as novel tools to study the physiological roles of the muscarinic cholinergic system. *Receptors Channels*. 2003;9(4):279–290.
7. Lanzafame AA, Christopoulos A, Mitchelson F. Cellular signaling mechanisms for muscarinic acetylcholine receptors. *Receptors Channels*. 2003;9(4):241–260.

8. Wess J. Molecular biology of muscarinic acetylcholine receptors. *Crit Rev Neurobiol*. 1996;10(1):69–99.
9. Veldhuis NA, Poole DP, Grace M, McIntyre P, Bunnett NW. The G protein-coupled receptor-transient receptor potential channel axis: molecular insights for targeting disorders of sensation and inflammation. *Pharmacol Rev*. 2015;67(1): 36–73.
10. Nilius B, Szallasi A. Transient receptor potential channels as drug targets: from the science of basic research to the art of medicine. *Pharmacol Rev*. 2014;66(3): 676–814.
11. Hurst R, Rollema H, Bertrand D. Nicotinic acetylcholine receptors: from basic science to therapeutics. *Pharmacol Ther*. 2013;137(1):22–54.
12. Aaron LA, Burke MM, Buchwald D. Overlapping conditions among patients with chronic fatigue syndrome, fibromyalgia, and temporomandibular disorder. *Arch Intern Med*. 2000;160(2):221–227.
13. Allen J, Murray A, Di Maria C, Newton JL. Chronic fatigue syndrome and impaired peripheral pulse characteristics on orthostasis—a new potential diagnostic biomarker. *Physiol Meas*. 2012;33(2):231–241.
14. Brenu EW, Ashton KJ, van Driel M, et al. Cytotoxic lymphocyte microRNAs as prospective biomarkers for chronic fatigue syndrome/myalgic encephalomyelitis. *J Affect Disord*. 2012;141(2–3):261–269.
15. Brenu EW, Huth TK, Hardcastle SL, et al. Role of adaptive and innate immune cells in chronic fatigue syndrome/myalgic encephalomyelitis. *Int Immunol*. 2014; 26(4):233–242.
16. Brenu EW, Staines DR, Baskurt OK, et al. Immune and hemorheological changes in chronic fatigue syndrome. *J Transl Med*. 2010;8:1.
17. Brenu EW, van Driel ML, Staines DR, et al. Longitudinal investigation of natural killer cells and cytokines in chronic fatigue syndrome/myalgic encephalomyelitis. *J Transl Med*. 2012;10:88.
18. Brenu EW, van Driel ML, Staines DR, et al. Immunological abnormalities as potential biomarkers in chronic fatigue syndrome/myalgic encephalomyelitis. *J Transl Med*. 2011;9:81.
19. DeLuca J, Johnson SK, Beldowicz D, Natelson BH. Neuropsychological impairments in chronic fatigue syndrome, multiple sclerosis, and depression. *J Neurol Neurosurg Psychiatry*. 1995;58(1):38–43.
20. Grafman J, Schwartz V, Dale JK, Scheffers M, Houser C, Straus SE. Analysis of neuropsychological functioning in patients with chronic fatigue syndrome. *J Neurol Neurosurg Psychiatry*. 1993;56(6):684–689.
21. Hoad A, Spickett G, Elliott J, Newton J. Postural orthostatic tachycardia syndrome is an under-recognized condition in chronic fatigue syndrome. *QJM*. 2008; 101(12):961–965.
22. Joyce E, Blumenthal S, Wessely S. Memory, attention, and executive function in chronic fatigue syndrome. *J Neurol Neurosurg Psychiatry*. 1996;60(5):495–503.
23. Klimas NG, Salvato FR, Morgan R, Fletcher MA. Immunologic abnormalities in chronic fatigue syndrome. *J Clin Microbiol*. 1990;28(6):1403–1410.
24. Krupp LB, Sliwinski M, Masur DM, Friedberg F, Coyle PK. Cognitive functioning and depression in patients with chronic fatigue syndrome and multiple sclerosis. *Arch Neurol*. 1994;51(7):705–710.
25. Lewis I, Pairman J, Spickett G, Newton JL. Clinical characteristics of a novel subgroup of chronic fatigue syndrome patients with postural orthostatic tachycardia syndrome. *J Intern Med*. 2013;273(5):501–510.
26. McDonald E, Cope H, David A. Cognitive impairment in patients with chronic fatigue: a preliminary study. *J Neurol Neurosurg Psychiatry*. 1993;56(7):812–815.
27. Medow MS, Stewart JM. The postural tachycardia syndrome. *Cardiol Rev*. 2007; 15(2):67–75.
28. Nisenbaum R, Reyes M, Mawle AC, Reeves WC. Factor analysis of unexplained severe fatigue and interrelated symptoms: overlap with criteria for chronic fatigue syndrome. *Am J Epidemiol*. 1998;148(1):72–77.
29. Ocon AJ, Medow MS, Taneja I, Clarke D, Stewart JM. Decreased upright cerebral blood flow and cerebral autoregulation in normocapnic postural tachycardia syndrome. *Am J Physiol Heart Circ Physiol*. 2009;297(2):H664–H673.
30. Khan F, Kennedy G, Spence VA, Newton DJ, Belch JJ. Peripheral cholinergic function in humans with chronic fatigue syndrome, Gulf War syndrome and with illness following organophosphate exposure. *Clin Sci*. 2004;106(2): 183–189.
31. Spence VA, Khan F, Kennedy G, Abbot NC, Belch JJ. Acetylcholine mediated vasodilatation in the microcirculation of patients with chronic fatigue syndrome. *Prostaglandins Leukot Essent Fatty Acids*. 2004;70(4):403–407.
32. Kawashima K, Fujii T, Moriwaki Y, Misawa H. Critical roles of acetylcholine and the muscarinic and nicotinic acetylcholine receptors in the regulation of immune function. *Life Sci*. 2012;91(21–22):1027–1032.
33. Kawashima K, Fujii T. Extraneuronal cholinergic system in lymphocytes. *Pharmacol Ther*. 2000;86(1):29–48.
34. Kawashima K, Fujii T. The lymphocytic cholinergic system and its biological function. *Life Sci*. 2003;72(18–19):2101–2109.
35. Sato KZ, Fujii T, Watanabe Y, et al. Diversity of mRNA expression for muscarinic acetylcholine receptor subtypes and neuronal nicotinic acetylcholine receptor subunits in human mononuclear leukocytes and leukemic cell lines. *Neurosci Lett*. 1999;266(1):17–20.
36. Marshall-Gradisnik SM, Staines DR, Smith P, Nilius B, Ramos SB, Brenu EW. Examination of single nucleotide polymorphisms in transient receptor potential ion channels in chronic fatigue syndrome. *Immunol Immunogenet Insights*. 2015:7;1–6.
37. Fukuda K, Straus SE, Hickie I, Sharpe MC, Dobbins JG, Komaroff A. The chronic fatigue syndrome: a comprehensive approach to its definition and study. International Chronic Fatigue Syndrome Study Group. *Ann Intern Med*. 1994;121(12): 953–959.
38. Oeth P, Beaulieu M, Park C, et al. iPLEX™ assay: increased plexing efficiency and flexibility for MassARRAY® system through single base primer extension with mass-modified terminators. Sequenom Application Note. 2005:8876-006.
39. Jurinke C, Denissenko MF, Oeth P, Ehrich M, van den Boom D, Cantor CR. A single nucleotide polymorphism based approach for the identification and characterization of gene expression modulation using MassARRAY. *Mutat Res*. 2005;573(1–2):83–95. Review.
40. Harvard. PLINK whole genome association analysis toolset. 2014. Available at: http://pngu.mgh.harvard.edu/purcell/pink/
41. Phelan KD, Shwe UT, Abramowitz J, et al. Canonical transient receptor channel 5 (TRPC5) and TRPC1/4 contribute to seizure and excitotoxicity by distinct cellular mechanisms. *Mol Pharmacol*. 2013;83(2):429–438.
42. Phelan KD, Mock MM, Kretz O, et al. Heteromeric canonical transient receptor potential 1 and 4 channels play a critical role in epileptiform burst firing and seizure-induced neurodegeneration. *Mol Pharmacol*. 2012;81(3):384–392.
43. von Spiczak S, Muhle H, Helbig I, et al. Association study of TRPC4 as a candidate gene for generalized epilepsy with photosensitivity. *Neuromolecular Med*. 2010;12(3):292–299.
44. Cartegni L, Krainer AR. Correction of disease-associated exon skipping by synthetic exon-specific activators. *Nat Struct Biol*. 2003;10(2):120–125.
45. Pagani F, Baralle FE. Genomic variants in exons and introns: identifying the splicing spoilers. *Nat Rev Genet*. 2004;5(5):389–396.
46. Ensembl. Human assembly and gene annotation. 2015. Available at: http://uswest.ensembl.org/Homo_sapiens/Info/Annotation. Accessed April 17, 2015.
47. Pan Q, Shai O, Lee LJ, Frey BJ, Blencowe BJ. Deep surveying of alternative splicing complexity in the human transcriptome by high-throughput sequencing. *Nat Genet*. 2008;40(12):1413–1415.
48. Wang H, Lu Y, Wang Z. Function of cardiac M3 receptors. *Auton Autacoid Pharmacol*. 2007;27(1):1–11.
49. Fedor MJ. Alternative splicing minireview series: combinatorial control facilitates splicing regulation of gene expression and enhances genome diversity. *J Biol Chem*. 2008;283(3):1209–1210.
50. Crawford DJ, Hoskins AA, Friedman LJ, Gelles J, Moore MJ. Single-molecule colocalization FRET evidence that spliceosome activation precedes stable approach of 5′ splice site and branch site. *Proc Natl Acad Sci U S A*. 2013;110(17): 6783–6788.
51. Shcherbakova I, Hoskins AA, Friedman LJ, et al. Alternative spliceosome assembly pathways revealed by single-molecule fluorescence microscopy. *Cell Rep*. 2013; 5(1):151–165.
52. Rigbi A, Kanyas K, Yakir A, et al. Why do young women smoke? V. Role of direct and interactive effects of nicotinic cholinergic receptor gene variation on neurocognitive function. *Genes Brain Behav*. 2008;7(2):164–172.
53. Ruggieri MR, Braverman AS. Gastric body cholinergic contractile signal transduction in M2 and M3 receptor knockout mice. *J Recept Signal Transduct Res*. 2013;33(4):249–254.
54. Meeus M, Nijs J. Central sensitization: a biopsychosocial explanation for chronic widespread pain in patients with fibromyalgia and chronic fatigue syndrome. *Clin Rheumatol*. 2007;26(4):465–473.
55. Ocon AJ, Messer ZR, Medow MS, Stewart JM. Increasing orthostatic stress impairs neurocognitive functioning in chronic fatigue syndrome with postural tachycardia syndrome. *Clin Sci*. 2012;122(5):227–238.
56. Smith AP, Thomas M, Borysiewicz L, Llewelyn M. Chronic fatigue syndrome and susceptibility to upper respiratory tract illness. *Br J Health Psychol*. 2010;4(4): 327–335.
57. Suhadolnik RJ, Reichenbach NL, Hitzges P, et al. Upregulation of the 2-5A synthetase/RNase L antiviral pathway associated with chronic fatigue syndrome. *Clin Infect Dis*. 1994;18(suppl 1):S96–S104.
58. Conti-Fine BM, Navaneetham D, Lei S, Maus AD. Neuronal nicotinic receptors in non-neuronal cells: new mediators of tobacco toxicity? *Eur J Pharmacol*. 2000;393(1–3):279–294.
59. Elwary SM, Hasse S, Schallreuter KU. M2 muscarinic acetylcholine receptor (mAchR) subtype is present in human epidermal keratinocytes in situ and in vitro. *J Invest Dermatol*. 2004;123(6):1206–1207.
60. Gahring LC, Rogers SW. Neuronal nicotinic acetylcholine receptor expression and function on nonneuronal cells. *AAPS J*. 2005;7(4):E885–E894.
61. Gupta V, Thompson EB, Stock-Novack D, et al. Efficacy of prednisone in refractory multiple myeloma and measurement of glucocorticoid receptors. A Southwest Oncology Group study. *Invest New Drugs*. 1994;12(2):121–128.
62. Kurzen H, Wessler I, Kirkpatrick CJ, Kawashima K, Grando SA. The nonneuronal cholinergic system of human skin. *Horm Metab Res*. 2007;39(2):125–135.

63. Macklin KD, Maus AD, Pereira EF, Albuquerque EX, Conti-Fine BM. Human vascular endothelial cells express functional nicotinic acetylcholine receptors. *J Pharmacol Exp Ther*. 1998;287(1):435–439.
64. Maus AD, Pereira EF, Karachunski PI, et al. Human and rodent bronchial epithelial cells express functional nicotinic acetylcholine receptors. *Mol Pharmacol*. 1998;54(5):779–788.
65. Mayerhofer A, Fritz S. Ovarian acetylcholine and muscarinic receptors: hints of a novel intrinsic ovarian regulatory system. *Microsc Res Tech*. 2002;59(6): 503–508.
66. Ndoye A, Buchli R, Greenberg B, et al. Identification and mapping of keratinocyte muscarinic acetylcholine receptor subtypes in human epidermis. *J Invest Dermatol*. 1998;111(3):410–416.
67. Parnavelas JG, Mione MC, Lavdas A. The cell lineage of neuronal subtypes in the mammalian cerebral cortex. *Ciba Found Symp*. 1995;193:41–58; discussion 9–70.
68. Fujii T, Takada-Takatori Y, Kawashima K. Regulatory mechanisms of acetylcholine synthesis and release by T cells. *Life Sci*. 2012;91(21–22):981–985.
69. Paldiharis P, Szelenyi JG, Nguyen TH, Hollan SR. Changes in the expression of the cholinergic structures of human lymphocytes-T due to maturation and stimulation. *Thymus*. 1990;16(2):119–122.
70. Benhammou K, Lee M, Strook M, et al. [H-3]nicotine binding in peripheral blood cells of smokers is correlated with the number of cigarettes smoked per day. *Neuropharmacology*. 2000;39(13):2818–2829.
71. Kuo YP, Lucero L, Michaels J, DeLuca D, Lukas RJ. Differential expression of nicotinic acetylcholine receptor subunits in fetal and neonatal mouse thymus. *J Neuroimmunol*. 2002;130(1–2):140–154.
72. Middlebrook AJ, Martina C, Chang Y, Lukas RJ, DeLuca D. Effects of nicotine exposure on T cell development in fetal thymus organ culture: arrest of T cell maturation. *J Immunol*. 2002;169(6):2915–2924.
73. Richman DP, Arnason BG. Nicotinic acetylcholine-receptor—evidence for a functionally distinct receptor on human-lymphocytes. *Proc Natl Acad Sci U S A*. 1979;76(9):4632–4635.
74. Skok MV, Kalashnik EN, Koval LN, et al. Functional nicotinic acetylcholine receptors are expressed in B lymphocyte-derived cell lines. *Mol Pharmacol*. 2003; 64(4):885–889.
75. Matteoli G, Boeckxstaens GE. The vagal innervation of the gut and immune homeostasis. *Gut*. 2013;62(8):1214–1222.
76. Richardson CE, Morgan JM, Jasani B, et al. Effect of smoking and transdermal nicotine on colonic nicotinic acetylcholine receptors in ulcerative colitis. *QJM*. 2003;96(1):57–65.
77. Gautam D, Han SJ, Hamdan FF, et al. A critical role for beta cell M3 muscarinic acetylcholine receptors in regulating insulin release and blood glucose homeostasis in vivo. *Cell Metab*. 2006;3(6):449–461.
78. Gromada J, Hughes TE. Ringing the dinner bell for insulin: muscarinic M3 receptor activity in the control of pancreatic beta cell function. *Cell Metab*. 2006; 3(6):390–392.
79. Morris G, Maes M. Mitochondrial dysfunctions in myalgic encephalomyelitis/chronic fatigue syndrome explained by activated immuno-inflammatory, oxidative and nitrosative stress pathways. *Metab Brain Dis*. 2014;29(1):19–36.
80. van Borren MM, Verkerk AO, Wilders R, et al. Effects of muscarinic receptor stimulation on Ca2+ transient, cAMP production and pacemaker frequency of rabbit sinoatrial node cells. *Basic Res Cardiol*. 2010;105(1):73–87.
81. Bruggmann D, Lips KS, Pfeil U, Haberberger RV, Kummer W. Rat arteries contain multiple nicotinic acetylcholine receptor alpha-subunits. *Life Sci*. 2003; 72(18–19):2095–2099.
82. Wang Y, Pereira EF, Maus AD, et al. Human bronchial epithelial and endothelial cells express alpha7 nicotinic acetylcholine receptors. *Mol Pharmacol*. 2001; 60(6):1201–1209.
83. Abbruscato TJ, Lopez SP, Mark KS, Hawkins BT, Davis TP. Nicotine and cotinine modulate cerebral microvascular permeability and protein expression of ZO-1 through nicotinic acetylcholine receptors expressed on brain endothelial cells. *J Pharm Sci*. 2002;91(12):2525–2538.
84. Changeux J, Edelstein SJ. Allosteric mechanisms in normal and pathological nicotinic acetylcholine receptors. *Curr Opin Neurobiol*. 2001;11(3):369–377.
85. Gotti C, Moretti M, Maggi R, et al. Alpha7 and alpha8 nicotinic receptor subtypes immunopurified from chick retina have different immunological, pharmacological and functional properties. *Eur J Neurosci*. 1997;9(6):1201–1211.
86. Hogg RC, Raggenbass M, Bertrand D. Nicotinic acetylcholine receptors: from structure to brain function. *Rev Physiol Biochem Pharmacol*. 2003;147:1–46.

Supplementary Data

Supplementary Table 1. Analysis of the frequency distribution and significance of AChR single nucleotide polymorphisms (SNPs) in CFS patients and nonfatigued controls in rank order of significance.

GENE	CHR	RefSNPID	BP	A1	A2	FREQUENCY_A	FREQUENCY_U	χ^2	*P*-VALUE
nAchα10	11	rs2672215	3670419	A	C	0.4607	0.36	3.399	0.06523
nAchα2	8	rs6474413	42695921	C	T	0.2308	0.1513	3.336	0.06778
mAchM3	1	rs10926008	239898823	G	A	0.3722	0.277	3.333	0.06792
nAchα2	8	rs2741343	27468610	C	T	0.5337	0.4324	3.317	0.06855
nAchα5	15	rs7178270	78628735	G	C	0.3571	0.4539	3.231	0.07226
nAchα5	15	rs4243084	78619330	G	C	0.3977	0.3026	3.227	0.07245
nAchα5	15	rs601079	78577237	A	T	0.3901	0.4868	3.155	0.07571
nAchα5	15	rs12911602	78599107	C	T	0.3901	0.4868	3.155	0.07574
nAchα5	15	rs588765	78573083	T	C	0.3846	0.4803	3.095	0.07854
nAchα5	15	rs680244	78578946	A	G	0.3846	0.4803	3.095	0.07857
nAchα5	15	rs6495306	78573551	G	A	0.3895	0.4863	3.01	0.08274
nAchα5	15	rs6495307	78597979	T	C	0.4111	0.5068	2.997	0.08341
mAchM3	1	rs12093821	239660948	A	G	0.489	0.3947	2.979	0.08434
mAchM3	1	rs16838637	239665050	G	A	0.4889	0.3947	2.957	0.08551
nAchα2	8	rs6997909	42705106	A	G	0.2333	0.1579	2.945	0.08617
nAchα10	11	rs2672216	3670794	C	T	0.4888	0.3947	2.934	0.08671
mAchM3	1	rs6429165	239896757	A	G	0.2473	0.1711	2.873	0.09007
nAchα2	8	rs891398	27467305	C	T	0.533	0.4392	2.872	0.09012
nAchα5	15	rs4366683	78619861	G	A	0.3956	0.4868	2.802	0.09413
nAchα2	8	rs6985052	42696176	C	T	0.2308	0.1579	2.774	0.0958
nAchα2	8	rs4950	42697490	C	T	0.2308	0.1579	2.774	0.0958
nAchα2	8	rs6474414	42705193	A	C	0.2308	0.1579	2.774	0.0958
nAchα2	8	rs6474415	42707795	G	A	0.2308	0.1579	2.774	0.0958
nAchα5	15	rs35211613	78597441	D	I	0.3889	0.48	2.771	0.09601
nAchα5	15	rs621849	78580519	G	A	0.3901	0.4803	2.745	0.09757
nAchα5	15	rs8025188	78619936	C	G	0.3901	0.4803	2.745	0.09757
nAchα2	8	rs10109429	42763247	T	C	0.05495	0.01974	2.745	0.09758
nAchα10	11	rs2231532	3671890	A	G	0.45	0.36	2.742	0.09777
nAchα5	15	rs6494211	32236220	T	A	0.3611	0.2763	2.713	0.09954
mAchM3	1	rs2083817	239670305	A	T	0.4551	0.3649	2.709	0.09976
nAchα4	20	rs3787137	63347748	G	A	0.4341	0.3467	2.629	0.1049
mAchM3	1	rs7551001	239681300	G	A	0.4833	0.3947	2.622	0.1054
nAchα5	15	rs7182583	78606868	C	G	0.3352	0.4211	2.609	0.1063
mAchM3	1	rs6690809	239810706	A	G	0.4121	0.5	2.584	0.1079
nAchα10	11	rs2741865	3670677	A	G	0.4828	0.3947	2.549	0.1104
nAchε	17	rs12602789	4902954	T	C	0.0989	0.05263	2.47	0.116
mAchM3	1	rs1867263	239644620	A	G	0.4231	0.34	2.396	0.1217
nAchα2	8	rs7845663	42753420	A	G	0.1868	0.125	2.371	0.1236
nAchα5	15	rs1878399	78619661	G	C	0.3833	0.4671	2.371	0.1236
nAchα2	8	rs7004381	42696018	A	G	0.2247	0.1579	2.342	0.1259
mAchM3	1	rs12090480	239650653	A	G	0.2667	0.3446	2.341	0.126
nAchα5	15	rs12914385	78606381	T	C	0.4341	0.3553	2.146	0.1429
nAchα2	8	rs1072003	42764858	G	C	0.1782	0.12	2.123	0.1451
mAchM3	1	rs6701181	239906887	T	C	0.4451	0.3667	2.089	0.1483

(*continued*)

Supplementary Table 1. (*Continued*)

GENE	CHR	RefSNPID	BP	A1	A2	FREQUENCY_A	FREQUENCY_U	χ^2	*P*-VALUE
mAchM3	1	rs16839070	239900650	T	A	0.2857	0.2171	2.055	0.1517
mAchM3	1	rs6692904	239653232	A	C	0.2637	0.3355	2.047	0.1525
mAchM3	1	rs6693851	239678897	C	T	0.431	0.3533	2.035	0.1537
nAchα5	15	rs3743074	78617138	C	T	0.3516	0.4276	2.017	0.1555
nAchα5	15	rs692780	78584163	G	C	0.3462	0.4211	1.972	0.1603
mAchM3	1	rs10926010	239900836	G	A	0.191	0.1333	1.969	0.1605
nAchε	17	rs4790235	4902757	A	C	0.01111	0.03333	1.946	0.163
mAchM2	7	rs17494846	136885565	G	A	0.2778	0.3487	1.936	0.1641
nAchα5	15	rs514743	78591885	T	A	0.3462	0.42	1.904	0.1676
mAchM3	1	rs10925941	239649238	A	G	0.456	0.3816	1.883	0.17
mAchM3	1	rs2165872	239663688	T	C	0.4341	0.36	1.879	0.1705
mAchM2	7	rs1424569	136884669	A	G	0.489	0.4145	1.856	0.1731
nAchα5	15	rs17408276	78589276	C	T	0.3462	0.4189	1.837	0.1753
nAchα5	15	rs615470	78593646	T	C	0.35	0.4236	1.835	0.1755
nAchα5	15	rs11637635	78584808	A	G	0.3483	0.42	1.774	0.1829
nAchα2	8	rs2304297	42753056	C	G	0.2363	0.1776	1.719	0.1898
nAchα5	15	rs3743075	78617110	A	G	0.3516	0.4211	1.688	0.1939
mAchM3	1	rs685548	239831606	T	G	0.4176	0.3487	1.659	0.1978
mAchM3	1	rs576386	239831989	C	G	0.4176	0.3487	1.659	0.1978
nAchε	17	rs12600861	7452302	A	C	0.01099	0	1.658	0.1978
nAchα5	15	rs6494212	32243752	T	C	0.2637	0.2039	1.64	0.2004
mAchM3	1	rs6700643	239635621	C	T	0.456	0.3867	1.62	0.2031
mAchM3	1	rs1867264	239681977	A	T	0.4286	0.36	1.614	0.2039
nAchα5	15	rs472054	78595652	T	C	0.3466	0.4145	1.598	0.2061
mAchM3	1	rs521328	239884722	A	G	0.3956	0.3289	1.587	0.2077
nAchα5	15	rs2869546	78615003	C	T	0.3462	0.4133	1.581	0.2086
nAchα5	15	rs660652	78595490	A	G	0.3516	0.4189	1.565	0.2109
nAchα5	15	rs12901300	78600610	A	G	0.4056	0.4737	1.555	0.2124
mAchM3	1	rs599855	239882678	A	T	0.4011	0.3355	1.526	0.2168
nAchα2	8	rs10107450	42774752	T	C	0.2253	0.1711	1.519	0.2178
mAchM3	1	rs10802810	239885325	A	G	0.3989	0.3333	1.502	0.2204
mAchM3	1	rs12057204	239643093	T	C	0.2278	0.2867	1.495	0.2214
mAchM3	1	rs10754677	239669800	A	G	0.4659	0.5333	1.473	0.2249
nAchα5	15	rs495956	78577588	G	A	0.3556	0.42	1.435	0.2309
mAchM3	1	rs10399860	239901238	G	A	0.3022	0.2434	1.434	0.2312
mAchM3	1	rs12036109	239902578	A	G	0.4396	0.375	1.427	0.2323
nAchα5	15	rs4887070	78623845	C	T	0.3333	0.3986	1.417	0.2339
nAchα2	8	rs2217732	42763303	G	A	0.2176	0.1667	1.294	0.2552
mAchM3	1	rs6701925	239648462	C	G	0.2363	0.2895	1.217	0.2699
mAchM3	1	rs485412	239776596	C	T	0.3077	0.2533	1.197	0.2739
nAchα5	15	rs11071503	32042753	T	C	0.2473	0.1974	1.184	0.2766
nAchα5	15	rs636842	33976415	C	A	0.3889	0.3311	1.174	0.2786
nAchα5	15	rs883473	32033473	T	C	0.3034	0.25	1.162	0.2812
mAchM3	1	rs12033608	239824351	A	G	0.1978	0.2467	1.145	0.2847
mAchM3	1	rs12097526	239637551	T	C	0.2418	0.2933	1.123	0.2893
mAchM3	1	rs6688537	239687288	C	A	0.4615	0.5197	1.123	0.2893
mAchM3	1	rs652349	239765167	C	T	0.3297	0.2763	1.112	0.2918

Supplementary Table 1. (*Continued*)

GENE	CHR	RefSNPID	BP	A1	A2	FREQUENCY_A	FREQUENCY_U	χ^2	*P*-VALUE
mAchM3	1	rs6703930	239761570	T	C	0.511	0.4527	1.11	0.292
nAchα5	15	rs6494223	32104256	T	C	0.4382	0.3816	1.085	0.2976
mAchM3	1	rs537277	239882649	T	A	0.3956	0.3421	1.016	0.3136
mAchM3	1	rs539364	239882940	G	A	0.3956	0.3421	1.016	0.3136
mAchM3	1	rs532718	239768318	C	A	0.3132	0.2632	1.006	0.3159
nAchα2	8	rs892413	42759235	A	C	0.1923	0.1513	0.9699	0.3247
mAchM3	1	rs3841063	154580041	D	I	0.2389	0.2867	0.9693	0.3249
mAchM3	1	rs714803	239631123	T	A	0.4945	0.4408	0.9594	0.3273
nAchα5	15	rs499167	34056137	T	G	0.2944	0.2466	0.9307	0.3347
mAchM3	1	rs536477	239882608	A	G	0.3956	0.3446	0.9084	0.3405
nAchα5	15	rs8029344	34056771	T	C	0.2967	0.25	0.9052	0.3414
nAchα5	15	rs579975	34057023	T	C	0.2967	0.25	0.9052	0.3414
mAchM3	1	rs2355237	239694224	G	A	0.5055	0.4533	0.8961	0.3438
nAchα1	2	rs2697782	232542806	C	G	0.3132	0.3618	0.8797	0.3483
nAchα2	8	rs2741337	27478381	T	A	0.2778	0.2333	0.8451	0.3579
mAchM3	1	rs2120241	239645191	T	A	0.4529	0.4014	0.8386	0.3598
nAchα4	20	rs1044396	63349782	C	T	0.4253	0.375	0.8285	0.3627
mAchM3	1	rs10802789	239669380	T	C	0.4176	0.4671	0.8244	0.3639
nAchα5	15	rs885071	32032373	A	C	0.1818	0.1447	0.8154	0.3665
mAchM2	7	rs12532094	136923178	T	C	0.3791	0.4276	0.8113	0.3677
nAchε	17	rs8834	4897899	G	A	0.3352	0.2895	0.8022	0.3704
nAchα10	11	rs142924694	3669531	D	I	0.15	0.1867	0.792	0.3735
mAchM3	1	rs12409845	239654201	T	C	0.427	0.3784	0.7917	0.3736
nAchα1	2	rs6761667	232542701	A	T	0.489	0.4408	0.7737	0.3791
mAchM3	1	rs10802815	239892999	G	A	0.4286	0.3816	0.7577	0.384
nAchα5	15	rs2651417	32299597	G	C	0.4725	0.5197	0.7385	0.3901
nAchα2	8	rs10087172	42761725	C	T	0.1868	0.1513	0.7374	0.3905
nAchα4	20	rs6090385	63360368	C	A	0.3736	0.3289	0.7237	0.3949
nAchα2	8	rs2741342	27472579	A	G	0.2363	0.2763	0.7003	0.4027
nAchα2	8	rs7819756	27473420	C	T	0.5058	0.4589	0.6958	0.4042
mAchM2	7	rs17497261	136927841	T	C	0.3846	0.4276	0.6364	0.425
mAchM3	1	rs474930	239891583	T	C	0.3077	0.3487	0.6331	0.4262
nAchα5	15	rs1909884	32297961	T	C	0.3571	0.3158	0.6327	0.4264
nAchα2	8	rs2741338	27478217	G	A	0.2747	0.2368	0.6217	0.4304
nAchα5	15	rs8035849	34058132	A	C	0.2921	0.2533	0.6155	0.4327
mAchM2	7	rs1364406	136923521	C	G	0.3736	0.4145	0.5797	0.4464
nAchα5	4	rs7669882	40348634	A	G	0.2967	0.3355	0.5792	0.4466
nAchα1	2	rs58091792	232524457	C	G	0.2802	0.2434	0.578	0.4471
mAchM3	1	rs6660775	154566078	G	C	0.08791	0.06579	0.5647	0.4524
nAchα1	2	rs1656388	232542094	G	C	0.1868	0.22	0.5622	0.4534
mAchM3	1	rs658842	239785335	A	T	0.4396	0.4803	0.5528	0.4572
nAchε	17	rs2302767	7447225	C	T	0.3407	0.3026	0.5474	0.4594
nAchε	17	rs2302762	7455542	T	C	0.3407	0.3026	0.5474	0.4594
nAchε	17	rs2302764	7456791	C	T	0.2308	0.1974	0.5464	0.4598
mAchM3	1	rs6691263	239648803	G	A	0.2473	0.2829	0.5422	0.4615
nAchα5	4	rs4469116	40335231	T	A	0.2473	0.2829	0.5422	0.4615
mAchM2	7	rs7807871	136985024	T	A	0.2582	0.2237	0.5384	0.4631

(*continued*)

Supplementary Table 1. (*Continued*)

GENE	CHR	RefSNPID	BP	A1	A2	FREQUENCY_A	FREQUENCY_U	χ^2	*P*-VALUE
nAchα4	20	rs6062900	63348773	C	T	0.1167	0.09155	0.5303	0.4665
nAchα1	2	rs1656389	232542095	A	G	0.2857	0.3224	0.5275	0.4677
mAchM3	1	rs6429152	239690837	G	A	0.4667	0.5067	0.5241	0.4691
nAchα5	15	rs8043009	78615812	C	G	0.2167	0.25	0.514	0.4734
mAchM3	1	rs644326	239777828	A	G	0.2989	0.2632	0.5102	0.475
nAchε	17	rs7210231	7454545	A	C	0.2159	0.1842	0.5099	0.4752
nAchα2	8	rs2565049	27472125	A	G	0.1374	0.1118	0.4907	0.4836
nAchα5	15	rs7177514	78615064	G	C	0.2143	0.2467	0.4885	0.4846
mAchM3	1	rs10802814	239890697	A	T	0.2747	0.3092	0.478	0.4893
nAchα5	15	rs11072768	78637136	T	G	0.1868	0.2171	0.474	0.4912
nAchα1	2	rs6433502	174760914	G	A	0.511	0.4733	0.4665	0.4946
mAchM3	1	rs525389	239769082	G	A	0.2967	0.2632	0.4609	0.4972
nAchα1	2	rs2853446	232524616	T	C	0.4231	0.46	0.455	0.5
nAchα10	11	rs55885673	62913240	T	G	0.06044	0.07895	0.4425	0.5059
nAchα10	11	rs2186410	62917945	A	G	0.06044	0.07895	0.4425	0.5059
nAchα5	15	rs7183604	78606871	T	C	0.2151	0.2466	0.4419	0.5062
nAchα5	15	rs11637890	78643077	G	C	0.3647	0.4014	0.4418	0.5063
mAchM2	7	rs7810473	136911710	G	A	0.4835	0.5197	0.4346	0.5097
nAchα2	8	rs2292974	27460874	T	C	0.4505	0.4867	0.4309	0.5115
nAchα5	4	rs4861065	40342378	C	T	0.3022	0.3355	0.4247	0.5146
nAchα2	8	rs2292975	27460908	A	C	0.4505	0.4865	0.4235	0.5152
nAchα5	15	rs6495308	78615314	C	T	0.2198	0.25	0.4226	0.5157
nAchα5	15	rs8042059	78615517	C	A	0.2198	0.25	0.4226	0.5157
nAchα5	15	rs8042374	78615690	G	A	0.2198	0.25	0.4226	0.5157
mAchM2	7	rs1424543	136947256	C	G	0.3864	0.4211	0.4081	0.5229
nAchε	17	rs12942540	4900778	C	G	0.09195	0.1133	0.4025	0.5258
mAchM3	1	rs543307	239764310	A	G	0.2944	0.2632	0.4	0.5271
nAchα5	15	rs569207	78580777	A	G	0.2143	0.2434	0.4	0.5271
mAchM3	1	rs1111249	239802010	G	A	0.4066	0.4408	0.397	0.5286
mAchM3	1	rs481036	239773282	A	G	0.3077	0.2763	0.3933	0.5306
mAchM2	7	rs6947206	136930364	C	G	0.4286	0.3947	0.3911	0.5317
mAchM3	1	rs643040	239784120	A	G	0.4396	0.4737	0.3889	0.5329
nAchα5	15	rs7175359	32283601	T	C	0.4725	0.5066	0.3844	0.5353
mAchM3	1	rs12063454	239808590	A	T	0.2527	0.2237	0.3841	0.5354
nAchα4	20	rs11698563	63360933	A	C	0.3895	0.3562	0.3755	0.5411
nAchε	17	rs3514	4898299	C	G	0.1099	0.1316	0.3696	0.5432
nAchα2	8	rs3824103	27466912	G	A	0.02198	0.01316	0.3652	0.5456
mAchM2	7	rs6944132	136885529	T	A	0.3132	0.2829	0.3623	0.5472
nAchα1	2	rs67583510	232540940	A	G	0.2588	0.2297	0.3618	0.5475
nAchα5	15	rs12440014	78634384	G	C	0.2191	0.2467	0.3475	0.5556
nAchα10	11	rs2067482	46385217	T	C	0.1538	0.1776	0.3407	0.5595
nAchα5	15	rs9920506	78638715	A	G	0.1538	0.1776	0.3407	0.5595
nAchα5	15	rs667282	78571130	C	T	0.2135	0.24	0.3276	0.5671
mAchM3	1	rs665159	239798702	T	C	0.4556	0.4868	0.3238	0.5693
mAchM2	7	rs7806357	136935405	T	C	0.3222	0.2933	0.3197	0.5718
mAchM3	1	rs2278642	239703843	T	G	0.4451	0.4145	0.3158	0.5741

Supplementary Table 1. (*Continued*)

GENE	CHR	RefSNPID	BP	A1	A2	FREQUENCY_A	FREQUENCY_U	χ^2	*P*-VALUE
mAchM3	1	rs2841040	239799881	T	G	0.456	0.4868	0.3154	0.5744
nAchα5	15	rs1948	78625057	T	C	0.3389	0.3684	0.3149	0.5747
nAchα5	4	rs6819385	40337557	A	G	0.511	0.4803	0.3128	0.5761
nAchα5	15	rs938682	78604205	C	T	0.2143	0.24	0.3109	0.5771
mAchM2	7	rs17411478	136916210	T	C	0.3846	0.4145	0.3081	0.5788
mAchM2	7	rs17412549	136928070	A	G	0.3846	0.4145	0.3081	0.5788
mAchM3	1	rs663927	239772051	A	G	0.2874	0.26	0.3026	0.5823
mAchM3	1	rs10925964	239739214	A	T	0.4444	0.4145	0.3019	0.5827
mAchM3	1	rs682355	239867099	A	G	0.4444	0.4145	0.3019	0.5827
mAchM3	1	rs4620530	239900521	T	G	0.4176	0.4474	0.2996	0.5841
mAchM3	1	rs2790328	239763515	C	T	0.2967	0.2697	0.2959	0.5864
nAchα4	20	rs11697662	63360653	C	T	0.1703	0.1933	0.2939	0.5877
nAchα5	4	rs4861323	40353798	G	A	0.1868	0.1645	0.2842	0.5941
mAchM2	7	rs1111418	136948957	C	T	0.2418	0.2171	0.2838	0.5942
mAchM2	7	rs2113550	136881786	G	A	0.3407	0.3684	0.2794	0.5971
nAchα5	4	rs10022491	40335891	T	C	0.456	0.4276	0.271	0.6027
mAchM3	1	rs6698105	239826290	G	T	0.3022	0.2763	0.2692	0.6038
mAchM3	1	rs714266	239713560	T	C	0.4121	0.44	0.2622	0.6086
mAchM2	7	rs7357341	137006706	T	C	0.3791	0.4067	0.2619	0.6088
nAchα5	15	rs28534575	78631503	G	T	0.2167	0.24	0.2536	0.6145
nAchα1	2	rs2278478	232526753	C	T	0.2816	0.2568	0.2505	0.6167
nAchα5	4	rs13105969	40341407	G	A	0.3077	0.3333	0.2487	0.618
mAchM3	1	rs12130403	154576994	C	T	0.2921	0.3176	0.2472	0.6191
nAchα1	2	rs13026409	232537797	T	C	0.2529	0.2292	0.2413	0.6233
nAchα2	8	rs2741341	27472769	C	T	0.4011	0.4276	0.2405	0.6239
mAchM3	1	rs6688548	239687338	C	A	0.467	0.4934	0.2311	0.6307
mAchM3	1	rs12406493	239689805	C	A	0.467	0.4934	0.2311	0.6307
mAchM2	7	rs17495182	136895009	C	T	0.382	0.4079	0.2299	0.6316
mAchM3	1	rs528011	239855007	A	C	0.4382	0.4122	0.224	0.6361
nAchα5	15	rs3825845	78617916	A	G	0.2088	0.2303	0.2236	0.6363
nAchε	17	rs7774	4897868	T	G	0.2967	0.2733	0.2199	0.6391
mAchM2	7	rs13246516	136908793	T	C	0.3764	0.4013	0.2143	0.6434
mAchM3	1	rs1544170	239744936	A	G	0.4725	0.4474	0.211	0.646
mAchM3	1	rs1155611	239734527	T	C	0.4506	0.4247	0.2102	0.6466
nAchε	17	rs2075763	4899390	T	C	0.05618	0.06849	0.21	0.6468
nAchα5	15	rs11637630	78607377	G	A	0.2222	0.2434	0.2079	0.6484
nAchα5	15	rs11636605	78636536	A	G	0.1868	0.2067	0.2058	0.6511
nAchα1	2	rs12463989	232530319	C	T	0.3833	0.36	0.1905	0.6625
nAchα5	15	rs8042524	33989422	C	T	0.2747	0.2961	0.1851	0.6672
nAchα5	4	rs10029313	40348130	T	G	0.3462	0.3684	0.1791	0.6722
nAchα10	11	rs2741864	3670891	T	C	0.4505	0.4737	0.1784	0.6728
mAchM3	1	rs12059546	239806797	G	A	0.25	0.2303	0.1755	0.6753
nAchα1	2	rs3828246	232533505	T	C	0.25	0.2303	0.1755	0.6753
nAchα5	15	rs4779656	34032402	G	A	0.1889	0.2067	0.1635	0.6862
mAchM3	1	rs2072661	154576404	A	G	0.2414	0.2222	0.1619	0.6874
mAchM2	7	rs4341110	137007215	C	T	0.3736	0.3947	0.1562	0.6927
nAchα5	15	rs1316971	78638168	A	G	0.1868	0.2039	0.1551	0.6937

(*continued*)

Supplementary Table 1. (*Continued*)

GENE	CHR	RefSNPID	BP	A1	A2	FREQUENCY_A	FREQUENCY_U	χ^2	*P*-VALUE
nAchα5	15	rs7179008	32302586	G	A	0.2637	0.2829	0.1534	0.6953
nAchα10	11	rs10897304	62922775	G	A	0.3132	0.3333	0.1528	0.6959
nAchα5	15	rs511422	33990781	C	T	0.3989	0.42	0.1503	0.6982
nAchα1	2	rs4973537	232527255	G	A	0.3889	0.3684	0.1466	0.7018
nAchα5	15	rs11636753	78636604	T	G	0.3833	0.4041	0.1459	0.7025
mAchM3	1	rs1431719	239717903	G	A	0.4286	0.4079	0.1454	0.7029
mAchM3	1	rs988231	239696190	C	T	0.4725	0.4934	0.1448	0.7035
nAchα2	8	rs2196128	42763143	C	T	0.2143	0.1974	0.1446	0.7037
nAchα5	15	rs12593950	78628593	C	G	0.2198	0.2368	0.1372	0.7111
nAchα5	15	rs4887072	78633093	G	A	0.2198	0.2368	0.1372	0.7111
nAchα1	2	rs1376866	174763128	C	T	0.1056	0.09333	0.1358	0.7125
nAchα5	15	rs12915695	32111144	A	G	0.3297	0.3487	0.1338	0.7146
mAchM3	1	rs1019882	239735556	G	A	0.4389	0.4189	0.1322	0.7162
mAchM3	1	rs891700	239718626	A	G	0.467	0.4868	0.1303	0.7181
mAchM3	1	rs717227	239719299	C	T	0.467	0.4868	0.1303	0.7181
mAchM3	1	rs6694220	239720316	A	G	0.467	0.4868	0.1303	0.7181
mAchM3	1	rs6657343	239728211	T	A	0.467	0.4868	0.1303	0.7181
mAchM3	1	rs11585281	239746351	T	C	0.4341	0.4145	0.1301	0.7183
mAchM3	1	rs622628	239837749	T	C	0.3407	0.3224	0.1249	0.7238
mAchM3	1	rs12751235	239706521	T	C	0.4333	0.4145	0.12	0.7291
nAchα5	15	rs1685119	33984852	G	A	0.4157	0.4342	0.1146	0.7349
nAchα4	20	rs6011779	63352965	C	T	0.1648	0.1513	0.1134	0.7363
nAchα1	2	rs1376865	174759869	G	A	0.1099	0.09868	0.1109	0.7392
mAchM2	7	rs10488598	136903096	C	T	0.382	0.4	0.1106	0.7395
nAchα10	11	rs11823728	62909330	T	C	0.03409	0.0411	0.1091	0.7411
nAchα10	11	rs544978	62917758	C	A	0.2667	0.2829	0.1091	0.7412
nAchα5	15	rs4779652	34003918	C	T	0.2088	0.2237	0.1086	0.7417
nAchα5	15	rs4887069	78616728	G	A	0.2278	0.2432	0.1081	0.7423
nAchα10	11	rs2075748	62920797	A	G	0.2389	0.2237	0.1069	0.7436
nAchα5	15	rs2611603	32149331	G	T	0.3722	0.3553	0.1023	0.7491
mAchM2	7	rs324650	137008914	T	A	0.4451	0.4276	0.1022	0.7492
mAchM2	7	rs1455858	136946956	A	G	0.2802	0.2961	0.1014	0.7502
mAchM2	7	rs12535371	136899328	C	T	0.3846	0.4013	0.09691	0.7556
mAchM2	7	rs7811061	136911836	A	G	0.3846	0.4013	0.09691	0.7556
nAchε	17	rs1053754	4897993	A	C	0.2614	0.2466	0.09195	0.7617
nAchα5	15	rs489832	33985706	A	G	0.4101	0.4267	0.09172	0.762
nAchε	17	rs4151134	7443804	C	T	0.4505	0.4671	0.09145	0.7623
nAchα9	4	rs4568270	40345218	G	C	0.4835	0.5	0.09005	0.7641
nAchε	17	rs3829603	7443723	A	C	0.3242	0.3092	0.08562	0.7698
mAchM3	1	rs497576	239862677	T	C	0.4157	0.4	0.08336	0.7728
nAchα5	15	rs7175823	34029325	A	C	0.1944	0.2067	0.07639	0.7823
nAchα1	2	rs112001880	232539050	D	I	0.3864	0.3716	0.07419	0.7853
nAchα5	15	rs17236700	33989532	T	C	0.2809	0.2945	0.07273	0.7874
nAchα5	15	rs8030094	34044681	A	G	0.1923	0.2039	0.07077	0.7902
nAchα1	2	rs3791729	232530587	T	C	0.3626	0.3487	0.07031	0.7909
nAchα10	11	rs56783086	62912718	D	I	0.06044	0.06757	0.0696	0.7919
mAchM3	1	rs16832150	239646675	A	T	0.2637	0.2763	0.06658	0.7964

Supplementary Table 1. (*Continued*)

GENE	CHR	RefSNPID	BP	A1	A2	FREQUENCY_A	FREQUENCY_U	χ^2	*P*-VALUE
mAchM3	1	rs1155612	239734405	A	G	0.4725	0.4867	0.06587	0.7974
nAchα10	11	rs1942499	62916053	C	T	0.3022	0.2895	0.0643	0.7998
mAchM3	1	rs2841036	239763732	C	T	0.2889	0.2763	0.06419	0.8121
mAchM2	7	rs324645	137007017	G	A	0.4611	0.4474	0.06277	0.8022
mAchM2	7	rs6948054	136996054	A	G	0.2865	0.274	0.06249	0.8026
nAchα5	15	rs12440298	78635247	G	T	0.01648	0.01316	0.06212	0.8032
CHRNA9	4	rs10009228	40354405	A	G	0.1813	0.1711	0.06002	0.8065
mAchM3	1	rs10802795	239707475	C	T	0.467	0.4539	0.05708	0.8112
mAchM2	7	rs1158586	136952389	G	A	0.2944	0.2829	0.05348	0.8171
mAchM2	7	rs1035609	137001124	C	T	0.3889	0.4013	0.05327	0.8175
nAchα2	8	rs623330	101281429	T	A	0.3956	0.4079	0.05208	0.8195
mAchM2	7	rs2350780	136908222	G	A	0.2912	0.3026	0.05182	0.8199
nAchα1	2	rs12466358	232532815	G	T	0.2473	0.2368	0.04887	0.8251
nAchα5	15	rs7175581	32093264	A	G	0.4444	0.4333	0.04101	0.8395
nAchα5	4	rs4264869	40353109	C	T	0.489	0.5	0.04001	0.8415
nAchα1	2	rs13018423	232543573	T	C	0.2472	0.2568	0.03928	0.8429
mAchM2	7	rs2113545	136977994	A	G	0.2667	0.2763	0.03883	0.8438
nAchα6	19	rs4239626	15717596	G	T	0.2527	0.2434	0.03858	0.8443
mAchM3	1	rs10495447	239888040	A	G	0.2473	0.2566	0.03827	0.8449
mAchM3	1	rs12131198	239889149	A	C	0.2473	0.2566	0.03827	0.8449
mAchM2	7	rs12537962	136894261	G	A	0.3846	0.3947	0.03569	0.8502
mAchM3	1	rs10495448	239892173	A	G	0.2802	0.2895	0.03485	0.8519
mAchM2	7	rs2350786	136991823	A	G	0.2389	0.2303	0.03411	0.8535
nAchα5	15	rs2702282	34023861	G	T	0.4111	0.4211	0.03353	0.8547
nAchα5	15	rs623941	34060377	C	A	0.314	0.3043	0.03305	0.8557
mAchM3	1	rs7513746	239699111	G	A	0.4231	0.4133	0.03207	0.8579
mAchM3	1	rs10802813	239889304	A	C	0.2582	0.25	0.02965	0.8633
nAchα1	2	rs2600686	174759035	G	A	0.1044	0.09868	0.02955	0.8635
mAchM2	7	rs1378646	136950254	G	A	0.2912	0.2829	0.02795	0.8672
mAchM3	1	rs1125489	239843813	A	G	0.3466	0.3378	0.02735	0.8686
nAchα1	2	rs2600685	174762320	G	A	0.4451	0.4539	0.02647	0.8708
nAchα5	15	rs7178075	34005294	T	C	0.2033	0.2105	0.02641	0.8709
CHRNE	17	rs7215056	7446731	A	C	0.2033	0.2105	0.02641	0.8709
mAchM3	1	rs685550	239761108	C	T	0.2111	0.2039	0.02569	0.8727
mAchM3	1	rs3738436	239709193	A	C	0.4231	0.4145	0.02518	0.8739
mAchM3	1	rs7511970	239719955	A	G	0.4231	0.4145	0.02518	0.8739
mAchM3	1	rs12743042	239725004	C	T	0.4231	0.4145	0.02518	0.8739
mAchM3	1	rs10802801	239739541	A	G	0.4231	0.4145	0.02518	0.8739
mAchM3	1	rs713117	239652741	G	A	0.2556	0.2632	0.02482	0.8748
nAchα5	4	rs12509596	40341020	T	A	0.4389	0.4474	0.02402	0.8768
nAchα5	4	rs10015231	40335549	T	C	0.2167	0.2237	0.02366	0.8778
mAchM3	1	rs536071	239792665	C	T	0.3989	0.4067	0.02054	0.8862
nAchα1	2	rs12185542	174762902	T	A	0.4396	0.4474	0.02046	0.8863
nAchα2	8	rs16891604	42763570	A	C	0.05556	0.05921	0.0204	0.8864
mAchM2	7	rs7800170	136939573	C	A	0.4222	0.4145	0.02033	0.8866
mAchM3	1	rs10802794	239707321	T	C	0.4615	0.4539	0.01923	0.8897
nAchα5	15	rs4779565	32248700	T	G	0.3941	0.3867	0.01859	0.8916

(*continued*)

Supplementary Table 1. (*Continued*)

GENE	CHR	RefSNPID	BP	A1	A2	FREQUENCY_A	FREQUENCY_U	χ^2	*P*-VALUE
mAchM2	7	rs1364403	136904080	T	C	0.2088	0.2027	0.0185	0.8918
mAchM3	1	rs2072660	154576245	T	C	0.2308	0.2368	0.01707	0.8961
mAchM3	1	rs6663632	239714421	A	C	0.4611	0.4539	0.01704	0.8961
nAchα4	20	rs2236196	63346204	G	A	0.2363	0.2303	0.01666	0.8973
nAchα5	15	rs646950	33999459	T	C	0.4011	0.4079	0.01588	0.8997
nAchα5	15	rs2611605	32149432	T	C	0.1813	0.1867	0.01568	0.9003
mAchM3	1	rs16832152	239648409	T	G	0.2692	0.2632	0.01563	0.9005
nAchα5	15	rs2337980	32302859	T	C	0.4341	0.4408	0.01522	0.9018
nAchε	17	rs12936083	4898592	G	A	0.2833	0.2895	0.01521	0.9018
nAchα5	4	rs4861307	40343222	A	G	0.5	0.4934	0.01427	0.9049
nAchε	17	rs33970119	4901607	A	G	0.04396	0.04667	0.01401	0.9058
mAchM3	1	rs602117	239843485	G	A	0.4722	0.4662	0.01176	0.9136
mAchM2	7	rs1364407	136925178	C	T	0.2778	0.2829	0.0107	0.9176
mAchM3	1	rs7533134	239761809	A	G	0.2747	0.2697	0.0104	0.9188
nAchα5	4	rs4861306	40343001	C	T	0.4945	0.5	0.00993	0.9206
mAchM3	1	rs1416789	239738345	G	A	0.4489	0.4539	0.008513	0.9265
mAchM3	1	rs10802812	239889122	A	G	0.2527	0.2566	0.00641	0.9362
mAchM3	1	rs2163546	239894660	A	G	0.489	0.4933	0.006147	0.9375
mAchM3	1	rs1431718	239716253	T	C	0.4121	0.4079	0.00602	0.9382
nAchα1	2	rs2767	232535364	C	T	0.3791	0.375	0.005987	0.9383
nAchα5	15	rs12903907	34029780	C	T	0.2033	0.2067	0.005734	0.9396
mAchM3	1	rs12037424	239635112	C	T	0.2667	0.2632	0.005208	0.9425
mAchM3	1	rs619288	239795368	T	C	0.4023	0.3986	0.004438	0.9469
nAchε	17	rs35400274	4900416	A	G	0.1111	0.1133	0.004059	0.9492
mAchM3	1	rs1134	239708872	T	C	0.4176	0.4145	0.003294	0.9542
mAchM3	1	rs6684622	239714237	C	G	0.4821	0.4789	0.003296	0.9542
nAchα10	11	rs542269	62918065	C	T	0.2802	0.2829	0.002931	0.9568
mAchM2	7	rs12155015	136961350	T	C	0.2874	0.2847	0.002676	0.9587
nAchα1	2	rs2853457	232533258	A	G	0.4176	0.42	0.001975	0.9646
mAchM2	7	rs324649	137008762	T	C	0.4111	0.4133	0.001668	0.9674
mAchM3	1	rs6429154	239713966	G	A	0.4222	0.42	0.001657	0.9675
mAchM3	1	rs10802802	239746642	A	G	0.4231	0.4211	0.001391	0.9702
mAchM3	1	rs12029701	239747301	C	T	0.4231	0.4211	0.001391	0.9702
mAchM3	1	rs7536133	239825549	T	C	0.2418	0.2434	0.001247	0.9718
mAchM2	7	rs324594	136970576	C	T	0.1923	0.1908	0.001232	0.972
mAchM2	7	rs10242108	136886960	T	C	0.3736	0.375	0.000667	0.9794
mAchM3	1	rs693948	239792376	G	A	0.4	0.4013	0.000594	0.9806
nAchα5	15	rs603152	34002436	A	C	0.4066	0.4079	0.000581	0.9808
mAchM2	7	rs1455857	136955194	A	G	0.2944	0.2933	0.000487	0.9824
mAchM3	1	rs2068941	239846641	G	A	0.3407	0.34	0.000159	0.9899
nAchα1	2	rs1530906	174756207	C	T	0.0989	0.09868	4.38E-05	0.9947
nAchα1	2	rs2600689	174756626	G	A	0.0989	0.09868	4.38E-05	0.9947
nAchα1	2	rs2646165	174756754	G	A	0.0989	0.09868	4.38E-05	0.9947
mAchM3	1	rs606709	239794842	T	C	0.3944	0.3947	2.95E-05	0.9957
mAchM3	1	rs2790336	239799386	G	A	0.3944	0.3947	2.95E-05	0.9957

Notes: SNP, single nucleotide polymorphism with 115 CFS/ME patients and 90 controls. Data presented for gene AChRs (muscarinic M3, nicotinic alpha 2, 5 and 10), chromosome location (CHR), reference SNP identification (RefSNPID), base pair (BP) location of SNP, alleles (A1 and A2), Allelic Frequency A (Frequency_A) of this allele in CFS cases, Frequency U (Frequency_U) of this allele in controls, χ^2 for basic allelic test (1df), and *P*-value for this test set at a significance of $P < 0.05$.