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Depression Case Control (DeCC) Study fails to support involvement of the muscarinic acetylcholine receptor M2 (*CHRM2*) gene in recurrent major depressive disorder

Sarah Cohen-Woods[1,*], Daria Gaysina[1], Nick Craddock[2], Anne Farmer[1], Joanna Gray[1], Cerisse Gunasinghe[1], Farzana Hoda[1], Lisa Jones[3], Jo Knight[1], Ania Korszun[4], Michael J. Owen[2], Abram Sterne[1], Ian W. Craig[1] and Peter McGuffin[1]

[1]MRC SGDP Centre, Institute of Psychiatry, King's College London, De Crespigny Park, London SE5 8AF, UK, [2]Department of Psychological Medicine, Cardiff University, School of Medicine, Heath Park, Cardiff CF14 4XN, UK, [3]Division of Neuroscience, Department of Psychiatry, University of Birmingham, The Barberry, 25 Vincent Drive, Edgbaston, Birmingham B15 2FG, UK and [4]Centre for Psychiatry, Wolfson Institute of Preventive Medicine, Barts and The London, Charterhouse Square, London EC1M 6BQ, UK

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It has been suggested that alteration in the muscarinic-cholinergic system is involved in modulation of mood. Three studies have reported linkage on chromosome 7 with major depressive disorder (MDD) in or close to a region containing the muscarinic receptor *CHRM2* gene. A haplotype of SNPs located in *CHRM2* (rs1824024–rs2061174–rs324650) has been significantly associated with MDD in a previous study. We report the first study investigating this gene in a large, adequately powered, clinical depression case–control sample (*n* = 1420 cases, 1624 controls). Our data fail to support association with the *CHRM2* polymorphisms previously implicated in the genetic aetiology of depression. It is possible our failure to replicate may be a consequence of differences in definition of the MDD phenotype and/or ethnic differences.

INTRODUCTION

The World Health Organisation suggests that as a leading cause of disability in adults, major depressive disorder (MDD) closely follows cardiovascular disease (1), but the pathogenesis and underlying aetiology of the disorder remain unclear (2). Much research has focused on aberrant function in neurotransmitter pathways, especially serotoninergic and noradrenergic systems; however, muscarinic–cholinergic pathways have also been implicated (3). The receptors in this pathway are G-protein-coupled receptors, central in the control of the acetylcholine response. Specifically, negative feedback on acetylcholine release from cholinergic terminals is mediated by the cholinergic muscarinic 2 receptor (CHRM2) (4,5).

The muscarinic–cholinergic system has been implicated in evaluating and learning the salience of sensory stimuli (6), leading to the suggestion that disruption may modify the perception of emotionally valenced events (7). A major presynaptic autoreceptor, the M_2 receptor, is highly expressed in the brain (8), and has been implicated in stress; corticosteroids can enhance M_2 receptor expression (mRNA, protein and function) (9). Similarly, the administration of a muscarinic agonist to mice produces a significant increase in corticosterone, void of parasympathomimetic side effects (10), whereas no increase in corticosterone levels is observed in M_2 receptor knockout mice (11). This indicates that the corticosterone response is mediated by the muscarinic M_2 receptors. Cholinergic hypersensitivity has also been observed in depression, with increased neurophysiological responses in individuals with current or remitted MDD, and exacerbation of depressive symptomotology following administration of muscarinic receptor agonists or acetylcholinesterase inhibitors (3,6,12–14).

*To whom correspondence should be addressed at: MRC SGDP Centre, Institute of Psychiatry, King's College London, Box PO82, De Crespigny Park, London, SE5 8AF, UK. Tel: +44 2078480856; Fax: +44 2078480866; Email: sarah.cohen@iop.kcl.ac.uk

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Located on 7q31–35, *CHRM2* has an intronless open reading frame, with a single coding sequence encoded by a part of exon 6 (15). *CHRM2* is a complex gene that possesses a large 5′-UTR that may produce tissue-specific alternative splicing patterns (4,16), and has been studied across phenotypes including Alzheimer's disease (17), cognition and memory (18–20), alcoholism and drug dependence (21,22), externalizing disorder (23) and MDD (21,22,24). Three studies have reported evidence of linkage with MDD with markers on chromosome 7 in, or close to, a region containing the *CHRM2* gene. An initial report identified linkage with alcohol dependence (25); however, these data were later re-analysed with MDD as the phenotype, and the same region showed significant LOD scores (26). Two further papers report evidence for linkage in the same chromosomal region with both alcohol dependence and MDD (21,27), providing further indication that this chromosomal region may be of importance in MDD.

There have been three association studies investigating polymorphisms in the *CHRM2* gene in association with mood disorders. Comings *et al.* (24) investigated one SNP (rs81919921), reporting an association with MDD in women. A more comprehensive investigation of the gene was undertaken by Wang *et al.* (21), studying six SNPs in the 5′-UTR and 5 in the 3′-UTR in the collaborative study on the genetics of alcohol (COGA) sample. Evidence for association with MDD was found with four SNPs in the 5′-UTR, and a three marker haplotype comprising of three of these SNPs. A third study (22) also took a comprehensive approach investigating many SNPs across the gene. However, this study was based upon broad phenotype (affective disorders, including bipolar disorder, unipolar disorder and seasonal affective disorders) and did not attempt to replicate the previously reported haplotype, including just two SNPs that Wang *et al.* (21) studied, neither of which were included in the haplotype the latter group pursued. No evidence for single SNP association was reported, however suggestive and modestly significant haplotypes were described in association with affective disorder.

Using a large clinical unipolar case–control sample from the UK, the aim of the current study was to investigate the four markers previously associated by Wang *et al.* (21) and the initial three-marker haplotype identified.

RESULTS

The genotypic distributions of the SNPs investigated within the *CHRM2* gene are in accordance with Hardy–Weinberg equilibrium (HWE) in the control and the unipolar sample, as a whole and when stratified by gender ($P > 0.05$). Complete genotypes were available for 97–99% of the individuals across the SNPs. It is possible to calculate an odds ratio of 1.59 [95% confidence interval (CI) 1.18–2.15] for the association observed by Comings *et al*. (24) on the basis of the frequency data reported in their published paper. The DeCC sample (see Materials and Methods for details) possesses 100% power to detect allelic association with all SNPs if there is a true odds ratio at the previously reported level of 1.59, and even at the more modest level of 1.50. Even if the true odds ratio is 1.30, power to detect allelic association remains above 90% with all the SNPs. However, this drops drastically if the true odds ratio is just 1.18, the lower bound of the 95% CI for the association reported by Comings *et al*. (24) (Table 1).

Table 1. Power of samples to detect allelic association with the SNPs in the *CHRM2* gene and recurrent unipolar depression (minor allele as risk allele)

SNP	OR	Total	Male	Female
rs1824024	1.18[a]	0.59	0.25	0.40
	1.30	0.94	0.54	0.79
	1.50	1.00	0.89	0.99
	1.59[b]	1.00	0.96	1.00
rs2061174	1.18[a]	0.58	0.25	0.39
	1.30	0.93	0.53	0.78
	1.50	1.00	0.88	0.99
	1.59[b]	1.00	0.96	1.00
rs2350786	1.18[a]	0.53	0.22	0.35
	1.30	0.90	0.47	0.75
	1.50	1.00	0.84	0.99
	1.59[b]	1.00	0.94	1.00
rs324650	1.18[a]	0.62	0.27	0.44
	1.30	0.95	0.57	0.82
	1.50	1.00	0.91	0.99
	1.59[b]	1.00	0.97	1.00

[a]Lower bound of the 95% CI of the odds ratio[b] calculated from Comings *et al*. (24).

Single marker analyses of *CHRM2*

The allelic distribution observed in this sample for the four SNPs is comparable to those reported by Wang *et al.* (21). Single-locus analyses yielded no significant genotypic or allelic association with recurrent unipolar depression. None of the 95% CIs of the odds ratios overlapped with the 1.59 odds ratio that we calculated for the association reported by Comings *et al*. (24) (Table 2).

Haplotype analyses of *CHRM2*

We tested the same three-marker haplotype as Wang *et al.* (21). SNP rs2350786 shows high LD with rs2061174 ($r^2 = 0.75$, D' = 1.00; Table 3) in agreement with previous reports (21) and was excluded. LD between the other SNPs, although comparable, is slightly greater in our sample. The haplotype analysed was rs1824024–rs2061174–rs324650, and yielded no significant global χ^2 statistic associated with MDD ($\chi^2 = 2.31$, $P = 0.889$). Over 99% of each sample (control and unipolar) was included in the haplotype analyses. The haplotype-specific analyses are shown in Table 4; the common haplotypes (T-T-T, G-C-A and T-T-A) make up 85.6% of the observed haplotypes in the unaffected sample. The haplotype with a frequency below 1% (T-C-T) was excluded from analyses, improving statistical power.

DISCUSSION

This study presents no evidence for association with the genetic variants genotyped in the *CHRM2* gene in depression. Two of the three previous studies investigating this gene in depression have utilized family samples originally collected

Table 2. Comparison of allelic and genotypic distributions of four SNPs within the *CHRM2* gene between individuals affected with unipolar depression and individuals unaffected

Genotypes/alleles	Control sample (%)	Unipolar depression (%)	OR (95% CI)
rs1824024	$n = 1612$	$n = 1412$	
GG	174 (10.8)	134 (9.5)	
GT	734 (45.5)	645 (45.7)	
TT	704 (43.7)	633 (44.8)	
χ^2 (*P*) (GG versus GT versus TT)	df = 2	1.49 (0.476)	
G	1082 (33.6)	913 (32.3)	
T	2142 (66.4)	1911 (67.7)	
χ^2 (Cochran-Armitage *P*)	df = 1	1.03 (0.302)	1.06 (0.95–1.18)
rs2061174	$n = 1606$	$n = 1379$	
CC	157 (9.8)	135 (9.8)	
CT	714 (44.5)	598 (43.4)	
TT	735 (45.7)	646 (46.8)	
χ^2 (*P*) (CC versus CT versus TT)	df = 2	0.39 (0.823)	
C	1028 (32.0)	868 (31.5)	
T	2184 (68.0)	1890 (68.5)	
χ^2 (Cochran-Armitage *P*)	df = 1	0.20 (0.657)	0.98 (0.88–1.09)
rs2350786	$n = 1591$	$n = 1361$	
CC	860 (54.1)	741 (54.5)	
CT	629 (39.5)	523 (38.4)	
TT	102 (6.4)	97 (7.1)	
χ^2 (*P*) (CC versus CT versus TT)	df = 2	0.81 (0.667)	
C	2349 (73.8)	2005 (73.7)	
T	833 (26.2)	717 (26.3)	
χ^2 (Cochran-Armitage *P*)	df = 1	0.02 (0.887)	1.02 (0.88–1.11)
rs324650	$n = 1607$	$n = 1389$	
AA	332 (20.7)	300 (21.6)	
AT	822 (51.1)	681 (49.0)	
TT	453 (28.2)	408 (29.4)	
χ^2 (*P*) (AA versus AT versus TT)	df = 2	1.34 (0.511)	
A	1486 (46.2)	1281 (46.1)	
T	1728 (53.8)	1497 (53.9)	
χ^2 (Cochran-Armitage *P*)	df = 1	0.01 (0.924)	1.00 (0.90–1.10)

Table 3. Pair-wise disequilibrium between SNPs in the *CHRM2* gene

SNP	rs1824024	rs2061174	rs2350786	rs324650
Control				
rs1824024		0.853	0.886	0.496
rs2061174	0.678		0.991	0.563
rs2350786	0.558	0.748		0.632
rs324650	0.143	0.172	0.165	
Unipolar				
rs1824024		0.829	0.880	0.524
rs2061174	0.656		1.000	0.586
rs2350786	0.575	0.786		0.607
rs324650	0.154	0.183	0.155	

D' values shown in the upper right triangle and r^2 values shown in the lower left triangle.

Table 4. Haplotype analysis with three SNPs in the *CHRM2* gene with MDD

Haplotype[a]	Unaffected	Unipolar	χ^2 Statistic (*P*-value)	OR
T-T-T	0.443	0.453	0.446 (0.504)	0.96
G-C-A	0.220	0.216	0.240 (0.625)	1.02
T-T-A	0.193	0.192	0.029 (0.865)	1.01
G-C-T	0.071	0.063	1.510 (0.219)	1.14
T-C-A	0.027	0.030	0.442 (0.506)	0.90
G-T-A	0.026	0.026	0.000 (0.994)	1.00
G-T-T	0.021	0.020	0.044 (0.833)	1.05

Unipolar: global $\chi^2 = 2.31$, df = 6, $P = 0.889$ excluding haplotypes with frequency below 1%.
[a]rs1824024–rs2061174–rs324650 haplotype.

for the study of alcohol- and/or drug-abuse or dependence: COGA (21) and MTFS (24). The third study had only a small ‘additional’ sample added of individuals diagnosed with MDD (22), otherwise it too was primarily geared to elucidating the genetic aetiology of alcohol- and drug-dependence. The study of Luo *et al*. (22) failed to present convincing evidence for association with affective disorder ($n = 137$), whereas those of Wang *et al*. (21) and Comings *et al*. (24) did ($n = 773$ MDD and 126 MDD, respectively). There have been no large clinical case–control studies investigating genetic variation in the *CHRM2* gene and its potential implication in the development of MDD specifically; this paper presents the first.

No single-marker or globally significant haplotypic association was observed in the DeCC sample with the three-marker haplotype (rs1824024–rs2061174–rs324650) previously associated in the COGA sample with MDD (21). Wang *et al*. reported over-transmission of haplotypes G-C-A and G-C-T, and under-transmission of the most common haplotype T-T-T, and a rare haplotype T-C-A. In contrast, haplotype-specific analyses yielded no significant results within the DeCC sample; again this was the case when genders were both combined for analyses, or when they were separated. It is possible that failure to replicate the findings of Wang *et al.* (21) is because the phenotype of the DeCC sample is not the same as that in the COGA sample. Depression occurring solely in relation to alcohol was an exclusion criterion in the DeCC sample, as was alcohol dependence; however, the COGA study was designed to investigate alcohol dependence and does not differentiate between individuals suffering depression as a consequence of alcohol, or those suffering independent of alcohol. The other single *CHRM2* marker study reporting an association with MDD also derived their study-group from an alcohol- and drug-dependence sample (22). Therefore, it is possible that an association with *CHRM2* exists only in depression comorbid with substance misuse.

It is also possible that population structure in previous studies produced false positive results. The DeCC sample was 100% self reported white European origin. In contrast, the COGA sample was 82% Caucasian, 15% African-American and 3% ‘other’. The report of Wang *et al.* (21) on the *CHRM2* gene and MDD in the COGA sample did not correct for population stratification, citing the comparatively small size of the African-American sample and the fact that internal controls were used in a family-based analysis. Indeed Luo *et al*. (22) reported that their observed association with alcohol- and drug-dependence with SNPs in the *CHRM2* gene was stronger within the African-American sample relative to the Caucasian sample. Unfortunately, they reported no such data on their mood-disorder group, as they were entirely of European-American origin. However, their findings generally support the hypothesis that ethnic differences are important as potential explanations of across-study disparities.

It is also worth-noting that the rare three-marker haplotype excluded in the haplotype analysis in the DeCC sample (T-C-T) shows a very different frequency (<1%) from that in the COGA sample (3.8%). No haplotype within the COGA sample falls below the 1% rare haplotype threshold. It is possible that this also is a consequence of ethnic differences. There are known small differences in the allelic distributions of these SNPs between the African-Americans and Caucasians, and larger differences are also observed with the Chinese population (www.ensembl.org) who could have been included with ‘other’ in the COGA sample. The haplotypic frequency disparity may also be a consequence of differences in LD in the samples. Consistent with this hypothesis is the observation that LD in the DeCC sample is slightly greater than that reported in the COGA sample, which could be the reason less individuals possess the T-C-T haplotype in the DeCC sample. This too could be attributed to population stratification in the US sample.

This paper presents the first clinical case–control study investigating *CHRM2* in recurrent unipolar depression that is not co-morbid with substance misuse. We have failed to replicate a previous study associating four SNPs in the *CHRM2* gene with MDD in the COGA sample (21), and present no evidence that these markers or a three-marker haplotype (rs1824024–rs2061174–rs324650) increase the risk of the disorder. This supports a previous study that failed to find an association with mood disorder and two of the SNPs, rs1824024 and rs3246560 (22); however, the variation covered within the gene through the investigation of these replication markers is 15%. Therefore, it cannot be completely ruled out that the CHRM2 locus is implicated in the genetic aetiology of MDD.

MATERIALS AND METHODS

Study subjects

Individuals ($n = 1420$; 435 men, 983 women and 2 sex unknown) with recurrent unipolar depression, mean age 47.17 years, were recruited from three clinical UK sites: London, Cardiff and Birmingham. Subjects were identified from psychiatric clinics, hospitals and general medical practices and from volunteers responding to media advertisements. Only subjects, of white European (‘Caucasian’) parentage, were included if they were over the age of 18 and had experienced 2 or more episodes of unipolar depression of at least moderate severity separated by at least 2 months of remission as defined by the Diagnostic and Statistical Manual 4th edition operational criteria (DSMIV) (28) or the International Classification of Diseases 10th edition operational criteria (ICD10), for unipolar depression (29). All subjects were interviewed using the Schedules for Clinical Assessment in Neuropsychiatry (SCAN) (30,31). At the time of interview, interviewers obtained 25 ml of whole blood collected in 37.5 ml (EDTA containing) monovettes. In addition, drops of blood were placed on a Guthrie blood spot card. The blood samples were labelled with a bar code, gently mixed and stored frozen upright in a -20°C freezer pending DNA extraction.

Subjects were excluded if they or a first-degree relative, ever fulfilled criteria for mania, hypomania or schizophrenia. Subjects were also excluded if they experienced psychotic symptoms that were mood incongruent or present when there was no evidence of a mood disturbance. Other exclusion criteria were intravenous drug use with a lifetime diagnosis of dependency; depression occurring solely in relation to alcohol or substance abuse or depression only secondary to medical illness or medication, and a clear diagnosis of bipolar disorder, schizophrenia, schizo-affective disorder or acute or transient psychotic disorders in first- or second-degree relatives.

The mean age of all the control individuals ($n = 1624$; 698 men, 922 women and 4 sex unknown) was 42.24 years. They were screened for lifetime ever absence of psychiatric disorder using a modified version of the Past History Schedule (32). Subjects were excluded if they, or a first-degree relative, ever fulfilled criteria for depression or any other psychiatric disorder. Subjects were also excluded if they scored 10 or above on the Beck Depression Inventory (33), did not return consent, or failed to return cheek swabs. Informed written consent was obtained and buccal mucosa swabs were collected for DNA via the mail. The swab containers were labelled with a bar code pending DNA extraction.

Phenotypic data analysis

All phenotypic information from SCAN interviews and questionnaires was coded by assigning a number to each subject, and removing any personal identifying information. The same codes were used on the blood sample or cheek swab tubes using a bar code system. The phenotypic information was entered into a data file created using Statistical Procedures for the Social Sciences (SPSS) version 12 for Windows for the statistical analyses.

Statistical analysis

To test for deviation from HWE, the Pearson (χ^2) test statistic and an exact statistic were calculated with the computer program FINETTI (available on http://ihg.gsf.de/cgi-bin/hw/hwal.pl). Cases and controls were considered separately. This program was also used for calculating allelic odds ratios with 95% CIs. Power was calculated using the PS program (34). In this program, the method of Schlesselman (35) is used with independent case and control groups for analysis using an uncorrected χ^2 test. When the case and control sample sizes are unequal, PS uses the generalization of Casagrande’s method proposed by Fleiss (36). The alternative hypothesis is specified in terms of odds ratios.

Single marker association analysis was carried out using standard contingency table analysis incorporating χ^2 tests of independence for each SNP, run in SPSS for Windows (Version 12.0). Two- and three-marker haplotype analyses, and conditional testing, were carried out using UNPHASED (37) and significance assessed utilizing a Monte Carlo method following 10 000 permutations.

DNA genotyping

Genomic DNA was extracted from bloods and cheek swabs collected as described previously (38,39). Genotyping data were obtained for 3830 individuals ($n = 1408$ unipolar

cases, 846 bipolar cases and 1576 controls) and was blind to all phenotypic information. A proportion of individuals ($n =$ 1222 cases and 832 controls) were externally genotyped for the four SNPs (rs1824024, rs2061174, rs2350786 and rs324650) using the KASPar system by KBioscience, UK (www.kbioscience.co.uk). The remaining individuals ($n =$ 186 cases and 744 controls) were genotyped at the MRC SGDP Centre using the Taqman® SNP genotyping platform (PE Applied Biosystems, Foster City, CA, USA). The Taqman® assays ordered from Applied Biosystems (Foster City, CA, USA) were: C_11626291_10 (rs2061174), C_1604562_10 (rs2350786), C_3036433_10 (rs1824024) and C_773390_10 (rs324650).

Conflict of Interest statement. None declared.

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REFERENCES

1. Murray, C.J. and Lopez, A.D. (1997) Global mortality, disability, and the contribution of risk factors: Global Burden of Disease Study. *Lancet*, **349**, 1436–1442.
2. Wong, M.L. and Licinio, J. (2001) Research and treatment approaches to depression. *Nat. Rev. Neurosci.*, **2**, 343–351.
3. Janowsky, D.S., Overstreet, D.H. and Nurnberger, J.I. Jr (1994) Is cholinergic sensitivity a genetic marker for the affective disorders? *Am. J. Med. Genet.*, **54**, 335–344.
4. Mash, D.C., Flynn, D.D. and Potter, L.T. (1985) Loss of M2 muscarine receptors in the cerebral cortex in Alzheimer's disease and experimental cholinergic denervation. *Science*, **228**, 1115–1117.
5. Zhou, C., Fryer, A.D. and Jacoby, D.B. (2001) Structure of the human M(2) muscarinic acetylcholine receptor gene and its promoter. *Gene*, **271**, 87–92.
6. McGaugh, J.L. (2004) The amygdala modulates the consolidation of memories of emotionally arousing experiences. *Annu. Rev. Neurosci.*, **27**, 1–28.
7. Phillips, M.L., Drevets, W.C., Rauch, S.L. and Lane, R. (2003) Neurobiology of emotion perception II: implications for major psychiatric disorders. *Biol. Psychiatry*, **54**, 515–528.
8. Hamilton, S.E., Loose, M.D., Qi, M., Levey, A.I., Hille, B., McKnight, G.S., Idzerda, R.L. and Nathanson, N.M. (1997) Disruption of the m1 receptor gene ablates muscarinic receptor-dependent M current regulation and seizure activity in mice. *Proc. Natl Acad. Sci. USA*, **94**, 13311–13316.
9. Jacoby, D.B., Xiao, H.Q., Lee, N.H., Chan-Li, Y. and Fryer, A.D. (1998) Virus- and interferon-induced loss of inhibitory M2 muscarinic receptor function and gene expression in cultured airway parasympathetic neurons. *J. Clin. Invest.*, **102**, 242–248.
10. Hemrick-Luecke, S.K., Bymaster, F.P., Evans, D.C., Wess, J. and Felder, C.C. (2002) Muscarinic agonist-mediated increases in serum corticosterone levels are abolished in m(2) muscarinic acetylcholine receptor knockout mice. *J. Pharmacol. Exp. Ther.*, **303**, 99–103.
11. Hemrick-Luecke, S.K., Bymaster, F.P., Evans, D.C., Wess, J. and Felder, C.C. (2002) Muscarinic agonist-mediated increases in serum corticosterone levels are abolished in m(2) muscarinic acetylcholine receptor knockout mice. *J. Pharmacol. Exp. Ther.*, **303**, 99–103.
12. Dilsaver, S.C. (1986) Pathophysiology of 'cholinoceptor supersensitivity' in affective disorders. *Biol. Psychiatry*, **21**, 813–829.
13. Janowsky, D.S., el Yousef, M.K., Davis, J.M. and Sekerke, H.J. (1972) A cholinergic-adrenergic hypothesis of mania and depression. *Lancet*, **2**, 632–635.
14. Risch, S.C., Kalin, N.H. and Janowsky, D.S. (1981) Cholinergic challenges in affective illness: behavioral and neuroendocrine correlates. *J. Clin. Psychopharmacol.*, **1**, 186–192.
15. Gentles, A.J. and Karlin, S. (1999) Why are human G-protein-coupled receptors predominantly intronless? *Trends Genet.*, **15**, 47–49.
16. Fenech, A.G., Billington, C.K., Swan, C., Richards, S., Hunter, T., Ebejer, M.J., Felice, A.E., Ellul-Micallef, R. and Hall, I.P. (2004) Novel polymorphisms influencing transcription of the human CHRM2 gene in airway smooth muscle. *Am. J. Respir. Cell Mol. Biol.*, **30**, 678–686.
17. Lai, M.K., Lai, O.F., Keene, J., Esiri, M.M., Francis, P.T., Hope, T. and Chen, C.P. (2001) Psychosis of Alzheimer's disease is associated with elevated muscarinic M2 binding in the cortex. *Neurology*, **57**, 805–811.
18. Baxter, M.G. and Chiba, A.A. (1999) Cognitive functions of the basal forebrain. *Curr. Opin. Neurobiol.*, **9**, 178–183.
19. Everitt, B.J. and Robbins, T.W. (1997) Central cholinergic systems and cognition. *Annu. Rev. Psychol.*, **48**, 649–684.
20. Dick, D.M., Aliev, F., Kramer, J., Wang, J.C., Hinrichs, A., Bertelsen, S., Kuperman, S., Schuckit, M., Nurnberger, J. Jr, Edenberg, H.J. *et al.* (2007) Association of CHRM2 with IQ: converging evidence for a gene influencing intelligence. *Behav. Genet.*, **37**, 265–272.
21. Wang, J.C., Hinrichs, A.L., Stock, H., Budde, J., Allen, R., Bertelsen, S., Kwon, J.M., Wu, W., Dick, D.M., Rice, J. *et al.* (2004) Evidence of common and specific genetic effects: association of the muscarinic acetylcholine receptor M2 (CHRM2) gene with alcohol dependence and major depressive syndrome. *Hum. Mol. Genet.*, **13**, 1903–1911.
22. Luo, X., Kranzler, H.R., Zuo, L., Wang, S., Blumberg, H.P. and Gelernter, J. (2005) CHRM2 gene predisposes to alcohol dependence, drug dependence and affective disorders: results from an extended case–control structured association study. *Hum. Mol. Genet.*, **14**, 2421–2434.
23. Dick, D.M., Aliev, F., Wang, J.C., Grucza, R.A., Schuckit, M., Kuperman, S., Kramer, J., Hinrichs, A., Bertelsen, S., Budde, J.P. *et al.* (2008) Using dimensional models of externalizing psychopathology to aid in gene identification. *Arch. Gen. Psychiatry*, **65**, 310–318.
24. Comings, D.E., Wu, S., Rostamkhani, M., McGue, M., Iacono, W.G. and MacMurray, J.P. (2002) Association of the muscarinic cholinergic 2 receptor (CHRM2) gene with major depression in women. *Am. J. Med. Genet.*, **114**, 527–529.
25. Reich, T., Edenberg, H.J., Goate, A., Williams, J.T., Rice, J.P., Van Eerdewegh, P., Foroud, T., Hesselbrock, V., Schuckit, M.A., Bucholz, K. *et al.* (1998) Genome-wide search for genes affecting the risk for alcohol dependence. *Am. J. Med. Genet.*, **81**, 207–215.
26. Foroud, T., Edenberg, H.J., Goate, A., Rice, J., Flury, L., Koller, D.L., Bierut, L.J., Conneally, P.M., Nurnberger, J.I., Bucholz, K.K. *et al.* (2000) Alcoholism susceptibility loci: confirmation studies in a replicate sample and further mapping. *Alcohol Clin. Exp. Res.*, **24**, 933–945.
27. Nurnberger, J.I. Jr, Foroud, T., Flury, L., Su, J., Meyer, E.T., Hu, K., Crowe, R., Edenberg, H., Goate, A., Bierut, L. *et al.* (2001) Evidence for a locus on chromosome 1 that influences vulnerability to alcoholism and affective disorder. *Am. J. Psychiatry*, **158**, 718–724.
28. American Psychiatric Association. (1994) *Diagnostic and Statistical Manual. 4*. American Psychiatric Press, Washington, D.C.
29. World Health Organisation. (1993) *The ICD-10 Classification of Mental and Behavioural Disorders Diagnostic Criteria for Research*. World Health Organisation, Geneva.
30. Wing, J.K., Babor, T., Brugha, T., Burke, J., Cooper, J.E., Giel, R., Jablenski, A., Regier, D. and Sartorius, N. (1990) SCAN. Schedules for clinical assessment in neuropsychiatry. *Arch. Gen. Psychiatry*, **47**, 589–593.
31. World Health Organisation. (1998) *Diagnosis and Clinical Measurement in Psychiatry. A Reference Manual for SCAN*. World Health Organisation, Geneva.
32. McGuffin, P., Katz, R. and Aldrich, J. (1986) Past and present state examination: the assessment of 'lifetime ever' psychopathology. *Psychol. Med.*, **16**, 461–465.
33. Beck, A.T. and Steer, R.A. (1984) Internal consistencies of the original and revised Beck Depression Inventory. *J. Clin. Psychol.*, **40**, 1365–1367.

34. Dupont, W.D. and Plummer, W.D. Jr (1990) Power and sample size calculations. A review and computer program. *Control Clin. Trials*, **11**, 116–128.
35. Schlesselman, J.J. (1982) *Case–Control Studies: Design, Conduct, Analysis.*, Oxford University Press, New York.
36. Fleiss, J.L. (1981) *Statistical Methods for Rates and Proportions*. John Wiley, New York.
37. Dudbridge, F. (2003) Pedigree disequilibrium tests for multilocus haplotypes. *Genet. Epidemiol.*, **25**, 115–121.
38. Freeman, B., Powell, J., Ball, D., Hill, L., Craig, I. and Plomin, R. (1997) DNA by mail: an inexpensive and noninvasive method for collecting DNA samples from widely dispersed populations. *Behav. Genet.*, **27**, 251–257.
39. Freeman, B., Smith, N., Curtis, C., Huckett, L., Mill, J. and Craig, I.W. (2003) DNA from buccal swabs recruited by mail: evaluation of storage effects on long-term stability and suitability for multiplex polymerase chain reaction genotyping. *Behav. Genet.*, **33**, 67–72.