Association of polymorphism in the human \(\mu\)-opioid receptor \(\text{OPRM1}\) gene with proinflammatory cytokine levels and health perception

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**Abstract**

Recent studies in psychoneuroimmunology have indicated that proinflammatory cytokines cause several diseases and behaviors that overlap symptomatically with depression. It is known that the endogenous opioid peptide \(\beta\)-endorphin regulates proinflammatory cytokine secretion from peripheral immune cells via \(\mu\)-opioid receptor-dependent mechanisms. Therefore, it is possible that the functional polymorphism of the \(\mu\)-opioid receptor gene (\(\text{OPRM1}, \text{SNP: A118G}\)) influences peripheral circulating proinflammatory cytokine levels and the health-related quality of life (QOL) even in healthy populations. In this study, we compared the serum concentrations of several proinflammatory cytokines (interleukin-2 (IL-2), interleukin-1\(\beta\) (IL-1\(\beta\)), tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)), and interferon-\(\gamma\) (IFN-\(\gamma\))) and the health-related QOL between \(\text{OPRM1}\) genotypes. Interestingly, serum concentrations of IL-6, TNF-\(\alpha\), and IFN-\(\gamma\) were significantly lower and the general health score was significantly higher in carriers of the G allele, who show a strong binding of \(\beta\)-endorphin to the \(\mu\)-opioid receptor as compared to individuals without the G allele. Correlation analysis indicated that the general health score was negatively correlated with the IL-6 serum concentration. These results suggest that the sensitive endogenous opioid system in carriers of the G allele may suppress proinflammatory cytokine secretion from peripheral immune cells; consequently, it may influence the health perception.

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**1. Introduction**

The central nervous, peripheral autonomic nervous, endocrine, and immune systems are known to be interrelated via complex biochemical pathways (Ader, 2000). Peripheral circulating proinflammatory cytokines, which are the immune signaling molecules that promote systemic inflammation, such as tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)), interleukin-1\(\beta\) (IL-1\(\beta\)), and interleukin-6 (IL-6), reach the brain via leaky regions in the blood–brain barrier, active transport molecules, and afferent nerve fibers (Raison et al., 2006; Dantzer et al., 2008). Cytokine signals induce a syndrome of sickness behavior whose features overlap with those of major depression, including anhedonia, anorexia, impaired sleep, and reduced locomotor activity (Raison et al., 2006; Dantzer et al., 2008). In addition, increased circulating proinflammatory cytokines may cause several diseases (Ridker et al., 2000; Lindmark et al., 2001; Parissis et al., 2009); therefore, circulating proinflammatory cytokine levels may be associated with the physical and mental health-related quality of life (QOL).

The endogenous opioid peptide \(\beta\)-endorphin is known to affect immune functions (Finley et al., 2008); it inhibits IL-6 secretion from the spleen in mice through a \(\mu\)-opioid receptor-dependent mechanism (Straub et al., 1997, 1998). The \(\mu\)-opioid receptor is a class of opioid receptors with a high affinity for \(\beta\)-endorphin (Trescot et al., 2008), and its activation by its ligands causes analgesia, euphoria, and sedation (Trescot et al., 2008). There is a single nucleotide polymorphism (SNP) at position 118 (A118G) in the coding region of the \(\mu\)-opioid receptor gene (\(\text{OPRM1}\)) in humans. This SNP codes for a change from asparagine (Asn) to aspartic acid (Asp) at position 40, resulting in a 3-fold stronger binding of \(\beta\)-endorphin to the \(\mu\)-opioid receptor (Bond et al., 1998). It is suggested that carriers of the G allele may show behavioral differences in responses mediated by \(\beta\)-endorphin, such as analgesia, euphoria, and sedation, because of the sensitive \(\mu\)-opioid receptor (Ray and Hutchison, 2004; Chou et al., 2006; Lütsch et al., 2006). For example, carriers of the G allele display a stronger alcohol-induced
euphoria than do individuals without the G allele (Ray and Hutchinson, 2004).

Therefore, it is suggested that the OPRM1 A118G polymorphism may be involved in the secretion of proinflammatory cytokines from peripheral immune cells and in health-related QOL even in a healthy population. Carriers of the G allele may have lower peripheral proinflammatory cytokine levels and higher QOL than individuals without the G allele; however, this has not been investigated in the healthy population. Therefore, we compared the serum concentrations of several proinflammatory cytokines (interleukin-2 (IL-2), IL-6, TNF-α, and interferon-γ (IFN-γ)) and health-related QOL between OPRM1 genotypes (AA, AG, and GG) in a healthy population.

2. Materials and methods

2.1. Participants

We recruited 123 healthy volunteers (65 males and 58 females; age range: 20–38 years) for the study. The participants were Japanese undergraduate and graduate students of Nagoya University, Mie University, and Mie Prefectural College of Nursing, and technical staff of Aichi Medical University. Participants were instructed not to eat 2 h before the blood sampling, but they were allowed to consume non-alcoholic and caffeine-free fluids. All the participants provided written informed consent in accordance with the Declaration of Helsinki. Participants were excluded if they were suffering from any chronic or oral illness, if they had taken medication known to influence immunity such as a steroid during the 3-month period before experiment, or if they used oral contraceptives. In addition, participants suffering from an infectious illness in the three weeks before the experiment were rescheduled. This study was approved by the Human Studies Committee of Aichi Medical University.

2.2. Genotyping

Genomic DNA was extracted from peripheral blood collected from the participants by using a DNA Extractor WB-Rapid Kit (Wako, Osaka, Japan). The DNA was amplified by real-time PCR using the ABI/PRISM 7000 sequence detection system (Applied Biosystems, Foster, CA). We detected the OPRM1 A118G SNP (dbSNP number rs1799971) using a TaqMan® SNP Genotyping Assay (C___8950074_1; Applied Biosystems). The call rate for the SNP was 94.6%.

2.3. Blood sampling and measurements of cytokine concentrations

Blood sampling was performed between 1400 and 1700 to minimize the influence of the circadian rhythm on cytokines. Further, women were examined during the late luteal and early follicular phases of their menstrual cycle when the secretion of female sex hormones is low, thus minimizing the influences of these hormones on the immune system. Blood samples were collected in serum-separator tubes and centrifuged at 3000g for 10 min; the serum was separated and then stored at −80°C until analysis. Several proinflammatory serum cytokine levels (IL-2, IL-6, TNF-α, and IFN-γ) were determined by a BDA® Cytometric Bead Array (Human Th1/Th2 Cytokine Kit II; BD Biosciences, San Diego, CA) according to the manufacturer’s instructions.

2.4. Measurement of health-related QOL

The Japanese-translated version of the Short-Form 36 Health Survey (SF-36), which is a generic instrument to measure eight QOL dimensions—physical functioning, physical role, bodily pain, general health, vitality, social functioning, emotional role, and mental health—was used to assess the impact on health-related QOL. This translated version has been studied extensively for its reliability and validity (Fukuhara et al., 1998a, 1998b). To perform the correlation analysis, we displayed the eight dimension scores with the raw values.

2.5. Statistical analyses of self-reported and physiological data

Results are expressed as means ± SEM. The effects of variations in OPRM1 and those of gender on cytokine levels and health-related QOL were examined with two-way analysis of variance (ANOVA) [gender (male and female) × genotype (AA, AG, and GG)]. Post hoc test (Tukey test) was performed to determine the significance of genotype effects. The effects of variations in OPRM1 on IL-2, TNF-α, and IFN-γ levels were also examined with Student’s t test. Furthermore, Pearson’s correlation coefficient was computed between the values of general health and IL-6 to examine the relationship between health perception and IL-6 levels. The effect of variations in OPRM1 on health status was also examined with analysis of covariance (ANCOVA). We entered the concentration of IL-6 as covariates.

3. Results

Genotype distribution for OPRM1 variation is as follows: Male, AA: 24 (36.9%), AG: 32 (49.2%), GG: 9 (13.9%); Female, AA: 12 (20.7%), AG: 27 (46.5%), GG: 19 (32.8%); Overall, AA: 36 (29.2%), AG: 59 (48.0%), GG: 28 (22.8%). The OPRM1 genotype distribution for the entire population was similar to that reported in previous studies in Japan (Nishizawa et al., 2006; Hayashida et al., 2008). There was no significant difference of age among these genotypes (Table 1).

To assess the effects of variations in OPRM1 on peripheral proinflammatory cytokine levels, we measured the serum concentrations of IL-2, IL-6, TNF-α, and IFN-γ. ANOVAs indicated no significant effects of gender and gender × genotype interaction on these cytokine levels; however, a significant effect of genotype was observed on the IL-6 serum concentration (F(2,120) = 5.85, p < 0.01, power = 0.87). Further statistical analysis revealed that the IL-6 serum concentration was significantly lower in AG (p < 0.01; Fig. 1a) and GG (p < 0.05; Fig. 1a) genotypes than that in the AA genotype. No significant effect of the genotype was observed on IL-2, TNF-α, and IFN-γ levels; however, the concentra-

![Table 1](https://example.com/table1.png)

<table>
<thead>
<tr>
<th>Age</th>
<th>AA (24.8 ± 0.09)</th>
<th>AG (25.0 ± 0.72)</th>
<th>GG (27.0 ± 1.23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokines (pg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-2</td>
<td>2.14 ± 0.23</td>
<td>1.66 ± 0.16</td>
<td>1.85 ± 0.25</td>
</tr>
<tr>
<td>TNF-α</td>
<td>2.78 ± 0.29</td>
<td>2.12 ± 0.21</td>
<td>1.99 ± 0.32</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>6.56 ± 0.67</td>
<td>4.85 ± 0.44</td>
<td>5.48 ± 0.75</td>
</tr>
<tr>
<td>SF-36 subscales</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical functioning</td>
<td>29.3 ± 0.21</td>
<td>29.3 ± 0.19</td>
<td>28.7 ± 0.46</td>
</tr>
<tr>
<td>Physical role</td>
<td>18.6 ± 0.38</td>
<td>18.6 ± 0.35</td>
<td>18.4 ± 0.69</td>
</tr>
<tr>
<td>Bodily pain</td>
<td>3.58 ± 0.29</td>
<td>3.70 ± 0.26</td>
<td>3.32 ± 0.39</td>
</tr>
<tr>
<td>Vitality</td>
<td>13.1 ± 0.46</td>
<td>13.6 ± 0.35</td>
<td>13.1 ± 0.48</td>
</tr>
<tr>
<td>Social functioning</td>
<td>8.50 ± 0.26</td>
<td>8.90 ± 0.21</td>
<td>8.71 ± 0.33</td>
</tr>
<tr>
<td>Emotional role</td>
<td>12.7 ± 0.43</td>
<td>12.9 ± 0.38</td>
<td>12.7 ± 0.57</td>
</tr>
<tr>
<td>Mental health</td>
<td>17.9 ± 0.56</td>
<td>18.7 ± 0.47</td>
<td>18.8 ± 0.60</td>
</tr>
</tbody>
</table>

Each result represents the mean ± SEM concentration or rating score.
4. Discussion

The present study aimed to reveal the effects of variations in OPRM1 on peripheral proinflammatory cytokine levels and health-related QOL in a healthy population. This study revealed that serum concentrations of IL-6, TNF-α, and IFN-γ were significantly lower and the general health score was significantly higher in carriers of the G allele than those in individuals without the G allele. Further, this study indicated that the general health score was negatively correlated with the IL-6 serum concentration. From these data, it is suggested that variations in OPRM1 may influence peripheral proinflammatory cytokine levels and health perception even in a healthy population. The sensitive endogenous opioid system in carriers of the G allele may suppress proinflammatory cytokine secretion from peripheral immune cells; consequently, it may influence the health perception.

There is growing evidence that acute psychosocial stressors such as public speaking increase circulating proinflammatory cytokine levels in humans (Ackerman et al., 1998; Altemus et al., 2001; Edwards et al., 2006; von Känel et al., 2006). Previous reports have suggested that elevated levels of circulating proinflammatory cytokine may be regulated by the sympathetic nervous and endocrine systems (van Gool et al., 1990; Zhou et al., 1993). Carriers of the OPRM1 G allele were shown to have a decreased response to a social stressor by means of suppression of the hypothalamic–pituitary–adrenal (HPA) axis activation (Chong et al., 2006). Hypothalamic corticotropin-releasing factor (CRF) neurons, which effect glucocorticoid release by stimulating adrenocorticotropic (ACTH) secretion from pituitary, are directly and indirectly inhibited by β-endorphin-producing neurons via the μ-opioid receptor (Johnson et al., 1992). Furthermore, it has been reported that β-endorphin can enhance the cytotoxicity of natural killer (NK) cells, which are a particular subset of lymphocytes capable of destroying virus-infected cells and tumor cells in an antibody-independent manner, through the μ-opioid receptor-mediated mechanism (Hsueh et al., 1996; Lang et al., 2003). On the basis of the previous observations, it is suggested that the cytotoxicity of NK cells may be higher in carriers of the OPRM1 G allele than in individuals without the G allele. NK cells are thought to be an important factor in resistance to viruses and tumor surveillance and are considered to be associated with health (Orange and Ballas, 2006). These previous observations may indicate the other reasons why carriers of the G allele are healthier than individuals without the G allele.

This study indicated that the general health score was negatively associated with the peripheral IL-6 levels and ANCOVA, on the general health score \( F(2,120) = 4.86, p < 0.01 \). Further statistical analysis revealed that the general health score was significantly higher in AG \( (p < 0.01; \text{Fig. 1b}) \) and GG genotypes \( (p < 0.05; \text{Fig. 1b}) \) than in the AA genotype. No significant effect of the genotype was observed in previous observations may indicate the other reasons why carriers of the G allele are healthier than individuals without the G allele.

This study indicated that the general health score was negatively associated with the peripheral IL-6 levels and ANCOVA,
which set IL-6 as covariate, indicated that genetic association with the general health was not statistically significant, suggesting the association of IL-6 with health status. Previous study has indicated that self-assessment of health status is associated with peripheral IL-6 levels (Parisis et al., 2009). It has also been indicated that lower positive emotional style was associated with greater objective and subjective markers of illness and these associations were decreased substantially by controlling for IL-6 but not for other cytokines (Doyle et al., 2006). Therefore, it is suggested that we may perceive that we are unhealthy when the peripheral circulating IL-6 level becomes high. However, the direction of the association cannot be determined from the current study. It is possible that a third variable could be the cause, such that when we are under excessive stress our health is negatively affected and our IL-6 levels increase. Furthermore, it has been reported that elevated peripheral circulating IL-6 levels are associated with several diseases. A recent study has demonstrated that apparently healthy men with high baseline circulating IL-6 levels had a greater than 2-fold higher risk to develop a myocardial infarction than did men with low IL-6 levels (Ridker et al., 2000). Similarly, circulating IL-6 levels can be used to predict the mortality in chronic heart failure patients (Lindmark et al., 2001; Parisis et al., 2009). Although the A118G SNP of OPRM1 has been reported to be associated with suicide (Hishimoto et al., 2008), no association between the A118B polymorphism and these heart diseases has been reported. However, it is highly possible that the A118G SNP of OPRM1 is also associated with the risk of developing several heart diseases.

Previous studies have indicated the gender and genotype interactions of the gene polymorphisms, such as the serotonin transporter gene-linked polymorphic region (5HTTLPR) and the Val66Met polymorphism of the brain-derived neurotrophic factor (BDNF) gene, in psychological and physiological activities (Mizuno et al., 2006; Shalev et al., 2009). In this study, no gender and genotype interactions of the A118G SNP of OPRM1 were observed in the measured indices. Previous studies of A118G SNP of the OPRM1 gene have also not indicated any gender and genotype interactions in physiological activity (Bart et al., 2006; Chong et al., 2006), suggesting that the A118G SNP may function similarly in both genders.

Certain limitations of this study must be recognized. First, the relatively small sample size (n = 123 samples) was insufficient to determine the effects of variations in OPRM1 on peripheral proinflammatory cytokine levels and health-related QOL. Further, in this study we did not examine the influences of body mass index, smoking state, and alcohol habit, which may change β-endorphin level. It is also possible that relatively small sample size caused the big difference in distribution of genotypes between women and men presented in this study. Therefore, a replication study with the same number of subjects for proinflammatory cytokines and SF-36 would be necessary. Second, the impact of population differences on the moderating effect of OPRM1 A118G on the proinflammatory cytokines and health-related QOL cannot be ruled out. A previous study has indicated that carriers of the G allele in European Americans had a significantly greater cortisol response to the opioid antagonist naloxone than those in Asians although the allele frequency did not differ by population (Hernandez-Avila et al., 2007). Thus, whether the present findings can be generalized must be further tested using different populations.

In conclusion, carriers of the OPRM1 G allele may be healthier than individuals without the G allele in healthy populations because of lower proinflammatory cytokine levels in the former. The sensitive μ-opioid receptor-mediated system in carriers may suppress the activation of the HPA axis and IL-6 secretion from peripheral immune cells, resulting in their high health perception. These results may expand the scope of clinical literature that addresses the links between gene polymorphisms and immunity.

Conflict of interest statement

All authors declare that there are no conflicts of interest.

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References


