Genetic variation in the choline O-acetyltransferase gene in depression and Alzheimer's disease: The VITA and Milano studies

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1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized pathologically by extensive neuronal cell loss (in particular within the hippocampus and neocortex) and by the accumulation of both senile plaques containing β-amyloid and of neurofibrillary tangles (NFTs) containing abnormally-phosphorylated tau protein (Chapman et al., 2001). Thus far, autosomal dominant mutations have been identified in three separate genes which contribute to autosomal early onset forms of this disease: amyloid precursor protein, presenilin 1 and presenilin 2 (Di Fede et al., 2009; Serretti et al., 2007), which account for an estimated 3–5% of all AD cases. Predominantly, this disease has late onset (>65 years of age) and does not show a clear Mendelian pattern of segregation. However, genetic factors play an important role in determining late onset AD (LOAD) risk. The apolipoprotein E (APOE) gene ε4 allele is the only known genetic variant that is unequivocally associated with increased late onset AD risk (Laws et al., 2003; Serretti et al., 2007). Yet fewer than half of all AD sufferers possess the ε4 allele and not all ε4 carriers...
develop disease (Bertram, 2009). Thus, because APOE does not account for all the estimated heritability in LOAD, additional genes must be implicated together with environmental factors. Linkage studies have identified several promising chromosomal regions to harbor additional AD genes, including chromosomes 12, 10, 9 and 6 (Kamboh, 2004). A broad linkage peak encompassing a ~60 cM region between chromosome 10q21 and 10q25 that influences both AD risk and age at onset has been suggested (Bertram et al., 2000; Ertekin-Taner et al., 2000; Li et al., 2002; Myers et al., 2002). There are more than 300 genes in this broad genomic region of chromosome 10 and thus the task of identifying the candidate gene is daunting. One approach is to focus on known biological candidate genes in the region. There are a number of promising candidate genes in this region that are involved either in the production, processing, or clearance of β-amyloid peptide and among these is the choline O-acetyltransferase (CHAT) gene (gene accession number NM_020549, X65685 partial).

CHAT-protein 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 end-stage AD, the most consistent losses throughout the progression of AD are seen in long projection neurons, including cholinergic neurons of the basal forebrain (Auld et al., 2002; Mufson et al., 2003). Cholinergic neurons within the nucleus basalis and the septal diagonal band provide the major source of cholinergic innervation to the cerebral cortex and hippocampus, respectively, and play a key role in memory and attentional function (Auld et al., 2002; Mufson et al., 2003). Cholinergic basal forebrain cortical projection neurons contain the pathological AD hallmark, NFTs, and undergo chemical phenotypic alterations during the progression of AD (Mufson et al., 2007).

Previously, some groups have examined the role of CHAT genetic variation with AD risk or age at onset, but the results have been equivocal (Ahn Jo et al., 2006; Cook et al., 2005; Harold et al., 2003; Kim et al., 2004; Mubumbila et al., 2002; Ozturk et al., 2006; Piccardi et al., 2007; Scacchi et al., 2009; Schwarz et al., 2003; Tang et al., 2008). Several studies examined multiple SNPs in the CHAT gene (Ahn Jo et al., 2006; Cook et al., 2005; Harold et al., 2003; Ozturk et al., 2006; Piccardi et al., 2007; Scacchi et al., 2009), while other studies examined only one SNP in exon 5, A120T (rs3810950) (Kim et al., 2004; Mubumbila et al., 2002; Schwarz et al., 2003), and most of these studies used relatively small sample sizes and thus fall short of providing conclusive results. The objective of this study was to use a broadly characterized longitudinal cohort study and an additional case-control study to examine the role of nineteen CHAT SNPs that previously showed suggestive associations with AD risk and quantitative trait of AD. As second aim, we examined the association of these SNPs to depression, since depression seems to intersect with AD (Grünblatt et al., 2008) and the case-control Milano sample. Briefly, the VITA study is a prospective community-based cohort study in which some subjects were 75.76 ± 0.45 years old at baseline recruitment coming from the geographical area of Vienna’s districts 21 and 22. Six hundred and six volunteers consented to participate in this study at baseline. The Milano sample comprises 354 subjects originating from Northern Italy including two hundred and ten patients with AD (probable AD) consecutively recruited at the Alzheimer Unit of Ospedale Maggiore Policlinico (Milan). All patients underwent a standard battery of examinations, including medical history, physical and neurological examination, screening laboratory tests, neurocognitive evaluation, brain Magnetic Resonance Imaging (MRI) or Computed Tomography (CT) and, if indicated, Positron Emission Computed Tomography (PET). Dementia severity was assessed by the Clinical Dementia Rating (CDR) and the Mini Mental Scale Examination (MMSE). Subjects with significant vascular brain damage were excluded (Hachinski Ischemic Score <4).

2. Methods

2.1. Patient recruitment and evaluation

The present study investigated two samples described earlier in detail, namely the VITA study cohort (Fischer et al., 2008; Grünblatt et al., 2008) and the case-control Milano sample. Briefly, the VITA study is a prospective community-based cohort study in which some subjects were 75.76 ± 0.45 years old at baseline recruitment coming from the geographical area of Vienna’s districts 21 and 22. Six hundred and six volunteers consented to participate in this study at baseline. The Milano sample comprises 354 subjects originating from Northern Italy including two hundred and ten patients with AD (probable AD) consecutively recruited at the Alzheimer Unit of Ospedale Maggiore Policlinico (Milan). All patients underwent a standard battery of examinations, including medical history, physical and neurological examination, screening laboratory tests, neurocognitive evaluation, brain Magnetic Resonance Imaging (MRI) or Computed Tomography (CT) and, if indicated, Positron Emission Computed Tomography (PET). Dementia severity was assessed by the Clinical Dementia Rating (CDR) and the Mini Mental Scale Examination (MMSE). Subjects with significant vascular brain damage were excluded (Hachinski Ischemic Score <4). The diagnosis of possible and probable AD was made by exclusion according to NINCDS-ADRDA criteria (McKhann et al., 1984). In the VITA study at 60 months follow-up 111 subjects had probable/probable AD, while 319 were considered as controls without AD. In the Milano sample, fifty AD patients had an early onset of disease (EOAD; <65 years) whereas remainders had a late onset AD (LOAD; >65 years). Conversely in the VITA cohort all AD subjects are LOAD. The Milano control group consisted of 144 subjects matched for ethnic background, age and gender without memory impairment (MMSE >28) and behavioral complaints.

2.2. Preparation of genomic DNA

DNA was prepared from 2 ml EDTA-blood as described previously (Grünblatt et al., 2005). For DNA extraction of the Milano samples, the Flexigene kit (Qiagen) was used, according to the manufacturer’s protocol. The DNA is then aliquoted into cryo-Vials (Nunk GmbH, Wiesbaden, Germany) and frozen at ~70 C till processing.

2.3. Genotyping

19 single-nucleotide polymorphisms (SNP) tagging the common allelic variation of the CHAT gene were investigated. Tagging SNPs were chosen with the Haploview Tagger (Barrett et al., 2005) function on the basis of the HapMap CEU population (Frazer et al., 2007). rs3810950 was genotyped using TaqMan technology as previously described (Grünblatt et al., 2008). All other 18 SNPs (rs10776585, rs2289305, rs3750752, rs12356649, rs11591558, rs8179894, rs1153783, rs1880676, rs12359885, rs10082479, rs17775758, rs7903612, rs7091005, rs2889759, rs4838391, rs11101186, rs10857520) were genotyped using the Sequenom MassArray iPLEX® platform. Multiplex PCR assays were designed using the Sequenom SpectroDESIGNER® software (version 3.0.0.3) (Supplementary Table S3). Briefly, 10 ng genomic DNA was amplified
in a 5 μl reaction containing 1 × Hot-Star Taq PCR buffer (Qiagen), 1.625 mM MgCl₂, 500 μM each dNTP, 100 nM each PCR primer, 0.5 U HotStar Taq (Qiagen). The reaction was incubated at 94 °C for 15 min followed by 44 cycles of 94 °C for 20 s, 56 °C for 30 s, 72 °C for 1 min, followed by 3 min at 72 °C and cooling at 4 °C for 4 min. Excess dNTPs were then removed from the reaction by incubation with 0.3 U shrimp alkaline phosphatase (Sequenom) at 37 °C for 20 min followed by 5 min at 85 °C to deactivate the enzyme. Single primer extension over the SNP was carried out in a final concentration of between 0.625 μM and 1.5 μM for each extension primer (depending on the mass of the probe), iPLEX® termination mix (Sequenom) and 1.35 U iPLEX® enzyme (Sequenom) and cycled using a two-step 200 short cycles program; 94 °C for 30 s followed by 40 cycles of 94 °C for 5 s, 5 cycles of 52 °C for 5 s, and 80 °C for 5 s, then 72 °C for 3 min. The reaction was then desalted by addition of 6 mg cation exchange resin followed by mixing 20 min and centrifugation to settle the contents of the tube. The extension product was then spotted onto a 384 well spectroCHIP® array. Automated spectra acquisition in the mass spectrometer (Bruker Autoflex, Bruker, Karlsruhe, Germany) was performed using SpectroACQUIRE® (Sequenom). We performed data analysis with the MassArray Typer® software version 3.4. Laboratory staff was blinded to case status of study participants. Additionally, genotypes for each subject were checked manually to ensure data quality.

2.4. Statistical analysis

Statistical analyses were carried out using SPSS for Windows version 16.0 (SPSS Inc., Chicago, IL, USA) and R 2.8.1 (R Development Core Team (2008)). Continuous data are described by mean, 95% confidence interval and standard deviation. For categorical data, frequencies and percentages are given. Continuous data were compared between controls and the different diagnosis groups of depression and dementia using Mann–Whitney-Test.
Table 1

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Significant p-values were set at < 0.0025. Nominal-p values bolded in Table 1 for details see Supplementary Table S6.

In total, nineteen SNPs were genotyped spanning a region of ~69.3 kb across the CHAT locus (Fig. 1a). The minor allele frequencies of the SNPs ranged from 0.021 to 0.497 (Supplementary Tables S4 & S5). All of the analyzed CHAT SNPs conformed to HWE, in the AD and controls of the VITA and the Milano study (Supplementary Tables S4 & S5).

For the 19 SNPs genotyped, no statistically significant differences were observed between genotype or allele frequencies for the different time points as well as the cross-sectional analysis in the VITA and the Milano study (Supplementary Tables S6 and S7) following Benjamini and Hochberg correction. Nominal significant results were only apparent for rs3810950 in the cross-sectional VITA study in probable AD versus controls (p = 0.038, genotype; OR = 1.66 (95% CI 1.03–2.66) p = 0.052 allele-wise; see Table 1). In the Milano study, this SNP did not replicate, although there was a trend (p = 0.080). When cases and controls of both samples were combined, rs3810950 was significantly associated with AD (χ² = 2df pcombined = 0.01634; the combined samples had a power of 82% to detect variants conveying a relative risk of 1.8 assuming a minor allele frequency of 5%; (Menashe et al., 2008)). Rs8178984 genotype showed both in the cross-sectional analysis as well as in the 60 months follow-up of the VITA study nominal significance (n = 0.047 and p = 0.015, respectively; Table 1). The G allele had a lower frequency in the AD group (defined as possible + probable AD) at both time points as compared to control (OR = 0.61 (95% CI 0.38–0.97) p = 0.045, cross-sectional; and OR = 0.41 (95% CI 0.22–0.70) p = 0.003, 60 months follow-up). This, again, was not replicated in the Milano study (p = 0.180, genotype; p = 0.201, allele-wise) but still showed a trend of similar frequency direction as the VITA. Rs1880676, previously reported to be associated with AD, showed no significant association in the VITA study (for all time points) as well the Milano study (Supplementary Tables S6 and S7).

In order to investigate associations reported in previous studies (Mubumbila et al., 2002; Ozturk et al., 2006; Scacchi et al., 2009; Tang et al., 2008), a subgroup of samples was stratified for APOE status (Table 2, for detailed information see Supplementary Tables S7 and S8). Restriction of the findings to APOE ε4 risk allele carriers resulted in a nominal significant association for rs3810950 with an AD diagnosis in the VITA study for the cross-sectional analysis as well as for the 60 months follow-up. Cross-sectional analysis resulted in a p-value = 0.009 for the genotypic association, and also the A-allele frequency was higher for the probable AD versus controls (OR = 3.21 (95% CI 1.43–7.19) p = 0.007). Similarly, nominal significance was found for 60 months...
3.2. Haplotype associations with AD

Analysis of variation across the CHAT locus in the VITA study sample (Fig. 1b and c) identified five haplotype blocks. Haplotype analysis revealed that low LD occurred between the SNPs investigated. Cases showed similar patterns of variation (data not shown).

Neither haplotype was associated with AD in any of the study groups (Supplementary Tables S9 and S10 and Fig. S1). The most common haplotype (Block 1/2 CA) was observed at a frequency of 83.7–87.6% in AD subjects and 80.4–90.6% in controls.

3.3. Single and multi-marker associations with depression

The minor allele frequencies of the SNPs ranged from 0.022 to 0.485 (Supplementary Table S11). All of the analyzed CHAT SNPs conformed to HWE, in the depression (minor + major) and control subjects of the VITA study (Supplementary Table S11). From the 19 SNPs genotyped, no statistically significant differences were observed between genotype or allele frequencies for the different time points of the VITA study (Supplementary Table S12). Furthermore, there was no global haplotype association with depression (Supplementary Table S13). Nominally significant associations were observed in the VITA study for block 2-haplotypes CC and CT (frequencies: depressed 50.1% vs. control 60.8%; p = 0.0084; depressed 41.7% vs. control 33.1%; p = 0.0294, respectively), and for block 4-haplotypes TG and CA (frequencies: depressed 42.8% vs. control 50.9%; p = 0.0491; depressed 19.2% vs. control 13.2%; p = 0.0429, respectively).

4. Discussion

In the current study we have conducted an association study investigating the contribution of genetic variation within the CHAT gene to the risk for AD as well as for depression in a longitudinal cohort study (VITA study) as well as in a replication case-control study (Milano study) using a marker set of 19 SNPs across the CHAT gene. In order to account for multiple testing, the permutation algorithm implemented in Haploview was used to obtain a measure of significance corrected for multiple testing. After this correction, no significant association was identified with any of the SNPs or haplotypes assessed in AD or depression in both study groups.

Nevertheless, we found a nominally significant and suggestive association of the rs3810950 with the risk of AD in the cross-sectional analysis of the VITA study. Originally, Mubumbila et al. (Mubumbila et al., 2002) reported a significant association of this SNP in 122 late onset AD cases and 112 controls in a French–German population, but replication attempts yielded inconsistent results (Ahn Jo et al., 2006; Cook et al., 2005; Grünblatt et al., 2008; Harold et al., 2003; Kim et al., 2004; Ozturk et al., 2006; Schwarz et al., 2003; Tang et al., 2008). Cumulative meta-analysis revealed a marginal influence of the CHAT rs3810950 A-allele on the risk of AD (OR = 1.17, 95% CI = 0.96–1.44), as pointed out by Bertram et al. (Bertram et al., 2007) in the AlzGene database. The APOE ε4 allele is an important genetic susceptibility factor for the development of sporadic AD and previous studies have reported a possible
association between CHAT polymorphism and AD in APOE ε4 carriers (Ahn Jo et al., 2006; Kim et al., 2004; Ozturk et al., 2006; Tang et al., 2008). Accordingly, in our study a nominally significant association of the rs3810950 A-allele and AD was found after stratification to APOE ε4 carriers (p = 0.007). In addition, the SNPs in the CHAT gene were investigated in further recent studies, but again, results were equivocal (Cook et al., 2005; Ozturk et al., 2006; Piccardi et al., 2007; Sacchì et al., 2009). In our study we could not find an association to AD with rs1879894 or rs1880676. However, nominally significance for rs1879894 to AD was found in the cross-sectional and the 60 months follow-up (p = 0.047 and p = 0.015, respectively), while for rs1880676 nominally significant association to AD was revealed only after stratifying to APOE ε4 carriers (p = 0.008). Additionally, these findings could not be confirmed in our replication dataset (Milano study), probably because of its high percentage of earlier onset cases in conjunction with a low frequency of APOE ε4 alleles.

Quantitative traits and markers also showed suggestive associations to some of the SNPs studied, e.g. cognitive function measured by MMSE score, which showed at baseline of the VITA study nominally significant decreased MMSE scored for A-allele carriers of the rs3810950 (p = 0.037). However, this association was not observed at later follow-up time points. Such an association was also suggested in previous publications, yet again this was not consistently replicated (Harold et al., 2006; Ozturk et al., 2006; Tang et al., 2008). Small sample sizes might account for this.

In summary, with the exception of CHAT rs3810950, our data do not seem to suggest that CHAT is a major susceptibility gene for AD in later life. However, the strong functional candidacy of CHAT and our data on rs3810950 recommended to be examined in larger and more powerful sample sets. It is furthermore possible that the perturbations to the cholinergic system observed in AD patients may reflect changes in mRNA or protein synthesis, stability, or post-translational modification due to non-genetic factors. Alternatively, this may also be due to selective vulnerability of specific cholinergic neuronal subtypes. Thus, despite the fact that we do not provide strong evidence that genetic variation of CHAT is implicated in AD, the cholinergic system still is a prime target of AD pathogenesis.

Acknowledgement

We thank the supported by the Ludwig Boltzmann Institute of Aging Research, Vienna, Austria and Fondazione Ospedale Maggiore Policlinico and Monzino Foundation, Milan, Italy. We thank also the DFG for its support. We thank all subjects taking part in the VITA study and their families. We thank Theresia Töpner and Miryame Hofmann for their excellent technical assistance.

Appendix. Supplementary data

Supplementary data related to this article can be found online at doi:10.1016/j.jspychires.2011.03.017.

References


