Research Article

Genetic Association between Akt1 Polymorphisms and Alzheimer’s Disease in a Japanese Population

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1. Introduction

The main pathological feature of Alzheimer’s disease (AD) is the senile plaque containing aggregated Amyloid β peptide (Aβ). Three genes have been identified as causative genes in familial AD: the amyloid precursor protein (APP), presenilin-1, and presenilin-2 genes. Apolipoprotein E (APO E) is recognized as a genetic risk factor for familial and sporadic AD [1]. In addition, variants of the sortilin-related receptor 1 (SORL1) gene have also been associated with the disease [1, 2]. There is widening recognition that the phosphatidylinositol-3-OH kinase (PI3K)-Akt-mTOR signaling pathway is directly affected by Aβ and especially Aβ oligomer modulate cell survival through PI3K-Akt pathway [3, 5–7]. An impaired insulin-mediated signal transduction is one of the pathological features of neurodegenerative diseases [8]. Epidemiological studies note that type II diabetes is risk factor for late-onset AD [9]. Insulin dysfunction might be associated with Aβ and tangles [10, 11]. Intraneuronal Aβ inhibits insulin receptors signaling in neurons by interfering with the association between Akt1 to preclude Akt1 activation [12]. In AD brains, level of PI3K-Akt-mTOR would be decreased [8]. Thus current reports revealed that the dysfunction of PI3K-Akt-mTOR system affect AD pathology [9]. Although genetic variability of PI3K has been reported to affect the risk for AD [13], there are few genetic researches about Akt and AD. In this study, the association between six single nucleotide polymorphisms (SNPs) covering the Akt1 gene and Japanese sporadic AD was investigated.

2. Materials and Methods

DNA was extracted from white blood cells using a standard method. Our sporadic Japanese AD cases (n = 180, Male : Female = 79 : 101) were obtained from department of psychiatry, Juntendo university hospital, Tokyo, Japan and department of psychiatry, Juntendo Koshigaya hospital, Saitama Japan. The mean age of the AD group (67.4, S.D. 6.2) was not significantly different from that of the control group.
Table 1: Genotypic frequencies of SNPs of the Akt1 gene.

| SNP           | Location | Genotype | AD | Controls | \(P\) value | HWE \(P\) value |
|---------------|----------|----------|----|----------|-------------|----------------|
| rs113021411   | intron 1 | A/A      | 3  | 3        |             |                |
|               |          | A/C      | 33 | 45       | 0.002*      | AD: 0.93       |
|               |          | C/C      | 142| 81       | Controls: 0.68|
| rs2494746     | intron 3 | C/G      | 72 | 85       | 0.00002*    | AD: 0.52       |
|               |          | G/G      | 29 | 22       | Controls: 0.04*|
| rs2494743     | intron 3 | C/T      | 71 | 85       | 0.00001*    | AD: 0.47       |
|               |          | T/T      | 29 | 22       | Controls: 0.04*|
| rs2494738     | intron 3 | A/G      | 79 | 68       | 0.3         | AD: 0.93       |
|               |          | G/G      | 71 | 41       | Controls: 0.91|
| rs3730344     | intron 6 | A/G      | 4  | 3        | 0.99        | AD: 0.99       |
|               |          | G/G      | 0  | 0        | Controls: 0.99|
| rs7140735     | intron 13| A/G      | 27 | 13       | 0.17        | AD: 0.88       |
|               |          | G/G      | 0  | 1        | Controls: 0.99|

\(P^* < 0.05\): Statistically significant (Fisher’s exact probabilities test).

P-values were calculated based on the Fisher's exact probabilities test.

Table 2: Linkage disequilibrium (\(D'\) value) between SNPs.

| SNP       | rs1130214 | rs2494746 | rs2494743 | rs2494738 | rs3730344 | rs7140735 |
|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| rs1130214 | 1         |           |           |           |           |           |
| rs2494746 |           | 0.9728    | 0.9933    |           |           |           |
| rs2494738 | 0.0554    | –0.4329   | –0.4273   | –0.9999   | 1         |           |
| rs3730344 | –0.9999   | 1         | 1         | 0.3476    | 0.2407    | 0.2742    |
| rs7140735 | 0.2407    | 0.2742    | 0.2838    | –1        | 1         |

(64.4, S.D. 6.7) by the Fisher’s exact probabilities test. All the AD cases were diagnosed according to the NINCDS-ADRDA criteria, and none had familial history of AD. The control cases (n = 130, Male : Female = 63 : 67) were obtained from healthy volunteers from among staff of our hospital with no history of dementia or other neuropsychiatric diseases. The purpose and significance of this study were explained in detail to each patient and his/her family, and all subjects provided informed consent. The study protocol was approved by the Ethics committee of the Juntendo University School of Medicine.

Information on the single nucleotide polymorphisms SNPs was obtained from the SNP database (dbSNP) established by the National Center for Biotechnology Information. We selected the SNPs to cover the entire gene, including tagging SNPs. The chosen SNPs were validated, according to the dbSNP and have minor allele frequencies (MAF) greater than 5%. Six SNPs of the Akt1 gene were genotyped using TaqMan technology on an ABI7500 system (Applied Biosystems, Calif, USA). All probes and primers were designed by the Assay-by-Design TM service of Applied Biosystems. A standard PCR reaction was carried out using the TaqMan universal PCR master mix reagent kit in a 10 μL volume. Hardy-Weinberg equilibrium (HWE) tests were carried out for all SNPs for both cases and controls. APO E genotypes for all the samples were determined according to a previous report [14]. Differences in the genotypic frequencies were evaluated using a case-control study design and applying the Fisher’s exact probabilities test.

Linkage disequilibrium (LD) between the SNPs as well as a haplotype analysis was performed using SNPAlyse version 5 (DYNACOM, Yokohama, Japan). LD, denoted as \(D'\), was calculated from the haplotype frequency using the expectation-maximization algorithm. SNPs were considered to be in LD if \(D'\) was greater than 0.75. A case-control haplotype analysis was performed using a permutation method to obtain the empirical significance. The global \(P\) values represent the overall significance of the observed versus expected frequencies of all the haplotypes considered together using the chi-squared test. The individual haplotypes were tested for association by grouping all others together and applying the chi-squared test with l df. \(P\) values were calculated on the basis of 10,000 replications. All \(P\) values reported are two tailed, and statistical significance was defined as \(< 0.05\). Logistic regression analyses were performed to estimate the relationship among onset of AD, APO E status, and six
SNPs using SPSS software ver. 17.0 for Windows; (Chicago, Ill., USA). A P value of <0.05 was considered statistically significant.

3. Results and Discussion

Our sample set has the power to detect an odds ratio of at least 1.40, assuming a significance level of 0.05, power of 0.70, and an exposure frequency of 0.25 in the controls. Although four SNPs were found to be in HWE, controls of rs2494746 and rs2494743 were not in HWE marginally. Genotypic distribution of the two polymorphisms showed significant difference between our cases and controls (Table 1). Other four SNPs Linkage disequilibrium examination showed strong LD from rs1130214 to rs2494743 and from rs3730344 to rs7140735 (Table 2). The frequency of the C-C-C-G-A haplotype was significantly higher in the AD group compared to controls (Table 3). Other two rare haplotypes also showed marginal association.

To date, this is the first study to clarify genetic associations between common SNPs of the Akt1 gene and AD. We found that two SNPs rs2494746 and rs2494743 studied here with Japanese population were not in HWE. Haplotype analysis seemed to be positive superficially. Multiple regression analysis suggested that six SNPs of the Akt1 gene did not associate with the risk for AD and logistic regression analysis for the Akt1 SNPs, APO E and the onset of AD showed no synergic association (data not shown). SNPs which were not in HWE make us suppose that the regions around such SNPs are highly polymorphic. These SNPs would be triallelic SNPs, or there are large deletions or insertions generally. Since we confirmed that the two SNPs are biallelic, potential large deletion or insertion might exist in the Akt1 gene. Our LD analyses also suggested that the gene consists of two distinct LD blocks. Rs2494738 was thought not to be involved in the two LD blocks. SNPs which are not in HWE with homozygote excess sometimes show positive generally. These SNPs suggest that there are potential polymorphisms including insertion or deletion associated with the disease. Reviewing our raw genotyping data, heterozygotes of control cases of those two SNPs are more frequent than estimated. Thus we guess the SNPs studied here did not affect the disease.

Previous genetic studies for schizophrenic patients are in debate [15–18]. An original report identified Akt1 as a potential schizophrenia susceptibility gene in families of European origins [19]. The additional multi-SNP haplotype analysis showed that specific haplotype is associated with lower Akt1 protein levels [20]. Controversial results have been issued for Japanese schizophrenic patients and Akt1 [15, 16, 21]. The detailed LD analysis from these Japanese studies suggested that there are two apparent LD blocks in the gene [21, 22]. These findings accord with our results. Thus the Akt1 gene is highly polymorphic, and functional SNPs might affect Akt1 levels potentially. We believe that the small size of our dataset may account for the negative results. Our dataset could detect the genetic association between APO E4 and AD. If the SNPs of the Akt1 gene affect the onset of AD, the effects would be smaller than those of APO E4. Previous studies and our findings revealed that the regions of the Akt1 gene are highly polymorphic for Japanese population.

4. Conclusion

Although our pilot study could not show a genetic association between Akt1 and AD, PI3K-Akt-mTOR system has an important role for pathophysiology of AD. Denser SNPing studies would be needed for clarifying the genetic association between Akt1 and AD. Since the relationship between Akt1 and AD remains inconclusive, a meta-analysis would be performed in the future.

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