The *p53* G72C and *MDM2* T309G single nucleotide polymorphisms in patients with Wegener's granulomatosis

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ABSTRACT

Objective. *Wegener's granulomatosis (WG) is a rare disease with unknown aetiology, but there is evidence for a complex genetic background. The tumor suppressor p53 and its most important negative regulator, MDM2, are positioned in the centre of a pathway that eliminates damaged cells through apoptosis. Furthermore, p53 is one of the most important negative regulators of the pro-inflammatory transcription factor nuclear factor kappa b (NFκB). In this respect the investigation of polymorphisms in the p53-network could be a promising approach contributing susceptibility of WG and its course of disease.*

Methods. *A case control study with 132 patients with WG and 512 healthy blood donors was conducted to evaluate an association of p53-SNP G72C or MDM2-SNP T309G with WG. SNPs were genotyped by polymerase chain reaction (PCR) and subsequent differential enzymatic restriction. All patients showed the clinical pathological findings of WG according to the ACR classification criteria of 1990.*

Results. *The p53 G72C and MDM2 T309G polymorphisms did not show any difference between WG patients and controls. The subgroup analysis of gender differences and earlier onset of WG (younger than median age of 51 years at diagnosis) did not show any differences in allelic or genotype frequencies of p53 G72C or MDM2 T309G SNP between WG patients and the control group.*

Conclusions. *Our study showed no association between the p53 SNP G72C and the MDM2 SNP T309G with susceptibility or course of disease in patients with WG. The data presented do not suggest that alterations in the p53-network play a key role in the pathogenesis of WG.*

Introduction

Wegener's granulomatosis (WG) is a systemic autoimmune disease characterized by nectrotizing granulomatous inflammation of the upper and lower respiratory tract, systemic necrotizing small-vessel vasculitis, and the presence of antineutrophile cytoplasmatic antibodies directed against Wegener's autoantigen proteinase 3 (*PR3)-ANCA*. WG is rare with a prevalence of only 1 to 3/100000 (1-3). The aetiology of the disease is still unknown, but there is evidence for a complex genetic background (4). Different single nucleotide polymorphisms (SNP) in the genes of certain cytokines like Interleukin 10 (IL-10), TNF alpha, and Interleukin 4 (IL-4) are suspected to contribute to both susceptibility and to disease formation of WG (5-7). In addition, the carriage of the defective allele *PI*Z* was observed as genetic risk factor for the development of *PR3-ANCA* associated vasculitis (8). Furthermore, allelic variations of *CTLA4* showed a genetic impact of WG formation (9). Interestingly, Jagiello *et al*. reported an extended association screen with 202 microsatellite markers representing apoptosis-related genes in patients with WG. The *HLA-DPB1/RXRB* genomic region on chromosome 6p21.3 was found significantly associated with the disease (10). This association was confirmed in an independent cohort recently and comprises the most consolidated genetic WG association detected so far (11). The latter study provided evidence that the association of this locus cannot be exclusively explained by *HLA-DPB1* alleles alone, but rather by the additional, partly independent effects of certain alleles of the *RXRB/RING1* region.

In the context of this potential link to apoptosis, it is interesting that *p53* autoantibodies were found in sera of

Table I. Main demographic and clinical characteristics of the WG patients (n=132) and control group (n=512).

Characteristics	Patients (n=132)	Controls (n=512)
Age (median in years)	61 (28-90)	44 (18-65)
Sex (female / male)	67 / 65	183 / 329
Disease duration (mean in years ± SD)	12 (± 3.1)	
Age at diagnosis (median in years)	51 (17-82)	
c-ANCA-status positive (%)	116 (87.9)	
Generalized status of WG (%)	120 (90.9)	

patients with WG (12). The tumor suppressor *p53* and its most important negative regulator, *MDM2*, are positioned in the centre of a pathway that eliminates damaged cells through apoptosis (13). In this context of negative regulation of *p53*-gene, the work by Bond and colleagues has recently shown that the presence of the *MDM2*-309 G allele is associated with higher *MDM2* protein levels and consequently, less functional *p53* in stressed cells (14). Other work has shown that the 72C allele of *p53* (resulting in a *p53* protein with a proline instead of an arginine at amino acid position 72) is linked to impaired apoptosis induction (15). Moreover, recent *in vitro* and mouse model studies have indicated that *p53* is one of the most important negative regulators of the pro-inflammatory transcription factor NFκB (16, 17). TNF-alpha and interleukin 1-beta are known to be subject to regulation by NFκB increasing expression of proinflammatory cytokines like TNF-alpha and different Interleukins in different rheumatic disorders (18). In this respect, the investigation of these polymorphisms could be a promising approach to further elucidate the genetic background of WG susceptibility and its clinical course. Here we have conducted a case control study to evaluate whether *p53*-SNP G72C or *MDM2*-SNP T309G are associated with WG.

Materials and methods

Participants

We recruited 132 patients with WG from the outpatient department at the Hospital of the University of Saarland Medical School and the Rheumaklinik Bad Bramstedt, Germany. All patients showed both the clinical pathological findings of WG according to the classification criteria of American College of Rheumatology 1990 and Chapel Hill Consensus Conference 1992 (19). The diagnostic procedure included physical examination, laboratory tests of c-ANCA, urine analysis and was completed by CT or MRI of different region as required in dependency on organ involvements of WG. All patients underwent a biopsy of the manifestation region of WG followed by histopathological preparation inclusive pertaining immunpathology. Blood donors (n=512) from the Institute for transfusion Medicine, University of Saarland Medical School served as controls. Both WG patients and control blood donors were of European Caucasian ethnicity. The ethics committees of medical association of the Saarland, Germany, approved the study, and all patients gave written informed consent. Table I summarizes the clinico-pathological characteristics of the study patients according to age, sex, disease duration, age at diagnosis, ANCA-status, and local versus generalized stage. Table II shows genotype frequencies inclusive allelic frequencies of the *p53*-G72C and *MDM2*-T309G SNP.

Genotyping and analysis of the polymorphisms

SNPs were genotyped by PCR and subsequent differential enzymatic restriction. PCRs were carried out using HotStart Taq polymerase (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The *MDM2* promotor SNP (T309G, rs2279744) was analyzed using the primers 5'-TTCAGGGTAAAGGTCACG-3' and 5'-GACTTAACTCCTTTTACTGCAGT-3'. PCR products were cut with MspA1I (New England Biolabs, Frankfurt, Germany) revealing fragments of 212bp for the T allele and 40+172bp for the G allele. The sequence surrounding the *p53* R72P SNP (rs1042522) was amplified with the primers 5'-TCCCAAGCAATGGATGATTT-3' and 5' TTGGCTGTCCCAGAATGC-3' and digested with Bsh1236I (Fermentas, St. Leon-Rot, Germany). An uncut fragment (251bp) was observed in case of the C allele, while the G allele resulted in two fragments of 107 and 144bp, respectively. All fragments were separated on agarose gels stained with ethidium bromide.

Statistical analysis

Data were analysed using SPSS [20]. The differences in genotype distribution and allele frequency among patients and controls were analysed using Fisher's exact test for 2×3 tables and Fisher's two-sided exact test for 2×2 tables, respectively. Differences in allele penetrance were quantified by odds ratios and 95% confidence intervals.

Results

Table I shows the demographic and clinical characteristics of the study population. Genotype and allele frequencies of *p53* and *MDM2* are shown in Table II. The *p53* 72C allele was not significantly more frequent in patients with WG compared to controls. The *MDM2* T309G polymorphism did not show any difference between WG patients and controls. The frequencies of the *p53* genotypes (C/C, C/G and G/G) were not significantly different in patients with WG. Furthermore, the *MDM2* genotype distribution did not show significant differences between WG and control group.

The mean of age at diagnosis was 51 years; a statistical analysis of the subgroup of patients younger than 51 years at diagnosis did not correlate with differences in allelic or genotype frequencies of *p53* SNP G72C or *MDM2* SNP T309G between WG patients (n=65 of 132) and age-matched control group (n=467 of 512) (Table III). The subgroup-analysis of gender differences in allelic or genotype frequencies of *p53* G72C or *MDM2* T309G SNP between WG patients (67 female *vs*. 65

Table II. Allele and genotype frequencies of *p53* G72C and *MDM2* T309G SNP in the WG patients and control group.

	WG patients n=132 (%)	Healthy controls n=512 (%)	*p*-value/odds-ratio
p53 Alleles			
G	193 (73.1)	762 (74.4)	0.777 [a]/1.07 (0.78-1.47) [b]
C	71 (26.9)	262 (25.6)	
MDM2-Alleles			
T	175 (66.3)	630 (61.5)	0.170 [c]/0.83 (0.62-1.11) [d]
G	89 (33.7)	394 (38.5)	
p53 Genotypes			
GG	70 (53.0)	283 (55.3)	0.891 [e]
GC	53 (40.2)	196 (38.3)	
CC	9 (6.8)	33 (6.4)	
MDM2-Genotypes			
TT	58 (43.9)	192 (37.5)	0.373 [f]
TG	59 (44.7)	246 (48.0)	
GG	15 (11.4)	74 (14.5)	

[a] [c] Controls versus patients, Fisher's exact test for 2×2 tables.
[b] [d] odds-ratio, 95% confidence-intervals.
[e] [f] Controls versus patients, Fisher's exact test for 2×3 tables.

Table III. Allele and genotype frequencies of *p53* G72C and *MDM2* T309G SNP in the WG patients younger than 51 years at diagnosis and age-matched control group.

	WG patients n=65 (%)	Healthy controls n=467 (%)	*p*-value/odds-ratio
p53 Alleles			
G	97 (74.6)	700 (74.9)	0.914 [g]/1.01 (0.81-1.23) [h]
C	33 (25.4)	234 (25.1)	
MDM2-Alleles			
T	88 (67.7)	575 (61.6)	0.209 [i]/0.81 (0.57-1.20) [j]
G	42 (32.3)	359 (38.4)	
p53 Genotypes			
GG	35 (53.8)	262 (56.1)	0.824 [k]
GC	27 (41.6)	176 (37.7)	
CC	3 (4.6)	29 (6.2)	
MDM2-Genotypes			
TT	30 (46.2)	176 (37.7)	0.414 [l]
TG	28 (43.1)	223 (47.8)	
GG	7 (10.7)	68 (14.5)	

[g] [i] Controls versus patients, Fisher's exact test for 2×2 tables.
[h] [j] odds-ratio, 95% confidence-intervals.
[k] [l] Controls versus patients, Fisher's exact test for 2×3 tables.

male) and sex-matched control group (183 female *vs*. 329 male) did not show any significant differences (data not shown).

Discussion

This study is the first attempt to evaluate the role of *p53* G72C and *MDM2* T309G SNP in the pathogenesis of WG, based on the rationale that the weakened *p53* response associated with both minor alleles may impact on the function of the pro-inflammatory, *p53*-modulated NFκB (17). Our study showed no association between the *p53* SNP G72C and the *MDM2* SNP T309G with susceptibility or course of disease in patients with WG. So far, only few data of the role of p53-network in the pathogenesis of WG exist. Only one study by Kuhn *et al*. obtained 17 sera with elevated autoantibodies-levels against *p53*-protein out of 73 patients with various autoimmune disease inclusive systemic lupus erythematodes (SLE) and WG (12). Concerning other rheumatic diseases, there are several studies that investigated the influence of *p53* on arthritis. Two representative studies observed in the *p53*-negative mouse increased activity of collagen-induced arthritis with consecutively elevated expression of collagenases 3 and interleukin 1-beta (21, 22). Another study investigated both the *p53* protein and *MDM2* protein levels in fibroblasts like synoviocytes (*FLS*) of synovialis after joint replacement surgery in patients with rheumatoid arthritis compared with osteoarthritis patients. The authors found increased *MDM2*-levels in cells from patients with rheumatoid arthritis compared to cells from osteoarthritis patients (23). For the *p53* G72C SNP, one investigation failed to find an association with the occurrence of rheumatoid arthritis in Italians but detected a significant positive association of radiological erosions after disease duration of five years with the C/C genotype (24). Our negative results, obtained with WG patients, thus suggest a different pathogenesis for arthritic rheumatic diseases and WG underlining previous studies (25). In conclusion, our findings indicate that the p53-network is unlikely to play a key role in the aetiology and pathogenesis of WG.

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