Clinical Study

KIF6 719Arg Carrier Status Association with Homocysteine and C-Reactive Protein in Amnestic Mild Cognitive Impairment and Alzheimer’s Disease Patients

Michael Malek-Ahmadi, Amar Patel, and Marwan N. Sabbagh

Banner Sun Health Research Institute, Cleo Roberts Center for Clinical Research, Sun City, AZ 85351, USA

Correspondence should be addressed to Michael Malek-Ahmadi; michael.ahmadi@bannerhealth.com

Received 27 June 2013; Revised 11 October 2013; Accepted 12 October 2013

Academic Editor: Francesco Panza

Copyright © 2013 Michael Malek-Ahmadi et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Recent research has demonstrated associations between statin use, KIF6 719Arg carrier status, and cholesterol levels and amnestic mild cognitive impairment (aMCI) and Alzheimer’s disease (AD) patients. The association between 719Arg carrier status with homocysteine (tHcy) and c-reactive protein (CRP) levels in aMCI and AD has not been previously investigated. Data from 175 aMCI and AD patients were used for the analysis. 719Arg carriers had significantly lower levels of tHcy than noncarriers ($P = 0.02$). No significant difference in CRP levels between 719Arg carriers and noncarriers was present ($P = 0.37$). Logistic regression yielded no significant effect for 719Arg status on CRP [OR = 1.79 (0.85, 3.83), $P = 0.13$] but did demonstrate a significant effect for tHcy [OR = 0.44 (0.23, 0.83), $P = 0.01$] after adjusting for ApoE ε4 status, age, gender, and statin use. This study is the first to explore the relationship between KIF6 719Arg carrier status with tHcy and CRP levels. 719Arg carriers were more likely to have normal tHcy levels after adjusting for ApoE ε4 status, age, gender, and statin use. These results suggest that the KIF6 gene might influence cardiovascular pathways associated with AD.

1. Introduction

The KIF6 gene is one of several molecular components involved in the intracellular transport of protein complexes, membrane organelles, and messenger ribonucleic acid along microtubules [1]. There are three polymorphisms of the KIF6 gene which include Arg/Arg, Trp/Arg, and Trp/Trp. Individuals carrying the Arg/Arg or Trp/Arg polymorphisms are classified as 719Arg carriers and have been linked to an increased risk for cardiovascular disease [1–6] relative to 719Arg noncarriers (Trp/Trp). Some of these studies also demonstrated a greater lipid lowering response from statin therapy among 719Arg carriers when compared to noncarriers [1–4]. Others have found no association between 719Arg carrier status and cardiovascular outcomes [7, 8]. A recent study by Sabbagh et al. [9] found that positive 719Arg carrier status combined with statin use was associated with lower total cholesterol (TC) and lower low density lipoprotein (LDL) levels in a sample of amnestic mild cognitive impairment (aMCI) and Alzheimer’s disease (AD) patients.

In terms of disease risk, cholesterol levels and statin use have garnered significant attention from aMCI/AD researchers [10–13]; however, homocysteine (tHcy) [14, 15] and c-reactive protein (CRP) [16–18] have also been implicated as risk factors for aMCI and AD. Troen and Rosenberg [19] describe several studies that established elevated tHcy as a risk factor for AD; however, several studies which found no association with tHcy and AD are also cited. Previous research has shown that elevated tHcy may lead to intraneuronal accumulation of Aβ42 as a result of neurotoxicity which inhibits cellular DNA repair mechanisms [20]. Irizarry et al. [21] found that circulating tHcy correlated with Aβ42 formation while Kok et al. [24] demonstrated that allelic variations in the CRP gene
are associated with differing levels senile plaque formation. In contrast, O'Bryant et al. [25] found that AD patients had significantly lower CRP levels relative to controls while Roberts et al. [26] found no association with elevated CRP and amnestic MCI [OR = 1.21; 95% CI (0.81, 1.82)]. Haan et al. [27] found that ApoE ε4 carriers had significantly lower CRP levels than noncarriers and that higher CRP levels were associated with a decreased risk of incident all-cause CIND/dementia among ApoE ε4 carriers [OR = 0.60; 95% CI (0.20, 0.91) P = 0.03]. Among cognitively normal older adults, Ravaglia et al. [28] found that ApoE ε4 carriers with normal CRP levels had a significantly lower risk of elevated tHcy levels [OR = 0.22; 95% CI (0.08, 0.59)] and were less likely to have elevated CRP levels in the presence of normal tHcy levels [OR = 0.51; 95% CI (0.31, 0.85)] when compared to noncarriers.

Since tHcy and CRP have demonstrated associations with cardiovascular disease [29, 30] and AD [14–18], we sought to investigate whether KIF6 719Arg carrier status is associated with tHcy and CRP in a group of clinically diagnosed AD and aMCI patients. Since KIF6 719Arg carrier status has been previously associated with cardiovascular disease [1–6], investigating its associations with tHcy and CRP in AD is of interest given the proposed cardiovascular pathways for AD pathogenesis [31, 32].

2. Method

2.1. Study Sample. Data from 175 patients (74 aMCI, 101 AD) between the ages of 53 and 97 who were seen in a neurology clinic in Sun City, AZ, were used for the analysis. All patients were of Caucasian ethnicity. Clinical diagnoses of aMCI and probable or possible AD were made based on medical history, social history, clinical laboratory results, mental status exam, assessment of daily functioning, MRI, and neuropsychological testing. The AD patients met NINCDS-ADRDA [33] criteria for a clinical diagnosis of probable or possible AD. Petersen criteria were used to classify aMCI patients [34]. This study was exempted from review by the Western IRB as it was a study that utilized existing data which was recorded in a way that prevented the identities of the patients from being known.

2.2. CRP, tHcy, KIF6 Genotype, and ApoE Genotype Processing. CRP was processed with an automated immunoturbidimetric assay using Roche Modular and tHcy was processed with an enzymatic assay using Roche Modular. KIF6 and ApoE genotyping were processed using real-time PCR (Berkeley Heart Lab, Berkeley, CA).

2.3. Statistical Analysis. Chi-square analyses were used to assess differences in frequency for gender, disease status (AD versus aMCI), 719Arg carrier status, and ApoE ε4 carrier status. For the Chi-square analyses, a Bonferroni adjusted P-value of 0.025 was used to correct multiple comparisons. Log transformations were performed on the raw data for tHcy and CRP in order to normalize their distributions. For log-transformed tHcy and CRP values, geometric means with 95% confidence intervals are reported. Group differences between 719Arg carriers and noncarriers for tHcy and CRP were assessed using a two-sample t-test. Additional two-sample t-tests were carried out for comparisons of gender, ApoE ε4 carrier status, and cholesterol drug (statin) use status on tHcy and CRP.

Logistic regression was used to analyze the associations between tHcy and CRP with 719Arg carrier status in order to provide an estimate of effect size and to provide a more practical interpretation of the associations than the two-sample t-tests can provide. For these analyses, the sample was dichotomized into elevated and normal groups using recommended clinical guidelines [35] for tHcy and CRP. Individuals with tHcy levels that were ≥14 μmol/L were classified as elevated while individuals with CRP levels that were >3.0 mg/L were classified as elevated. For the logistic models, elevated/normal status for tHcy and CRP was used as the outcome variable with 719Arg carrier status as the predictor variable. Although dichotomizing continuous variables can result in a loss of statistical power, when the cut-point used has clinical significance this approach is justified [36]. Age, gender, ApoE ε4 carrier status, and statin use were used as covariates.

Four different logistic regression models were carried out for both tHcy and CRP using elevated versus normal status as the outcome (8 models total). The first model used only 719Arg carrier status as the predictor variable in order to assess the crude associations with tHcy and CRP. The second model included ApoE ε4 carrier status, age, and gender in order to account for their effects. The third model adjusted for statin use in addition to age, gender, and ApoE ε4 carrier status. The fourth model used a multiplicative interaction term for 719Arg carrier status and ApoE ε4 carrier status (719Arg × ApoE ε4) as the predictor variable while adjusting for age, gender, and statin use. The fourth model was intended to be an exploratory analysis to further rule out the effect of ApoE ε4 carrier status on the association between 719Arg carrier status with tHcy and CRP.

3. Results

The study sample was comprised of 82 females and 93 males with an average age of 77.92 ± 8.23 years. Clinical and demographic characteristics of the study sample are displayed in Table 1. For tHcy and CRP, the means and standard deviations of the raw values are reported in addition to the geometric means and their 95% confidence intervals. There was no significant association between 719Arg carrier status, ApoE ε4 carrier status, gender, or statin use. There was no significant difference for age or CRP between 719Arg carriers and noncarriers; however, 719Arg carriers had significantly lower tHcy levels. We also analyzed tHcy and CRP by gender, ApoE ε4 carrier status, and statin use status (Table 2). The only significant differences noted were for gender and tHcy where males were significantly higher and for ApoE ε4 carrier status and CRP where ε4 noncarriers were significantly higher than carriers.
Table 1: Demographic and clinical characteristics.

<table>
<thead>
<tr>
<th></th>
<th>719Arg carriers</th>
<th>719Arg noncarriers</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>106</td>
<td>69</td>
<td>—</td>
</tr>
<tr>
<td>Males/females (n)</td>
<td>54/52</td>
<td>39/30</td>
<td>0.47</td>
</tr>
<tr>
<td>ApoE ε4 carrier/noncarrier (n)</td>
<td>59/47</td>
<td>40/29</td>
<td>0.76</td>
</tr>
<tr>
<td>Statin use/no statin use (n)</td>
<td>59/47</td>
<td>46/23</td>
<td>0.15</td>
</tr>
<tr>
<td>Age</td>
<td>77.18 ± 8.58</td>
<td>79.06 ± 7.58</td>
<td>0.13</td>
</tr>
<tr>
<td>tHcy (μmol/L)</td>
<td>13.64 ± 4.59</td>
<td>15.12 ± 4.42</td>
<td>—</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>4.32 ± 8.49</td>
<td>3.46 ± 8.60</td>
<td>—</td>
</tr>
</tbody>
</table>

Mean ± standard deviation.

Table 2: Analysis of confounding factors for tHcy and CRP.

<table>
<thead>
<tr>
<th></th>
<th>tHcy (μmol/L)</th>
<th>CRP (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoE ε4 carrier status</td>
<td>Carrier: 13.43 (12.68, 14.22)</td>
<td>Carrier: 1.08 (0.80, 1.44)</td>
</tr>
<tr>
<td>Gender</td>
<td>Males: 14.32 (13.52, 15.21)</td>
<td>Males: 1.15 (0.84, 1.56)</td>
</tr>
<tr>
<td></td>
<td>Females: 12.68 (11.78, 13.68)</td>
<td>Females: 1.66 (1.25, 2.20)</td>
</tr>
<tr>
<td>Statin use status</td>
<td>Statin—yes: 13.15 (12.05, 14.32)</td>
<td>Statin—yes: 1.57 (1.09, 2.28)</td>
</tr>
<tr>
<td></td>
<td>Statin—no: 13.80 (13.06, 14.59)</td>
<td>Statin—no: 1.24 (0.97, 1.59)</td>
</tr>
</tbody>
</table>

Geometric mean (95% confidence interval).

A post-hoc power analysis for the two-sample t-tests found that our sample was large enough to detect a medium effect size (d = 0.50) with 90% statistical power [37]. An additional power analysis for the logistic regression analyses demonstrated that our sample achieved 91% power.

Using methods described by Rodriguez et al. [38], we determined whether the frequency of KIF6 and ApoE genotypes were consistent with the Hardy-Weinberg equilibrium. The frequency of KIF6 genotypes did not violate the Hardy-Weinberg equilibrium (χ² = 1.44, df = 2, P = 0.49). The genotype frequency for ApoE ε4 noncarriers did not violate the Hardy-Weinberg equilibrium (χ² = 0.30, df = 2, P = 0.86). However, the genotype frequency for ApoE ε4 carriers did violate the Hardy-Weinberg Equilibrium (χ² = 40.09, df = 2, P < 0.001). The latter is likely due to the fact AD studies tend to have a greater proportion of ApoE ε4 carriers when compared to the genotype's population prevalence [39]. Specifically, the frequency of individuals with the 3/4 genotype (n = 80) was substantially greater than the frequencies of the 2/4 (n = 4) and 4/4 (n = 15) genotypes. The prevalence of the 2/4 genotype (2%) in our study is consistent with population prevalence estimates proposed by Raber et al. [40]; however, the prevalence of the 3/4 (46%) and 4/4 (9%) genotypes in our study are higher than what would be expected in the general population. Given the ε4 genotype's well known association with AD, the violation of the Hardy-Weinberg Equilibrium described above is not surprising. Fisher exact test results found that there was no significant difference in genotype frequency between aMCI and AD patients for KIF6 (P = 0.09) and ApoE (P = 0.99).

Results of the logistic regression models for tHcy and CRP are displayed in Table 3. 719Arg carrier status showed no significant association with elevated CRP but did demonstrate a significant association with elevated tHcy status even after adjusting for ApoE ε4 carrier status, age, gender, and statin use. The association between 719Arg carrier status and elevated tHcy showed that 719Arg carriers were less likely to have elevated tHcy than noncarriers. The models that tested the KIF6 × ApoE ε4 interaction showed no significant association with elevated tHcy or elevated CRP. We carried out additional logistic regression analyses to assess the associations for ApoE ε4 carrier status with tHcy and CRP as the outcomes. Both analyses yielded nonsignificant results: tHcy [OR = 0.99 (0.54, 1.80), P = 0.97]; CRP [OR = 0.58 (0.30, 1.16), P = 0.12].

4. Discussion

This study is the first to assess the associations between KIF6 719Arg carrier status with tHcy and CRP in a sample of
Table 3: KIF6 719Arg carrier status association with elevated tHcy and CRP.

<table>
<thead>
<tr>
<th>Model</th>
<th>tHcy</th>
<th>CRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1—no adjustment</td>
<td>0.41 (0.22, 0.75)</td>
<td>1.86 (0.90, 3.87)</td>
</tr>
<tr>
<td>2—adjusted for ApoE</td>
<td>0.43 (0.23, 0.82)</td>
<td>1.90 (0.90, 4.01)</td>
</tr>
<tr>
<td>3—adjusted for ApoE, age, and gender</td>
<td>0.44 (0.23, 0.83)</td>
<td>1.80 (0.85, 3.83)</td>
</tr>
<tr>
<td>4—KIF6 × ApoE 4 interaction adjusted for age, gender, and statin use</td>
<td>0.59 (0.30, 1.14)</td>
<td>0.80 (0.38, 1.68)</td>
</tr>
</tbody>
</table>

Odds ratio (95% confidence interval). $P$ value.

Table has suggested that the C677T polymorphism of MTHFR is associated with AD independently of ApoE e4 carrier status [46] while Anello et al. [47] reported that the effect of homocysteine as a risk factor for AD was exacerbated when both ApoE e4 and the MTHFR 677T allele were present. Given the results of our study, it would be interesting to see if positive carrier status for KIF6 719Arg mitigates this association since 719Arg carriers in our study had lower overall tHcy levels and a lower risk of having elevated tHcy relative to 719Arg noncarriers. This finding may also be of value to studies in the area of cardiology given the association between elevated homocysteine and cardiovascular disease [48].

Since increased tHcy has been linked to the pathophysiology of AD [49], the role of KIF6 in this pathway may be of importance. Although it is unlikely that KIF6 plays a direct role in AD pathogenesis, it is possible that KIF6 is responsible for certain mediating effects along tHcy-related pathways for AD. The results of this study suggest that carriers of the KIF6 719Arg allele have a preferential tHcy profile that may provide some level of protection along AD-related cardiovascular pathways involving tHcy.

Conflict of Interests

The authors have no conflict of interests to disclose.

Acknowledgments

The authors are grateful to the Banner Sun Health Research Institute Brain and Body Donation Program of Sun City, Arizona. The Brain and Body Donation Program is supported by the National Institute of Neurological Disorders and Stroke (U24 NS072026 National Brain and Tissue Resource for Parkinson's Disease and Related Disorders), the National Institute on Aging (P30 AG19610 Arizona Alzheimer's Disease Core Center), the Arizona Department of Health Services (contract 211002, Arizona Alzheimer's Research Center), the Arizona Biomedical Research Commission (contracts 4001, 0011, 05-901, and 1001 to the Arizona Parkinson's Disease Consortium), and the Michael J. Fox Foundation for Parkinson's Research.

References


