Association of single-nucleotide *NR3C1* gene polymorphisms with glucocorticosteroid responsiveness in patients with pemphigus vulgaris

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Abstract

The glucocorticosteroid (GC) is crucial when managing patients with pemphigus vulgaris (PV). Polymorphisms in the gene encoding the nuclear receptor subfamily 3, group C, member 1 (*NR3C1*) protein (the GC receptor) may explain the variations in treatment efficacy. We evaluated the effects of 10 single nucleotide polymorphisms (SNPs) in the *NR3C1* gene and the correlations with the GC responsiveness in patients with PV. The accumulative GC doses were recorded, and patients were assessed for the Pemphigus Disease Activity Index (PDAI) scores until the GC doses would be tapered. Whole blood samples at the initial visit were genotyped using TaqMan SNP Genotyping. In the *NR3C1* gene, SNPs were detected in 6 (rs17209237, rs11745958, rs7701443, rs41423247, rs33388, and rs6196); the genotypes rs17209237 *AA*, rs11745958 *CC*, and rs6196 *AG* may be associated with a need for a lower accumulative GC dose; rs17209237 *AA* and rs6196 *AG* with shorter times to commencement of tapering; and rs17209237 *AA* and rs11745958 *CC* with shorter times to attainment of 50 and 25% PDAI scores. Thus, *NR3C1* gene variations may predict GC responsiveness in PV patients.

Introduction

Pemphigus vulgaris (PV) is a blistering autoimmune disease typically affecting the skin and mucosa. The incidence of PV varies ethnically, ranging from 0.76 to 16.1 new cases per million subjects per year.[1,2] In the pre-corticosteroid era, the mortality rate was 70%.[3] During the 1950s, corticosteroids were approved and have been the backbone of PV treatment and have reduced mortality to about 30%.[4] Although many other immunosuppressive agents are available, glucocorticosteroids (GCs) remain the first-line treatment according to most of the PV management guidelines.[5-7] Although GC side effects are of concern, GC efficacy has become increasingly recognized. Nevertheless, GC responsiveness may vary between subjects. In patients with diseases responding to GCs, some will effectively respond to GC treatment, meanwhile some will exhibit "GC resistance"; they require higher drug doses for longer times, or other immunosuppressive agents. The longer usage as well as the higher accumulative amount of GC may lead to adverse effects. Therefore, it is necessary to have a predictive marker for GC responsiveness during PV treatment and follow-up.

GCs exert their biological effects via GC receptor (GR), which regulates the expression of GC-target genes.[8,9] Earlier studies reported that several *NR3C1* single nucleotide polymorphisms (SNPs) (rs6189, rs6190, rs6195, rs6196, rs6198, and rs41423247) might correlate with GC responsiveness in patients with inflammatory bowel disease, rheumatoid arthritis, asthma, and idiopathic nephrotic syndrome.[10-16] For PV patients, Fang *et al.*[17] were the first to report associations between *NR3C1* SNPs and GC effectiveness; rs11745958 C/T and rs17209237 A/G may be associated with increased risks of GC resistance, but rs33388 A/T and rs7701443 A/G with decreased risks. Taken together, we hypothesized that the polymorphisms of *NR3C1* may be differed in patients with PV in terms of GC responsiveness. However, it remains unclear how these SNPs affect GC dose requirements and Pemphigus Disease Activity Index (PDAI) scores, and whether these parameters vary environmentally, ethnically, or genetically. We thus evaluated the effects of 10 SNPs (rs 17209237, rs11745958, rs7701443, rs33388, rs41423247, rs6189, rs6190, rs6195, rs6196, and rs6198) in Vietnamese PV patients.

Materials and Methods

Patients' recruitment

There were 26 patients treated between December 2018 and June 2019 as inpatients of the Department of Dermatology and Venereology of Ho Chi Minh City Hospital, Vietnam. All subjects were diagnosed with PV based on clinical and histological findings or direct immunofluorescence. All exhibited active disease (new skin or mucosal blisters or erosions) and required a

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Contributions: Le TVT drafted the outline study proposal, the manuscript and supervised the whole process. Nguyen DQ recruited patients, wrote the manuscript, and prepared samples for genetic analysis. Tran ND wrote the manuscript and analyzed data. Trinh HKT edited the manuscript and checked the data analysis.

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return to the initial dose of oral GCs (usually about 1 mg/kg). All subjects gave written informed consent; this study was approved by the ethics committee of the University of Medicine and Pharmacy at Ho Chi Minh City, Viet Nam. Patients with systemic diseases, who could not receive optimal dose GC (such as patients with liver or renal failure), those who received immunosuppressive agents other than GCs within the prior 6 months, and patients who did not adhere to treatment, were excluded.

Assessment of GC responsiveness

Patients were monitored at the time of the first tapering dose. The PDAI was used to assess disease severity in week 1 and every 1–2 weeks thereafter until the PDAI scores attained 50 and 25%. The disease severity was classified as moderate and severe using the PDAI score cut-off at 15-45 and >45, respectively.

The GC doses were tapered when the old blistering lesions declined in the absence of new blisters, followed by 10% reduction in doses every 10 days. We recorded the time from the first GC dose to the first tapering dose and the total amount of GCs prescribed.

NR3C1 SNP genotyping

Based on the literature review, we chose 10 SNPs reported to affect GC efficacy in patients with various diseases: rs17209237, rs11745958, rs33388, rs7701443, rs41423247, rs6189, rs6190, rs6195, rs6196, and rs6198.[10-17] Whole blood samples from all patients were collected in tubes containing ethylenediamine tetra-acetic acid (EDTA) and sent to our Biotechnology Center for gDNA isolation and TaqMan SNP genotyping.[18]

Statistical analysis

EPI DATA ver. 3.1 and STATA ver. 14.0 software were used to manage and analyze all data. Possible associations between *NR3C1* polymorphisms and GC efficacy were explored using the Kruskal-Wallis and Wilcoxon rank-sum tests. The significance level was set to $p<0.05$.

In silico analysis

An *in silico* tool was used to search for functional SNPs in linkage disequilibrium (LD) with other SNPs of the HapMap JPT and CHB populations.[19] ESEfinder (http://rulai.cshl.edu/cgi-bin/tools/ESE3/esefinder.cgi?process=home) was used to evaluate whether SNPs lay in exonic splicing enhancers (ESEs). ESEs bind Ser/Arg-rich proteins (SR proteins) that play multiple roles prior to mRNA splicing.

Results

Characteristics of the subjects

At the end of the study, 15 patients with PV, who satisfied the study criteria, were included for statistical analysis. There were 2 (13.33%) males and 13 (86.67%) females, of mean age 49.33±16.10 years. The mean PDAI score on the first hospital day was 44.2 (±15.45). Five patients (33.3%) had moderate disease and 10 (66.66%) severe disease.[20] The median GC commencement dose was 1.00 (0.98–1.02) mg/kg/day, the minimum 0.89 mg/kg/day, and the maximum 1.16 mg/kg/day. Ten subjects remained on their starting doses at the time of start of tapering but the remaining five (all with severe disease) required higher doses for symptoms management

GC responsiveness

Table 1 lists the clinical responses to GCs in terms of PDAI score changes and GC doses. We recorded the time required to attain 50 and 25% of the initial PDAI scores; the median values were 23 and 36 days, respectively. One patient required only 7 days to attain the 25% PDAI score but another 91 days. The median time to tapering was 1 month, but one patient required more than 3 months.

Frequencies of NR3C1 SNPs

Of the 10 sites, SNPs were detected in 6: rs17209237, rs11745958, rs33388, rs7701443, rs41423247, and rs6196. The SPNs rs41423247 and rs6196 featured only two genotypes; the other four SNPs each featured three genotypes (Table 2).

Table 1. Clinical responses to glucocorticosteroids.

GC responsiveness	Median (25–75th percentile)	Range
Time to attain the 50% PDAI score (days)	23.0 (15.0–35.0)	7–40
Time to attain the 25% PDAI score (days)	36.0 (27.0–50.0)	7–91
Time to start of tapering dose (days)	28.0 (20.0–38.0)	14–99
The average accumulative amount of GCs (mg) per patient	1,395 (1,120–2,065)	630–5,940

GC, glucocorticosteroids; PDAI, Pemphigus Disease Activity Index.

Table 2. Distribution of NR3C1 SNPs among patients with pemphigus vulgaris.

NR3C1 SNP	Genotype frequency, n (%)			Allele frequency, n (%)	
rs17209237	AA 10 (66.67)	AG 4 (26.67)	GG 1 (6.67)	A 24 (80)	G 6 (20)
rs11745958	TT 1 (6.67)	TC 3 (20.00)	CC 11(73.33)	C 25 (83.33)	T 5 (16.67)
rs33388	TT 7 (46.67)	AT 8 (53.33)	AA 0	T 22 (73.33)	A 8 (26.66)
rs7701443	AA 3 (20.00)	AG 7 (46.67)	GG 5 (33.33)	A 13 (43.33)	G 17 (56.66)
rs41423247	GG 8 (53.33)	GC 7 (46.67)	CC 0	C 7 (23.33)	G 23 (76.67)
rs6196	AA 12 (80.00)	AG 3 (20.00)	GG 0	A 27 (90.00)	G 3 (10.00)

NR3C1, Nuclear Receptor Subfamily 3 Group C Member 1; SNP, single nucleotide polymorphism; PV, pemphigus vulgaris.

Associations between NR3C1 SNPs and GC responsiveness

We found significant positive correlations between SNPs rs17209237, rs11745958, and rs6196 (Table 3). The associations between the accumulative amounts of GC required and the genotypes of the six SNPs are shown in Figure 1. For rs17209237, the accumulative amount of GCs (mg), the time to dose tapering (days), and the time to the PDAI 50% score of the genotype *AA* group were lower than those of the genotype non-*AA* group ($p<0.05$), reflecting a better GC responsiveness status in the *AA* group (Figure 1A). Similarly, for rs11745958, the accumulative number of GCs (mg), the time to dose tapering (days), and the time to the PDAI 50% score of the genotype *CC* group were lower than those of the genotype non-*CC* group ($p<0.05$) (Figure 1B). For rs6196, the total amount of GCs (mg) and the time to dose tapering (days) of the genotype *AG* group were lower than those of the genotype *AA* group ($p<0.05$) (Figure 1C).

In silico analysis

Of the three SNPs significantly associated with GC efficacy in PV patients, only rs6196 lies in an *NR3C1* exon; rs17209237 and rs11745958 lie in introns. We searched *in silico* for functional SNPs in LD with each SNP. For rs17209237 and rs11745958, we discovered 17 SNPs in each position that were in LD ($r^2>0.8$) in the CHB and JPT populations. However, none was predicted to affect function. For rs6196, 31 SNPs were in LD ($r^2>0.8$) in the CHB and JPT populations. Of these, rs6194 was predicted to be functional by the SNPinfo Web Server. Although rs6196 was predicted to exhibit no function, rs6194 affected splicing; this may be of functional significance.

Discussion

This is the first study evaluating the association between *NR3C1* polymorphisms with GC responsiveness in patients with PV. In this study, we found correlations between three SNPs (rs17209237, rs117458958, and rs6196) and the clinical response to GCs. rs17209237 and rs117458958, located in introns, were first shown to correlate with GC responsiveness in PV patients by Fang *et al.*[17] However, of the 17 SNPs for each position in LD ($r^2>0.8$) in the CHB and JPT populations, none was predicted to be functional; further research is needed.

The rs6196 genotype AA patients scored higher on all four indices of GC efficacy. The rs6196 genotype significantly affected the time to tapering (days) and total amount of GCs (mg) ($p=0.0429$ and 0.0208, respectively). Rs6196 AA patients with idiopathic nephrotic syndrome were at increased risk of steroid resistance.[16] Rs6196 lies in exon 9α of the transcriptionally active form of GCs.[21] Rs6196 SNP is a synonymous codon substitution. *In silico*, rs6196 was predicted to have no function. However, this SNP and rs6194 are in ligand-binding domains important in terms of protein–protein interactions.[22] Niu *et al.* investigated the functional impacts of com-

Table 3. Correlations between SNP genotypes and other measures of glucocorticosteroids efficacy.

	Genotype	N.	Time to 50% PDAI score (days)	Time to 25% PDAI score (days)	Time to tapering dose (days)	Accumulative number of GCs (mg)
rs17209237	AA	10	18.0 (14.0–23.0)	30.0 (22.0–37.0)	21.0 (18.0–28.0)	1205.0 (840.0–1400.0)
	AG & GG	5	36.0 (30.0–36.0)	43.0 (36.0–50.0)	31.0 (30.0–38.0)	2065.0 (2000.0–3070.0)
	p**		*0.0228*	*0.0572*	*0.0370*	*0.0199*
	AA & AG	14	22.0 (15.0–35.0)	35.0 (27.0–50.0)	25.5 (20.0–31.0)	1342.5 (1120.0–2000.0)
	GG	1	36.0 (36.0–36.0)	36.0 (36.0–36.0)	38.0 (38.0–38.0)	2065.0 (2065.0–2065.0)
	p**		0.2010	0.9077	0.3537	0.3541
rs11745958	TT & TC	4	36.0 (28.5–38.0)	46.5 (39.5–70.5)	34.0 (30.0–68.5)	2567.5 (2032.5–4505.0)
	CC	11	21.0 (14.0–30.0)	32.0 (22.0–37.0)	21.0 (18.0–31.0)	1260.0 (840.0–1400.0)
	p**		*0.0305*	*0.0498*	0.0671	*0.0130*
	TT	1	36.0 (36.0–36.0)	36.0 (36.0–36.0)	38.0 (38.0–38.0)	2065.0 (2065.0–2065.0)
	TC & CC	14	22.0 (15.0–35.0)	35.0 (27.0–50.0)	25.5 (20.0–31.0)	1342.5 (1120.0–2000.0)
	p**		0.2010	0.9077	0.3537	0.3541
rs7701443	AA	3	35.0 (21.0–36.0)	36.0 (28.0–54.0)	28.0 (15.0–38.0)	1120.0 (750.0–2065.0)
	AG	7	30.0 (15.0–36.0)	36.0 (22.0–50.0)	30.0 (21.0–31.0)	1395.0 (840.0–3070.0)
	GG	5	15.0 (14.0–23.0)	32.0 (27.0–37.0)	21.0 (20.0–39.0)	1400.0 (1290.0–1400.0)
	p*		0.2212	0.7652	0.9447	0.6188
	AA&AG	10	32.5 (21.0–36.0)	36.0 (28.0–50.0)	29.0 (21.0–31.0)	1272.5 (840.0–2065.0)
	GG	5	15.0 (14.0–23.0)	32.0 (27.0–37.0)	21.0 (20.0–39.0)	1400.0 (1290.0–1400.0)
	p**		0.0967	0.4998	0.9511	0.4620
	AA	3	35.0 (21.0–36.0)	36.0 (28.0–54.0)	28.0 (15.0–38.0)	1120.0 (750.0–2065.0)
	AG&GG	12	22.0 (14.5–32.5)	35.0 (24.5–46.5)	26.5 (20.5–35.0)	1397.5 (1205.0–2535.0)
	p**		0.24769	0.6128	0.7724	0.3861
rs6196	AG	3	21.0 (7.0–35.0)	28.0 (7.0–34.0)	15.0 (14.0–23.0)	750.0 (630.0–1150.0)
	AA	12	23.0 (15.0–35.5)	36.5 (29.5–50.0)	30.0 (21.0–38.5)	1400.0 (1275.0–2567.5)
	p**		0.4685	0.1117	*0.0429*	*0.0208*
rs33388	TT	7	23.0 (21.0–35.0)	36.0 (28.0–37.0)	21.0 (18.0–31.0)	1290.0 (1120.0–1400.0)
	AT	8	18.0 (11.0–35.5)	38.5 (20.0–50.0)	30.0 (22.0–46.5)	1700.0 (995.0–3072.5)
	p**		0.4158	1.00	0.2235	0.3850
rs41423247	GC	7	35.0 (21.0–36.0)	36.0 (28.0–50.0)	30.0 (15.0–38.0)	2000.0 (750.0–3070.0)
	GG	8	19.0 (14.5–26.5)	34.0 (24.5–43.5)	24.5 (20.5–35.0)	1342.5 (1190.0–1400.0)
	p**		0.1813	0.6428	1.00	0.7282

Data were presented as median (range). P values were analyzed by *Kruskal-Wallis test and **Wilcoxon rank-sum test. Italics values indicated significant P values.

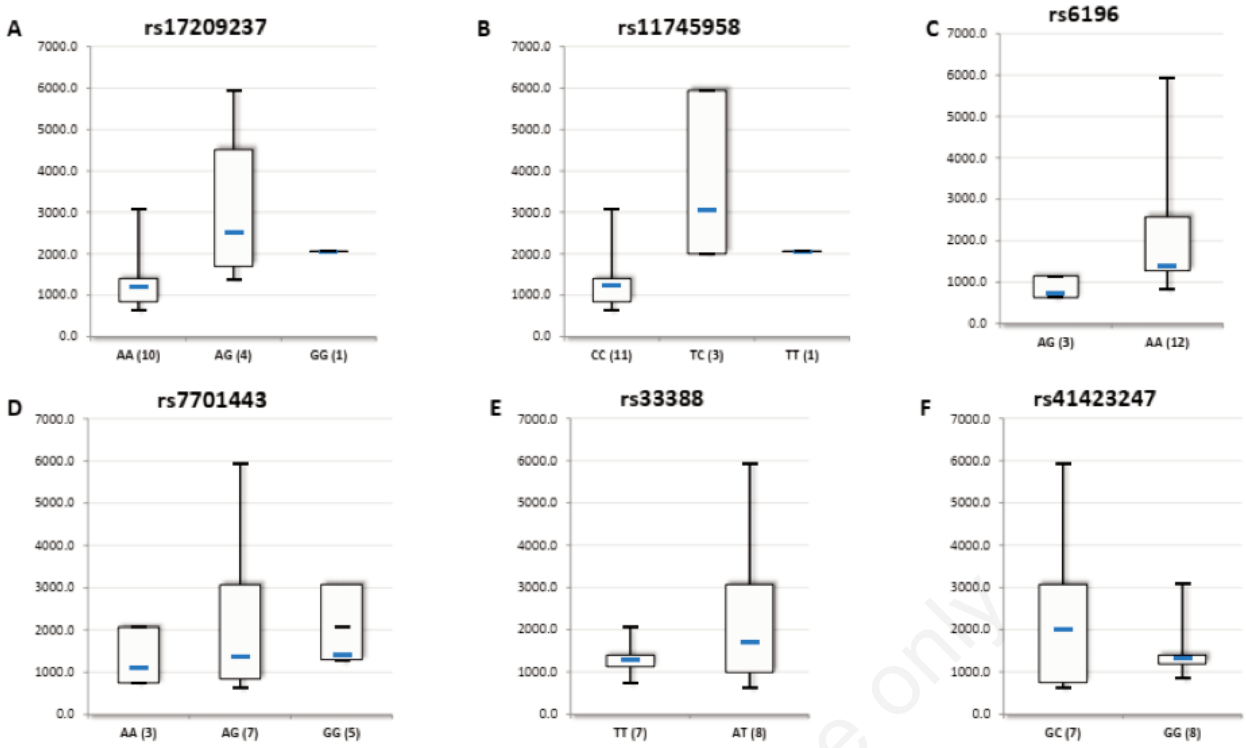

Figure 1. Correlations between SNP genotypes and accumulative amounts of GCs required (mg). A) A marginal correlation between rs17209237 genotypes and total GC amounts (p=0.0666). B,C) Significant associations between rs11745958 and rs6196 genotypes and total GC amounts (p=0.0199 and 0.0453, respectively). D-F) No correlations between rs7701443, rs33388, and rs41423247 genotypes and total GC amounts. p<0.05 comparing between the indicated groups.

mon NR3C1 SNPs (including rs6196 and rs6194) in COS-1 cells; the protein expression levels were higher than that of the wild type.[23]

None of the 15 PV patients exhibited the rs6189, rs6190, rs6195, or rs6198 SNPs associated with GC efficacy in patients with inflammatory bowel disease and rheumatoid arthritis;[10-12,15] our sample size may have been too small. A Chinese study found significant relationships between rs17209237, rs11745958, rs33388, and rs7701443 and GC efficacy in PV patients.[17] We also identified these SNPs; the genotype and allele frequencies were similar to those in Chinese patients. We found that rs17209237 (AA) and rs11745958 (CC) were protective in terms of time to the 50 and 25% PDAI scores (days), GC dose required (mg/kg), time to tapering, and average daily GC dose (mg/kg/day). Fang *et al.*[17] found correlations between rs33388 and rs7701443 and GC efficacy. Although we also identified these polymorphisms, we failed to detect any correlation with GC efficacy.

Rs41423247 featured the genotypes CC and GC (53 and 47%). Rs41423247 (Bcl1) correlated with GC efficacy in patients with inflammatory bowel disease and rheumatoid arthritis.[10-12,15] In Chinese PV patients, Fang *et al.*[17] failed to find an SNP at this site; rs41423247 in PV patients was first reported in the present study. However, we found no significant association between this SNP and any measure of GC responsiveness.

Conclusions

SNPs in *NR3C1* may affect GC responsiveness in PV patients to some extent. The associations between *NR3C1* polymorphism and GC responsiveness may indicate a potential marker to predict the GC efficacy during PV treatment. Further study is required.

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