Clinical outcome in anti-neutrophil cytoplasmic antibody-associated vasculitis and gene variants of 11 beta-hydroxysteroid dehydrogenase type 1 and the glucocorticoid receptor

Hessels, Arno C; Tuin, Janneke; Sanders, Jan Stephan F; Huitema, Minke G; van Rossum, Elisabeth F C; Koper, Jan W; van Beek, André P; Stegeman, Coen A; Rutgers, Abraham

Published in:
Rheumatology

DOI:
10.1093/rheumatology/key319

Publication date:
2019

RHEUMATOLOGY

Rheumatology 2019;58:447–454
doi:10.1093/rheumatology/key319
Advance Access publication 14 November 2018

Original article

Clinical outcome in anti-neutrophil cytoplasmic antibody–associated vasculitis and gene variants of 11β-hydroxysteroid dehydrogenase type 1 and the glucocorticoid receptor

Arno C. Hessels[1], Janneke Tuin[1], Jan Stephan F. Sanders[1], Minke G. Huitema[2], Elisabeth F. C. van Rossum[3], Jan W. Koper[3], André P. van Beek[4], Coen A. Stegeman[1] and Abraham Rutgers[2]

Abstract

Objectives. We aimed to investigate whether five potential functional haplotypes of the glucocorticoid receptor (*GR*) gene and a single-nucleotide polymorphism of 11β-hydroxysteroid dehydrogenase type 1 (*HSD11B1*) are associated with clinical outcome in ANCA-associated vasculitis.

Methods. Patients diagnosed with ANCA-associated vasculitis ($n = 241$) were genotyped for five polymorphisms of the *GR* gene and one polymorphism of the *HSD11B1* gene. *GR* gene haplotypes were predicted based on genotyping results. Relapse-free survival, mortality, renal survival, metabolic adverse events and infections were compared between carriers and non-carriers of *GR* haplotypes and the *HSD11B1* genotype.

Results. Carriers of haplotype 4 (ER22/23EK + 9β+TthIII1) of *GR* had a significantly higher 5-year mortality risk [hazard ratio (HR) 4.5 (95% CI 1.6, 12.8)] and had a higher risk of developing end-stage renal disease [HR 7.4 (95% CI 1.9, 28.7)]. Carriers of a minor variant of *HSD11B1* more frequently experienced relapse [HR 2.5 (95% CI 1.5, 4.1)] except if they also carried haplotype 1 (*BclI*) of *GR*. Homozygous carriers of haplotype 1 had a higher risk of developing dyslipidaemia [HR 4.1 (95% CI 1.8, 9.6)]. The occurrence of infections did not differ between *GR* haplotypes and *HSD11B1* genotypes.

Conclusion. Haplotypes 1 and 4 of *GR* and a polymorphism of the *HSD11B1* gene were associated with clinically relevant inflammatory and metabolic outcomes in ANCA-associated vasculitis.

Key words: anti-neutrophil cytoplasm antibody, biomarkers, epidemiology, genetics, inflammation, metabolic disease, microscopic polyangiitis, vasculitis, Wegener's granulomatosis

Rheumatology key messages

- The 11β-hydroxysteroid dehydrogenase type 1 genotype affects the risk of relapse in ANCA-associated vasculitis.
- The glucocorticoid receptor haplotype affects renal survival, mortality and the development of dyslipidaemia in ANCA-associated vasculitis.

Introduction

ANCA-associated vasculitis (AAV) is a group of autoimmune diseases associated with inflammation of small and medium-sized blood vessels. Granulomatosis with polyangiitis, microscopic polyangiitis and eosinophilic granulomatosis with polyangiitis are diagnoses within this group [1].

Regardless of further induction and maintenance therapy, AAV patients are treated with high initial doses (up to 1 mg/kg/d) of the exogenous glucocorticoid (GC) prednisolone, because of its effectiveness in inducing disease remission [2]. Unfortunately, high-dose GCs are associated

[1]Department of Internal Medicine/Nephrology and [2]Department of Rheumatology and Clinical Immunology, University of Groningen, University Medical Center Groningen, Groningen, [3]Department of Endocrinology, Erasmus Medical Center, University Medical Center Rotterdam, Rotterdam and [4]Department of Endocrinology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

Submitted 3 April 2018; accepted 6 September 2018

Correspondence to: Arno Christiaan Hessels, Division of Nephrology, Department of Internal Medicine (HPC AA52), University Medical Center Groningen, 9713 GZ Groningen, The Netherlands.
E-mail: a.c.hessels@umcg.nl

CLINICAL SCIENCE

with many immunological, cardiometabolic and other adverse effects [3–5]. Furthermore, GC sensitivity differs widely between patients [6], which may affect treatment efficacy and toxicity in individual patients.

In recent years, personalized medicine has received more attention in AAV research. One approach has been to identify disease subsets and to target specific pathways involved in the pathogenesis of AAV [7]. Examples include the identification of different genetic variants associated with either PR3- or MPO-ANCA-positive patients [8] and a clinical trial for a drug that targets the C5a receptor in the alternative complement pathway [9]. Another approach to personalized medicine is pharmacogenetics (i.e. identification of genetic polymorphisms related to drug efficacy and/or toxicity) [10]. Examples for AAV include cytochrome P450 polymorphisms for CYC [11] and Fcγ receptor polymorphisms for rituximab therapy [12].

Hydroxysteroid dehydrogenase 11β type 1 (HSD11B1) is an enzyme involved in the regulation of local GC levels. *In vivo*, it predominantly converts inactive cortisone into active cortisol [13]. Due to disadvantageous cardiometabolic effects of cortisol, inhibition of HSD11B1 has been investigated as a potential treatment for diabetes and obesity [14]. In contrast, HSD11B1 deficiency may worsen inflammatory diseases or hinder their resolution [14, 15]. The latter has been demonstrated in animal studies but not yet in a clinical setting [14–16]. A common genetic polymorphism of *HSD11B1*, rs11119328, is hypothesized to result in reduced expression of the enzyme [17]. The influence of rs11119328 on inflammation has not yet been studied.

Cortisol and prednisolone bind to the glucocorticoid receptor (GR) in the cytoplasm. After binding, the resulting complex moves to the nucleus where it affects gene expression; these genomic effects influence cardiometabolic effects and inflammation [6]. Several polymorphisms have been described for the GR encoding gene. Some of these polymorphisms, such as N363S (rs6195) and *Bcl*I (rs41423247) are associated with an increased sensitivity to GCs. Other polymorphisms, such as ER22/23EK (rs6189 and rs6190) and 9β (rs6198), are associated with relative GC resistance [6]. The polymorphism TthIII1 (rs10052957) is thought to have no influence on GC sensitivity by itself but is strongly associated with the simultaneous presence of the ER22/23EK polymorphism [18].

In the general population, GR polymorphisms have been associated with distinct clinical phenotypes. The N363S and *Bcl*I polymorphisms have been associated with obesity, insulin resistance and a reduced risk of inflammatory diseases such as RA [6, 19, 20]. In contrast, ER22/23EK has been associated with higher insulin sensitivity, lean mass and muscle strength and the ER22/23EK and 9β polymorphisms have been associated with an increased risk of RA [6, 19, 20]. In multiple sclerosis and RA, the ER22/23EK polymorphism in particular has been associated with a more severe disease phenotype [21, 22]. Several studies have suggested associations between GR polymorphisms and GC treatment response [20, 23–26].

The aim of this study was to explore the influence of five GR polymorphisms and one polymorphism of *HSD11B1* (rs11119328) on the efficacy and toxicity of high-dose prednisolone therapy in AAV. Given their functional consequences, we hypothesized that N363S and *Bcl*I would be associated with a higher risk of cardiometabolic adverse events and that ER22/23EK, 9β and rs11119328 would be associated with more severe disease activity and a higher risk of relapse.

Methods

Study population

All 421 consecutive AAV patients diagnosed from 1990 to 2015 receiving treatment and follow-up at the University Medical Center Groningen (UMCG) were considered for inclusion. Patients were included if they received treatment with GCs combined with another immunosuppressive drug as initial treatment, according to the EULAR/European Renal Association–European Dialysis and Transplant Association recommendations [27]. Prednisolone was slowly tapered according to the local protocol of the UMCG starting 6 weeks after initiation of treatment. Eosinophilic granulomatosis with polyangiitis (formerly Churg–Strauss syndrome) patients were excluded because they have a different clinical course, with frequent asthma and ENT exacerbations often requiring chronic GC therapy [28]. This study is part of a large cohort study investigating biomarkers in relation to clinical outcomes in AAV. All patients gave written informed consent. The protocol for the biomarker study was approved by the local medical ethical committee (NL29354.042.10) and adheres to the principles of the Declaration of Helsinki.

Data collection

Clinical data were collected from the patients' medical records. Disease activity at diagnosis and first relapse were scored using the BVAS version 1 [29]. Comorbidity at diagnosis relevant for mortality risk was scored using the Charlson comorbidity index [30]. The primary endpoint of the study was 10 year relapse-free survival. Secondary endpoints were 10 year mortality, 1 year renal survival, risk of infections (overall, severe, opportunistic) in the first year after diagnosis and occurrence of hypertension, diabetes and dyslipidaemia. Definitions of study endpoints are included as Supplementary Table S1, available at *Rheumatology* online. Data were collected up to October 2017.

Genotyping and determination of haplotypes

Genotyping has been performed at the Laboratory of Clinical and Experimental Endocrinology, Department of Internal Medicine, Erasmus Medical Center, Rotterdam, The Netherlands. All genotypes were determined in one batch. The following five GR polymorphisms on chromosome 5 were determined using TaqMan allelic discrimination assays (Applied Biosystems, Foster City,

CA, USA): *Bcl*I (rs41423247), Tth111I (rs10052957), 9β(rs6198), N363S (rs6195) and ER22/23EK (rs6189 and rs6190). Additionally, the presence of the *HSD11B1* (rs11119328) polymorphism on chromosome 1 was determined. Haplotypes were determined based on combinations of GR polymorphisms using the computer programme PHASE [31, 32]. The haplotypes were defined as follows: haplotype 0 (reference)—major variant of all polymorphisms; haplotype 1—minor variant of the *Bcl*I polymorphism; haplotype 2—minor variant of the Tth111I and *Bcl*I polymorphisms; haplotype 3—minor variant of the 9β and Tth111I polymorphisms; haplotype 4—minor variant of the ER22/23EK, 9β and Tth111 polymorphisms; haplotype 5—minor variant of the N363S polymorphism. Detailed methods are described elsewhere [33]. As rs11119328 was the only gene polymorphism from chromosome 1 included in this study, it was not analysed as part of a haplotype. In the results, it will be called *HSD11B1* minor for simplicity.

Statistical analysis

Data were analysed using SPSS Statistics version 23 (IBM, Armonk, NY, USA). The proportionality assumption for Cox regression was tested in R version 3.2.3 (R Project for Statistical Computing, Vienna, Austria) using scaled Schoenfeld residuals [34]. Correction for multiple comparisons was performed in R using the Benjamini–Hochberg (BH) procedure with a false discovery rate of 0.05 [35]. BH-adjusted *P*-values were calculated, with *P*-values <0.05 indicating statistical significance. Missing data were handled using complete case analysis. Baseline characteristics were compared between carriers and non-carriers of each haplotype and *HSD11B1* minor using Fisher's exact test for categorical variables and the Mann–Whitney *U* test for continuous variables. Deviation from Hardy–Weinberg equilibrium was tested using a χ^2 test [36]. Univariable survival analysis was performed for relapse-free, overall, renal and adverse event-free survival using the log rank test. In case of significant differences between *GR* haplotypes or *HSD11B1* genotypes, Cox regression was performed to quantify the differences. Multivariable relapse-free and overall survival analyses were performed using Cox regression with adjustment for previously reported risk factors of relapse [37, 38] and mortality [5, 39], respectively. Interactions between GR haplotypes and *HSD11B1* minor were checked by adding the product of both variables as an interaction term to the regression model. Lastly, differences regarding the occurrence of infections in the first year after diagnosis were analysed using Fisher's exact test.

Results

Baseline results

Baseline characteristics of the 241 patients in the study are shown in Table 1, as well as differences between carriers and non-carriers of the different haplotypes of the GR and the rs11119328 polymorphism of *HSD11B1* (*HSD11B1* minor). Patients received a median initial prednisolone dose of 60 mg/d [interquartile range (IQR) 60–60]. After 6 months the median dose was 7.5 mg/d (IQR 5–10 mg/d). Discontinuation of prednisolone within 1 year after diagnosis was achieved for 60% of patients. GC exposure did not differ between GR haplotypes or between carriers and non-carriers of *HSD11B1* minor. A flowchart of inclusion is shown in Supplementary Fig. S1, available at *Rheumatology* online.

Genotype and haplotype frequencies

The minor allele frequencies of the *GR* gene and the *HSD11B1* gene were the following: 40% for *Bcl*I, 32% for Tth111I, 17% for 9β, 5% for N363S, 4% for ER22/23EK and 19% for rs11119328 (*HSD11B1* minor). These were comparable to minor allele frequencies found in the general population [6].

Haplotypes were derived for the GR gene polymorphisms. The haplotype frequency was 38.6% for haplotype 0 (all major), 24.7% for haplotype 1 (minor *Bcl*I), 15.8% for haplotype 2 (minor Tth + *Bcl*I), 13.1% for haplotype 3 (minor Tth + 9β), 3.1% for haplotype 4 (minor ER22 + 9β + Tth), and 4.8% for haplotype 5 (minor N363S). This is comparable to haplotype frequencies reported by Wester *et al*. [40] based on a large general population cohort.

Relapse-free survival

In total, 129 of 241 (54%) patients experienced a relapse within 10 years after diagnosis. Cumulative relapse-free survival was 92% after 1 year, 53% after 5 years and 38% after 10 years. The median BVAS at relapse was 12 (IQR 7–16). The most frequent organ manifestations were kidneys (56%), ENT (53%) and systemic (84%). Patients most frequently received CYC induction therapy (68%) and AZA maintenance therapy (47%) in addition to prednisolone in a high initial dose. Plasmapheresis was required for 7% of patients and 5% needed (temporary) haemodialysis therapy.

When accounting for multiple comparisons, there was no statistically significant difference in the risk of relapse between carriers and non-carriers of rs11119328 (BH adjusted $P = 0.18$; Fig. 1), haplotype 1 ($P = 0.97$), haplotype 2 ($P = 0.68$), haplotype 3 ($P = 0.91$), haplotype 4 ($P = 0.77$) and haplotype 5 of *GR* ($P = 0.77$).

In the Cox regression, the *HSD11B1* genotype showed a significant interaction with haplotype 1. Carriers of *HSD11B1* minor without a concomitant haplotype 1 [$n = 42/227$ (19% of patients)] had a significantly higher risk of relapse [hazard ratio (HR) 2.5], while this risk was compensated (HR 1.0) by the simultaneous presence of haplotype 1 [$n = 38/227$ (17% of patients)]. This remained true after correction for age, gender, serum creatinine, ENT and pulmonary involvement, ANCA specificity and AZA maintenance therapy (Table 2, Fig. 1).

Mortality

In total, 58 of 241 (24%) patients died within 10 years of diagnosis. Cumulative survival was 97% after 1 year (*n* at

risk = 241), 88% after 5 years ($n = 209$) and 72% after 10 years ($n = 136$). The log rank test showed no significant differences in the risk of mortality between carriers and non-carriers of haplotype 1 (BH adjusted $P = 0.97$), haplotype 2 ($P = 0.97$), haplotype 3 ($P > 0.99$), haplotype 4 ($P = 0.35$) and haplotype 5 ($P = 0.99$) or the *HSD11B1* genotype ($P = 0.42$). In univariable Cox regression, haplotype 4 did not meet the proportionality assumption. After stratification by time, haplotype 4 was a significant predictor of mortality only in the first 5 years following diagnosis. In multivariable Cox regression, after adjusting for age, gender, serum creatinine, BVAS, requirement of plasmapheresis, AZA maintenance therapy and ANCA specificity, haplotype 4 remained a significant predictor of 5 year mortality (Table 3, Fig. 2).

Renal survival

In total, 26 of 241 patients (11%) developed end-stage renal disease within 10 years after diagnosis. Relatively many events (10/26) of end-stage renal disease occurred in the first year after diagnosis. Carriers of haplotype 4, even after correction for multiple comparisons, had a significantly worse 1 year renal survival compared with non-carriers [HR 7.4 (95% CI 1.9, 28.7), BH adjusted $P = 0.007$] (see Supplementary Fig. S2, available at *Rheumatology* online). One-year renal survival did not differ significantly between carriers and non-carriers of haplotype 1 (BH adjusted $P = 0.68$), haplotype 2 ($P = 0.96$), haplotype 3 ($P = 0.97$) or haplotype 5 ($P = 0.60$), nor between carriers and non-carriers of a minor variant of *HSD11B1* ($P = 0.97$). Because of the limited number of events, multivariable Cox regression was not performed.

Infections

Overall, 103/241 (43%) patients had an infection requiring antimicrobial treatment during the first year after diagnosis. A severe infection occurred in 48 (20%) patients, an opportunistic infection in 49 (20%), varicella zoster in 9 (4%) and CMV in 21 patients (9%). There were no significant differences between *GR* haplotypes or *HSD11B1* genotypes regarding the occurrence of any severe or opportunistic infections in the first year after diagnosis.

Metabolic adverse events

There were no significant differences between haplotypes of the *GR* or *HSD11B1* genotypes for prevalence of diabetes or hypertension at diagnosis, although homozygous carriers of haplotype 1 tended to have diabetes more frequently at baseline [4/15 (27%)] compared with heterozygous [6/89 (7%)] and non-carriers [10/135 (7%)].

After 10 years of follow-up, 156 patients (67%) had hypertension (77 new onset), 75 (31%) had diabetes (57 new onset) and 68 (29%) had dyslipidaemia (49 new onset). When accounting for multiple comparisons, haplotype 1 was significantly related to the development of new-onset dyslipidaemia (BH adjusted log rank $P = 0.007$), but not hypertension ($P = 0.18$) or type 2 diabetes ($P = 0.68$). Homozygous carriers of haplotype 1 [HR 4.1 (95% CI 1.8, 9.6)], but not heterozygous carriers [HR 0.9 (95% CI 0.5, 1.6)], had an increased risk of new-onset dyslipidaemia compared with non-carriers (see Fig. 3). There were no significant differences in the risks of new-onset hypertension, diabetes or dyslipidaemia between other *GR* haplotypes or *HSD11B1* genotypes.

Table 1 Baseline characteristics

Variable	All ($n = 241$)	HT 1 ($n = 104$)	HT 2 ($n = 70$)	HT 3 ($n = 60$)	HT 4 ($n = 15$)	HT 5 ($n = 22$)	rs11119328 ($n = 80$)[a]
Male, %	56	58	49	60	60	55	65
Age at diagnosis, median (IQR), years	56 (44–66)	57 (44–66)	53 (43–65)	55 (44–64)	59 (50–77)	62 (46–71)	55 (44–65)
Follow-up, median (IQR), years	10 (6–15)	10 (6–14)	9 (6–15)	10 (5–17)	12 (3–13)	10 (8–17)	9 (6–19)
GPA, %	75	73	79	72	73	68	75
PR3 ANCA, %	73	71	80	70	73	64	74
CYC induction, %	88	88	87	92	100	82	89
Plasmapheresis, %	19	26	16	13	40	14	16
Haemodialysis, %	8	11	9	7	13	9	10
AZA maintenance, %	60	64	63	58	60	64	51
ENT activity, %	63	64	67	60	53	41	63
Pulmonary activity, %	46	52	40	38	33	50	50
Renal activity, %	69	70	73	70	80	73	70
Serum creatinine, median (IQR), μmol/l	100 (79–227)	106 (77–247)	112 (72–234)	105 (80–209)	180 (100–350)	101 (87–215)	106 (81–236)
BVAS at diagnosis, median (IQR)	18 (12–24)	19 (12–25)	20 (13–25)	16 (12–22)	18 (13–20)	19 (15–24)	18 (13–26)
Charlson index score	1 (0–3)	2 (0–3)	1 (0–3)	1 (0–3)	2 (1–4)	2 (0–3)	2 (0–3)

[a]Available for 227 of 241 patients. GPA: granulomatosis with polyangiitis; HT: haplotype.

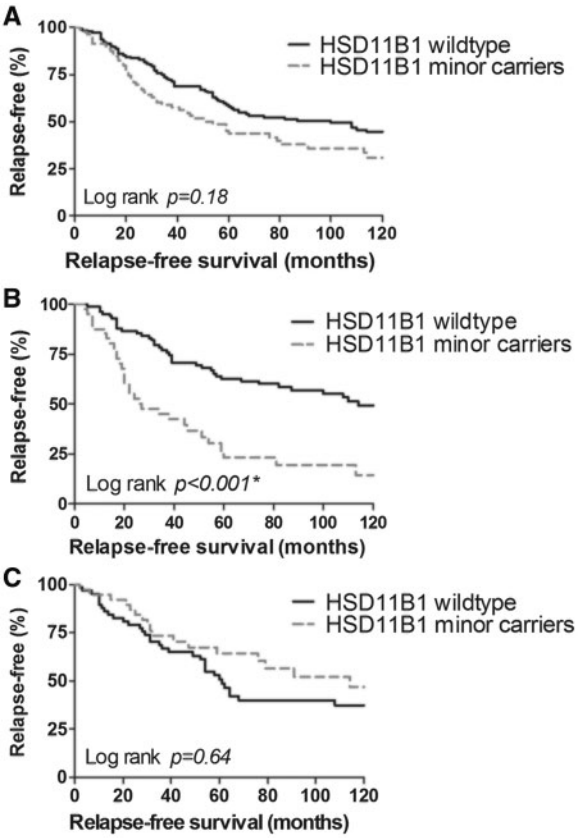
Discussion

In this retrospective study in a large cohort of AAV patients, we found that haplotype 4 of the *GR* gene (ER22/23EK + 9β + Tth111I), related to relative GC resistance, was associated with an increased risk of 5 year mortality and the development of end-stage renal disease. Furthermore, the rs11119328 polymorphisms of the gene encoding *HSD11B1*, hypothesized to be associated with reduced local GC activation, in the absence of haplotype 1 (*BclI*) of the *GR* gene, was associated with an increased risk of disease relapse. Lastly, homozygous carriers of haplotype 1, previously shown to be associated with increased GC sensitivity, had an increased risk of developing dyslipidaemia after diagnosis of vasculitis.

GC resistance due to haplotype 4 seems to be a two-sided coin. In the general population, it is associated with improved overall survival [41]. This might be mediated by beneficial metabolic effects of GC resistance (i.e. higher insulin sensitivity, lower cholesterol levels) [42]. In case of an inflammatory disease, haplotype 4 is potentially disadvantageous. This has been shown for RA [21], multiple sclerosis [22] and now for AAV. In RA patients, patients with the ER22/23EK polymorphism more frequently had erosive disease and more frequently required anti-TNF therapy [21]. This is in line with our finding that haplotype 4 carriers more frequently developed end-stage renal disease and had a higher risk of 5 year mortality, suggesting more severe inflammation in these patients. Contradictory to these results, we found no association of haplotype 4 with relapse-free survival. This could be explained, at least in part, by the higher risk of severe renal disease in haplotype 4 carriers, which has been associated with a lower risk of relapse in AAV [37]. This might counteract an increased risk of relapse due to GC resistance in haplotype 4, resulting in no net effect on relapse. Also, as haplotype 4 is relatively uncommon ($n = 15$) and is associated with an increased risk of mortality in our population, the number of haplotype 4 carriers with long-term data on relapse might not be sufficient to detect an association.

To our knowledge, we are the first to report the relation between a genetic polymorphism of *HSD11B1* and clinical outcomes of an autoimmune inflammatory disease in humans. While *GR* gene polymorphisms have also been studied for RA and multiple sclerosis patients [21, 22], polymorphisms of *HSD11B1* have not previously been studied in human patients with an inflammatory disease. In a mouse model of inflammatory arthritis, HSD11B1-deficient mice had earlier onset and later resolution of inflammation [16]. This is in accordance with the increased risk of relapse of patients with an *HSD11B1* minor

Fig. 1 Relapse-free survival per genotype of *HSD11B1*



Relapse-free survival for carriers (grey dashed line) and non-carriers (black solid line) of the *HSD11B1* minor allele. *P*-values shown are BH adjusted. (**A**) Overall relapse-free survival. (**B**) Non-carriers of haplotype 1 (glucocorticoid receptor). (**C**) Carriers of haplotype 1. *Significant after adjustment for multiple comparisons.

Table 2 Cox regression analysis for 10 year relapse-free survival

Variable	Model 1		Model 2[a]	
	HR (95% CI)	BH adjusted *P*-value	HR (95% CI)	BH adjusted *P*-value
HSD11B1 minor carrier	2.9 (1.8, 4.7)	<0.001*	2.5 (1.5, 4.1)	0.005*
HT1 carrier	1.5 (0.9, 2.4)	0.42	1.4 (0.8, 2.2)	0.60
HT1 * *HSD11B1* minor	0.2 (0.1, 0.5)	0.005*	0.3 (0.1, 0.6)	0.02*

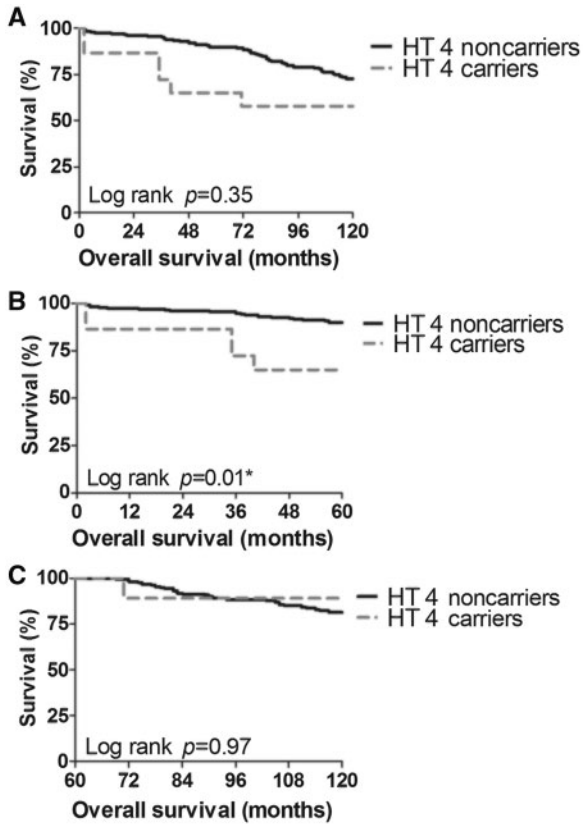
Cox regression analyses of the *HSD11B1* genotype, haplotype 1 (*BclI*) of the GR and their interaction for 10 year relapse-free survival. [a]Adjusted for age, gender, serum creatinine, ENT and pulmonary involvement, ANCA specificity and AZA maintenance therapy. *Significant after adjustment for multiple comparisons. HT: haplotype.

Table 3 Cox regression analysis for 10 year overall survival

Variable	Model 1		Model 2[a]	
	HR (95% CI)	BH adjusted *P*-value	HR (95% CI)	BH adjusted *P*-value
Haplotype 4 (0–5 years)	4.3 (1.6, 11.3)	0.03*	4.5 (1.6, 12.8)	0.03*
Haplotype 4 (5–10 years)	0.6 (0.1, 4.5)	0.97	1.0 (0.1, 7.7)	>0.99

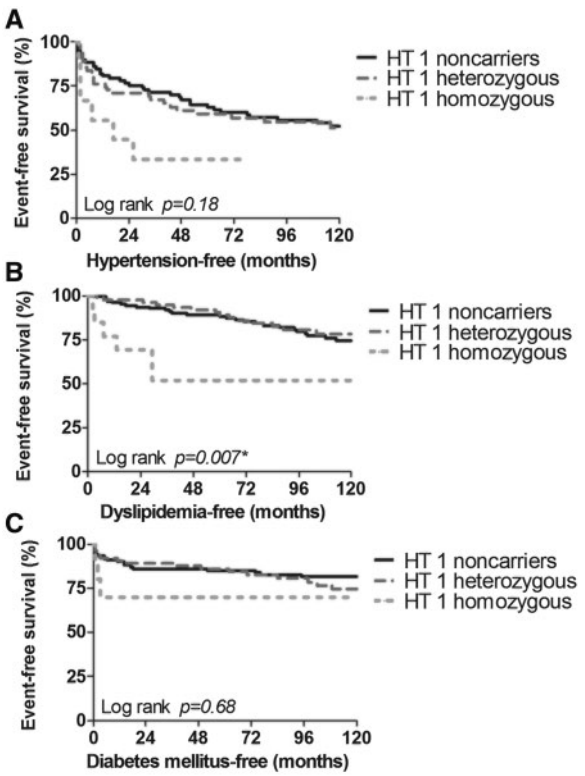
Cox regression analyses of haplotype 4 (ER22/23EK + 9β+TthIII1) of the GR, stratified by time, for 10 year overall survival. [a]Adjusted for age, gender, serum creatinine, BVAS score, requirement of plasmapheresis, AZA maintenance therapy and ANCA type. *Significant after adjustment for multiple comparisons.

Fig. 2 Overall survival for carriers and non-carriers of haplotype 4 (ER22 + 9β+Tth)



Kaplan–Meier curve for overall survival of carriers (grey dashed line) and non-carriers (black solid line) of haplotype 4 of GR. *P*-values shown are BH adjusted. (**A**) 10 year mortality. (**B**) 5 year mortality. (**C**) Mortality between 5 and 10 years of follow-up. *Significant after adjustment for multiple comparisons. HT: haplotype.

Fig. 3 Adverse event-free survival and haplotype 1 (*BclI*)



Kaplan–Meier curves for 10 year event-free survival *vs* haplotype 1 of the GR. *P*-values shown are BH adjusted. (**A**) Hypertension-free survival. (**B**) Dyslipidaemia-free survival. (C) Diabetes-free survival. *Significant after adjustment for multiple comparisons. HT: haplotype.

(rs11119328) genotype in our study, which suggests a pro-inflammatory phenotype due to reduced local activation of cortisone in carriers of this polymorphism. Interestingly, we found that the increased risk of relapse by the minor variant of *HSD11B1*, which would theoretically decrease local availability of cortisol to bind GR [15], can be compensated by the simultaneous presence of haplotype 1, which is associated with increased sensitivity of GR to active GC [43]. Nevertheless, 19% of the study population belonged to the group with increased risk (i.e. rs11119328 and no haplotype 1), which is a considerable number of patients.

Our finding of an increased risk of dyslipidaemia in homozygous carriers of haplotype 1 is in accordance with similar findings in the literature [19, 44, 45], although associations of the *BclI* polymorphism with metabolic

outcomes were not consistently found in all studies [20]. Based on results from this study and the aforementioned previous studies, two copies of haplotype 1 are required for significant effects on metabolic adverse events (i.e. heterozygous carriers did not have an increased risk of developing dyslipidaemia).

This study has several limitations. First, this retrospective study spans more than a decade. Therefore differences in treatment exist between patients according to each patient's time of diagnosis. In particular, some patients were diagnosed before the introduction of AZA maintenance therapy. Although we studied a cohort of >200 AAV patients from a single centre, the cohort size might be too small for some analyses, especially for comparing heterozygous *vs* homozygous carriers of and testing for interactions with relatively uncommon haplotypes (i.e. haplotypes 4 and 5). Furthermore, we did not include a validation cohort to confirm our findings. Therefore the results should be verified in other cohort studies. Nevertheless, our analyses were based on pre-specified hypotheses, correction was performed for multiple comparisons and our findings are consistent with previous literature.

The findings of this study provide insight into the influence of GC sensitivity on clinical outcomes in AAV. They indicate proneness to relapse in carriers of a minor variant of *HSD11B1*, more severe renal disease and mortality in carriers of haplotype 4 and an increased risk of dyslipidaemia in homozygous carriers of haplotype 1 of *GR*. This might prove useful in guiding treatment for individual patients. Examples of potential applications, after appropriate validation of these results, include the use of more intensive induction therapy for carriers of haplotype 4 with renal disease activity, longer duration of maintenance therapy for carriers of an *HSD11B1* variant without haplotype 1 or more emphasis on GC-sparing treatment in homozygous carriers of haplotype 1.

Funding: This work was supported by the Dutch Kidney Foundation (13OKJ39 to J.S.F.S.) and the Netherlands Organization of Scientific Research (907-14-542 to J.S.F.S., 917-16-453 to E.F.C.R.).

Disclosure statement: The authors have declared no conflicts of interest.

Supplementary data

Supplementary data are available at *Rheumatology* online.

References

1 Jennette JC. Overview of the 2012 revised International Chapel Hill Consensus Conference nomenclature of vasculitides. Clin Exp Nephrol 2013;17:603–6.

2 Kallenberg CG. Pathogenesis and treatment of ANCA-associated vasculitides. Clin Exp Rheumatol 2015;33:11–4.

3 Wong L, Harper L, Little MA. Getting the balance right: adverse events of therapy in anti-neutrophil cytoplasm antibody vasculitis. Nephrol Dial Transplant 2015;30:i164–70.

4 Robson J, Doll H, Suppiah R *et al*. Glucocorticoid treatment and damage in the anti-neutrophil cytoplasm antibody-associated vasculitides: long-term data from the European Vasculitis Study Group trials. Rheumatology (Oxford) 2015;54:471–81.

5 Little MA, Nightingale P, Verburgh CA *et al*. Early mortality in systemic vasculitis: relative contribution of adverse events and active vasculitis. Ann Rheum Dis 2010;69:1036–43.

6 Quax RA, Manenschijn L, Koper JW *et al*. Glucocorticoid sensitivity in health and disease. Nat Rev Endocrinol 2013;9:670–86.

7 van der Geest KSM, Brouwer E, Sanders JS *et al*. Towards precision medicine in ANCA-associated vasculitis. Rheumatology (Oxford) 2018;57:1332–9.

8 Lyons PA, Rayner TF, Trivedi S *et al*. Genetically distinct subsets within ANCA-associated vasculitis. N Engl J Med 2012;367:214–23.

9 Jayne DRW, Bruchfeld AN, Harper L *et al*. Randomized trial of C5a receptor inhibitor avacopan in ANCA-associated vasculitis. J Am Soc Nephrol 2017;28:2756–67.

10 Tavakolpour S, Darvishi M, Ghasemiadl M. Pharmacogenetics: a strategy for personalized medicine for autoimmune diseases. Clin Genet 2018;93:481–97.

11 Schirmer JH, Bremer JP, Moosig F *et al*. Cyclophosphamide treatment-induced leukopenia rates in ANCA-associated vasculitis are influenced by variant CYP450 2C9 genotypes. Pharmacogenomics 2016;17:367–74.

12 Cartin-Ceba R, Indrakanti D, Specks U *et al*. The pharmacogenomic association of Fcγ receptors and cytochrome P450 enzymes with response to rituximab or cyclophosphamide treatment in antineutrophil cytoplasmic antibody-associated vasculitis. Arthritis Rheumatol 2017;69:169–75.

13 Tomlinson JW, Walker EA, Bujalska IJ *et al*. 11β-hydroxysteroid dehydrogenase type 1: a tissue-specific regulator of glucocorticoid response. Endocr Rev 2004;25:831–66.

14 Gathercole LL, Lavery GG, Morgan SA *et al*. 11β-hydroxysteroid dehydrogenase 1: translational and therapeutic aspects. Endocr Rev 2013;34:525–55.

15 Chapman KE, Coutinho AE, Zhang Z *et al*. Changing glucocorticoid action: 11β-hydroxysteroid dehydrogenase type 1 in acute and chronic inflammation. J Steroid Biochem Mol Biol 2013;137:82–92.

16 Coutinho AE, Gray M, Brownstein DG *et al*. 11β-Hydroxysteroid dehydrogenase type 1, but not type 2, deficiency worsens acute inflammation and experimental arthritis in mice. Endocrinology 2012;153:234–40.

17 Dekker MJ, Tiemeier H, Luijendijk HJ *et al*. The effect of common genetic variation in 11β-hydroxysteroid dehydrogenase type 1 on hypothalamic-pituitary-adrenal axis activity and incident depression. J Clin Endocrinol Metab 2012;97:E233–7.

18 van Rossum EF, Roks PH, de Jong FH *et al*. Characterization of a promoter polymorphism in the glucocorticoid receptor gene and its relationship to three

other polymorphisms. Clin Endocrinol (Oxf) 2004;61:573–81.

19 Koper JW, van Rossum EF, van den Akker EL. Glucocorticoid receptor polymorphisms and haplotypes and their expression in health and disease. Steroids 2014;92:62–73.

20 Manenschijn L, van den Akker EL, Lamberts SW, van Rossum EF. Clinical features associated with glucocorticoid receptor polymorphisms. An overview. Ann NY Acad Sci 2009;1179:179–98.

21 van Oosten MJ, Dolhain RJ, Koper JW *et al*. Polymorphisms in the glucocorticoid receptor gene that modulate glucocorticoid sensitivity are associated with rheumatoid arthritis. Arthritis Res Ther 2010;12:R159.

22 van Winsen LM, Manenschijn L, van Rossum EF *et al*. A glucocorticoid receptor gene haplotype (TthIII1/ER22/23EK/9β) is associated with a more aggressive disease course in multiple sclerosis. J Clin Endocrinol Metab 2009;94:2110–4.

23 Zou YF, Xu JH, Wang F *et al*. Association study of glucocorticoid receptor genetic polymorphisms with efficacy of glucocorticoids in systemic lupus erythematosus: a prospective cohort study. Autoimmunity 2013;46:531–6.

24 Quax RA, Koper JW, Huisman AM *et al*. Polymorphisms in the glucocorticoid receptor gene and in the glucocorticoid-induced transcript 1 gene are associated with disease activity and response to glucocorticoid bridging therapy in rheumatoid arthritis. Rheumatol Int 2015;35:1325–33.

25 Gabryel M, Skrzypczak-Zielinska M, Kucharski MA, Slomski R, Dobrowolska A. The impact of genetic factors on response to glucocorticoids therapy in IBD. Scand J Gastroenterol 2016;18:1–12.

26 Eipel O, Hegyi M, Csordas K *et al*. Some GCR polymorphisms (N363S, ER22/23EK, and Bcl-1) may influence steroid-induced toxicities and survival rates in children with all. J Pediatr Hematol Oncol 2016;38:334–40.

27 Yates M, Watts RA, Bajema IM *et al*. EULAR/ERA-EDTA recommendations for the management of ANCA-associated vasculitis. Ann Rheum Dis 2016;75:1583–94.

28 Groh M, Pagnoux C, Baldini C *et al*. Eosinophilic granulomatosis with polyangiitis (Churg-Strauss) (EGPA) Consensus Task Force recommendations for evaluation and management. Eur J Intern Med 2015;26:545–53.

29 Luqmani RA, Bacon PA, Moots RJ *et al*. Birmingham Vasculitis Activity Score (BVAS) in systemic necrotizing vasculitis. QJM 1994;87:671–8.

30 Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis 1987;40:373–83.

31 Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 2001;68:978–89.

32 Stephens M, Scheet P. Accounting for decay of linkage disequilibrium in haplotype inference and missing-data imputation. Am J Hum Genet 2005;76:449–62.

33 Dekker MJ, van den Akker EL, Koper JW *et al*. Effect of glucocorticoid receptor gene polymorphisms in Guillain-Barre syndrome. J Peripher Nerv Syst 2009;14:75–83.

34 Bellera CA, MacGrogan G, Debled M *et al*. Variables with time-varying effects and the Cox model: some statistical concepts illustrated with a prognostic factor study in breast cancer. BMC Med Res Methodol 2016;10:20.

35 Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Series B Stat Methodol 1995;57:289–300.

36 Rodriguez S, Gaunt TR, Day IN. Hardy-Weinberg equilibrium testing of biological ascertainment for Mendelian randomization studies. Am J Epidemiol 2009;169:505–14.

37 Walsh M, Flossmann O, Berden A *et al*. Risk factors for relapse of antineutrophil cytoplasmic antibody-associated vasculitis. Arthritis Rheum 2012;64:542–8.

38 Pagnoux C, Hogan SL, Chin H *et al*. Predictors of treatment resistance and relapse in antineutrophil cytoplasmic antibody-associated small-vessel vasculitis: comparison of two independent cohorts. Arthritis Rheum 2008;58:2908–18.

39 Flossmann O, Berden A, de Groot K *et al*. Long-term patient survival in ANCA-associated vasculitis. Ann Rheum Dis 2011;70:488–94.

40 Wester VL, Koper JW, van den Akker EL *et al*. Glucocorticoid receptor haplotype and metabolic syndrome: the Lifelines cohort study. Eur J Endocrinol 2016;175:645–51.

41 van Rossum EF, Feelders RA, van den Beld AW *et al*. Association of the ER22/23EK polymorphism in the glucocorticoid receptor gene with survival and C-reactive protein levels in elderly men. Am J Med 2004;117:158–62.

42 van Rossum EF, Koper JW, Huizenga NA *et al*. A polymorphism in the glucocorticoid receptor gene, which decreases sensitivity to glucocorticoids in vivo, is associated with low insulin and cholesterol levels. Diabetes 2002;51:3128–34.

43 van Rossum EF, Koper JW, van den Beld AW *et al*. Identification of the BclI polymorphism in the glucocorticoid receptor gene: association with sensitivity to glucocorticoids in vivo and body mass index. Clin Endocrinol (Oxf) 2003;59:585–92.

44 Geelen CC, van Greevenbroek MM, van Rossum EF *et al*. *Bcl*I glucocorticoid receptor polymorphism is associated with greater body fatness: the Hoorn and CODAM studies. J Clin Endocrinol Metab 2013;98:E595–9.

45 van Moorsel D, van Greevenbroek MM, Schaper NC *et al*. *Bcl*I glucocorticoid receptor polymorphism in relation to cardiovascular variables: the Hoorn and CODAM studies. Eur J Endocrinol 2015;173:455–64.