



# Correlation between polymorphisms in the glucocorticoid receptor gene *NR3C1* and susceptibility to asthma in a Chinese population from the Henan Province

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**ABSTRACT.** The aim of this study was to investigate the association between four single nucleotide polymorphisms in *NR3C1* (*Tth111I*, *BcII*, ER22/23EK, and N363S), which encode the glucocorticoid receptor, and asthma susceptibility in patients from the Henan Province of China. Three hundred and twenty-eight patients with asthma and 60 healthy volunteers were recruited to this study. The target SNPs were genotyped by polymerase chain reaction (PCR)-high resolution melting and PCR-restriction fragment length polymorphism. The frequencies of the AA (8.84%) and GG (30.79%) genotypes of *Tth111I* were higher, and that of the AG genotype was lower (60.37%), in the asthma patients compared to that seen in healthy controls (5.00, 26.67, and 68.33%, respectively). On the other hand, asthma patients showed higher frequencies of the AA genotype (78.05%) of N363S, and lower

frequencies of the AG and GG genotypes (15.55 and 6.40%), compared to healthy volunteers (71.67, 18.33, and 10.00%, respectively). Neither of these differences were found to be statistically significant. Moreover, we observed no significant differences in the genotype or allele frequencies of the *BclI* and ER22/23EK SNPs between the patient and control groups. In conclusion, SNPs in *NR3C1* were not significantly associated with asthma in patients from the Henan Province. Patients showed higher frequencies of the AA and GG genotypes of *Tth111I* and the AA genotype of the N363S SNP compared to healthy volunteers, although these differences were not significant.

**Key words:** *NR3C1*; Polymorphism; *Tth111I*; *BclI*; ER22/23EK; N363S; Asthma

## INTRODUCTION

Asthma is a serious global health problem affecting people of all ages (Bateman et al., 2008). The constant deterioration in environmental quality, specifically the increase in air pollution and haze in the Henan province, has resulted in a drastic increase in the incidence of asthma.

Glucocorticosteroids (GCs) are commonly used in the treatment of asthma as key elements of complex therapy. However, the biological actions of GCs are complicated, and their effects are determined individually; therefore, some patients can have side effects despite the use of low doses of GCs, while others show no such effects even with high doses of GCs. Accordingly, a wide range of doses is used to treat and control asthma. Approximately 2.3 and 6.6% of a healthy population is resistant and sensitive to GCs, respectively (Lamberts et al., 1996; van der Velden, 1998; Ito et al., 2006; Adcock et al., 2008). However, recent studies have shown a considerable decrease in the efficacy of GCs in treating asthma, because of occurrence of mutations and single nucleotide polymorphisms (SNP) in *NR3C1*. These mutations induce a decrease in the expression of GR as well as excessive production of transcription factors, which in turn weakens the binding ability of GCs to DNA and their ligands (Luisi et al., 1991; Chanez et al., 2000; DeRijk et al., 2002; Kino et al., 2003; Leung and Bloom, 2003).

Previous studies have indicated that polymorphisms in the *GR* gene could play a major role in the pathogenesis of asthma and the response of asthmatic patients to GC treatment (Ito et al., 2006). Four SNPs in the *GR* gene that are responsible for various disorders (*Tth111I*, *BclI*, ER22/23EK, and N363S) have also been reported to influence GR-sensitivity.

*Tth111I* is a restriction fragment length polymorphism (RFLP) that is coupled with the ER22/23EK SNP (rs6189 and rs6190) (Detera-Wadleigh et al., 1991; Rosmond et al., 2000; DeRijk and de Kloet, 2008; Nicolaidis et al., 2010; Panek et al., 2013a). The C/G SNP in *BclI* (rs41423247) is associated with increased cortisol suppression (van Rossum et al., 2003; Stevens et al., 2004), while ER22/23EK (rs6189 and rs6190) (Koper et al., 1997) is believed to be associated with decreased GR sensitivity (van Rossum et al., 2002, 2004). On the other hand, the N363S A/G SNP (rs6195) affects the degree of phosphorylation of GR, via increased transactivation of gene coding, as well as increased cortisol suppression and insulin response (Koper et al., 1997; Huizenga et al., 1998).

In this study, we investigated the effect of SNPs of *Tth111I*, *BclI*, ER22/23EK, and N363S SNPs in *NR3C1* on TGF- $\beta$ 1 mRNA expression in asthmatic patients in the Henan

province of China, in order to identify the molecular diagnostic methods and anti-inflammatory drugs for asthma therapy based on the regional incidence of this disease.

## MATERIAL AND METHODS

### Patients

Three hundred and twenty-eight patients (212 males and 116 females; age: 18-65; average age:  $43.37 \pm 20.67$ ) diagnosed with asthma, based on the guidelines of the Global Initiative for Asthma (GINA) report (Bousquet, 2000), between February 1, 2005 and October 31, 2011 by two doctors (with >20 years of experience) affiliated to the Respiratory and Critical Care Medicine department of the First Affiliated Hospital of Zhengzhou University were recruited to this study. The patient group was composed of 76 patients with intermittent asthma, 130 with mildly continuous asthma, 100 with moderately continuous asthma, and 22 with severely continuous asthma. Patients were selected based on a total immunoglobulin E (IgE) count > 0.2 IU/L. The control group was composed of 60 healthy volunteers (31 males and 29 females; age: 20-65; average age:  $43.91 \pm 19.64$ ).

None of the patients or volunteers had any family history of asthma or any other disease, including lung disease (chronic obstructive pulmonary disease or pulmonary fibrosis), allergic inflammations (allergic rhinitis, atopic dermatitis, allergic purpura), diabetes, nephrotic disease, systemic lupus erythematosus, or psoriasis. Patients with asthma who were treated with rifampicin, phenobarbital, phenytoin, or ephedrine, or those with infection-induced asthma were not included in this study.

Informed consent was obtained from all patients and volunteers. This study was approved by the medical ethics committee of the First Affiliated Hospital of Zhengzhou University.

### DNA isolation

Venal blood samples were collected from all patients and volunteers in EDTA (Thermo Fisher Scientific, Waltham, MA, USA) anticoagulant tubes. Genomic DNA was isolated using the TGuide M16 DNA extraction kit (Tiangen Biotech Co., Ltd., Beijing, China) according to the manufacturer instructions, and stored at  $-20^{\circ}\text{C}$  until further use.

### Genotyping the *Tth111I*, *BcII*, ER22/23EK, and N363S polymorphisms in *NR3C1*

The genotypes of the *Tth111I*, *BcII*, ER22/23EK and N363S SNPs were identified by the TaqMan allelic discrimination assay (Thermo Fisher Scientific), designed using the Assay-by-Design service (Applied Biosystems, Foster City, CA, USA). The primer sequences, incubation temperatures, and restriction enzymes used to amplify the *Tth111I*, *BcII*, ER22/23EK, and N363S SNPs, as well as the resultant sequence lengths, are summarized in Table 1. The *Tth111I*, *BcII*, ER22/23EK, and N363S polymorphisms were exponentially amplified according to the PCR system detailed in Table 2.

The PCR mixture was composed of 2  $\mu\text{L}$  5 ng genomic DNA, 200 nM probe, 0.9  $\mu\text{M}$  primers, and 2X Taqman PCR master mix (ABgene; Thermo Fisher Scientific). The DNA fragments were identified by melting curve analysis (Panek et al., 2013b), and the fragments of interest were genotyped by restriction fragment length polymorphism (RFLP) using the

LightScanner High Sensitivity Master Mix (Idaho Technology, Salt Lake City, UT, USA). The RFLP products were separated by electrophoresis on an 8% polyacrylamide gel, and stained with ethidium bromide for observation under a UV light (Pharmacia Biotech ImageMaster VDS; BioSurplus, Inc., San Diego, CA, USA). Typical homozygotes and heterozygotes were sequenced and used as internal controls.

**Table 1.** Primer sequences, incubation temperatures, and restriction enzymes used to genotype the *Tth1111*, *BclI*, ER22/23EK, and N363S single nucleotide polymorphisms.

	Forward primer	Reverse primer	Incubation temperature (°C)	Restriction enzyme
<i>Tth1111</i>	5'-GGATGAATCCCTATCTGAGTG-3'	5'-GGCCACAACAATAACCCAGTA-3'	37	<i>PstI</i>
<i>BclI</i>	5'-GAGAAATTCACCCTACCAAC-3'	5'-AGAGCCCTATTCTCAAAC-3'	37	<i>BclI</i>
ER22/23EK	5'-TGCATTCGGAGTTAACTAAAA-3'	5'-ATCCCAGGTCATTCCCATCA-3'	37	<i>MnlI</i>
N363S	5'-CCAGTAATGTAACACTGCC-3'	5'-TTCGACCAGGGGAAGTTCAGA-3'	65	<i>TaqI</i>

**Table 2.** Polymerase chain reaction amplification system.

Step	Temperature (°C)	Time	Cycle number
Pre-denaturation	95	15 min	40 cycles
Denaturation	95	15 s	
Annealing	60	60 s	
Extension	60	60 s	
Terminal extension	72	7 min	1 cycle
Storage	4	→∞	

## Statistical analysis

SPSS 19.0 (IBM, Armonk, NY, USA) was used to analyze the data. The genotype and allele frequencies of the GR SNPs were analyzed using the chi-square test. The conformance of the genotypes and alleles of all SNPs with the Hardy-Weinberg equilibrium (HWE) was confirmed using the online calculator developed by Michael H. Court. Differences with P values < 0.05 were considered to be statistically significant.

## RESULTS

### Genotype and single polymorphic allele frequencies of the *NR3C1* SNPs

The 4 SNP sites were in accordance with the HWE in both the case and control groups ( $P > 0.05$ ), indicating the representativeness of the sample subset. The genotype and single polymorphic allele frequencies of the *Tth1111*, *BclI*, ER22/23EK, and N363S SNPs in *NR3C1* are summarized in Table 3. We observed no significant differences in the genotype and allele frequencies of the *Tth1111*, *BclI*, ER22/23EK, and N363S SNPs between the cases and controls. However, the frequencies of the AA and GG genotypes were higher, and that of the AG genotype was lower, in the asthma patients than that in the healthy volunteers. Specifically, a greater percentage of asthma patients expressed the AA frequency compared to the healthy volunteers, while the genotype frequencies of AG and GG were lower in the asthma patients.

**Table 3.** Frequencies of the genotypes and single polymorphic alleles of the *Tth111I*, *BcII*, ER22/23EK, and N363S single nucleotide polymorphisms.

NR3C1 SNP			Cases group (N = 328)		Control group (N = 60) (%)		P
			N	Frequency (%)	N	Frequency (%)	
<i>Tth111I</i>	Genotype	AA	29	8.84	3	5.00	0.089
		AG	198	60.37	41	68.33	
		GG	101	30.79	16	26.67	
	Allele	A	256	39.02	47	39.17	
		G	400	60.98	73	60.83	
<i>BcII</i>	Genotype	CC	20	6.10	3	5.00	0.532
		CG	90	27.44	15	25.00	
		GG	218	66.46	42	70.00	
	Allele	C	130	19.82	21	17.50	
		G	526	80.18	99	82.50	
ER22/23EK	Genotype	AA	0	0.00	0	0.00	0.806
		AG	15	4.57	3	5.00	
		GG	313	95.43	57	95.00	
	Allele	A	15	2.29	3	2.50	
		G	641	97.71	117	97.50	
N363S	Genotype	AA	256	78.05	43	71.67	0.103
		AG	51	15.55	11	18.33	
		GG	21	6.40	6	10.00	
	Allele	A	563	85.82	97	80.83	
		G	93	14.18	23	19.17	

## DISCUSSION

Asthma is a complex disease, incorporating the secretion of several substances, such as cytokines, by various cells. Asthma is a chronic inflammatory disease of the respiratory system; this disease is determined by a number of factors, including interactions between hereditary and environmental factors, resulting in the development of various complications such as bronchial hyper-responsiveness, recurrent episodes of wheezing, cough, and dyspnea (Panek et al., 2013a,b).

It is important to determine the level of hypersensitivity of a patient to GCs in clinical practice. Asthma is induced in the presence of a high concentration of cortisol in the blood, characterized by a high 24-h excretion of free cortisol, but without the symptoms of Cushing's syndrome.

Resistance to GCs is related to mutations in *NR3C1*, which induce changes in the amino acid content in the structural receptor regions, whose functional domains play important roles in the biological action of related proteins. Asthmatic patients showing a lack of or very slight response to GCs have been shown to express normal levels of cortisol (Lipsett et al., 1985; DeRijk et al., 2002; Kino et al., 2003; Bray and Cotton, 2003; Adcock et al., 2008).

Patients that received inefficient GC treatment displayed an increase in the density of complement receptors on monocytes, while the expression of GR did not change in these patients compared to that in patients who were sensitive to GCs (Carmichael et al., 1981). On the other hand, patients that did not receive GC treatment showed activation of the p38 mitogen-activated protein kinase (p38MAP) due to the chronic inflammatory effect of cytokines interleukin (IL)-2 and IL-4. The affinity of GR to GCs is also influenced by the activity of other kinases, including the mitogen-activated protein kinase (MAPK). Patients

displaying low sensitivity to GCs have been reported to display increased cellular activity of the c-Jun N-terminal kinase (JNK). These findings indicated that phosphorylation pathways dependent on p38MAPK and JNK inhibited GC activity (Barnes, 2006).

Therefore, we attempted to elucidate the significance of the effect of *NR3C1* SNPs in asthma, but observed no significant differences in the genotype and allele frequencies between asthma patients and healthy individuals in this study. However, asthma patients showed higher frequencies of the AA or GG genotype, and lower frequencies of the AG genotype, compared to healthy volunteers. Although this difference was not statistically significant, the higher incidence of the AA and GG genotypes indicated a possible relationship between the *Tth1111* SNP and asthma incidence.

Previous studies (van Rossum and Lamberts, 2004; De Iudicibus et al., 2011; Panek et al., 2013a) reported an association between the *Tth1111* SNP and increased cortisol level in the blood; additionally, these studies reported a decrease in the cortisol secretion following the administration of 1 mg dexamethasone (DEX). Additionally, carriers of the *Tth1111* SNP who also express the ER22/23EK SNP have been shown to induce resistance to GCs and show a normal metabolic profile (Van Rossum et al., 2004; Nicolaides et al., 2010; Panek et al., 2012a). Other studies have confirmed that the N363S SNP imparts the GC-GR complex with an increased ability to transactivate its target genes (Huizenga et al., 1998; Panek et al., 2012a,b). In this study, patients with asthma presented higher frequencies of the AA variant, and lower frequencies of the AG and GG variants, of the N363S SNP, compared to the healthy volunteers, which was consistent with the results of previous studies (Huizenga et al., 1998; Panek et al., 2012a,b, 2013a). Some reports have also indicated that the AG or GG variants at codon 363 of *NR3C1* are correlated with increased anti-inflammatory effects during GC treatment, and lower risk of uncontrolled asthma; however, this effect was not significant in this study. These differences could be attributed to the small sample size or the regional limitation.

In conclusion, the *Tth1111* and N363S SNPs of *NR3C1* could be related to asthma in a Chinese population specific to the Hebei province. Patient-specific molecular diagnostic tests, and anti-inflammatory drugs and targeted therapy for the treatment of asthma must be identified and confirmed in future investigations.

## Conflicts of interest

The authors declare no conflict of interest.

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