Genetic Polymorphism of ITGA2 C807T Collagen Receptor Encoding Gene of Aspirin Therapy among Javanese-Indonesian Healthy Respondents

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Abstract

BACKGROUND: Aspirin is an antiplatelet drug commonly administered as primary and secondary prophylaxis to prevent thromboembolic events. However, there has been a common incidence of aspirin resistance that leads to a recurrent cerebrovascular disease. One of the causes of such event is the genetic polymorphisms of the integrin alpha-2 (ITGA2) gene that encodes the glycoprotein Ia (GPIa) receptor in the pharmacodynamics of aspirin.

AIM: This study analyzed the genetic polymorphism of ITGA2 as the GPIa collagen receptor encoding gene of aspirin therapy among healthy Javanese, the largest ethnic group in Indonesia.

METHODS: This cross-sectional study involved 100 respondents who met the inclusion criteria with their blood sample taken for DNA isolation. Identification of genetic polymorphism in the target SNPs was done using the PCR-RFLP method with 5'-CCTTAAAGCTACCGGCCCATGT-3' forward primer and 5'-TTGGCCTATTAGCACAAACTTACC-3' reverse primer as well as Hpy188I restriction enzyme to fragment the target at position 244 in the C base.

RESULTS: This study found that the dominant genotype and allele were CT (51%) and C (66.5%), respectively.

CONCLUSION: The allele frequency of ITGA2 gene in this study was similar to that of the populations in other Asian countries. Further research regarding the effects of ITGA2 C807T polymorphism on the pharmacodynamics of aspirin as an antiplatelet is recommended to minimize atherothrombotic events and examine its interactions as a biomarker of the risk and prognosis of some cancer types.
of aspirin target action as an antiplatelet. Numerous studies focusing on the polymorphism of ITGA2 C807T gene that encodes the GPIa collagen receptor have proved its correlation with aspirin resistance. A study of Han Chinese patients with acute ischemic stroke shows that the T allele significantly correlates with aspirin resistance (OR 4.86) [7]. Similarly, a meta-analysis reveals that rs1126643 (C807T) polymorphism becomes the genetic variation associated with aspirin insensitivity [8], risk of ischemic stroke among the Asian populations [9], and risk of elevated levels of serum cholesterol [10]. In addition, a comprehensive meta-analysis of 60 studies suggests that ITGA2 C807T with the Ser allele of HPA-3 and B allele of glycoprotein Ibα simultaneously correlates with an increased risk of ischemic stroke [11]. Pharmacogenetic research on the pharmacodynamics of aspirin that involves the Indonesian population has never been done to date. Therefore, this study aims to determine the distribution of ITGA2 C807T allele frequency among the Indonesian population, particularly in the Javanese as the major ethnic group in Indonesia.

Materials and Methods

Research subjects

This cross-sectional study used the stored biological samples in the form of isolated DNA collected from the 3 ml of blood of 100 healthy subjects of a previous study. The respondents were categorized as Javanese according to their previous three generations of Javanese-Indonesian. This research has passed the ethical review from the Ethics Committee of the Faculty of Medicine of Universitas Islam Indonesia with protocol Number 4/Ka.Kom.Et/D/KE/XII.

Genotype analysis of ITGA2 C807T gene

The genotype analysis of the target polymorphism involved the PCR-RFLP method with forward primer 5'-CCTAAAGCTACGGGCAATTGT-3' and reverse primer 5'-TTGGCCTATTAGCACCACAAAACCTAC-3' followed by digestion of the amplicons using the Hpy188I enzyme. The PCR conditions for amplification included pre-denaturation at a temperature of 95°C for 2 min, 35 cycles of denaturation at the same temperature for 30 s, annealing at 57°C for 30 s, and extension at 72°C for 30 s with the final extension at 72°C for 5 min. The visualization of amplification products was prepared using agarose gel electrophoresis with 2.5% agarose concentration at 70 volts for 90 min. The digestion of 288 bp amplicon resulted in 244 bp and 44 bp fragments of wild-type CC genotype, also 288 bp, 244 bp, and 44 bp of heterozygous CT genotypes, and 288 bp of mutant TT genotype. The genotype and allele frequencies were determined based on the Hardy-Weinberg principle as follows [12], [13].

\[
\text{Genotype frequency} = \frac{\text{Number of individuals with a specific genotype}}{\text{Total number of individuals}}
\]

\[
\text{C Allele frequency} = \frac{2 \times \text{Number of CC Individuals}}{2 \times \text{Total Number of Individuals}}
\]

\[
\text{T Allele frequency} = \frac{2 \times \text{Number of TT Individuals}}{2 \times \text{Total Number of Individuals}}
\]

Results

There were 100 healthy respondents from Javanese-Indonesian ethnic group involved in this study of the SNPs frequency of ITGA2 rs1126643 C>T gene, consisting of men and women in equal number. The characteristics of the research subjects are presented in Table 1.

<table>
<thead>
<tr>
<th>Patient characteristic</th>
<th>Male (n = 50)</th>
<th>Female (n = 50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>21.26 ± 1.21</td>
<td>21.14 ± 1.43</td>
</tr>
<tr>
<td>Mean BMI (kg/m²)</td>
<td>22.73 ± 4.09</td>
<td>21.49 ± 3.41</td>
</tr>
<tr>
<td>Type of ITGA2 rs1126643 C&gt;T genotype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>19</td>
<td>22</td>
</tr>
<tr>
<td>CT</td>
<td>28</td>
<td>23</td>
</tr>
<tr>
<td>TT</td>
<td>3</td>
<td>5</td>
</tr>
</tbody>
</table>

The subject characteristics in terms of both phenotypic factor, which includes mean age and BMI, and genotypic factor related to genetic variants in the target SNPs indicate no significant differences between men and women (p > 0.05). Overall, this study found that the majority of genotypic variants in the ITGA2 rs1126643 C>T gene were CT type and C allele. Figure 1 shows the electrophoretic display of the enzyme digestion products for detecting the target polymorphism.

Discussion

The frequency of genotypic variants in ITGA2 C807T as the GPIa protein-encoding gene does not significantly differ between the men and the women in this study. In contrast to the GPIIb-IIIa receptors, the GPIa collagen receptor shows no different expression based on sex [14]. However, differences

https://oamjms.eu/index.php/mjms/index
in the response to aspirin as primary cardioprotection between men and women have been discussed by a gender-specific meta-analysis [15]. Similarly, the WHI long-term study has found aspirin to be effective for primary prevention of stroke in female patients but ineffective for that of myocardial infarction (MI). In contrast, aspirin is effective as a primary prevention of MI in men. However, there is no clarity as to what causes such differences, making it necessary to probe for further explanation [16]. In addition, in conjunction with clinical studies that involve only a small number of female subjects, especially in Phase 1, and recent findings other than pharmacokinetic studies, gender-related differences have proved to influence the pharmacodynamics and pharmacogenomics of drugs, including aspirin. Consequently, before such studies are translated into a clinical setting, a gender-based approach is required to draw a feasible conclusion for both genders so as to adjust the administration of aspirin according to individual needs [17]. If further studies can provide evidence to reinforce the presence of significant differences in the pharmacokinetics and pharmacodynamics of aspirin between the two genders, then the pharmacogenomic studies of aspirin, in addition to the study designs that involve a control group and adequate sample size, are required to employ equal proportion of both genders to provide more accurate data analysis and results.

A number of studies with the same SNPs targets as this study and with the pharmacodynamic impacts of aspirin have been carried out among various populations (Table 2).

Table 2 describes the studies published as original articles with the majority of them finding no correlations between ITGA2 C807T and an increased

<table>
<thead>
<tr>
<th>Population/ Race</th>
<th>Number of samples</th>
<th>Genotype frequency (%)</th>
<th>Finding</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original article</td>
<td>100</td>
<td></td>
<td></td>
<td>Present study</td>
</tr>
<tr>
<td>Caucasian</td>
<td>2369 patients with VTE, 1460 healthy subjects</td>
<td>Case 34.6 Control 36.0</td>
<td>The frequency of GP1a C807T mutant allele was lower than that of C allele</td>
<td>Kvasnicka et al. (2015) [18]</td>
</tr>
<tr>
<td>Han Chinese</td>
<td>503 patients with ST-elevation myocardial infarction</td>
<td>Data not shown</td>
<td>There was no difference in the frequency of GP1a C807T polymorphism between VTE and the control, but there was a significantly increasing risk among 732 VTE patients with a family history of myocardial infarction and/or stroke</td>
<td>Zhang et al. (2016) [19]</td>
</tr>
<tr>
<td>Jordanian</td>
<td>584 patients</td>
<td>Aspirin responders 38.4</td>
<td>There was no association between ITGA2 C807T polymorphism in the platelet receptor encoding gene and the risk of ischemic stroke and bleeding incidence</td>
<td>Al-Azzam et al. (2013) [20]</td>
</tr>
<tr>
<td>Ukrainian</td>
<td>54 patients with stable angina pectoris II-III and ACS with history of PCI</td>
<td>Aspirin responders 63.2</td>
<td>Declined sensitivity to aspirin as an antiplatelet was correlated with ITGA2 C807T polymorphism in patients with ACS after PCI. IHD patients with T allele had a lower platelet response especially in patients who received antiplatelet therapy, including aspirin</td>
<td>Liahotitsa (2017) [21]</td>
</tr>
<tr>
<td>Caucasian</td>
<td>179 stroke patients, 172 control</td>
<td>Stroke with 3 subtypes (CT/TT) 55.9</td>
<td>The integrin α2 C807T polymorphism did not affect the development of ischemic stroke Together with rs1062635 SNPs, rs1126643 polymorphism was associated with the prognosis of cardiovascular diseases, especially in high-risk patients</td>
<td>Cole et al. (2003) [22]</td>
</tr>
<tr>
<td>German</td>
<td>941 patients with stable CAD</td>
<td>Stroke with 3 subtypes</td>
<td>Present study</td>
<td>Rath et al. (2017) [23]</td>
</tr>
</tbody>
</table>

Table 2: Genotype frequencies of GP1a C807T in various populations in the world
<table>
<thead>
<tr>
<th>Population/Race</th>
<th>Number of samples</th>
<th>Genotype frequency (%)</th>
<th>Finding</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasian</td>
<td>286 healthy subjects, 160 patients with hereditary mucocutaneous bleeding</td>
<td>Data not shown</td>
<td>CT+TT Patients with bleeding 67.1</td>
<td>Martinez et al. (2009) [24]</td>
</tr>
<tr>
<td>Chinese</td>
<td>350 patients with ischemic stroke patients 300 control</td>
<td></td>
<td>Control 65.6</td>
<td></td>
</tr>
<tr>
<td>Dutch</td>
<td>1327 patients with primary PCI who received aspirin-clopidogrel combination therapy</td>
<td></td>
<td>Group without primary event (n=886) Group without primary event (n=1241) 34.3.</td>
<td></td>
</tr>
<tr>
<td>Chinese</td>
<td>1544 patients (cohort 2) who received CABG (Coronary Artery Bypass Graft) and follow-up for 72.8 years</td>
<td></td>
<td>Cohort 1 Case 18.8 Control 51.2 Cohort 2 Case 9.2 Control 90.8</td>
<td></td>
</tr>
<tr>
<td>Czech</td>
<td>73 patients with acute or chronic IHD who experienced bleeding complications within 30 days after cardiac catheterization (CAG) or PCI, 331 patients without bleeding as the control</td>
<td></td>
<td>Total (404) 43.3 12.4</td>
<td></td>
</tr>
<tr>
<td>Han Chinese</td>
<td>97 patients with acute ischemic stroke; aspirin sensitivity (AS) group with 54 subjects, aspirin resistance (AR) group with 43 subjects</td>
<td></td>
<td>AS 42.6 AR 27.9</td>
<td></td>
</tr>
<tr>
<td>Japanese</td>
<td>110 healthy subjects</td>
<td></td>
<td>Aspirin responders 40.0 Aspirin non-responders 35.0</td>
<td></td>
</tr>
<tr>
<td>Chinese</td>
<td>307 patients with gastric cancer (case), 307 control</td>
<td></td>
<td>Case 45.9 Control 55.4 Patients with iSSNHL 35.6 Control 52.2</td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>118 patients with idiopathic sudden sensorineural hearing loss (ISSNHL), 161 control</td>
<td></td>
<td>Patients with ISSNHL 56.2 Patients with ISSNHL 52.5 Control 40.4</td>
<td></td>
</tr>
<tr>
<td>Russian</td>
<td>46 full-term newborns with arterial and venous thrombosis, 57 healthy newborns as the control</td>
<td></td>
<td>Case 45.6 Control 42.1</td>
<td></td>
</tr>
<tr>
<td>Russian</td>
<td>446 preeclampsia patients</td>
<td></td>
<td>Case 32.5 Control 49.4</td>
<td></td>
</tr>
<tr>
<td>German</td>
<td>433 colorectal cancer patients</td>
<td></td>
<td>Patient 40.6 Control 56.6</td>
<td></td>
</tr>
<tr>
<td>Malaysian</td>
<td>300 patients with nasopharyngeal carcinoma (NPC)</td>
<td></td>
<td>57.0 34.3 8.7</td>
<td></td>
</tr>
<tr>
<td>Greece</td>
<td>32 fetuses with fetal growth restriction (FGR) and the mothers</td>
<td></td>
<td>46.9 43.7 9.4</td>
<td></td>
</tr>
<tr>
<td>Greek</td>
<td>18 fetuses as the control at corresponding gestational age and the mothers</td>
<td></td>
<td>Control 50.0 Control 59.0</td>
<td></td>
</tr>
</tbody>
</table>

(Cont...)

References
- [33] Golovchenko et al. (2020) [33]
- [34] Genger et al. (2009) [34]
- [36] Simo et al. (2017) [36]
risk of some events, including ischemic stroke, aspirin insensitivity, vein thromboembolism, recurrent attacks of MI, and bleeding. In contrast to the three meta-analyses, which draw relatively similar conclusion that SNPs are associated with the incidence of ischemic stroke and aspirin insensitivity, another meta-analysis conducted before 2010 shows that such polymorphisms are not a risk factor, either alone or in combination with other major cardiovascular risk factors, of the incidence of coronary artery disease [37]. It is interesting that nearly all of the studies linking ITGA2 rs1126643 C>T to clinical conditions other than cerebrocardiovascular disease have found significant correlations, including those associated with some types of cancer.

It is acknowledged that the ITGA gene or ITGA2 C807T gene found among the healthy Javanese-Indonesian subjects in this study is a novelty. The findings reveal that the frequency of the T allele in the ITGA2 C807T gene is lower than that of the C allele, which is 33.5%. Further research is necessary to analyze the correlation between such polymorphisms and their implications for the pharmacodynamic variability of aspirin as well as the risk and the prognosis of some cancer types.

**Conclusion**

The allele frequency in the ITGA2 C807T gene found among the healthy Javanese-Indonesian subjects in this study is a novelty. The findings reveal that the frequency of the T allele in the ITGA2 C807T gene is lower than that of the C allele, which is 33.5%. Further research is necessary to analyze the correlation between such polymorphisms and their implications for the pharmacodynamic variability of aspirin as well as the risk and the prognosis of some cancer types.

**References**


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**Table 2: (Continued)**

<table>
<thead>
<tr>
<th>Category</th>
<th>Subjects</th>
<th>Finding</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meta-analysis of 15 studies with a case–control design or cohort design consisting of 11 studies in Asian race and four studies involving Caucasians</td>
<td>2242 case and 2408 control</td>
<td>There was a relationship between ITGA2 C807T polymorphism and the risk of ischemic stroke in all Asian population and hospital-based population, but not in the Caucasian population and non-hospitalized individuals.</td>
<td>Wu et al. (2014) [8]</td>
</tr>
<tr>
<td>Meta-analysis of 16 studies with case–control, nested case–control, or cohort designs</td>
<td>2586 case and 2698 control with 16 studies in Asian race and Caucasians</td>
<td>Together with the other two polymorphisms, the TT genotype or T allele in GPIa C807T was correlated with the increased risk of ischemic stroke.</td>
<td>Liu et al. (2017) [11]</td>
</tr>
<tr>
<td>Meta-analyses of seven studies with case–control, nested case–control, or cohort designs</td>
<td>209 patients receiving aspirin and 676 control</td>
<td>The ITGA2 rs1126643 SNPs indicated a significant correlation with aspirin insensitivity. The subgroup analysis showed that these SNPs were potentially higher among patients with semi-resistance aspirin than in the aspirin resistance group.</td>
<td>Weng et al. (2013) [8]</td>
</tr>
</tbody>
</table>

PMID:23993980

PMID:28948649

PMID:24244288

PMID:24397542

PMID:25207618

PMID:28004990


PMID:9134654

PMID:23688555

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