

Association of the glucocorticoid receptor D641V variant with steroid-resistant asthma: a case-control study

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Objectives Several mutations of the glucocorticoid receptor (GR) gene cause malfunction of the protein, resulting in steroid resistance. In diseases other than asthma, the GR variants I559N, D641V, and V729I have been linked to steroid resistance. The aim of this study was to evaluate the link of these GR variants in steroid-resistant (SR) asthma in the Chinese Han population.

Methods GR polymorphisms were determined in 64 SR asthma patients, 217 steroid-sensitive (SS) asthma patients and 221 healthy control (CTR) individuals. The analysis of the GR variants was performed using PCR-sequence specific primers according to the European Molecular Biology Laboratory database (NC_000005.8). In addition, ligand binding and serum cortisol levels were determined.

Results Compared with SS asthma patients and CTRs, a significant lower frequency of the GR D641V variant AA genotype ($P = 0.003$, 0.014 , respectively) and the A allele ($P = 0.001$, 0.009 , respectively) was found in SR asthma patients. Furthermore, the equilibrium dissociation constant (K_d) of GR ligand binding in SR asthma patients with the GR D641V variant AA genotype was significantly lower compared with the AT or the TT genotype carriers ($P = 0.006$,

0.016 , respectively). There was no significant difference between the I559N and V729I GR variants on comparing SR asthma patients with SS asthma patients or CTRs.

Conclusion This study suggests that the D641V variant of the GR is probably associated with SR asthma in the Chinese Han population. *Pharmacogenetics and Genomics* 25:289–295 Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.

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Introduction

Glucocorticoids are the most prescribed and most effective drugs for the treatment of chronic inflammatory diseases such as asthma. However, ~10% of asthmatic patients do not respond well to a regular dose of inhaled corticosteroids (ICSs). About 1% of these asthma patients had to accept oral corticosteroids because of the poor response to ICSs. A small proportion of these patients showed no improvement after a high dose of oral corticosteroids; this type of asthma has been named as steroid-resistant (SR) asthma [1,2]. All these participants who respond less well to corticosteroids account for ~50% of the total healthcare costs of asthma [3].

Glucocorticoids exert their effect by binding to and activating cytosolic glucocorticoid receptor (GR), which subsequently enters the nucleus and acts as a positive or negative transcription regulator [4,5]. Previous studies showed that the reduction of steroid responsiveness in asthma patients may result from the loss of GR availability, caused by reduced expression, altered ligand

affinity, decreased ability of the GR to bind to DNA, reduced expression and/or activity of corepressor proteins, or the increased expression of inflammatory transcription factors, such as nuclear factor- κ B and activator protein-1 [1,4,5]. Furthermore, reduced activity of GR can be due to a low action of preceding signaling proteins, including p38 mitogen-activated protein kinase (MAPK)- α , p38 MAPK- γ , and c-Jun N-terminal kinase 1, which results in low phosphorylation of MAPK phosphatase 1 and protein phosphatase A2 [1]. On the DNA binding level, reduced GR activity was linked to increased GR- β expression competing with the activated GR- α [6,7], or with reduced expression of histone deacetylase-2, which modifies the DNA binding affinity of the active GR- α to the glucocorticoid response element [8].

The human GR gene is located on chromosome 5q31 and consists of nine exons encoding a protein of ~80 kb [9]. Two competing GR proteins can be generated on splicing: GR- α and GR- β , with only the GR- α isoform

exerting the anti-inflammatory gene regulating hormone effect [10,11]. Further analysis showed that single nucleotide polymorphisms (SNPs) of the GR gene can lead to functional loss and cause steroid resistance in asthma [12,13]. However, not all studies found association between GR polymorphisms and the response to glucocorticoids in asthma [14].

In other diseases, three GR- α polymorphisms, I559N, D641V, and V729I, which are located in GR exons 5, 7, and 9, respectively, had been identified as additional causes of steroid resistance [12,15–17]. The GR variant I559N is caused by the replacement of thymidine by adenosine, substituting isoleucine by asparagine [16,17]. GR variant D641V results from an exchange of adenosine by thymidine, replacing aspartic acid by valine [15,16]. The GR variant V729I in exon 9 is due to the replacement of guanosine by adenine, substituting valine by isoleucine [15,16]. All three of these single nucleotide exchanges disrupt gene transcription, decrease GR transactivation or ligand affinity, and thereby reduce corticosteroid responsiveness, which results in partial or total steroid resistance [17].

In a SR patient who presented with hypertension and oligospermia, the GR- α I559N variant showed normal ligand affinity of the GR, but a 50% reduction in binding sites [18]. In another study, the equilibrium dissociation constant (K_d) of the D641V variant was three-fold higher relative to wild type (WT) GR in transfected COS-7 cells [19]. The GR- α V729I variant was associated with steroid resistance in a young Hispanic boy and showed decreased affinity for ^3H -dexamethasone in COS-1 cells, resulting in 4- to 12-fold reduced GR activity [20,21]. However, all these studies were focused on the structure of GR gene, and the results in the above studies were obtained from familial, sporadic cases or cell lines. Therefore, large clinical trials are needed to support these viewpoints. Until now, no study has been published on the association of GR- α polymorphisms with SR asthma in the Chinese population. Therefore, it is important to clarify the reason of decreased GR function in each SR asthma patient.

In our study the GR- α polymorphisms of I559N, D641V, and V729I were analyzed in the Chinese Han population, and the association between steroid resistance and GR- α variants in asthma patients was investigated. These results may be helpful in elucidating the mechanism and determining a predisposition of steroid resistance in asthma.

Materials and methods

Patients

All patients were recruited from Xijing hospital, Xi'an Central Hospital and Xi'an Electricity Staff-worker Hospital in China. The characteristics of the study population are shown in Table 1. All study participants

were ethnic Chinese Han and were recruited from Shaanxi Province of China between August 2006 and June 2012.

The asthma patients were diagnosed according to the American Thoracic Society criteria, based on clinical asthma symptoms and lung function tests. To qualify for this study, baseline forced expired volume in 1 s (FEV_1) had to be less than or equal to 70% of the predicted value. All asthma patients confirmed an improvement of at least 12% in FEV_1 after albuterol inhalation. No patient had a postbronchodilator FEV_1 /forced vital capacity ratio less than 70% to indicate that the airway obstruction was reversible and no persistent airway obstruction existed, thus excluding chronic obstructive pulmonary disease [22].

A total of 64 of the asthma patients were classified as having SR asthma due to the failure to improve their prebronchodilator morning FEV_1 by more than 15% after a course of oral prednisone equal to or exceeding 40 mg/day over a 7-day period [23].

A total of 217 patients were classified as having steroid-sensitive (SS) asthma, as their prebronchodilator morning FEV_1 was increased by more than 30% after oral prednisone.

No chronic cough or sputum production was declared by any of the asthma patients, and none of them had experienced upper respiratory tract infection for at least 6 weeks before the recruitment. None of the patients received medications interfering with glucocorticoid metabolism.

A total of 221 healthy Chinese adults with no history of allergic disease were enrolled as healthy controls (CTRs). All participants were nonsmokers.

The study protocol was approved by the Ethics Committees of all participating hospitals. The study was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants before entry into the study.

Determination of genotypes and serum cortisol

Anticoagulated peripheral blood was collected between 8:00 and 9:00 a.m.; serum was separated and stored at -20°C . Serum cortisol was determined with radioimmunoassay provided by the Beijing North Biotechnology Research Institute.

Genomic DNA was extracted from peripheral blood mononuclear cells (PBMCs) using a DNA Extraction Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

We selected three GR gene SNPs, which had been associated with steroid resistance, including I559N, D641V, and V729I. SNP sequence data are available through the European Molecular Biology Laboratory

Table 1 Description of study population

	SR	SS	CTR	P
N	64	217	221	
Sex				
Male	36	115	103	P = 0.256
Female	28	102	118	
Age (years)	41.4 ± 10.1	42.3 ± 10.3	40.7 ± 11.5	P ^a = 0.577, P ^b = 0.622, P ^c = 0.118
Course of disease (years)	4.9 ± 7.1	4.1 ± 6.6	–	P ^a = 0.393
Baseline FEV ₁ (% predicted)	52.89 ± 9.31	51.29 ± 7.95	93.61 ± 8.17	P ^a = 0.172, P ^b = 8.870E-136, P ^c = 7.274E-210
FEV ₁ change (% after albuterol)	19.50 ± 4.95	20.82 ± 4.92	–	P = 0.061
FEV ₁ improvement (% after prednisone)	3.34 ± 4.83	39.67 ± 5.86	–	P ^a = 1.173E-83

The participant numbers and the sex distribution are expressed as direct counts. Other data are expressed as mean ± SD.

Sex distribution in SR asthma patients, SS asthma patients and CTRs were analyzed by the χ^2 -test. Comparisons of parameters (course of disease, FEV₁ change after albuterol, and FEV₁ improvement after prednisone therapy) between SR and SS asthma patients were made using unpaired Student's *t*-test. One-way analysis of variance and least significant difference *t*-test were used to compare age distribution and baseline FEV₁ (% predicted) among all three groups.

CTR, control; FEV₁, forced expired volume in 1 s; SR, steroid resistant; SS, steroid sensitive.

^aSR versus SS.

^bSR versus CTR.

^cSS versus CTR.

(EMBL) databases (NC_000005.8), and a fragment of each SNP was generated using PCR-sequence specific primers (PCR-SSP), as listed in Table 2, according to the method in the publication of Mayor *et al.* [24]. PCR reactions were performed with 1 µg/µl of DNA, 25 mmol of each dNTP, 1.5 U of Taq DNA polymerase (Promega, Madison, Wisconsin, USA), buffer, and 20 pmol of forward and reverse primers. The cycle conditions were as follows: 94°C for 5 min and then 35 cycles; 94°C for 30 s; primer-specific annealing temperature: 60°C (I559N WT), 65°C (I559N SNP), 58°C (D641V WT), 63°C (D641V SNP), 53°C (V729I WT), and 57°C (V729I SNP) for 30 s, followed by extension at 72°C for 30 s; and a final extension step of 1 min at 72°C, in a MyCycler Thermal Cycler (Bio-Rad, Berkeley, California, USA). The amplified products were fractionated with agarose gel electrophoresis and visualized using ethidium bromide staining.

Ligand affinity assay

An assay for ³H-dexamethasone radioligand-binding and Scatchard analysis were used to determine the GR-binding parameters of PBMCs [25]. Endogenous cortisol was removed from PBMCs (10⁷ cells/ml) by three

washes for 30 min with PBS, after which PBMCs were incubated with increasing concentrations (1, 2, 4, 8, 16, 32, 64, and 128 nmol/l) of ³H-dexamethasone (Radiochemical Center, Amersham, UK) in the presence or absence of a 1000-fold excess of unlabeled dexamethasone (Sigma-Aldrich, Steinheim, Germany). Radioactivity was quantified with a β -spectrometer (Beckman 2000, Beckman Coulter Inc., Brea, California, USA), and specific binding was calculated as the difference between the radioactivity in the cell pellet and the nonspecific radioactivity at each dexamethasone concentration. The *K_d* was calculated on the basis of Scatchard analysis [26].

Statistical analysis

The statistic software package SPSS (SPSS Inc., Chicago, Illinois, USA), version 19.0, was used for data analysis.

The sex distribution in SR asthma patients, SS asthma patients, and CTRs was analyzed using the χ^2 -test. Data are expressed as mean ± SD.

The GR genotype distribution and the allele frequencies were assessed using Fisher's exact test in all three groups. In addition, the genotype and allele frequencies

Table 2 Primers for GR- α PCR-SSP genotyping

Specificity	Primer sequence	Primer location	Product size (bp)
I559N WT (1676T)	Forward: 5'-TCCAGACTCAACTTGGAGGAT-3' Reverse: 5'-ACACAAAACGAGGACTAGACT-3'	1656–1676 1862–1882	227
I559N SNP (1676A)	Forward: 5'-TCCAGACTCAACTTGGAGGAA-3' Reverse: 5'-ACACAAAACGAGGACTAGACT-3'	1656–1676 1862–1882	227
D641V WT (1922A)	Forward: 5'-ACTCTACCCTGCATGTACGA-3' Reverse: 5'-TCCTTTTCGGTAACAGTTCT-3'	1902–1922 2091–2110	208
D641V SNP (1922T)	Forward: 5'-ACTCTACCCTGCATGTACGT-3' Reverse: 5'-TCCTTTTCGGTAACAGTTCT-3'	1902–1922 2091–2110	208
V729I WT (2185G)	Forward: 5'-TTGGATTCTATGCATGAAGTGG-3' Reverse: 5'-AGTGGTTAGTCTATGGTTTAT-3'	2164–2185 2270–2291	128
V729I SNP (2185A)	Forward: 5'-TTGGATTCTATGCATGAAGTGA-3' Reverse: 5'-AGTGGTTAGTCTATGGTTTAT-3'	2164–2185 2270–2291	128
Control band	Forward: 5'-TTTCTGCGTCTTACC-3' Reverse: 5'-CCACCGTTACACTTAA-3'	122–137 532–547	426

GR- α , glucocorticoid receptor- α ; SNP, single nucleotide polymorphism; SSP, sequence specific primer; WT, wild type.

were calculated and tested for the Hardy–Weinberg equilibrium.

The relative risk for the association of the SR group with the genotype and allele frequency was estimated as odds ratio (OR), with a 95% confidence interval (CI) under an additive genetic model. To adjust for age and sex, logistic regression analysis was used to identify the risk of the genotypes for steroid resistance.

Unpaired *t*-test was used to compare course of disease (years), FEV₁ change after albuterol, and FEV₁ improvement after prednisone between SR asthma patients and SS asthma patients.

One-way analysis of variance (ANOVA) and the least significant difference-*t* (LSD-*t*) test were used for the comparisons among the three groups for age, baseline FEV₁ (% predicted), K_d value, and serum cortisol level. K_d value and serum cortisol level among different GR variant genotypes were also analyzed using one-way ANOVA and the LSD-*t* test.

Statistical significance was defined as *P* value less than 0.05.

Results

In all study participants, sex, age, and course of disease were not significantly different on comparing SR asthma patients with SS asthma patients or CTRs. Baseline FEV₁ predicted and FEV₁ change after albuterol were not significantly different on comparing SR asthma patients with SS asthma patients.

Our analysis showed that all three GR- α polymorphisms, I559N, D641V, and V729I, were present in the Chinese Han population. The genotypes were distributed according to the Hardy–Weinberg equilibrium in all three groups ($P > 0.05$). The genotypes and allele frequencies of the I559N, D641V, and V729I polymorphisms in SR asthma patients, SS asthma patients, and CTRs are summarized in Table 3. No statistically significant difference was found for the distribution of I559N and V729I polymorphisms in SR asthma patients, SS asthma patients, or CTRs under an additive genetic model (Table 3). Importantly, the GR- α D641V polymorphism was associated with SR asthma patients under an additive genetic model (Table 3). Furthermore, in D641V SR asthma patients the AA genotype and A allele frequency was significantly lower than that in SS asthma patients or CTRs, whereas no differences were observed on comparing SS asthma patients with CTRs (Table 3). Logistic regression analysis revealed that asthma patients with D641V variant AT and TT genotypes had significantly higher risk for steroid resistance compared with AA genotype adjusted for age and sex under an additive genetic model (AT vs. AA, OR 1.853, 95% CI 1.016–3.379, $P = 0.044$; TT vs. AA, OR 2.131, 95% CI 1.354–3.352, $P = 0.003$). However, there was no

significant difference between different genotypes in I559N and V729I variants, with logistic regression analysis adjusted for age and sex.

We next determined the K_d for the GR in all participants. As shown in Table 4, in all SR asthma patients, the K_d values were significantly higher compared with SS asthma patients or with CTRs ($P = 5.130E - 15$, $P = 1.427E - 67$, respectively). It was also observed that the K_d values in SS asthma patients were higher than that in CTRs ($P = 2.551E - 57$). This indicates that SR asthma patients have the lowest GR affinity among the three groups (Table 4).

Our data showed a significantly higher K_d for the GR- α D641V variant AT and TT genotypes than for AA genotype in SR asthma patients under an additive genetic model ($P = 0.006$, 0.016 , respectively), indicating a lower affinity for glucocorticoids compared with the wild type GR (Table 4). No statistically significant difference was observed for the GR K_d among the AA, AT, and TT genotypes of the GR D641V variant in SS patients and CTRs. Similarly, no significant differences for the GR K_d were found between the TT genotype and the TA genotype of the GR I559N variant, nor for the AA, GA, and GG genotypes of the GR V729I variant in any group.

Correlating the serum cortisol levels with GR- α variant genotypes, we found no statistically significant difference in serum cortisol levels on comparing the different GR genotypes of I559N, D641V, and V729I, as all calculations based on one-way ANOVA and the LSD-*t* test resulted in *P* value more than 0.05 among all different genotypes in each variant (Table 5). In addition, we compared the serum cortisol levels between all SR asthma patients and SS asthma patients and CTRs and found no statistically significant differences among the three groups (SR vs. SS, $P = 0.407$; SR vs. CTR, $P = 0.984$; SS vs. CTR, $P = 0.228$), thus cortisol serum levels are not affected by the GR variant alleles (Table 5).

Discussion

In this study we show for the first time that presence of the GR D641V variant correlates with steroid resistance in asthma patients of the Chinese Han population. We furthermore provide data indicating that the GR-binding capacity in SS asthma patients is reduced compared with CTRs. Therefore, we confirmed the link between reduced glucocorticoid binding in asthma patients and GR D641V in the Chinese Han population.

Previous studies reported two types of steroid resistance in asthma patients, which could be subdivided on the basis of the affinity and the number of GRs expressed by PBMCs [3,23,27]. Type I SR asthma was more common and was characterized by reduced GR affinity, but normal serum cortisol level and normal GR number. Furthermore, the reduced GR affinity in type I SR

Table 3 Distribution of genotypes in SR asthma patients, SS asthma patients, and CTRs

Polymorphism	Genotype	Frequency [n (%)]			P			OR (95% CI)		
		SR (n = 64)	SS (n = 217)	CTR (n = 221)	P ^a	P ^b	P ^c	OR ^a	OR ^b	OR ^c
I559N	TT	58 (90.6)	203 (93.5)	212 (95.9)	0.414	0.112	0.291	1.00	1.00	1.00
	TA	6 (9.4)	14 (6.5)	9 (4.1)	—	—	—	1.45 (0.58–3.63)	2.30 (0.85–6.23)	1.58 (0.70–3.58)
	AA	0 (0)	0 (0)	0 (0)	—	—	—	—	—	—
D641V	A allele	6 (4.7)	14 (3.2)	9 (2.0)	0.421	0.116	0.297	1.45 (0.57–3.70)	2.30 (0.84–6.35)	1.58 (0.69–3.62)
	AA	30 (46.9)	143 (65.9)	135 (61.1)	0.003	0.014	0.581	1.00	1.00	1.00
	AT	26 (40.6)	67 (30.9)	78 (35.3)	—	—	—	1.46 (1.03–2.05)	1.27 (0.91–1.77)	0.87 (0.67–1.14)
V729I	TT	8 (12.5)	7 (3.2)	8 (3.6)	—	—	—	4.51 (1.75–11.66)	3.76 (1.51–9.37)	0.83 (0.31–2.24)
	T allele	42 (32.8)	81 (18.7)	94 (21.3)	0.001	0.009	0.353	1.76 (1.28–2.42)	1.54 (1.14–2.10)	0.88 (0.67–1.15)
	GG	61 (95.3)	204 (94.0)	198 (89.6)	1.000	0.450	0.257	1.00	1.00	1.00
	GA	3 (4.7)	12 (5.5)	21 (9.5)	—	—	—	0.84 (0.25–2.90)	0.49 (0.15–1.59)	0.58 (0.29–1.15)
	AA	0 (0)	1 (0.5)	2 (0.9)	—	—	—	—	—	0.49 (0.05–5.34)
	A allele	3 (2.3)	14 (3.2)	25 (5.7)	0.774	0.164	0.101	0.73 (0.21–2.49)	0.41 (0.13–1.35)	0.57 (0.30–1.08)

The participant number and frequency in different groups and genotypes are expressed as direct counts.

P values were calculated using Fisher's exact test.

OR indicates odds ratio with the 95% confidence interval (CI) were calculated under an additive genetic model.

CTR, control; SR, steroid resistant; SS, steroid sensitive.

^aSR versus SS.

^bSR versus CTR.

^cSS versus CTR.

Table 4 The K_d of the GR variant genotypes in SR asthma patients, SS asthma patients, and CTRs

Polymorphism	Genotype	K_d		
		SR	SS	CTR
I559N	TT	28.53 ± 6.62	20.72 ± 7.55	9.89 ± 3.61
	TA	25.91 ± 7.79	24.64 ± 6.11	9.73 ± 4.06
	AA	0	0	0
D641V	AA	25.85 ± 6.13	20.80 ± 7.65	9.80 ± 3.48
	AT	30.11 ± 5.36	21.24 ± 7.32	10.01 ± 3.96
	TT	31.44 ± 4.31	21.90 ± 7.44	10.12 ± 2.70
V729I	GG	28.07 ± 6.76	21.14 ± 7.57	10.03 ± 3.71
	GA	32.62 ± 4.68	17.97 ± 6.26	8.47 ± 2.24
	AA	0	23.25	10.29 ± 5.64
Mean		28.28 ± 6.71	20.97 ± 7.51	9.88 ± 3.62
P		P ^c = 5.130E-15	P ^d = 1.427E-67	P ^e = 2.551E-57

Data are expressed as mean ± SD.

One-way analysis of variance and the least significant difference *t*-test were used to compare the K_d values among all three groups and between different GR variant genotypes under an additive genetic model.

CTR, control; GR, glucocorticoid receptor; K_d , the equilibrium dissociation constant; SR, steroid resistant; SS, steroid sensitive.

^aComparison of D641V genotype AT to AA.

^bComparison of D641V genotype TT to AA.

^cSR versus SS.

^dSR versus CTR.

^eSS versus CTR.

asthma patients was reversible in isolated PBMCs incubated without serum. Either a higher glucocorticoid dosage, extended duration, or other anti-inflammatory agents may improve the condition of type I of SR asthma [27–29]. In contrast, the less common type II SR asthma was characterized by reduced GR numbers in the mono-nuclear cells fraction and normal K_d of the GRs. Thus, type II is a form of primary steroid resistance [6,23].

Earlier studies reported that the GR I559N, D641V, and V729I variants were associated with steroid resistance in familial, sporadic cases, or cell line studies, but were not

Table 5 Serum cortisol levels in SR asthma patients, SS asthma patients, and CTRs

Polymorphism	Genotype	Serum cortisol (nmol/l)		
		SR	SS	CTR
I559N	TT	307.45 ± 77.39	298.98 ± 75.50	305.56 ± 59.63
	TA	295.00 ± 70.50	285.36 ± 77.89	318.44 ± 68.07
	AA	—	—	—
D641V	AA	298.50 ± 69.69	298.40 ± 76.89	308.09 ± 61.14
	AT	313.65 ± 80.33	299.94 ± 74.21	302.56 ± 59.29
	TT	311.50 ± 93.68	274.43 ± 65.33	306.63 ± 47.48
V729I	GG	306.67 ± 77.78	298.57 ± 75.31	307.26 ± 59.10
	GA	298.33 ± 43.73	290.58 ± 85.64	295.38 ± 69.75
	AA	—	293.00	302.50 ± 27.58
Mean		306.28 ± 76.33	298.11 ± 75.54	306.09 ± 59.88
P		P ^a = 0.407	P ^b = 0.984	P ^c = 0.228

Data are expressed as mean ± SD.

One-way analysis of variance and least significant difference *t*-test were used to compare the serum cortisol levels among all three groups and GR variant genotypes.

CTR, control; SR, steroid resistant; SS, steroid sensitive.

^aSR versus SS.

^bSR versus CTR.

^cSS versus CTR.

confirmed in large clinical trials [18–21]. In our study, type I SR asthma patients were found to have a lower binding affinity of the GR to glucocorticoids, but had the same serum cortisol levels as SS asthma patients and CTRs. These findings suggest that the majority SR asthma patients of the Chinese Han population belong to type I SR asthma. The impact of these findings is based on the fact that the type I SR asthma is reversible and clinical symptoms may be alleviated by aggressive treatment strategies [30].

Different polymorphisms of the GR, including R23K, N363S, R477H, I559N, D641V, G679S, V729I, and I747M, had been described, and some of them can lead to either decreased or loss of gene transcription or decreased or abolished biological activity of the GR

protein. Thus, these mutations decreased the number of GR expressed by cells or reduced ligand affinity, both leading to steroid resistance [3,16].

In our study, the GR D641V variant was found in multiple SR asthma patients, not only in family members. Therefore, we suggest that it is the alleviation of the anti-inflammatory effect of glucocorticoids in patients carrying the GR D641V variant that results in steroid resistance in our asthma patients. The decreased GR ligand affinity was thought to be the main reason for steroid resistance, and our results support this conclusion [31]. In asthma patients who carried the GR D641V variant, TT genotype and T allele was significantly higher in SR asthma patients than in SS asthma patients or CTRs. In SR asthma patients it was confirmed that the GR affinity in the GR D641V variant AT and TT genotypes was much lower than that in the AA genotype. Thus, our data suggest that the T allele of the GR D641V variant accounts for decreased ligand binding affinity and therefore may create a condition that leads to poor glucocorticoid response or steroid resistance in Chinese Han asthma patients. In this research we observed lung function change for only 7 days after oral prednisone for asthma patients, thus we do not know if asthma patients carrying GR D641V variant TT and AT genotype have more severe symptoms, such as wheeze, shortness of breath and cough, or high frequency of exacerbations, compared with asthma patients with GR D641V variant AA genotype. We can search for such parameters in future studies.

In contrast to our study, others did not find an association between GR variants and the response to glucocorticoids in asthma patients [14,32–34]. The reasons for the difference may be (i) the different GR variants detected, (ii) the different ethnic origin of the study participants, or (iii) the different recruitment criteria for SR asthma. Furthermore, the results from these investigations did not report whether the study participants were suffering from other pulmonary diseases, or if they were cigarette smokers. In our study population, the GR variants I559N and V729I also did not correlate with steroid resistance, which was in accordance with the results of GR ligand affinity analysis. However, the molecular mechanism on how the GR D641V variant alleviates the anti-inflammatory effect of glucocorticoids is yet to be determined.

Conclusion

Our results showed that the GR I559N, D641V, and V729I variants are present in the Chinese Han population, and the GR D641V variant is associated with steroid resistance in asthma patients. To improve the treatment of SR asthma it might therefore be advisable to determine the GR variant and adjust the treatment strategy accordingly.

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Conflicts of interest

There are no conflicts of interest.

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