NOTE

A Polymorphism in the Promoter Region of the Glucocorticoid Receptor Gene Is Associated with Its Transcriptional Activity

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Abstract. Since glucocorticoid exerts its biological effects by binding to its receptor, the expression efficiency of the glucocorticoid receptor (GR) gene could influence glucocorticoid sensitivity. We found a polymorphism of cytosine/adenine (−22 C/A) in the upstream region of the GR gene. There was no difference in the allelic frequency between normal and type 2 diabetic subjects. The promoter activity determined by luciferase assay was significantly lower in the −22 A allele than in the −22 C allele in both HepG2 (A allele, 4.19 ± 0.15; C allele, 6.07 ± 0.27, p < 0.001) and human embryonic kidney 293 cell lines (A allele, 0.93 ± 0.16; C allele, 1.51 ± 0.32, p < 0.001). This polymorphism is associated with transcription of the GR gene, which could be related to glucocorticoid sensitivity through an alteration in tissue GR number.

Key Words: Glucocorticoid receptor, Gene polymorphism, Transcriptional activity

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from outpatients and inpatients treated in our department. Diagnosis of diabetes mellitus and IGT were made according to the criteria of the World Health Organization (WHO). All subjects were Japanese who resided in the same area (Kochi Prefecture, Japan). They gave their informed consent to participate in the study. All serum and plasma samples were stored at -80°C, and genomic DNA was stored at -20°C.

Variations upstream from the GR (−147/+159) gene were examined in members of our staff by means of a cycle sequencing method. The DNA fragment was amplified using a PCR method employing a sense primer (5'-CGTCGGGGACGGATTCTGTG-3') and an antisense primer (5'-CGGCCACCGTCCGCAGTTCC-3') with genomic DNA as a template. We used the GenBank accession numbers to refer to the sequence of the GR gene, M73050 and U10430, and the transcriptional start site according to the latter. A PCR fragment separated by electrophoresis in agarose gel was recovered and purified using an available commercial kit (QIAquick Gel Extraction Kit, QIAGEN). The sequence of the PCR fragment was detected using a commercial kit and analyzer (BigDye Terminator Cycle Sequencing FS Ready Reaction Kit and ABI PRISM™310 Genetic Analyzer, PE Applied Biosystems, Foster City, CA), and the same sense or antisense primer of the PCR was used for the sequencing primer. For screening of −22 C/A polymorphism in healthy volunteers and patients with diabetes or IGT, a PCR and RFLP method was performed. Briefly, the DNA fragment was amplified using the first PCR product as a template with the same sense primer (indicated above) and another antisense mismatched primer (5'-CATTGCCCAGCTGGATAGCC-3'), which produced a new BsrBR1 restriction site in the −22 A allele.

For the reporter gene assay, a DNA fragment of the promoter region of the GR gene (−328/+107) was amplified by the PCR method using a sense primer (5'-GGGGTACCGGAGCGTGAGTG-3') and an antisense primer (5'-TCCCCCGGGCCGAGAGGACGGGAGG-3'). The underlined areas indicate the sequences added as the restriction sites of Kpn I and Sma I, respectively. The DNA fragment was introduced into the firefly luciferase expression vector, pGL3-Basic (Promega), and subcloned into JM109. The introduced fragments were confirmed using the cycle sequencing method employing the primers described for pGL3-Basic. Transient transfection into the cells was performed using a cationic lipid method employing Tfx™.20 for HepG2 cells and TransFast™ for human embryonic kidney 293 (HEK293) cells according to the manufacturer's instructions (Promega). The pRL-TK vector which expressed Renilla luciferase (Promega) was co-transfected as an internal control. Both luciferase activities were measured using the Dual-Luciferase® Reporter Assay System (Promega). Transcriptional activity was expressed as firefly luciferase activity divided by Renilla luciferase activity.

Results of the reporter gene assay were presented as mean±SEM, and other data were expressed as mean±SD. Comparison of two groups was estimated using the unpaired t-test.

Results

The −22 C/A polymorphism was detected in the upstream fragment (−147/+159), which has not been reported before (Fig. 1). Three of 83 normal subjects (3.6%), one of 14 IGT subjects (7.1%), and 5 of 130 diabetic patients (3.8%) were −22 CA heterozygotes. No −22 AA homozygote was detected in our study population. No significant differences were seen in the mean BMI between subjects with −22 CC and those with −22 CA (normal subjects: 22.9±3.3 vs. 22.6±3.3 kg/m²; IGT subjects: 27.9±7.4 vs. 26.0 kg/m²; diabetic patients: 24.6±4.7 vs. 22.9±2.5 kg/m², respectively), although the number of subjects with −22 CA was very small in every group.

The luciferase activity of the −22 A allele was significantly lower than that of the −22 C allele in the HepG2 cell line (Fig. 2a). We performed the assay 3 times; the mean activity of the −22 A allele was 0.69-0.77-fold lower than that of the −22 C allele. The same result was obtained in the HEK293 cell line (Fig. 2b).

Discussion

We identified the −22 C/A polymorphism of the GR gene and found that this polymorphism was associated with the transcription of GR in HepG2 cells, as well as in HEK293 cells. A Tth1111 polymor-
polymorphism on the 5'-flanking region has been reported [7], and Rosmond et al. showed that this polymorphism was associated with basal cortisol secretion [2]. The reported polymorphism is apparently different from that which we report here because the sequence including -22 C/A showed no identity with the Tth111I restriction site, and the influence of the Tth111I polymorphism on promoter activity has not been examined. The promoter region of the GR gene has been reported to have several GC boxes [8]. The sequence of the -22 C/A polymorphism is next to a consensus sequence of the Sp1 binding site, which was the GC box nearest to the transcriptional start site. It is not known whether this Sp1 element plays an important role in transcription of the GR gene; however, the -22 C/A polymorphism could possibly be involved in the regulation of GR gene transcription by affecting the association between the GC box and transcription factors.

An Asn363Ser polymorphism, a single nucleotide difference (A1218G) in exon 2 of the GR gene, has been reported to be associated with increased glucocorticoid sensitivity and overweight in a Caucasian population [3, 4]. Therefore, we also investigated the Asn363Ser polymorphism in this study population (n=227) by means of PCR and Tsp509I digestion [4], but no Ser363 variant was identified (data not shown). Therefore, this polymorphism seems to be extremely rare in the Japanese population.

In this study, no differences in BMI were seen between subjects with -22 CC and those with -22 CA. However, the numbers of the -22 CA variant

![Figure 1](image_url)

**Fig. 1.** The -22 C/A polymorphism upstream from the glucocorticoid receptor gene. The GenBank accession numbers, M73050 and U10430, were used to refer to the sequence of the GR gene, and the transcriptional start site according to the latter.

![Figure 2](image_url)

**Fig. 2.** The dual luciferase activities of the upstream region in the -22 C allele (n=10) and the -22 A allele (n=10) of the GR gene. Data represent mean±SEM. *p<0.001, unpaired t-test. pGL3: pGL3-Basic without introducing DNA.
detected were too small for statistical comparison, hence a study in a larger population is necessary to clarify this. The clinical significance of this polymorphism of the GR gene is unknown. Therefore, further work is required to establish a causative link between the observed changes in GR gene transcription and glucocorticoid sensitivity.

References