Lack of Association between Tumour Necrosis Factor Receptor Superfamily Gene Polymorphisms and the Risk of Alzheimer’s Disease in a Chinese Population

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Introduction

Alzheimer’s disease (AD) is a progressive neurodegenerative disease characterised by the deposition of amyloid plaques and the formation of neurofibrillary tangles. Mutations in

Abstract

Objective: To investigate the association of polymorphisms in the tumour necrosis factor receptor 2 (TNFR2) and tumour necrosis factor superfamily, member 6 (TNFRSF6, FAS) genes and the risk of Alzheimer’s disease (AD) in a Chinese population.

Patients and Methods: One hundred and fifty Chinese AD patients and 155 cognitively intact control subjects were recruited for the study. Genotypes of TNFR2+196, FAS-670, and FAS-1377 were investigated in this study by polymerase chain reaction–restriction fragment length polymorphisms.

Results: The TNFR2+196 TT genotype was more prevalent in the AD group than in the controls. However, no significant difference in genotypic and allelic frequencies between AD and control groups (p = 0.68) was observed.

Conclusion: We suggested that TNFR2+196, FAS-670, and FAS-1377 genotypes were not associated with the risk of AD in our Chinese population. However, evidence suggests involvement of tumour necrosis factor–alpha pathway in the pathogenesis of AD and a more comprehensive study may be required to identify the underlying associations.

Key words: Alzheimer disease; Genetic predisposition to disease; Polymorphism, genetic; Receptors, tumor necrosis factor

摘要

目的：探討腫瘤壞死因子受體2（TNFR2）和腫瘤壞死因子超家族成員6（TNFRSF6，FAS）基因多態性與華裔老年癡呆症發病風險。

患者與方法：使用聚合酶鏈反應—限制性片段長度多態性（PCR-RFLP）法檢測150名AD病人和155名認知功能正常的對照組的TNFR2+196，FAS-670及FAS-1377多態性分佈情況。

結果：AD病人的TNFR2+196 TT基因頻率高於對照組，但是這幾個單核苷酸多態性的基因型分佈頻率在統計學上並沒有顯著性差異（p = 0.68）。

結論：TNFR2+196，FAS-670及FAS-1377的多態性與華裔AD病人沒有明顯相關性。但是，有證據顯示TNFα的信號途徑與AD的發病機制有密切關係，所以我們需要一個更詳細的研究策略去探討與TNF有關的基因的多態性。

關鍵詞：老年癡呆症、遺傳易感性、基因多態性、腫瘤壞死因子受體
genes like amyloid precursor protein (APP), presenilin-1 (PSEN1), and presenilin-2 (PSEN2) were suggested as causal for familial early-onset type of AD, which only contributes to about 5% of all AD cases.\textsuperscript{4} For the sporadic-type AD, both genetic and environmental factors may be involved in its aetiology.\textsuperscript{1} Several hypotheses for the pathogenesis of AD have been proposed, including the amyloid cascade,\textsuperscript{2} axonal transport dysfunction,\textsuperscript{3} cholesterol and apolipoprotein E (ApoE),\textsuperscript{4} and inflammation.\textsuperscript{5,6}

Increased levels of tumour necrosis factor alpha (TNFα) were observed in AD patients\textsuperscript{2} and Collins et al\textsuperscript{8} reported that the TNFα haplotype (TNF-308, TNF-238, and TNFa) was associated with AD in Caucasians. However, TNF-238 polymorphism is absent in the Chinese population\textsuperscript{7} and so we selected another 3 single nucleotide polymorphisms (SNPs) for this genetic association study. Our previous findings showed that TNFα polymorphisms (TNF-857, TNF-863, and TNF-1031) were associated with the risk of AD.\textsuperscript{9} Therefore, genes related to the TNFα pathway might be implicated in the pathogenesis of AD. The biological effects of TNF are mediated by binding to its 2 main receptors, TNFR1 (p55 TNF receptor) and TNFR2 (p75 TNF receptor). TNFR2 is localised on chromosome 1p36.2 and has a higher affinity for TNF than TNFR1. It is widely expressed in the brain and is responsible for proinflammatory response signalling.\textsuperscript{11} Since TNFR2 is the major receptor for TNFα, polymorphisms in this receptor may affect the binding of TNFα and the downstream pathway involved in inflammation. The TNF superfamily, member 6 (TNFRSF6, FAS) encodes the Fas receptor, which is important in signal transduction that leads to apoptosis. In addition, the FAS pathway is an important mediator of amyloid-β-induced neuronal death.\textsuperscript{12} Increased levels of FAS protein have been reported in the brain,\textsuperscript{13} serum,\textsuperscript{14} and cerebrospinal fluid\textsuperscript{15} of AD patients. Such evidence suggests that the TNF signalling / pathway may be important in the pathogenesis of AD and members of the pathway might be implicated in the disease.

Tumour necrosis factor family of receptors orchestrates many aspects of immune cell function, including lymphoid organ development, acute inflammation, and lymphocyte co-stimulation.\textsuperscript{16} Association of TNFR2 polymorphisms has been reported in diseases related to chronic inflammation such as rheumatoid arthritis,\textsuperscript{17} Crohn’s disease,\textsuperscript{18} and systemic lupus erythematosus.\textsuperscript{19} FAS polymorphisms were reported to be associated with the risk of AD,\textsuperscript{20,21} but the results remain controversial.\textsuperscript{22,23} Here, we investigated the association of the common polymorphisms in TNFR2 and FAS and the risk of AD in the Chinese population.

### Patients and Methods

A total of 150 Chinese patients (mean age 79, SD 7, range 65-95 years; 81% women) diagnosed for probable and possible AD (according to the National Institute of Neurological and Communicative Diseases and Stroke and the Alzheimer’s Disease and Related Disorders Association criteria) were recruited from the Academic Psychogeriatric Unit of the Department of Psychiatry at the Prince of Wales Hospital in Hong Kong. As controls for comparison, 155 Chinese elderly subjects (mean age 73.8, SD 6.3, range 65-94 years; 80% women) were recruited from local elderly social centres. All the controls were evaluated by the Chinese versions of the Mini-Mental State Examination,\textsuperscript{24} Mattis Dementia Rating Scale\textsuperscript{25} as well as by specialist psychiatrists as being cognitively intact. Both the AD patients and non-demented controls originated from the Guangdong province in Southern China.

Genomic DNA was extracted from peripheral blood samples using DNA extraction kits according to the manufacturer’s instruction (Roche, US). Mismatched polymerase chain reaction (PCR)–restriction fragment length polymorphisms were used to genotype the polymorphic sites at TNFR2+196, FAS-670, and FAS-1377 (primers shown in Table 1). The polymerase chain reaction was performed in 25 µl reactions; the contents comprised 0.25 µM of each primer pair, 2 mM MgCl\textsubscript{2}, 0.6 units of Ampli Taq Gold Polymerase (Applied Biosystems) and PCR buffer (10 mM Tris-HCl, pH 8.3; 50 mM KCl). The reaction mixture was initially heated to 96°C for 15 min to activate the polymerase, and DNA amplification was achieved by 35 cycles of 96°C for 30 s, annealing for 45 s and 72°C for 45 s. The final elongation step was performed at 72°C for 7 min. For restriction enzyme digestion, 7 µl of the PCR product was digested by 5 units of the required enzyme in the presence of the accompanying buffer, in a final volume of 14 µl incubated at the temperature with optimal activity of the enzyme for overnight. The polymorphism was visualised by separating the DNA in a 4% agarose gel and stained with ethidium bromide. To validate the genotyping

Table 1. Primer sequence for the analysis of single nucleotide polymorphisms in TNFR2 and FAS genes.

<table>
<thead>
<tr>
<th>Polymorphisms</th>
<th>Primer sequences</th>
<th>Restriction enzymes</th>
<th>Alleles</th>
<th>Size of fragments (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFR2+196 (rs1061622)</td>
<td>CCATCCCTGGGAATGCAAACAA AACTGGAAGAGCGAAGTCGC</td>
<td>StyI</td>
<td>T/G</td>
<td>T: 20,200 G: 220</td>
</tr>
<tr>
<td>FAS-670 (rs1800682)</td>
<td>TAGCTGGGCGCTATGCGGATT GTCCGCGCTGGGTACAGGAG</td>
<td>BstNI</td>
<td>A/G</td>
<td>A: 255 G: 137,117</td>
</tr>
<tr>
<td>FAS-1377 (rs2234767)</td>
<td>TGTGTGCACAAAGGCTGGgAC CTTCTGAGCCTTGTGgGc</td>
<td>BsaHI</td>
<td>G/A</td>
<td>G: 20,175 A: 195</td>
</tr>
</tbody>
</table>
Table 2. TNFR2 and FAS polymorphisms in Alzheimer’s disease in our Chinese population.

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Alzheimer’s disease</th>
<th>χ² (p Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFR2+196</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>8 (6%)</td>
<td>3 (3%)</td>
<td>0.26</td>
</tr>
<tr>
<td>GT</td>
<td>34 (26%)</td>
<td>21 (21%)</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>87 (67%)</td>
<td>78 (76%)</td>
<td></td>
</tr>
<tr>
<td>FAS-670</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>34 (28%)</td>
<td>34 (33%)</td>
<td>0.68</td>
</tr>
<tr>
<td>AG</td>
<td>58 (48%)</td>
<td>44 (43%)</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>30 (25%)</td>
<td>25 (24%)</td>
<td></td>
</tr>
<tr>
<td>FAS-1377</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>34 (22%)</td>
<td>25 (17%)</td>
<td>0.49</td>
</tr>
<tr>
<td>AG</td>
<td>76 (50%)</td>
<td>75 (51%)</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>42 (28%)</td>
<td>46 (32%)</td>
<td></td>
</tr>
</tbody>
</table>

results, genotyping experiments were repeated and direct sequencing was performed in 10% of the samples. The ApoE genotyping was performed as described earlier.10 Statistical analysis of the genotype distribution and allele frequencies was performed by Chi-square test using the Statistical Package for the Social Sciences for Windows version 11.5.

Results

The genotype distributions for TNFR2+196, FAS-670 and FAS-1377 are presented in Table 2. No significant deviation of genotype frequencies from the Hardy-Weinberg Equilibrium was noted in both the case and control groups. As expected, the APOE ε4 allele was over-represented in the AD group and ε4+ genotypes were significantly associated with AD (p = 0.001). The TNFR2+196 TT genotype was more prevalent in the AD group compared to the controls; although the difference did not reach statistical significance (76% vs 67%, p = 0.26). Neither the genotype frequencies nor the allelic frequencies between AD and control groups for the TNFR2+196, FAS-670, and FAS-1377 genotypes reached statistical significance. In addition, there was no statistical difference between AD and control groups in the distribution of genotypes for these 3 SNPs after stratification by age and ApoE genotypes.

Discussion

The TNFR2 and FAS genes were mapped to regions of linkage to late-onset AD26,27 and their involvement in the TNF signalling pathway supported the possible association with the risk of AD. The 3 SNPs selected for this study were reported to be functional. TNFR2 itself can induce cytokine production, cytotoxicity, and NF-κB activation and proliferation.28 TNFR2+196 is located in exon 4 and the nucleotide substitution of this polymorphism caused the non-conserved amino acid change from Met to Arg. However, our result showed TNFR2+196 was not associated with the risk of AD.

FAS-1377 and FAS-670 were 2 common SNPs investigated in the FAS gene. Polymorphism at -670 position of the FAS gene caused nucleotide substitution within the potential binding site of the transcription element Gamma interferon activation site for the transcription factor termed signal transducer and activator of transcription 1. The binding activity might be higher for FAS-670 G allele than that for the FAS-670 A allele. In addition, the transcriptional activity of the G allele is greater than that of the A allele.29 However, results from previous association studies on the FAS gene in AD remain controversial. In this study, we were unable to show an association of FAS gene polymorphisms and the risk of AD. Recently, He et al27 showed lack of association of FAS-670 and the risk of AD in the Chinese population. Our data also suggested that promoter polymorphisms in the FAS gene were not associated with the risk of AD.

As a preliminary study, we only selected SNPs reported in the previous literature, regardless of their functional consequences for the gene. Adaptation of these SNP selection criteria for genes like TNFR2 and FAS which span 42kb and 25kb, respectively, might not be a comprehensive way to investigate them. In addition, novel polymorphisms in the gene among the Chinese might be missed. Our results suggest that the SNPs in the TNFR2 and FAS genes we investigated were not associated with the risk of AD. However, there is evidence for the involvement of the TNF pathway in AD pathology and therefore possible roles played by these genes in its pathogenesis cannot be excluded. More extensive and comprehensive experimental designs such as increasing the SNPs to be genotyped, using haplotype and linkage disequilibrium information should therefore be incorporated into future genetic association studies of AD.

Acknowledgement

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References

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