Single-Nucleotide Nr3C1 Gene Polymorphisms Affect Glucocorticosteroid Treatment Efficacy In Patients With Pemphigus Vulgaris

Le Thai Van Thanh (thanh.ltv@umc.edu.vn)
Ho Chi Minh City University of Medicine and Pharmacy https://orcid.org/0000-0002-3222-758X
Nguyen Duy Quan
Ho Chi Minh City University of Medicine and Pharmacy
Tran Ngoc Dang
Ho Chi Minh City University of Medicine and Pharmacy
Duy Le Pham
Ho Chi Minh City University of Medicine and Pharmacy

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Abstract

Background

in recent years, the effectiveness of glucocorticosteroid (GC) treatment has become a central issue 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 observed variations in treatment efficacy. We aim to evaluate the effects of 10 SNPs (rs 17209237, rs11745958, rs7701443, rs33388, rs41423247, rs6189, rs6190, rs6195, rs6196, and rs6198) in Vietnamese PV patients.

Methods

we studied 10 sites in the NR3C1 gene (selected using published data) and sought correlations between single nucleotide polymorphisms (SNPs) in these regions and the clinical responses to GCs in 15 PV patients. Whole blood samples from all patients were collected in tubes containing ethylenediamine tetra-acetic acid (EDTA) and were genotyped using TaqMan SNP Genotyping assay.

Results

Of the 10 sites 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 total 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% Pemphigus Disease Activity Index scores.

Conclusions

NR3C1 gene variations may predict GC efficacy in PV patients. However, larger, randomized controlled trials are required.

Background

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]. In the time since such drugs were approved in the 1950s, corticosteroids 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 PV management guidelines [5], [6], [7]. Although GC side-effects are of concern, GC efficacy has become increasingly recognized. In patients with diseases

responding to GCs, some always exhibit “GC resistance”; they require higher drug doses for longer times, or other immunosuppressive agents.

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 efficacy in patients with inflammatory bowel disease, rheumatoid arthritis, asthma, and idiopathic nephrotic syndrome [10], [11], [12], [13], [14], [15], [16]. For PV patients, Fang et al. [17] were the first to report associations between NR3C1 SNPs and GC efficacy; 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. 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.

Methods

Subjects

We enrolled 15 patients treated between December 2018 and June 2019 as inpatients of the Department of Dermatology and Venereology of Ho Chi Minh City Hospital. 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 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 of Ho Chi Minh City. Patients were monitored to 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%. We recorded the time from the first GC dose to the first tapering dose and the total amount of GCs prescribed. 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.

NR3C1 SNP genotyping

Based on published data, 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], [11], [12], [13], [14], [15], [16], [17]. Whole blood samples from all patients were collected in tubes containing ethylenediamine tetra-acetic acid (EDTA) and sent to our Biotechnology Center for 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

We enrolled 15 PV patients, 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 (PDAI score 15–45) and 10 (66.66%) severe disease (PDAI score > 45) [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 to the time of start of tapering but the remaining five (all with severe disease) required higher doses.

GC efficacy

Table 1 lists the clinical responses to GCs in terms of PDAI score changes and GC doses. We recorded the times 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.

Table 1
Clinical responses to glucocorticosteroids

GC efficacy	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
Total amount of GCs (mg)	1,395 (1,120–2,065)	630–5,940

Frequencies of NR3C1 SNPs

Of the 10 sites, SNPs were detected in 6: rs17209237, rs11745958, rs33388, rs7701443, rs41423247, and rs6196. Rs41423247 and rs6196 featured only two genotypes; the other four SNPs each featured three

genotypes (Table 2).

Table 2
NR3C1 SNPs

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

Associations between NR3C1 SNPs and GC efficacy

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

Table 3
Correlations between SNP genotypes and other measures of GC efficacy

	Genotype	n	Time to 50% PDAI score (days)	Time to 25% PDAI score (days)	Time to tapering dose (days)	Total amount 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)

* *Kruskal-Wallis test*

	Genotype	n	Time to 50% PDAI score (days)	Time to 25% PDAI score (days)	Time to tapering dose (days)	Total amount of GCs (mg)
	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)

* *Kruskal-Wallis test*

	Genotype	n	Time to 50% PDAI score (days)	Time to 25% PDAI score (days)	Time to tapering dose (days)	Total amount of GCs (mg)
	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*

* *Kruskal-Wallis test*

** Wilcoxon rank-sum test

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

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 efficacy 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.

We are the first to report an association between rs6196 and GC efficacy in PV patients. Rs6196 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 located in ligand-binding domains important in terms of protein–protein interactions [22]. Niu et al. [23] investigated the functional impacts of common NR3C1 SNPs (including rs6196 and rs6194) in COS-1 cells; the protein expression levels were higher than that of the wild type.

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], [11], [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 times 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], [11], [12], [15]. In Chinese PV patients, Fang et al. [17] failed to find an SNP at this site; rs41423247 in PV patients is first reported in the present study. However, we found no significant association between this SNP and any measure of GC efficacy.

Conclusion

SNPs in NR3C1 may affect GC efficacy in PV patients. We found significant correlations between rs17209237, rs11745958, and rs6196 and several aspects of the clinical response to GCs. Further study is required.

Abbreviations

Glucocorticosteroid (GC)

Pemphigus Vulgaris (PV)

Single Nucleotide Polymorphisms (SNPs)

The Nuclear Receptor Subfamily 3, group C, member 1 (NR3C1)

Pemphigus Disease Activity Index (PDAI)

Ethylenediamine tetra-acetic acid (EDTA)

Linkage Disequilibrium (LD)

HapMap: The International HapMap Project

JPT and CHB: populations including in HapMap, 44 unrelated Japanese individuals from Tokyo, Japan (JPT) and 45 unrelated Han Chinese individuals from Beijing, China (CHB)

Exonic Splicing Enhancers (ESEs).

Ser/Arg-rich proteins (SR proteins)

Declarations

Ethics approval and consent to participate

This study was approved by the ethics committee of the University of Medicine and Pharmacy of Ho Chi Minh City by reference number 425/ĐHYD-HĐĐĐ.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

LTVT: Conceptualization, Methodology, Data curation, Writing- Reviewing and Editing. **NDQ**: Data curation, Data analysis, Writing- Original draft preparation. **TND**: Data analysis, Writing- Reviewing and Editing. **DLP**: Writing- Reviewing and Editing

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Figures

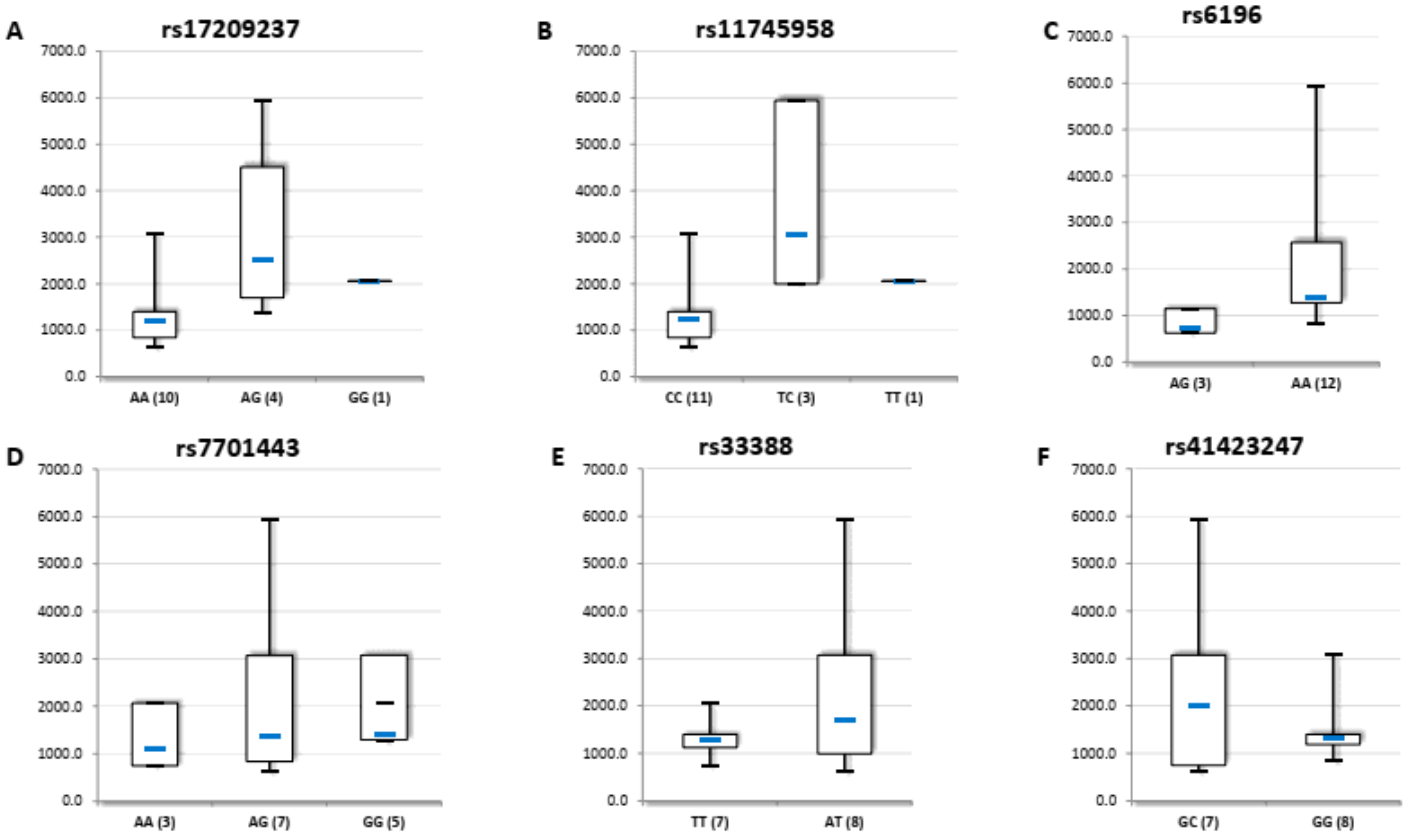

Figure 1

Correlations between SNP genotypes and total 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.