Mineralocorticoid receptor gene – 2G/C polymorphism in central serous chorioretinopathy and relation of polymorphism with plasma cortisol levels

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SCR1 中盐皮质激素受体基因 – 2G/C 的多态性及其与血浆皮质醇水平的关系

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Abstract

• AIM: To evaluate the mineralocorticoid receptor (MR) gene – 2G/C single nucleotide polymorphism in central serous chorioretinopathy (CSCR), polymorphism and plasma cortisol level relationship.

• METHODS: Sixty CSCR patients and 50 controls were included in the study. Inclusion criteria for patients were acute manifestation of CSCR characterized by serous retinal detachment, RPE detachment or dysfunction without evidence of any other possible cause of fluid exudation, such as choroidal neovascularization, inflammation or infiltration. Peripheric blood sample was collected from the participants between 8 and 10 a.m. to avoid the diurnal changes of cortisol levels. MR (NR3C2) gene polymorphism (rs2070951) and plasma cortisol levels were studied.

• RESULTS: The genotype frequencies in CSCR group were G/C (46.6%), G/G (26.7%) and C/C (26.7%). There was no statistically significant difference in terms of genotype distribution among groups (P = 0.96). The plasma cortisol levels were also studied and the results were 401.2 ± 162.1 nmol/L in the CSCR group and 296.8 ± 130.1 nmol/L in the control group and the difference was statistically significant (P < 0.01). The plasma cortisol levels also did not differ between G/C (345.0 ± 137.0 nmol/L), G/G (369.2 ± 165.3 nmol/L) and C/C (395.3 ± 188.8 nmol/L) genotype differences (P = 0.50).

• CONCLUSION: The MR (NR3C2) gene polymorphism is not associated with CSCR and the plasma cortisol levels.

• KEYWORDS: mineralocorticoid; macula; central serous chorioretinopathy; polymorphism; genetics

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C entral serous chorioretinopathy (CSWR) is a disorder characterized by the detachment of the neurosensory retina due to the accumulation of the fluid in the subretinal space. Generally, it is believed to be due to a focal leak from retina pigment epithelium (RPE). Although the etiology is unclear glucocorticoids, pregnancy, hypertension, type A personality are some of the mostly recognized risk factors. Recent findings suggest that the pathology is the dysregulation or focal thrombosis of the choroidal blood flow, which results in ischemia, vasodilation, and hyperpermeability of the choroidal vessels. As a result, choroid gets thicker and the increased hydrostatic pressure overwhelms the barrier function of the RPE and this result with the leak of fluid from the choroid to the subretinal space.

Exogenous or endogenous steroids are believed to have a critical role in the development of CSWR. They show their affect thru glucocorticoid receptor (GR) and mineralocorticoid receptor (MR). MR has similar high affinities to both mineralocorticoid and glucocorticoid and is typically expressed in kidney cells, hypothalamus-pituitary-adrenal axis and vascular system. Zhao et al. have found that MR was also present in neuroretina, muller glial cells and choroid. They showed that MR activation in rats resulted in chorio capillary vasodilation and focal leakage which in turn resulted in choroidal thickening. In clinical practice, the studies performed with MR antagonists revealed promising results in CSWR patients that further increased the interest in these receptors. Studies performed with MR also revealed that they are involved in stress coping, behavioral adaptation and modulating brain plasticity. From this point on numerous studies were performed to evaluate the polymorphism of the MR gene in neuro-psychiatric disorders involving depression, attention deficit hyperactivity disorder, bipolar disease and schizophrenia. Type A personality which can be accepted as stressful condition is also frequently seen in CSWR patients and associated with higher levels of plasma cortisol and catecholamine levels probably due to the difference in the perception of stress. Therefore, due to both possible involvement of MR in type A personality and established role of MR antagonists in the treatment of CSWR, we evaluated single nucleotide polymorphisms (SNPs) that could be associated with CSWR. In this particular study, we investigated the MR (NR3C2) gene polymorphism (rs2070951) in CSWR patients.

SUBJECTS AND METHODS
Patients who were diagnosed as CSWR in a tertiary referral hospital between Jan. 2013 and Aug. 2013 were enrolled.

The samples were previously studied for 4G/5G polymorphism of plasminogen activator inhibitor (PAI - 1) gene. Institutional Review Board and ethics committee approval was obtained and informed consent were gathered (Ethics committee of Balikesir University School of Medicine, 2013/56). Sixty CSWR patients and 50 healthy individuals were included in the study. Body mass index (BMI) was calculated according to the World Health Organisation guidelines. Inclusion criteria for patients were acute manifestation of CSWR (first attack or acute attack of recurrent disease) characterized by serous retinal detachment, RPE detachment or dysfunction without evidence of any other possible cause of fluid exudation, such as choroidal neovascularization, inflammation or infiltration. Detailed ophthalmic examination including best - corrected visual acuity, slit - lamp biomicroscopy, and dilated fundus examination was performed to all patients. CSWR diagnosis was confirmed by fluorescein angiography and neurosensory or RPE detachment was also documented with optical coherence tomography imaging (Cirrus HD-OCT Model 4000, Carl Zeiss Meditec Inc., Dublin, CA, USA).

Subjects with concurrent ocular or retinal disease, history of coagulation abnormalities such as thromboemobolism, pregnancy, congestive heart failure, diabetes mellitus, coronary artery disease, uncontrolled arterial hypertension, smoking, hyperlipidemia, cancer, autoimmune inflammatory diseases, renal and hepatic abnormalities, endocrine pathology, and concomitant treatment affecting fibrinolysis metabolism (such as glucocorticoids and oral contraceptives), drug and/or alcohol intake were excluded from the study. Peripheric blood sample were collected from the patients and controls between 8 and 10 a.m. to avoid the diurnal changes of cortisol levels.

2G/C Polymorphism Genotyping Genomic DNA was extracted from a 200 µL peripheral venous blood sample according to the standard protocol by using GenJet DNA Purification Kit (Thermo Fisher Scientific Inc., USA). DNA samples were stored at -20°C until use. Light Cycler Nano® (Roche Diagnostics GmbH, Mannheim Germany) were used for analysing the 2G/C polymorphism in the MR (NR3C2) gene. This polymorphism is amplified with specific primers and detected with hydrolysis probes (TIB MOLBIOL GmbH, Berlin, Germany). Single - nucleotide polymorphism amplification assays were performed according to the manufacturer’s instructions. Cycling conditions for amplification of the MR (NR3C2) gene were initial denaturation at 95°C for 10 min, followed by 45 cycles with at 95°C for 10 s, at 40°C for 10 s and at 72°C for 15 s.

Biochemical Analysis Serum cortisol level was measured with the electrochemiluminescence immunoassay method by cobas e 411 immunoassay analysers. (Roche Diagnostics GmbH, Mannheim Germany, Kit Measuring Range 1 - 1750 nmol/L.) The assays were performed according to the manufacturer’s recommendations. Serum samples were obtained from the patients within one week after the diagnosis
was confirmed with fluorescein angiography and optical coherence tomography. Peripheral venous blood was collected in the morning and was centrifuged at 1500g for 15 min at 4°C. After centrifugation, serum was separated from the sediment and stored frozen (−80°C) until use.

**Data Analyses** Quantitative data was described as mean ± standard deviation (SD) and qualitative data was described as percentage. SPSS for Windows software (version 11.0, SPSS, Inc., USA) was used for statistical analysis. Statistical analyses of the differences in gender and genotype frequencies between groups were analysed with Chi-square test. Normality of the quantitative data sets was assessed by the Shapiro–Wilk test. Student’s t-test for parametric analysis and Mann–Whitney U test for nonparametric analysis was used for searching intergroup difference. One-way ANOVA test was used for comparison of plasma cortisol levels among 3 different polymorphism types.

**RESULTS**
There were not statistically significant between the groups on age, gender and the BMI. The demographic characteristics of the groups were presented in Table 1. The genotype frequencies in CS5R group were G/C (46.6%) , G/G (26.7%) and C/C (26.7%). The frequencies in the control group were G/C (44.0%), G/G (28.0%) and C/C (28.0%). There was no statistically significant difference in terms of genotype distribution among groups (P = 0.96). The allele frequencies were 52% for G and 48% for C in the CS5R group and 49% for G and 51% for C allele in the control group (P > 0.05).

The plasma cortisol levels were also studied and the results were 401.2 ±162.1 nmol/L in the CS5R group and 296.8 ± 130.1 nmol/L in the control group and the difference was statistically significant (P < 0.01). The plasma cortisol levels also did not differ between G/C (345.0 ±137.0 nmol/L), G/G (369.2 ±165.3 nmol/L) and C/C (395.3 ±188.8 nmol/L) genotypes (P = 0.50).

**DISCUSSION**
CS5R is the 4th most common retinopathy after age related macular degeneration, diabetic retinopathy, and branch retinal vein occlusion [2]. To better understand the disease, many studies were conducted for epidemiology, pathophysiology, systemic associations, risk factors and treatment options [2,4,13–16]. The possible genetic contribution in the disease development was evaluated with some familial CS5R reports in the literature [17–20]. Additionally the racial predilection to white, Hispanic and Asian populations also supports a genetic tendency. However, the genetic background of the disease was not exclusively studied and the literature search reveals limited number of studies mostly of familial involvement basis [12,21–22]. In a recent review, this issue is also addressed and the need for further studies investigating SNPs was expressed [3]. Studies of SNPs might help identifying the individuals at risk and be useful to predict the ones to progress to chronic form of the disease.

The results of the current study revealed that there is no statistically significant difference between CS5R patients and healthy controls in terms of the MR – 2G/C polymorphism. The rates were very similar and nearly every 1 of 2 cases had 2G/C polymorphism in both CS5R and controls. The allelic frequency also did not differ between the groups. MR is critical in hypothalamic–pituitary–adrenal (HPA) axis and response to clinical stress which we believe might cause increased cortisol levels in type A personality CS5R patients. As expected, we found that plasma cortisol levels were significantly higher in the CS5R patients. However, the cortisol levels were not significantly different among G/G, G/C and C/C polymorphisms. Contrary to our findings, previous studies have found a relationship between polymorphisms and plasma cortisol levels. Muhitz et al. [23] investigated how MR gene variants affected basal cortisol secretion in 133 healthy adults and have found that MR – 2G/G gene polymorphism was associated with higher plasma cortisol levels in healthy adults. They emphasized that this polymorphism might have a role in interindividual variability in stress responsiveness and be involved in stress related disorder. Another study conducted on 166 school teacher, a stressful occupation, has found that subjects having C/C polymorphism has highest plasma cortisol levels [5]. Apart from chronic perceived stress, this study also performed acute experimental psychosocial stress test and found that individuals with C/C polymorphism showed higher salivary cortisol, plasma cortisol, adrenocorticotropic hormone (ACTH) levels and heart rate responses. Two different studies conducted on the effect of the MR gene polymorphism on cortisol levels found higher cortisol levels with homozygote allele carriers but one found C/C and the other found G/G to be related. In our study we failed to find any association of the MR – 2G/C polymorphisms with plasma cortisol levels. Therefore, the hypothesis that type A personality, one of the earliest risk factor for developing CS5R with higher levels of cortisol ( x 40) compared to type B personality, and MR gene might be related and MR gene polymorphism might have a role in the development of CS5R is needed to be further investigated.

The literature search has resulted with only 3 studies for SNPs in CS5R which were published recently. One of these studies belonged to us in which we investigated plasminogen activator inhibitor (PAI-1) gene 4G/5G polymorphism in the same patient group. The study has failed to find any difference between CS5R and controls in terms of PAI-1 gene 4G/5G

### Table 1 Characteristics of groups

<table>
<thead>
<tr>
<th>Features</th>
<th>CS5R</th>
<th>Control</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>60</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Age range (a)</td>
<td>29–62</td>
<td>36–59</td>
<td></td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>40/20</td>
<td>32/18</td>
<td>0.46</td>
</tr>
<tr>
<td>Mean age±SD (a)</td>
<td>46.72±8.39</td>
<td>45.82±8.13</td>
<td>0.73</td>
</tr>
<tr>
<td>BMI</td>
<td>24.93±2.04</td>
<td>25.36±1.30</td>
<td>0.28</td>
</tr>
</tbody>
</table>
polymorphism. The second study was related with cadherin 5 (CDH5) protein which stands as the major cell to cell adhesion molecules in the vascular endothelium [21]. The study confirmed that CDH5 protein was down-regulated with steroids and also found significant association of four common CDH5 SNPs with CSCR in male patients. They proposed that genetic variation in CDH5 protein might create a tendency for CSCR with triggering events such as hypercortisolism. The third study investigated the complement H protein that binds adenomedullin, a strong vasodilator in the choroidal vasculature [22]. They found an association between CSCR and common complement H SNPs and imply that some variants in this protein may act as susceptibility elements for CSCR development. Both CDH5 and complement H studies were performed with higher number of patients and controls (400 and 140 CSCR patients and 1400 and 934 controls respectively). The small sample size in our study is the major limitation and might have an impact on our results. As a conclusion, we did not find any difference in genotypes of MR gene (rs2070951) polymorphism between CSCR patients and healthy controls. Our current study is first to examine the association between the MR gene polymorphisms and CSCR in Turkish population. Similar studies with larger sample size are needed to reveal the genetic predispositions in CSCR.

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