Association of CHRNA4 polymorphism with depression and loneliness in elderly males

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The cholinergic receptor, nicotinic, alpha 4 (CHRNA4) gene encodes the neuronal nicotinic acetylcholine receptor alpha-4 subunit. Recent research has shown that a variation in CHRNA4 (rs1044396) affects attention and negative emotionality in normal adults. To determine the link between CHRNA4 variation and cognitive function/depressed mood, this study conducted a genotype–phenotype correlation analysis between the common CHRNA4:rs1044396 variant and several baseline parameters of cognition and depressed mood in 192 elderly male subjects without major psychiatric disorders or dementia. Study findings identified a significant link between the CHRNA4:rs1044396 polymorphism and depression and loneliness in the aged. Compared to carriers of at least one T-allele, carriers of the homozygous C/C genotype described themselves as more depressed and lonely. This is the first evidence which may implicate CHRNA4 in depressed emotions in the elderly.

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There are two main types of cholinergic receptors widely distributed throughout the brain: nicotinic acetylcholine receptors (nAChRs) and muscarinic acetylcholine receptors. nAChRs belong to a superfamily of ligand-gated ion channels which exist in many different subtypes, constructed from a myriad of possible subunit combinations. Evidence has suggested that nAChRs may be involved in the pathogenesis of depression (Mineur & Picciotto 2010; Philip et al. 2010). First, nAChRs are widely distributed in neuroanatomic regions implicated in depression, including basal ganglia, amygdala and ventral tegmental area. Through their actions in these areas, they modulate the release of other monoamine neurotransmitters, particularly dopamine, which is strongly implicated in affect regulation and reward processing (Albuquerque et al. 2009). Second, in addition to their role in cholinergic neurotransmission, nAChRs are also involved in neuroendocrine systems implicated in depression, particularly the hypothalamic–pituitary–adrenal axis (Mello 2010; Yu et al. 2010). Third, nAChRs are the primary target in the brain for nicotine ingested by smoking. Previous literature has well established the connection between smoking and depression, suggesting that the intake of nicotine has an effect on mood. Inhibition of nAChRs may also, in part, mediate the therapeutic actions of many antidepressants (Shytle et al. 2002). Fourth, varenicline and sazetidine-A, partial agonists of α4β2 nAChRs, have antidepressant-like properties in mice (Turner et al. 2010).

Depression and loneliness are common and distressing problems for many older people (Cohen 2000; Luanaigh & Lawlor 2008). In populations of elderly Caucasians, the prevalence of major depression ranges from 0.9% to 9.4% in private households, and from 14% to 42% in institutional living (Djernes 2006). A previous study on elders from rural areas in Sweden showed that 35% of the persons reported experienced loneliness (by the simple question: ‘Do you experience loneliness?’) (Holmén et al. 1992). A study in Israel based on home interviews of 70 year olds showed that around 46.3% of females and 21.3% of males reported being lonely (Stessman et al. 1996). This study also demonstrated that ‘being depressed’ is one of the major factors associated with loneliness. Loneliness and depression often correlate with physical illness and disability, life events and social isolation, which also associate with aging processes (Lawhorne 2005).

Although psychosocial issues play important roles in aged depression, studies of the past decade began to explore the biological factors related to susceptibility to aged depression (Halbreich & Lumley 1993). Several reports have identified genetic variants related to geriatric depression. In a cross-sectional study using a community sample of 3752 men aged 70 years or older, a methylenetetrahydrofolate reductase C677T genetic variant associated with depression (Almeida et al. 2008). Recently, our group found an association between brain-derived neurotrophic factor Val66Met polymorphism and Chinese geriatric depression, chiefly in males (Hwang et al. 2006). Another study, in a Caucasian aged population, replicated this finding (Taylor et al. 2007). To the authors’ knowledge, there is still no study of nAChR genetic variants related to depression in the aged population.
nAChRs belong to a superfamily of ligand-gated ion channels which exist in many different subtypes, constructed from a myriad of possible subunit combinations. Among them, the human CHRNA4 gene encodes the neuronal nAChRs alpha-4 subunit and is located on chromosome 20q13.2-13.3. The gene is ~17 kb in size and comprises six exons (Steinlein et al. 1996). Steinlein and coworkers first described a same sense thymine-to-cytosine polymorphism at cDNA position 1629 (rs1044396; initially named T1545C) within the CHRNA4 gene (Steinlein et al. 1997). Subsequent research associated this polymorphism with nicotine addiction (Breitling et al. 2009; Feng et al. 2004), visual attention (but not working memory) (Greenwood et al. 2009), attention in the elderly (Reinvang et al. 2009) and attentional network function as assessed using functional magnetic resonance (Winterer et al. 2007). In a recent genetic association study of 574 healthy, white participants, Markett et al. (2011a) demonstrated that the presence of the CHRNA4:rs1044396 polymorphism associates with common variances of conceptualizations of negative emotionality. Compared to T-allele carriers, C/C homozygotes described themselves as more anxious and emotionally unstable in various psychometric personality questionnaires. Considering this finding and the possible role of CHRNA4 in depressed mood, this study examined the relationship between the CHRNA4:rs1044396 polymorphism with depression and loneliness in the elderly. This polymorphism also relates to attention/cognitive function in normal adults, therefore procedures also tested its association with attention/cognitive function in the sample population. To minimize the confounding effects of gender, a homogeneous group, composed entirely of healthy aged Han Chinese males, was used for comparison.

Methods

Subjects

The subjects were 192 males aged between 63 and 98 years (mean = 80.0; SD = 5.0), with an average of 5.2 years of education (SD = 4.6; ranged from 0 to 16 years of schooling). They were recruited from a Veteran’s Home in northern Taiwan. When the Chinese civil war ended in 1949, many of the nationalist armed forces relocated from mainland China to Taiwan. After resigning from the armed forces, many of these men remained in Taiwan. A large part never married and continues to live in Veteran Homes.

For each case, an initial evaluation including self-reported current and past medical status and participants’ medical records were checked by a trained research nurse. Each case then received a neurological check-up and a diagnostic structured interview, the Mini-International Neuropsychiatric Interview, by a trained research assistant (Sheehan et al. 1998). Daily activities and cognitive functions were assessed using the Clinical Dementia Rating Scale (CDR). Exclusion criteria included the following: (1) presence of diagnoses on Axis I of the DSM-IV; (2) severe medical illness (malignancy, heart failure); (3) neurobiological disorders (stroke, Parkinson’s disease); (4) illiteracy; (5) subjects with CDR > 0.5 or Cognitive Abilities Screening Instrument Chinese version (CASI C-2.0) (Teng et al. 1994) >50 in order to exclude possible dementia. A study by Liu et al. (1994) in elderly Chinese subjects found that, using a cut-off score of ≤50 for dementia, the sensitivity was 0.88 and the specificity was 0.94. Application of these criteria resulted in the recruitment of a group of non-demented elderly subjects.

For evaluation of depression, all participants filled in a Geriatric Depression Scale-short form (GDS-SF). The GDS-SF is a commonly used instrument consisting of 15 yes/no questions and a translated and adapted Chinese version has been developed (Lee et al. 1993). Scores on the GDS-SF showed a high correlation with those from the original form (Burke et al. 1991). The University of California, Los Angeles (UCLA) Loneliness Scale (version 3) has previously been used to measure loneliness (Russell 1996). This scale is one of the most widely used loneliness measures and has reputable reliability and validity. It contains 20 items and participants rated how often they feel the way described in the items using a four point Likert scale ranging from never to often. Cognitive functions were assessed using the CASI C-2.0 test and the Wechsler Digit Span Task test (forward and backward). The CASI test, which is a 100-point cognitive test and provides quantitative assessment in nine domains of cognitive function (long-term memory, short-term memory, attention, concentration/mental manipulation, orientation, abstraction and judgment, language, visual construction and list-generating fluency), was designed for cross-cultural studies and for individuals with little or no formal education (Teng et al. 1994). It has been adapted into Chinese and we used the Chinese version (Tsai et al. 2007). This study was conducted in accordance with the Declaration of Helsinki and was approved by the local Institutional Review Board. Informed consent was obtained from all subjects prior to commencement.

Genotyping of the CHRNA4:rs1044396 polymorphism

Peripheral venous blood was taken from the study subjects for genotyping of the CHRNA4:rs1044396 polymorphism. Genomic DNA was isolated using the PUREGENE DNA purification system (Genta Systems, Minneapolis, MN, USA). For DNA quality examination, all the samples were genotyped for eight unrelated SNPs. The samples were diluted onto 96-well plates, and only the plate with an average successful genotyping rate greater than 95% for the eight SNPs were used for further study. Primers and probes were designed with SpectroDESIGNER software version 2.0 (SEQUENOM, San Diego, CA, USA). A multiplex polymerase chain reaction was performed, and unincorporated double stranded nucleotide triphosphate bases were dephosphorylated with shrimp alkaline phosphatase (Hoffman-LaRoche, Basel, Switzerland) followed by primer extension. The purified primer extension reaction was spotted on to a 384-element silicon chip (SpectroCHIP, Sequenom) and analyzed in the Bruker Biflex III MALDI-TOF SpectroREADER mass spectrometer (SEQUENOM). The resulting spectra were processed with SpectroTYPER (SEQUENOM). All the experiments were done by investigators who were blind to the phenotypes.

Statistical analysis

Differences in continuous variables were compared for individual genotypes using one-way analysis of variance, followed by the Bonferroni post hoc test for between-group comparisons. Data are presented as mean (SD), with the level of significance set at $P < 0.05$ for all tests.

Results

The CHRNA4:rs1044396 genotype distribution for the 192 subjects was as follow: C/C = 115, C/T = 67, T/T = 10. The distributions of the genotype did not differ significantly according to the Hardy–Weinberg equilibrium ($P = 0.953$). An earlier report suggested that the T-allele behaves dominantly with respect to negative emotionality (Markett et al. 2011a), therefore this study grouped T-allele carriers (C/T and T/T) together for comparison with the C/C genotype.

There were no significant differences in age and years of education between T-allele carriers and C/C homozygote carriers (Table 1). C/C homozygote carriers had significantly higher GDS-SF ($P = 0.019$) and Loneliness...
Scale (P = 0.034) ratings than T-allele carriers. There was a high correlation between GDS-SF and Loneliness Scale (r = 0.533; P < 0.001).

In cognitive function tests the mean CASI score was 85.9 ± 10.3 (range = 57–100). The CHRNA4:rs1044396 polymorphism did not significantly associate with total CASI score, the attention domain of CASI, and the forward/backward digit span tests (Table 1). Similarly, after adjustment for age and education years, there were no significant differences in the cognitive test findings between the CHRNA4:rs1044396 polymorphism and T-allele carrier groups (data not shown).

This sample included 68 (35.4%) never smokers, 57 (29.7%) ex-smokers and 67 (34.9%) current smokers. No association between smoking status and CHRNA4:rs1044396 polymorphism was still a significant predictor of GDS-SF (P = 0.186). This study indicates that, in the sample of elderly Tsai et al., the CHRNA4:rs1044396 polymorphism was a significant predictor of GDS-SF (P = 0.016; for smoking habit, P = 0.744; for smoking habit * CHRNA4:rs1044396 polymorphism, P = 0.286) and Loneliness Scale (P = 0.017; for smoking habit, P = 0.683; for smoking habit * CHRNA4:rs1044396 polymorphism, P = 0.186).

Discussion

The results of this study indicate that, in the sample of elderly Chinese males, subjects bearing the CHRNA4:rs1044396 C/C genotype have higher levels of depression compared to the T-allele carriers (Table 1 and Fig. 1a). This association is not affected by smoking status. To the authors’ knowledge, this is the first genetic study of nAChR genetic variants in depression. The findings have several implications. First, CHRNA4 may be involved in the pathogenesis of depression. Previous research on animals has also shown that partial agonists of α4β2 nAChRs have antidepressant-like properties (Turner et al. 2010). The study sample consisted of normal elderly males therefore association of the CHRNA4:rs1044396 polymorphism with geriatric depression in elderly females warrants further exploration.

Second, the study findings correlate with those of Markett et al. (2011a) in that, compared to T-allele carriers, C/C homozygotes described themselves as more anxious and emotionally unstable on various psychometric personality questionnaires. A previous study in Chinese males suggested that the CHRNA4:rs1044396 polymorphism associated with smoking status and the strength of nicotine addiction with the T-allele of this polymorphism appearing to be a resilience factor for smoking status and strength of nicotine dependence (Feng et al. 2004). This study’s findings indicate that increasing feelings of depression or loneliness in C/C homozygotes may increase the self-medicating use of nicotine as a means of relieving these negative feelings.

The CHRNA4:rs1044396 polymorphism is a synonymous polymorphism. Whether this polymorphism has a direct effect on CHRNA4 expression/function or in combination with a CHRNA4 functional polymorphism needs further exploration. Finally, it is possible that the study findings arose by chance, and subsequent confirmation in an independent sample is needed.

Another major finding of this study is that, similar to severity of depression, subjects bearing the CHRNA4:rs1044396 C/C allele have higher loneliness scores (Table 1 and Fig. 1b). In this study, there was a high correlation between GDS-SF and Loneliness Scale which in line with earlier report that ‘being depressed’ is one of the major factors associated with loneliness (Stessman et al. 1996). The study by Markett et al. (2011a) in adult subjects suggested that the C/C carriers were more anxious and emotionally unstable according to personality questionnaire responses. The subjects in our study all lived in the same Veteran’s Home, which may decrease the confounding effect of living conditions. It is possible that subjects with such personality traits are more likely to experience loneliness and/or depression due to the life events and social isolation associated with the aging processes.

Earlier studies had found that the CHRNA4:rs1044396 polymorphism is associated with visual attention (but not working memory) (Greenwood et al. 2009), attention in the elderly (Reinvang et al. 2009) and attentional network function as assessed using functional magnetic resonance (Winter et al. 2007). Some of these studies, such as an investigation

### Table 1: Depressed, loneliness and cognitive scores among the CHRNA4:rs1044396 genotypic groups in 192 elderly males without dementia

<table>
<thead>
<tr>
<th></th>
<th>C/C (n = 115)</th>
<th>T-carriers (n = 77)</th>
<th>t-Value</th>
<th>df</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>80.5 (5.0)</td>
<td>80.7 (4.8)</td>
<td>−1.653</td>
<td>190</td>
<td>0.100</td>
</tr>
<tr>
<td>Education (years)</td>
<td>5.1 (4.6)</td>
<td>5.3 (4.6)</td>
<td>−0.308</td>
<td>190</td>
<td>0.758</td>
</tr>
<tr>
<td>GDS-SF</td>
<td>3.2 (2.9)</td>
<td>2.3 (2.2)</td>
<td>2.317</td>
<td>187</td>
<td>0.019</td>
</tr>
<tr>
<td>Loneliness Scale</td>
<td>33.1 (8.1)</td>
<td>30.3 (8.6)</td>
<td>2.135</td>
<td>190</td>
<td>0.034</td>
</tr>
<tr>
<td>Total CASI</td>
<td>85.9 (10.3)</td>
<td>85.6 (10.5)</td>
<td>0.079</td>
<td>190</td>
<td>0.937</td>
</tr>
<tr>
<td>Attention</td>
<td>7.1 (1.0)</td>
<td>7.1 (1.0)</td>
<td>−0.087</td>
<td>190</td>
<td>0.931</td>
</tr>
<tr>
<td>Wechsler Digit Span Task</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forward</td>
<td>11.7 (2.9)</td>
<td>11.1 (3.2)</td>
<td>1.296</td>
<td>190</td>
<td>0.197</td>
</tr>
<tr>
<td>Backward</td>
<td>3.5 (2.0)</td>
<td>3.3 (2.2)</td>
<td>0.911</td>
<td>190</td>
<td>0.363</td>
</tr>
</tbody>
</table>

Data are presented as mean (SD).
by Hudson et al. (2010), showed negative findings. In this study, the polymorphism did not link to global cognitive function, attention or memory. In a recent report, Markett et al. (2011a) used exactly the same measure and found no significant effect as well. These negative findings may have arisen due to the use of different cognitive tests, different ethnic background or smaller sample size as compared with the earlier studies. There is other evidence that CHRNA4 does not exert a direct influence on working memory but in interaction with the dopaminergic system (Markett et al. 2010; Parasuraman et al. 2005). As shown in the report of Markett et al. (2010), there is a significant interaction between CHRNA4:rs1044396 polymorphism and a haplotype block covering all three dopaminergic polymorphisms on working memory capacity.

The current work is based on a hypothesis derived from a previous association study (Markett et al. 2011a). Our findings further demonstrated that CHRNA4:rs1044396 polymorphism may be associated with negative mood. This study is limited by its cross-sectional design which did not allow the determination of interaction of the CHRNA4:rs1044396 polymorphism with life events and any subsequent effects on depression or loneliness in the elderly. Subjects consisted of a population of Chinese elderly males in a Veteran’s Home, therefore any association between the CHRNA4:rs1044396 polymorphism and depression/loneliness in adults, females or other populations requires further confirmation. Another limitation is the small sample size. Depression and loneliness are both complex phenotypes, where each of the individual genetic contributions can be expected to be minor. Our findings, while nominally significant, did not survive correction for multiple testing. Initial findings of the genetic associations in a sample as small as this require replication in other, preferably larger, samples sets. Finally, only one CHRNA4 polymorphism was tested in this study. Further study of other CHRNA4 polymorphisms, particularly functional ones, is needed to fully evaluate the role of CHRNA4 in depression/loneliness.

Figure 1: The genetic effects of the CHRNA4:rs1044396 polymorphism on the scores of GDS-SF (a) and the UCLA Loneliness Scale (b). Error bars indicate standard error of the mean. (*P < 0.05).

References


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