A single nucleotide polymorphism in *CHAT* influences response to acetylcholinesterase inhibitors in Alzheimer's disease

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Background **Alzheimer's disease (AD) is a devastating neurodegeneration with a characteristic deficit in cholinergic neurotransmission. Treatment with acetylcholinesterase (AChE) inhibitors aims to reverse this deficit and does ameliorate the decline in cognition in some AD patients, although response is variable.**

Objective **To examine whether sequence variation in the gene encoding choline acetyltransferase (*CHAT*), which encodes the major catalytic enzyme of the cholinergic pathway, predicts response to AChE inhibitors.**

Methods **Alzheimer's disease patients (121) were treated with cholinesterase inhibitors and the effect of treatment on cognition was measured using the Mini Mental State Examination (MMSE). Six polymorphisms in *CHAT* were analysed for association with change in MMSE score.**

Results **After correction for multiple testing, we found one SNP, rs733722, in a promoter region of *CHAT*, is associated with response of AD patients to cholinesterase inhibitors ($P=0.03$) and accounts for 6% of the variance in response to AChE inhibitors.**

Conclusion **Rs733722 represents a putative marker of response to AChE inhibitors in AD patients.**
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Introduction

Alzheimer's disease (AD) is the most common form of dementia with an estimated more than 400 000 people affected in the UK. The common form of the disease primarily affects older people where the natural history is one of a gradual and highly distressing decline in brain function. Specific treatments are limited in number and efficacy, with acetylcholinesterase (AChE) inhibitors and memantine, an *N*-methyl-D-aspartate antagonist, being the only recognized effective treatments. However, there is marked variability in response of AD patients to treatment making it desirable to develop methods for identifying individuals likely to derive most benefit. This need was highlighted in the recent National Institute for Clinical Excellence (UK) consultation document (http://www.nice.org.uk/page.aspx?o = 245908).

AD is characterized by a widespread degeneration of the basal forebrain acetylcholinergic system [1–3]. The biosynthesis of acetylcholine is catalysed by choline acetyltransferase (ChAT), an enzyme that is characteristically reduced in AD [4,5] to an extent that correlates with the severity of dementia [6]. ChAT is imported into synaptic vesicles by the vesicular acetylcholine transporter encoded by the gene *SLC18A3*, which is located within an intron of the CHAT gene, and both genes are co-ordinately regulated [7]. Although not necessarily a primary pathogenic event, reduced cholinergic activity is thought to play an important role in mediating the cognitive deficits associated with the disease. This hypothesis forms the rationale underpinning the use of AChE inhibitors in AD which are thought to exert their therapeutic effects by at least partially and temporarily reversing the cholinergic deficit by preventing the degradation of synaptic acetylcholine [8].

Reasoning that the efficacy of AChE inhibitors might depend upon the amount of acetylcholine being synthesized and transported into synaptic vesicles, we investigated whether response to AChE treatment can be

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predicted by variants in *CHAT*, the gene encoding ChAT. In order to achieve this we analysed six polymorphic markers spanning *CHAT* and the gene encoding the vesicular acetylcholine transporter *SLC18A3* which is contained in the same locus, that we had previously identified in a study of AD susceptibility [9], in a sample of 121 AD patients treated with AChEs. Neither ourselves nor subsequently others [10] have found evidence that this locus influences risk of AD *per se*.

Methods

Participants were 121 white AD patients (mean age at treatment initiation 75.2 ± 7.4 years; mean Mini Mental State Examination (MMSE) 20.9 ± 4.1, 53% female) living in Northern Ireland. All individuals had MMSE measures at approximately six monthly intervals. At initiation, 70 were prescribed donepezil, 41 galantamine and 10 rivastigmine: 8 patients had their medication changed during the study but were included in the analysis as the change was to another AChE inhibitor. Response to treatment was measured by rates of decline in MMSE scores (points/year) from the first to the last available point: 12 individuals had measurements at two time points, 62 at three time points and 47 at four time points: the average duration in the study for all AD cases was approximately 15 months. The study was approved by The Research Ethics Committee, Queen's University, Belfast. The control population used to check whether any association was response to treatment or differences in the natural history of the disease was 176 AD individuals from a second UK AD sample, diagnosed with probable AD according to NINCDS-ADRDA criteria (as detailed in Harold *et al.* [9]), who had not taken AChE inhibitors and who had MMSE > 0 at collection. Collection of this sample was approved by the Multi-Centre Research Ethics Committee for Wales. Decline in MMSE score was calculated by using MMSE score at collection subtracted from an assumed score of 25 at onset of disease (age at onset 77.2 ± 6.3 years, age at collection 83.4 ± 5.7 years; 18.8% males, average disease duration about 6 years).

Our previous study detected 17 single nucleotide polymorphisms (SNPs) in the CHAT locus, 14 in *CHAT* and three in *SLC18A3* [9]. We restricted genotyping to SNPs with a minor allele frequency of > 0.15 to reduce redundancy. Where pairs of markers were in strong LD ($r^2 > 0.9$), only one was typed. Five previously genotyped [9] SNPs met these criteria, all in *CHAT*, and were examined for association with treatment efficacy. The rs8178981 SNP was identified by the same method as those in our previous study [9] but was not genotyped in that study as it had not been identified at the time thus allele frequency information was unavailable. Rs8178981 was located in a putative enhancer element of one of the multiple *CHAT* promoters. Genotyping assays were as described [9]. Rs817981 was genotyped by restriction fragment length polymorphism assay, using *Mly*I to differentiate between alleles.

To examine the relationship between MMSE score and polymorphisms in the CHAT gene we performed a quantitative association analysis [11] using qtphase [12]. To minimize the number of tests done we focused on additive effects only. In most quantitative genetic studies additive effects account for the vast majority of the genetic variance [13] and the most powerful tests for quantitative association analysis are commonly 1 degree of freedom additive model tests. *P*-values were corrected for multiple testing by permutation. These empirical *P*-values were based upon randomly permuting trait scores and recalculating the test-statistic over 20 000 replicates.

Results and discussion

Of the six SNPs examined only SNP rs733722 showed a significant association with decline in MMSE score in our sample (Table 1). Rs733722 explained almost 6% of the variance in response ($P = 0.0065$), with the T allele associated with less decline in MMSE score compared with the C allele. Permutation analysis to allow for multiple testing gave an empirical *P*-value for the association of 0.03. The additive effect of having one copy of the C allele at this locus is a decline of 1.66 MMSE points/year relative to the T allele (95% CI: 0.421–2.831 MMSE points/year). Thus subjects with CC genotype at this locus had a mean MMSE score decline of 3.24 MMSE points/year compared with TT homozygotes. In absolute terms, TT homozygotes on treatment showed a negligible decline in MMSE score.

Marker rs8178981 had a frequency of 2% in our population and although it showed a large difference in rate of decline between alleles, the 95% CI were also very wide. Thus we cannot exclude this polymorphism from having an effect on cholinesterase efficacy. However, marker rs733722 is present in around 33% of the population and rs8178981 in < 4% of the population so the effect of rs733722 on treatment efficacy will be important for a larger proportion of the population with AD.

To ensure that the association was to response to treatment, rather than a main effect of genotype on the natural history of disease progression, we genotyped 176 AD individuals from a second UK AD sample, who had not taken AChE inhibitors and who had MMSE > 0 at collection (average MMSE at collection 12.0 ± 6.6). Decline in MMSE score was calculated by using MMSE score at collection subtracted from an assumed score of 25 at onset of disease (age at onset 77.2 ± 6.3 years, age at collection 83.4 ± 5.7 years; 18.8% males, average disease duration about 6 years). The power of this sample to detect the effect size seen in the drug treated sample at

Table 1 Quantitative association analysis of ChAT genotype and MMSE score in AD patients treated with AChE inhibitors

Marker	Allele	Allele counts (frequency)	MMSE change/year	*P*-value (95% CI)
−5293	C	198 (0.82)	−1.84	0.0065
rs733722	T	44 (0.18)	−0.18	(0.421–2.831)
−4501	C	237 (0.98)	−1.48	0.1586
rs8178981	T	5 (0.02)	−4.46	(−2.175–9.417)
−44	C	188 (0.79)	−1.58	0.9864
rs7903315	G	50 (0.21)	−1.59	(−1.095–1.183)
1882	G	182 (0.76)	−1.71	0.3337
rs1880676	A	58 (0.24)	−1.16	(−1.533–0.504)
11604	G	201 (0.83)	−1.52	0.8308
rs868750	A	41 (0.17)	−1.66	(−0.787–1.471)
41388	A	124 (0.51)	−1.12	0.0827
rs7094421	G	118 (0.49)	−1.98	(−0.050–1.765)

Markers are numbered as in Harold *et al.* [9]. The 95% CI is on the difference between having the 1 allele versus having the 2 allele.

the 5% level is 89% [14]. However, no evidence for association was found between rate of decline in MMSE score and rs733722 ($P = 0.4593$). Carrying out the same analysis using only those subjects with MMSE > 10 at collection (mean MMSE 15.4 ± 4.4), to ensure that any floor effect in MMSE was excluded, similarly gave no evidence for association of rate of decline with rs722733 or any other polymorphism studied: this reduced the sample size to 115 with a 73% power to detect an effect size of 6% at the 5% level. The rates of decline at 1.6 MMSE points/year in the treated population and 2.0 MMSE points/year in the untreated control sample are in line with previously reported rates of decline in MMSE in AD [15,16] and indicate that similar rates of decline were prevalent in both populations studied here. These results suggest that the finding of association in the drug-treated group is not related to the natural history of the disorder but to the treatment.

Our data therefore suggest that rs733722, which lies in a putative promoter region of *CHAT*, is a marker of response to AChE treatment. Given the design of our study, it is *a priori* more likely that the association is the result of linkage disequilibrium between rs733722 and the true functional variant. It therefore follows that although the predictive power of rs733722 is low, the true functional variant may predict a much higher proportion of the response. More detailed molecular analyses at the CHAT locus are now warranted, as are analyses in other genes influencing acetylcholine neurotransmission. Finally, we would stress that prior to clinical exploitation of the findings, independent replication is required. Assuming that can be achieved, at present, the finding only accounts for a small proportion of the response to treatment.

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