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# Choline Acetyltransferase Variants and Their Influence in Schizophrenia and Olanzapine Response

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**Choline acetyltransferase (ChAt) is extensively distributed throughout the CNS where, by catalyzing acetylcholine synthesis, it participates in modulating wide-ranging cholinergic-dependent functions including cognitive performance, sleep, arousal, movement, and visual information processing. Recently, compelling evidence has mounted implicating ChAt in schizophrenia. In particular, studies have identified significant reductions in ChAt activity in the nucleus accumbens and pontine tegmentum of such patients, which furthermore correlate significantly with measures of cognitive performance in the disorder. Similarly, elevated levels of choline, the acetylcholine precursor, have been identified among patients, implicating altered ChAt activity in these individuals. We sought to investigate the potential contribution of three ChAt gene polymorphisms in schizophrenia, and uncovered evidence for significant association between one of these, rs1880676G/A, and disease susceptibility among Basque individuals (genotypewise $\chi^2 = 20.7$, $P = 0.00003$; allelewise $\chi^2 = 10.1$, $P = 0.002$). A similar trend for association with susceptibility was observed for a second SNP, rs3810950G/A, (genotypwise $\chi^2 = 6.4$, $P = 0.05$; allelewise $\chi^2 = 3.75$, $P = 0.05$). Evidence was also uncovered for a potential influence of these polymorphisms on olanzapine treatment outcome among Spanish patients (F-statistic = 5.02, $P = 0.03$; F-statistic = 6.53, $P = 0.02$ respectively), and on improvements in positive symptoms in the case of rs3810950 (F-statistic = 5.3, $P = 0.03$) and general psychopathology in the case of rs1880676 and rs3810950 (F-statistic = 5.24, $P = 0.03$; F-statistic = 5.31, $P = 0.03$ respectively) during therapy. While more comprehensive studies are warranted to determine the precise contribution of ChAt mediated mechanisms in schizophrenia, our findings tentatively implicate a genetic influence of ChAt in the disorder's susceptibility and treatment.**

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## INTRODUCTION

Choline acetyltransferase (ChAt) forms an integral part of the cholinergic system where it is responsible for catalyzing the synthesis of acetylcholine. Within the CNS ChAt is synthesized in the perikaryon of cholinergic neurons, and transported to nerve terminals where it exists in soluble and membrane-bound forms [Oda, 1999]. By modulating levels of acetylcholine, ChAt influences a wide range of cholinergic-dependent neurophysiological functions including cognitive performance, arousal, sleep, movement, and the processing of visual information [Whittaker, 1988; Selden et al., 1998; Bacciottini et al., 2001; Beelke and Sannita, 2002]. Modulatory effects on mesocorticolimbic dopaminergic pathways involved in cognitive and affective function, [Gronier et al., 2000], and a regulatory influence on serotonergic, glutamatergic, and histaminergic neurotransmitter activity, have also been demonstrated [Bacciottini et al., 2001; Dean, 2001].

The potential contribution of altered ChAt activity in schizophrenia has long been established. This involvement is exemplified in early studies which identified significant alterations to normal ChAt function in the septal regions of such patients [McGeer and McGeer, 1977], subsequent to which reductions in ChAt concentration in the nucleus accumbens, and pontine tegmentum have been reported [Bird et al., 1977; Karson et al., 1993]. Interestingly, decreases in pontine tegmentum ChAt activity have been found to correlate with measures of cognitive performance in schizophrenia such as orientation and reasoning, findings which highlight the potential phenotypic consequence of ChAt-related changes in the disorder [Karson et al., 1996]. Studies in magnetic resonance spectroscopy have also identified elevated levels of choline, the precursor to acetylcholine, in thalamic, anterior cingulate, and caudate nucleus regions of antipsychotic-naive patients [Bustillo et al., 2002; Theberge et al., 2004], implicating the presence of impaired ChAt function. The origins of altered ChAt activity in schizophrenia are unknown, and their consequences on the pathology and course of the disorder remain to be elucidated. In light of the existing evidence, we sought to investigate the potential for changes in ChAT gene integrity to influence susceptibility to schizophrenia, and the clinical outcome of such patients to antipsychotic drug therapy.

## MATERIALS AND METHODS

### Patient Samples

Patient samples comprised 252 individuals from Navarre, Northern Spain, and surrounding areas of whom 168 were Basque and 84 were Spanish. The samples, selected from an epidemiologically isolated population, ensured high levels of sample homogeneity within each ethnic group, thereby maximizing the likelihood of identifying genetic traits of importance. All were diagnosed by structured interview with schizophrenia according to DSM-IV criteria, and were undergoing olanzapine treatment at the time of DNA collection [detailed description given in Staddon et al., 2005]. Treatment response was assessed prospectively by at least two independent clinicians at the start of the treatment and after at least 3 months as a categorical variable according to the Global Assessment of Function (GAF) scales [Endicott et al., 1976]. The scale ranges between 1 and 100 where 1 represents the most adversely affected individual while 100 represents the healthiest. Patients were rated for overall function at the start of treatment and again after at least 3 months, and an overall score increase of 20 or more points used as the cut-off point to define good response. Response was also assessed as a continuous variable (quantitative trait) using the Positive and Negative Symptoms Scales (PANSS) [Kay et al., 1987]. DNA samples comprising 197 unaffected individuals of Basque (N = 124) and Spanish (N = 73) origin were also collated from the population, who at the time were assessed by a clinician to be free from any prior or existing history of psychiatric or neurological illness. Estimates of sample power were determined for each group using the computer software program Epi Info 6 (version 6.04b). A 95% confidence level was used in each instance. For the Basque case-control (schizophrenia) studies, the sample was calculated to be large enough to give a statistical power of at least 80% to detect an effect with an odds ratio $\geq$3.2 for any polymorphisms which exist at a frequency of approximately 8% or more in the general population. For the Spanish case-control (schizophrenia) association studies, the sample was sufficiently sized to give a statistical power of at least 80% to detect an effect with an odds ratio $\geq$3.8 for any polymorphisms which exist at a frequency of approximately 10% or more in the general population. In the case of drug response, the Basque sample was sufficient to detect an effect with an odds ratio $\geq$3.5 for any polymorphisms which exist at a frequency of approximately 10% or more, given the same statistical power. The Spanish sample was sufficient to detect an effect with an odds ratio $\geq$5.1 for any polymorphisms which exist at a frequency of approximately 12% or more, given the same statistical power. All samples were collected with informed consent, and ethical approval for the study was granted by the ethical committees of the Fundacion Argibide, Navarre, Spain and the Institute of Psychiatry, Kings College London, UK.

### SNP Selection and Sample Genotyping

Identification and selection of SNPs was facilitated by bioinformatic screening of sequences encoding the ChAt gene (accession number AF305907) using the databases ENSEMBL (version 26.35.1) and NCBI (build 35). Three SNPs were selected for examination; selection was based primarily on the relatively informative allele frequencies of each SNP, and the high correlation between alleles at these three loci and other polymorphisms located along this region of the ChAt gene, as documented in data available through the HapMap project [International HapMap Project, 2003]. One SNP (NCBI dbSNP ID rs733722C/T) is located at position 50486949 of chromosome 10; NCBI dbSNP ID rs1880676G/A is located at position 50494123; and NCBI dbSNP ID rs3810950G/A is a non-synonymous transition located at position 50494625 (exon 2). Genotyping was performed externally (KBiosciences, Hertforshire, UK) using propriety competitive allele specific based PCR (KASPar); confirmation of genotype data was performed in-house for each polymorphism on at least thirty samples using the SNaPshot genotyping assay in accordance with the manufacturers recommendations (Applied Biosystems, Warrington, UK). Due to sample depletion, in some instances it was not possible to genotype all the individuals described earlier.

### Statistical Analysis

Tests for chi-square and regression analysis (as F-statistic) were performed using Epi Info (version 3.2.2, 2004) and SPSS for Windows (Release version 12.0.1, 2003); based on the existence of an a priori hypothesis for the involvement of these specific polymorphisms in disease susceptibility and treatment response, correction for multiple testing was not performed; haplotype analyses were performed using the software package GeneCounting (version 1.3, 2003; [Zhao et al., 2002]).

## RESULTS

We investigated the influence on disease susceptibility of three ChAt gene polymorphisms, rs1880676G/A, rs733722C/T, and rs3810950G/A, and summarize our results in Table I. Investigation of rs1880676 revealed highly significant association genotypewise between this polymorphism and disease susceptibility among individuals of Basque origin ($\chi^2 = 20.7$, $P = 0.00003$), with a significant difference observed in the frequency of the G and A alleles among patients (frequency = 0.7 and 0.30) compared with controls (frequency = 0.57 and 0.43, respectively); (allelic association $\chi^2 = 10.07$, $P = 0.002$; Odds Ratio 1.75, $1.22 < \text{OR} < 2.52$). This association was however not observed when we investigated the polymorphism in our Spanish cohort (genotypic $\chi^2 = 3.34$, $P = 0.2$; allelic $\chi^2 = 1.14$, $P = 0.3$). Genotype frequencies for rs1880676 were seen to lie in Hardy–Weinberg equilibrium in both sample populations, (tests for heterogeneity; Basque patients and controls $\chi^2 = 1.79$, $P = 0.41$ and $\chi^2 = 0.40$, $P = 0.14$ respectively; Spanish patients and controls $\chi^2 = 0.34$, $P = 0.84$ and $\chi^2 = 0.83$, $P = 0.7$ respectively). Upon combining Basque and Spanish samples under stratified analysis (weighted for ethnicity), significant association was identified genotypewise between rs1880676 and disease susceptibility (Wald statistic = 14.8, $P = 0.001$; sample heterogeneity $P = 0.8$). Allelewise a trend towards association was observed (Mantel-Haenszel $\chi^2 = 3.62$, $P = 0.06$; Mantel-Haenszel weighted odds ratio = 1.33; Cornfield 95% CI $0.99 < 1.33 < 1.78$). A similar trend with disease susceptibility was observed for rs3810950 in our Basque cohort, genotypewise $\chi^2 = 6.4$, $P = 0.05$; allelewise $\chi^2 = 3.75$, $P = 0.05$. However this association was not seen in the Spanish cohort (genotypwise $\chi^2 = 4.09$, $P = 0.13$; allelewise $\chi^2 = 0.08$, $P = 0.8$), nor upon stratified analysis of combined Basque and Spanish populations (genotypewise Wald statistic = 1.76, $P = 0.42$, allelewise Mantel-Haenszel $\chi^2 = 1.71$, $P = 0.2$; Mantel-Haenszel weighted odds ratio = 0.81; 0.81; Cornfield 95% CI $0.59 < 0.81 < 1.11$). Analysis of rs733722 in the Basque sample did not reveal any significant differences in genotypic or allelic frequency distribution among patients and controls, though a trend towards association genotypewise in the Spanish cohort was observed ($\chi^2 = 6.04$, $P = 0.05$; see Table I). No influence on disease susceptibility was observed for this polymorphism upon combining Basque and Spanish populations. To investigate potentially important differences between patients and controls in the distribution of haplotype frequencies, genotype data for the three polymorph-

TABLE I. ChAt Gene Polymorphisms, Disease Susceptibility, and Olanzapine Response

| | Tests for disease susceptibility | | | | | | Tests for treatment outcome | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| rs1880676 G/A | Genotype frequencies N (%) | | | | Allele frequencies | | | | | |
| | G/G | G/A | A/A | $\chi^2$ | G | A | $\chi^2$ | diffPANSS | diffPPANSS | diffNPANSS | diffGPPANSS |
| Basques | | | | | | | | | | | |
| Patients (N = 159) | 83 (52.2) | 57 (35.8) | 19 (12) | $\chi^2 = 20.7, P = 0.000032$ | 0.70 | 0.30 | $\chi^2 = 10.1, P = 0.0015$ | F = 0.66, P = 0.42 | F = 2.35, P = 0.13 | F = 2.24, P = 0.14 | F = 1.01, P = 0.32 |
| Controls (N = 124) | 33 (26.6) | 76 (61.3) | 15 (12.1) | | 0.57 | 0.43 | | | | | |
| Spanish | | | | | | | | | | | |
| Patients (N = 84) | 39 (46.4) | 34 (40.5) | 11 (13.1) | $\chi^2 = 3.34, P = 0.19$ | 0.67 | 0.33 | $\chi^2 = 1.14, P = 0.29$ | F = 5.02, P = 0.030 | F = 1.78, P = 0.19 | F = 4.06, P = 0.05 | F = 5.24, P = 0.027 |
| Controls (N = 67) | 33 (49.3) | 31 (46.3) | 3 (4.4) | | 0.72 | 0.28 | | | | | |
| rs733722 C/T | Genotype frequencies N (%) | | | | Allele frequencies | | | | | | |
| | C/C | C/T | T/T | $\chi^2$ | C | T | $\chi^2$ | diffPANSS | diffPPANSS | diffNPANSS | diffGPPANSS |
| Basques | | | | | | | | | | | |
| Patients (N = 150) | 90 (60) | 53 (35.3) | 7 (4.7) | $\chi^2 = 0.0, P \geq 0.9$ | 0.78 | 0.22 | $\chi^2 = 0.0, P \geq 0.9$ | F = 0.0, P ≥ 0.9 | F = 1.64, P = 0.21 | F = 0.55, P = 0.46 | F = 0.32, P = 0.57 |
| Controls (N = 121) | 73 (60.3) | 42 (34.7) | 6 (5) | | 0.78 | 0.22 | | | | | |
| Spanish | | | | | | | | | | | |
| Patients (N = 60) | 40 (66.7) | 19 (31.7) | 1 (1.6) | $\chi^2 = 6.04, P = 0.049$ | 0.83 | 0.17 | $\chi^2 = 1.95, P = 0.16$ | F = 0.21, P = 0.89 | F = 0.01, P = 0.92 | F = 0.0, P ≥ 0.09 | F = 0.14, P = 0.71 |
| Controls (N = 70) | 57 (81.4) | 10 (14.3) | 3 (4.3) | | 0.89 | 0.11 | | | | | |
| rs3810950 G/A | Genotype frequencies N (%) | | | | Allele frequencies | | | | | | |
| | G/G | G/A | A/A | $\chi^2$ | G | A | $\chi^2$ | diffPANSS | diffPPANSS | diffNPANSS | diffGPPANSS |
| Basques | | | | | | | | | | | |
| Patients (N = 168) | 92 (54.8) | 60 (35.7) | 16 (9.5) | $\chi^2 = 6.4, P = 0.04$ | 0.73 | 0.27 | $\chi^2 = 3.75, P = 0.053$ | F = 0.01, P = 0.92 | F = 1.91, P = 0.17 | F = 7.06, P = 0.01 | F = 0.05, P = 0.82 |
| Controls (N = 99) | 49 (49.5) | 30 (30.3) | 20 (20.2) | | 0.65 | 0.35 | | | | | |
| Spanish | | | | | | | | | | | |
| Patients (N = 66) | 36 (54.5) | 20 (30.3) | 10 (15.2) | $\chi^2 = 4.09, P = 0.13$ | 0.70 | 0.30 | $\chi^2 = 0.1, P = 0.78$ | F = 6.53, P = 0.015 | F = 5.3, P = 0.027 | F = 3.88, P = 0.056 | F = 5.31, P = 0.027 |
| Controls (N = 73) | 36 (49.3) | 32 (43.8) | 5 (6.9) | | 0.71 | 0.29 | | | | | |

diffPANSS, difference in PANSS assessed before and after treatment; diffPPANSS, difference in positive symptoms assessed before and after treatment; diffNPANSS, difference in negative symptoms assessed before and after treatment; diffGPPANSS, difference in general psychopathology assessed before and after treatment.

isms was combined and simultaneously examined in independent Basque and Spanish populations using a permutated likelihood ratio test (number of permutations tested = 10,000). In Basques the most marked difference in frequency was observed for haplotype rs1880676A, rs733722C, and rs3810950G among cases (frequency = 0.29) and controls (frequency = 0.02); LRTMean = 72.5, $P = 0.0001$. In the Spanish sample this same haplotype combination was seen to differ most significantly between cases (frequency = 0.61) and controls (frequency = 0.04); LRTMean = 73.5, $P = 0.0001$.

When the three polymorphisms were investigated in relation to olanzapine response according to GAF assessment, a significant increase in the frequency of the A allele was identified for rs1880676 among Spanish patients who responded to treatment (frequency = 0.47), compared to those who failed to respond (frequency = 0.27); $\chi^2 = 4.4$, $P = 0.04$. This association was observed genotypewise when response was assessed as a continuous variable according to PANSS criteria (F-statistic = 5.02, $P = 0.03$), but not according to GAF criteria ($\chi^2 = 4.36$, $P = 0.11$) (see Table I; GAF response data omitted for brevity). A similar relationship was observed among these patients for rs3810950, where a trend towards an increase in the A allele was identified among responders (frequency = 0.39) compared with poor responders (frequency = 0.23) according to GAF criteria ($\chi^2 = 3.51$, $P = 0.061$). Again when response was assessed according to PANSS criteria this association was observed genotypewise (F-statistic = 6.53, $P = 0.015$), but not according to GAF criteria ($\chi^2 = 4.36$, $P = 0.11$). Investigation of these patients also revealed significant association between genotype at rs3180950 and improvements in positive symptoms (PPANSS) (F-statistic = 5.3, $P = 0.027$), and general psychopathology (GPPANSS) for rs1880676 and rs3180950 (F-statistic = 5.24, $P = 0.03$; F-statistic = 5.31, $P = 0.03$ respectively). Analysis of genotypes at these two loci also revealed a trend towards association with improvements in negative symptoms (NPANSS) (F-statistic = 4.06, $P = 0.05$; F-statistic = 3.88, $P = 0.06$ respectively; refer to table).

## DISCUSSION

We investigated the potential for polymorphisms in ChAt to influence susceptibility to schizophrenia, and uncovered tentative evidence for a contribution of this gene when we identified significant association between a 5′ polymorphism, rs1880676G/A, and disease susceptibility among Basque patients (genotypewise $P = 0.00003$, allelewise $P = 0.002$). Interestingly this association was not observed among Spanish patients, a disparity in findings which we postulate may in part be attributed to the differences in allele frequency that exist for this polymorphic region throughout populations. In North American individuals (N = 120) from Utah with ancestry from Northern and Western Europe, for example, genotypes G/G, G/A, and AA for rs1880676 are found at a prevalence of approximately 57, 40, and 3% respectively. Among Han Chinese from Beijing, China (N = 88); Japanese from Tokyo, Japan (N = 88); and Yoruba from Ibadan, Nigeria (N = 120); corresponding frequencies are (G/G) 80, 57, and 100%; (G/A) 18, 36, and 0%; and (A/A) 2, 7, and 0%, respectively [International HapMap Project, 2003]. Respective allele frequencies in North American, Han Chinese, Japanese, and Nigerian populations are 0.77, 0.89, 0.75, and 1.0 (G allele); and 0.23, 0.11, 0.25, and 0 (A allele). Given then the level of association observed between rs1880676 and disease susceptibility among Basque individuals, we postulate that this polymorphism may represent a marker for other, putatively functional, polymorphisms located in linkage disequilibrium (LD) that confer an important and more direct influence on

disease susceptibility. This is more so given that rs1880676 resides outside the postulated regulatory region of this gene, as determined from data recently published in ENSEMBL [Birney et al., 2006]. Further investigation of other potential LD polymorphisms in sample populations of larger statistical power is therefore warranted to determine this extent. At the same time the location of these polymorphisms on chromosome 10q11.23, which falls in proximity to a schizophrenia susceptibility locus implicated in a number of genome-wide studies [Fallin et al., 2003; Liu et al., 2003; Faraone et al., 2006], lends further support for a potential contribution of loci in this region to the disease. A second polymorphism, rs3810950G/A, was found to be moderately associated with disease susceptibility among Basques. As with rs1880676 this association was not identified in our Spanish population, a finding which again, in light of the underlying ethnic differences that exist in the distribution of this polymorphism, may be explained by the reasons put forth previously [refer to International HapMap Project, 2003]. At the same time it is conceded that differences in size between the Basque and Spanish samples may also be a contributing factor, with the possibility of lack of power existing in the Spanish sample. Given the purported functional role of rs3810950, where the G → A transition induces an alanine to threonine residue change in a consensus sequence implicated to be important for attenuating the translational activity of ChAt messenger RNA [Mubumbila et al., 2002], further work is warranted to determine the contribution of this SNP in the disorder. Tentative evidence was also uncovered for a possible influence of ChAt on outcome to olanzapine therapy amongst patients of Spanish origin, but not of Basque origin. Similar to our earlier finding of disparity for disease susceptibility between these groups, this observation may arise from differences in distribution of allele frequency that appear to occur for this polymorphic region throughout populations. In the case of Spanish patients, we postulate that these polymorphisms may therefore be in close LD with polymorphisms more strongly associated with drug efficacy, while in the case of Basque individuals they may be more closely linked to loci that influence disease susceptibility. The precise mechanisms underlying a potential contribution of ChAt to treatment outcome are however unclear since the enzyme is not known to directly participate in the mechanisms of action of antipsyhcotic compounds. Nevertheless olanzapine possesses highest affinity for acetylcholine dependent muscarinic 1 receptor target sites, where polymorphisms that alter ChAt expression and/or function might conceivably bear a significant impact on the functions of this system. At the same time the system is believed to have a modulatory effect on other neurotransmitter systems targeted by olanzapine, particularly mesocorticolimbic dopaminergic pathways involved in cognitive and affective function, and on serotonin, glutamine and histamine meditaed neurotransmitter pathways. It therefore remains to be determined whether molecular changes in ChAt gene integrity relate to the alterations in ChAt activity that have been extensively documented in schizophrenia, and whether through this process they impact on the disorder's susceptibility or treatment. At the same time the influence that these mechanisms bear on other symptoms pertinent to schizophrenia in which ChAt may be involved, particularly cognitive dysfunction, remains to be fully established.

## SUMMARY

In summary, we have investigated the potential contribution of three ChAt gene polymorphisms in schizophrenia, and uncovered evidence for an influence of a 5′ promoter polymorphism, rs1880676A/G, on susceptibility to the disorder among individuals of Basque origin. Evidence for a similar, though less significant, influence has also been uncovered for a second polymorphism of putative function, rs3810950. At the same time our findings implicate both polymorphisms in influencing treatment outcome to olanzapine among Spanish patients. Although further work remains, our findings tentatively suggest a genetic influence of ChAt mediated mechanisms in schizophrenia and it's treatment.

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