

# Lack of association between tumour necrosis factor receptor types 1 and 2 gene polymorphism and severe acute alcoholic hepatitis

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**Background/aims** Tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) is involved in the physiopathology of severe acute alcoholic hepatitis (AAH) binding with TNF receptor types TNFR1 and TNFR2, whose serum concentrations are elevated. We studied the role of TNFR1 and TNFR2 gene polymorphism in AAH patients.

**Methods** One hundred and ninety-two patients (58 AAH with Maddrey score  $\geq 32$ , 44 non-AAH cirrhotics, 90 healthy individuals) were genotyped for A36G TNFR1 and T676G TNFR2 using polymerase chain reaction–restriction fragment length polymorphism technique. Serum sTNFR1 and sTNFR2 were assayed.

**Results** The AAH and two control groups did not differ for genotype distribution. In three groups, A (36 TNFR1) and T (676 TNFR2) allelic frequencies were similar, at 0.47, 0.47, 0.44 and 0.78, 0.81, 0.80, respectively. The 36 TNFR1, 676 TNFR2 genotypes did not influence on prognostic scores (Maddrey, Child–Pugh), nor in response to corticosteroids or 6-month survival. sTNFR1 levels were higher in AAH than healthy group ( $3.07 \pm 1.14$  vs.  $1.17 \pm 0.27$  ng/ml,  $P < 0.001$ ) and sTNFR2 levels were

higher in AAH than cirrhosis ( $3.6 \pm 1.02$  vs.  $3.1 \pm 1.03$ ,  $P < 0.05$ ) and healthy groups ( $3.6 \pm 1.02$  vs.  $1.91 \pm 0.54$ ,  $P < 0.001$ ). However, sTNFR1 and sTNFR2 levels did not vary significantly according to genotypes.

**Conclusion** These results did not support an association between 36 TNFR1, 676 TNFR2 gene polymorphisms and AAH. *Eur J Gastroenterol Hepatol* 00:000–000 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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**Keywords:** 36 TNFR, 676 TNFR, acute alcoholic hepatitis, genetic polymorphism, Maddrey score, TNF-alpha, TNF receptor

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## Introduction

Severe acute alcoholic hepatitis (AAH) is a serious form of alcoholic liver disease (ALD) with a 1-month mortality rate of 35% [1–3]. Tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) is involved in the physiopathology of AAH [4]. Several studies have reported high serum TNF- $\alpha$  levels [5,6], in one series, a significant association with the 8-month mortality (82%) [6]. TNF- $\alpha$ 's activity is mediated by two types of cell receptor TNFR1 (p55) and TNFR2 (p75), both of which are involved in the regulation and expression of other cytokines through the activation of NF- $\kappa$ B [7,8]. The TNFR1 has an intracellular death domain that is capable of inducing apoptosis, whereas TNFR2 appears rather to have a regulatory role in TNF- $\alpha$ 's binding to TNFR1 [7,8]. The role of the soluble TNF receptors, sTNFR1 and sTNFR2, generated by cleavage of the corresponding membrane receptors [4,9] is subject to debate. The serum concentrations of these receptors were significantly higher in patients with ALD at different stages of severity [10], and reached a peak in

cirrhosis patients with severe AAH [10–12]. In one study, elevated serum sTNFR1 levels were significantly associated with 3-month mortality in AAH patients [13].

In an earlier study, we showed the influence of gene polymorphism of the –308 TNF- $\alpha$  promoter region in AAH patients [14]. The involvement of high serum levels of sTNFR1 and sTNFR2 in the same context makes these parameters potential candidates for genetic susceptibility markers. Several single nucleotide polymorphisms (SNPs) have been described for TNFR1 (A36G and T609G) and TNFR2 (T196G, T676G and A1466G) [15–18]. Hence, the 36 TNFR1 SNP may have a protective role in familial rheumatoid arthritis (RA) [19]. Conversely, –196 TNFR2 has been described as a susceptibility factor in the same condition [19,20]. In another series, 676 TNFR2 was associated with modified circulating levels of sTNFR2 [21]. In systemic lupus erythematosus, the –196 TNFR2 polymorphism has been associated with the disease [22,23]. Lastly, in

Crohn's disease, the 36 TNFR1 and -196 TNFR2 SNPs were negatively associated with the stricturing and colitic phenotypes, respectively [24], whereas the TNFR2 AT haplotype (TNFR2 A1466G and TNFR2 C1493T) increased susceptibility [25].

To the best of our knowledge, there is very little data on the influence of these gene polymorphisms in severe AAH. A recent study did not find a significant association between the -578 TNFR2 SNP and clinical expression in an ALD population, but without severe AAH, according to the Maddrey score [26].

The goal of this study was to study the role of several SNPs, 36 TNFR1 and 676 TNFR2, in the occurrence of AAH. We sought to determine the prevalence of the different genotypes in AAH patients in comparison with a control group of non-AAH cirrhosis patients and a group of cirrhosis-free, nonalcoholic, healthy controls and then compared the genotypes with the clinical and biochemical expression of the disease, the response to corticotherapy [27] and serum levels of sTNFR1 and sTNFR2.

## Patients and methods

### Patients

One hundred and ninety-two Caucasian patients were included in the study. Fifty-eight had severe AAH (the AAH group), 44 had cirrhosis but no AAH (the cirrhosis group) and 90 individuals constituted the healthy control group. AAH was defined as (i) chronic alcohol intake (more than 50 g per day) for the past 6 months, (ii) a Maddrey discriminant function (DF) of at least 32 [2] and (iii) compatible liver histology results [3]. Nonalcoholic cirrhosis was systematically ruled out by screening the hepatitis B and C virus serologies and the serum assay results for iron, transferrin and ferritin saturation, ceruleoplasmin,  $\alpha$ -1-antitrypsin deficiency and antinuclear, antismooth muscle, anti-LKM1 and antimitochondria antibodies. Patients with hepatocellular carcinoma and/or HIV were excluded. All the complications of cirrhosis were recorded. In addition, oesogastroduodenal fibroscopy and a liver ultrasound scan were performed in all patients, to identify any ascites or oesophageal varicela. Liver function was assessed through determination of the prothrombin time (PT), serum albumin and total bilirubin, enabling calculation of the Child-Pugh and Maddrey scores [2].

The two control groups comprised (i) 44 patients with alcohol-related cirrhosis in the absence of AAH (DF < 32, diagnosed either by the liver histology results or a self-consistent set of clinical, biochemical, echographic and endoscopic features) and (ii) 90 healthy, cirrhosis-free, nonalcoholic individuals. None of the patients and controls were related, all came from the same region of France and none had a history of chronic inflammatory disease or infectious disease involving TNF- $\alpha$ .

### Analytical method for determination of TNF- $\alpha$ promoter polymorphism

A column-based technique (Qiagen, Hilden, Germany) was used to extract DNA from peripheral blood mononuclear cells (sampled in an EDTA tube) or from buccal mucosa cells (sampled inside the cheek with a cotton wool swab). Genotype determination for the TNFR1 36 A/G, and TNFR2-676 T/G polymorphisms was performed using a polymerase chain reaction-restriction fragment length polymorphism procedure. The primers used were as follows: for 36, forward 5'-GAG CCC AAA TGG GGG AGT GAG AGG 3' and reverse 5'-ACC AGG CCC GGG CAG GAG AG 3'; for 676, forward 5'-ACT CTC CTA TCC TGC CTG CT 3' and reverse 5'-TTC TGG AGT TGG CTG CGT GT3' [15-18]. DNA amplification was performed in 50  $\mu$ l of KCl buffer solution (Bioline, London, UK) containing 200  $\mu$ mol/l of dNTP, 2.5  $\mu$ mol of each primer, 1  $\mu$ g of DNA, and 2 U of Taq polymerase (Bioline), for 35 cycles at 94°C for 1 min, 59°C for 1 min and 70°C for 45 s, followed by a 10-min cycle at 70°C. The PCR products were then digested at 37°C by *MspAII* and *NlaIII*, (for detecting the TNF-R1 36 A/G and TNF-R2 676 T/G polymorphisms, respectively). Genotypes were discriminated through differential migration (as a function of fragment size) in a 3% agarose gel (Sigma Aldrich, Saint Quentin Fallavier, France) with chemiluminescent detection under a UV lamp.

The genotyping was performed by two independent operators who were blinded to the clinical data and the patient groups. In the event of disagreement on the polymorphism, a consensus was reached.

### Serum sTNFR1 and sTNFR2 assays

Serum sTNFR1 and sTNFR2 levels were determined using an ELISA (the Quantikine Human sTNF RI and RII/TNFRSF1B immunoassay from R&D Systems, Minneapolis, USA).

### Statistical analyses

Results are given as the mean  $\pm$  standard deviation. Student's *t*-test was used to compare quantitative data. Intergroup comparisons of allelic and genotype frequencies were performed using the  $\chi^2$  test or (for small sample sizes) Fisher's exact test. The statistical significance threshold was set at a *P* value less than 0.05, with a Bonferroni's correction for multiple tests. The study was approved by the Picardy region's Ethics Committee. All individuals gave their informed, written consent to participate in the study and use of their personal genetic information. The study was carried out in accordance with the principles of the Declaration of Helsinki (2000).

## Results

The study population characteristics are summarized in Table 1. The patients in the AAH group had significantly worse liver function than the control cirrhosis, as

evidenced by Child–Pugh and Maddrey scores of  $12 \pm 1.5$  and  $54 \pm 17$ , respectively, for the AAH group and  $7 \pm 2$  and  $16 \pm 11$  in the cirrhosis group, together with significantly higher 6-month mortality in the AAH group. The two patient groups did not differ significantly in terms of age, sex ratio and alkaline phosphatase and  $\gamma$ -glutamyltransferase (GGT) levels.

**Polymorphism results for the promoter regions of TNFR1 and TNFR2**

The polymorphism results for the promoter regions of TNFR1 and TNFR2 are summarized in Table 2. We did not find any genotypic or allelic differences between the AAH group and the two control groups in terms of TNFR1 36 and TNFR2 676 polymorphisms. For the TNFR1 36 polymorphism, the allelic frequencies were 0.53, 0.53 and 0.56 for A and 0.47, 0.47 and 0.44 for G,

for the AAH, cirrhosis and healthy control groups, respectively. For the TNFR2 676 polymorphism, the allelic frequencies were 0.78, 0.81 and 0.80 for T and 0.22, 0.19 and 0.20 for G, for the AAH, cirrhosis and healthy control groups, respectively (Fig. 1).

**TNFR1 36 and TNFR2 676 polymorphisms and the clinical and biochemical status of AAH patients**

Comparison of AAH patients with a TNFR1 36 A/A genotype ( $n = 16$ ) with those with a TNFR1 36 A/G ( $n = 30$ ) or TNF- $\alpha$  36 G/G ( $n = 12$ ) genotype did not show any significant clinical and biochemical differences (in terms of age, sex ratio, total bilirubin, transaminases, GGT, creatinine, white blood cells, lymphocytes, platelets and the Maddrey and Child–Pugh scores; results not shown), apart from a difference in the PT for patients with a TNFR1 36 A/A genotype versus 36 G/G ( $36.7 \pm 8$  vs.  $30.1 \pm 5.2\%$ ,  $P = 0.01$ ) and the TNFR1 36 A/G genotype versus 36 G/G ( $37 \pm 10.3$  vs.  $30.1 \pm 5.2\%$ ,  $P = 0.03$ ). Albumin levels were significantly lower for patients with the TNFR1 36 A/A genotype versus 36 A/G, with  $24 \pm 4.6$  versus  $27.3 \pm 5.8$  g/l ( $P = 0.04$ ), respectively. Finally, there were differences in alkaline phosphatase levels for patients with the TNFR1 36 A/A genotype versus 36 A/G ( $234 \pm 147$  vs.  $151.3 \pm 44.4$  IU/l,  $P = 0.007$ ) and the TNFR1 36 A/A genotype versus 36 G/G ( $234 \pm 147$  vs.  $126.9 \pm 67.6\%$ ,  $P = 0.03$ ).

Similarly, comparison of the results for patients in the AAH group with a TNFR2 676 T/T ( $n = 36$ ) genotype versus TNFR1 36 T/G ( $n = 18$ ) and TNF- $\alpha$  36 G/G ( $n = 4$ ) did not show significant clinical and biochemical differences (in terms of age, sex ratio, PT, total bilirubin, albumin, GGT, creatinine, white blood cells, lymphocytes, platelets, the Maddrey and Child–Pugh scores and 6-month mortality; results not shown), apart from a difference in transaminases for patients with the TNFR2 676 T/G genotype versus 676 G/G ( $133.7 \pm 39.8$  vs.  $85.2 \pm 37.3$  IU/l,  $P = 0.03$ ). Finally, there was a difference in alkaline phosphatase levels for the patients with the

**Table 1 Clinical and biochemical characteristics of the study population**

	AAH ( <i>n</i> =58)	Cirrhosis, no AAH ( <i>n</i> =45)	Healthy controls ( <i>n</i> =90)	<i>P</i> value
Age in years	51.9 ± 8.2	56.7 ± 10.6	51.9 ± 8.2	
Median	53.5	55	53.5	0.83
Range	31–68	39–80	31–68	
Male sex, <i>n</i> (%)	32 (55.2)	31 (69)	32 (55.2)	0.15
Prothrombin time (%)	35.5 ± 9.1	63.5 ± 18.5		<0.001
Albuminaemia (g/l)	26.2 ± 5.8	32.4 ± 6.6		<0.001
Bilirubinaemia (μmol/l)	218 ± 110	58.35 ± 138.4		<0.001
Transaminases (U/l)	123.8 ± 50.8	90.4 ± 92.6		0.02
Gamma-glutamyltransferase (U/l)	297 ± 278	280 ± 337		0.70
Alkaline phosphatases (U/l)	168 ± 95.3	152 ± 87.5		0.39
Creatininaemia (μmol/l)	79.5 ± 38.7	96.7 ± 61.8		0.08
White cells (/mm <sup>3</sup> )	10433 ± 8178	6848 ± 3598		0.005
Lymphocytes (/mm <sup>3</sup> )	1338 ± 703.5	1243 ± 770		0.54
Platelets × 10 <sup>3</sup> /mm <sup>3</sup> )	118 ± 83	145 ± 75		0.09
Alpha-fetoprotein (ng/ml)	5.2 ± 2.2	4.6 ± 3.5		0.39
Maddrey score	54.26 ± 17	17.8 ± 23.1		<0.001
Child–Pugh score	11.7 ± 1.5	7.3 ± 2		<0.001
6-Month mortality, <i>n</i> (%)	18 (31)	5 (11)		0.01

Data represented are 'mean ± SD', otherwise specified. AAH, severe acute alcoholic hepatitis.

**Table 2 Frequencies of TNFR1 and TNFR2 promoter polymorphisms**

Position of polymorphism	<i>N</i>	Genotype, <i>n</i> (%)			OR (95% CI)	<i>P</i> value	Allele frequency		OR (95% CI)	<i>P</i> value
		A/A	A/G	G/G			A	G		
TNFR1 A36G										
AAH	58	16 (28)	30 (52)	12 (21)	0.88 (0.33–2.33) <sup>a</sup>	0.77	0.53	0.47	1.00 (0.55–1.81) <sup>a</sup>	0.99
Cirrhosis, no AAH	44	11 (25)	25 (57)	8 (18)	0.95 (0.43–2.14) <sup>b</sup>	0.90	0.53	0.47	1.11 (0.68–1.83) <sup>b</sup>	0.65
Healthy controls	90	24 (27)	53 (59)	13 (14)	1.09 (0.44–2.71) <sup>c</sup>	0.83	0.56	0.44	1.12 (0.65–1.92) <sup>c</sup>	0.67
TNFR2 T676G										
AAH	58	36 (62)	18 (31)	4 (7)	1.31 (0.53–3.25) <sup>a</sup>	0.52	0.78	0.22	1.21 (0.58–2.54) <sup>a</sup>	0.59
Cirrhosis, no AAH	44	30 (68)	11 (25)	3 (7)	1.16 (0.55–2.44) <sup>b</sup>	0.66	0.81	0.19	1.16 (0.63–2.12) <sup>b</sup>	0.61
Healthy controls	90	59 (66)	26 (29)	5 (6)	0.89 (0.38–2.02) <sup>c</sup>	0.76	0.80	0.20	0.96 (0.48–1.90) <sup>c</sup>	0.89

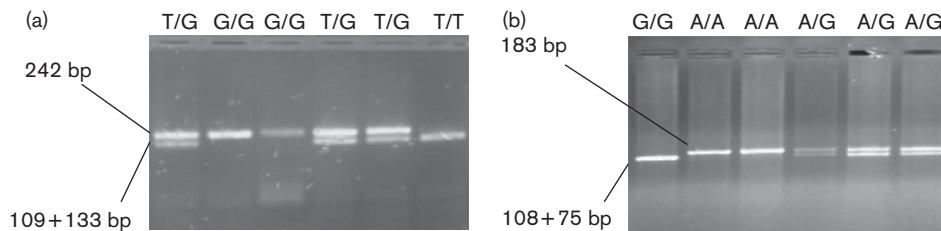
AAH, severe acute alcoholic hepatitis; CI, confidence interval; OR, odds ratio; TNFR1, tumour necrosis factor receptor type 1; TNFR2, tumour necrosis factor receptor type 2.

<sup>a</sup>Comparison between AAH and cirrhosis (no AAH).

<sup>b</sup>Comparison between AAH and healthy controls.

<sup>c</sup>Comparison between cirrhosis (no AAH) and healthy controls.

Fig. 1



Gel agarose images for 36 TNFR1 and 676 TNFR2 polymorphisms. (a) 3% agarose gel electrophoresis of NlaIII restriction fragment length polymorphism (RFLP) for T-G substitution of 676 TNFR2 gene. When T allele was present, the 242 bp PCR product was digested with NlaIII into two fragments of 133 and 109 bp. (b) 3% agarose gel electrophoresis of MspI RFLP for A-G substitution of 36 TNFR1 gene. When G allele was present, the 183 bp PCR product was digested with MspI into two fragments of 108 and 75 bp.

TNFR2 676 T/T genotype versus 676 T/G ( $151.5 \pm 51.7$  vs.  $216.9 \pm 146.3$  IU/l,  $P = 0.02$ ).

We did not observe any clinical and biochemical differences between the AAH patients with a wild-type TNFR1 36 A/A or TNFR2 676 T/T ( $n = 6$ ) haplotype versus all the haplotypes with at least one mutation (TNFR1 36 A/G, G/G or TNFR2 676 T/G, G/G,  $n = 52$ , data not shown).

**Serum sTNFR1 and sTNFR2 levels in the AAH, cirrhosis and healthy control groups and according to the observed genotypes**

Table 3 summarizes the serum levels of sTNFR1 and sTNFR2 for the three groups. For each soluble TNF- $\alpha$  receptor, serum levels were higher in the AAH group than in cirrhosis group or the healthy control group; the differences were statistically significant, apart from the AAH versus cirrhosis comparison for sTNFR1 ( $P = 0.42$ ). Serum levels of sTNFR1 and sTNFR2 were both significantly higher in the cirrhosis group than in the healthy control group.

Within the AAH group (Table 4), we did not observe any impact of the TNFR1 36 A/A, A/G and G/G genotypes on the serum level of sTNFR1, with  $3.07 \pm 1.0$ ,  $3.2 \pm 1.2$  and  $2.5 \pm 0.8$  ng/ml, respectively ( $P = NS$ ). Similarly, the same genotypes were not significantly correlated with the sTNFR2 serum level, with  $2.8 \pm 1.7$ ,  $2.7 \pm 1.7$  and  $2.4 \pm 1.9$  ng/ml, respectively ( $P = NS$ ) (Table 5). Furthermore, we observed no effect of the TNFR2 676 T/T, T/G and G/G genotypes on serum levels of sTNFR2 [ $3.7 \pm 1.14$ ,  $3.35 \pm 0.75$  and  $2.64 \pm 0.64$  ng/ml respectively, ( $P = NS$ )] or sTNFR1 [with  $3.3 \pm 1.3$ ,  $2.9 \pm 0.8$  and  $2.14 \pm 0.25$  ng/ml, respectively ( $P = NS$ )]. Finally, there were no differences between the TNFR1 36 A/A and TNFR2 676 T/T wild-type haplotypes ( $n = 6$ ) and all other haplotypes presenting at least one mutation ( $n = 52$ ) for serum levels of sTNFR1 ( $3.8 \pm 1.4$  vs.  $3.0 \pm 1.1$  ng/ml,  $P = 0.21$ ) or sTNFR2 ( $3.1 \pm 2.8$  vs.  $3.1 \pm 1.4$  ng/ml,  $P = 0.97$ ).

**Table 3 Serum levels of sTNFR1 and sTNFR2 in each group of patients**

Serum level (ng/ml)	Healthy controls (n=90)	Cirrhosis (n=45)	AAH (n=58)
sTNFR1	$1.17 \pm 0.27^{**}$	$2.8 \pm 1.7^{***}$	$3.07 \pm 1.14^{***}$
sTNFR2	$1.91 \pm 0.54^{**}$	$3.10 \pm 1.03^{***}$	$3.6 \pm 1.02^{***}$

TNFR1, tumour necrosis factor receptor type 1; TNFR2, tumour necrosis factor receptor type 2.  
 $*P = 0.42$ .  
 $**P < 0.001$ .  
 $***P < 0.05$ .

**Table 4 TNFR1 A36G polymorphisms and serum levels of TNFRs1 and TNFRs2**

Group	TNFR1 A36G genotypes	n (%)	sTNFR1 (ng/ml)	sTNFR2 (ng/ml)
AAH	A/A	16 (28)	$3.07 \pm 1.08$	$2.8 \pm 1.7$
	A/G	30 (52)	$3.2 \pm 1.2$	$2.7 \pm 1.7$
	G/G	12 (21)	$2.5 \pm 0.8$	$2.4 \pm 1.9$
Cirrhosis, no AAH	A/A	11 (25)	$2.35 \pm 0.57$	$2.53 \pm 1.94$
	A/G	25 (57)	$2.55 \pm 1.7$	$2.80 \pm 1.42$
	G/G	8 (18)	$3.85 \pm 2.07$	$3.75 \pm 1.21$
Healthy controls	A/A	24 (27)	$1.14 \pm 0.26$	$1.87 \pm 0.61$
	A/G	53 (59)	$1.2 \pm 0.26$	$1.97 \pm 0.58$
	G/G	13 (14)	$1.03 \pm 0.26$	$1.62 \pm 0.36$

sTNFR1 and sTNFR2 according to the A/A vs. A/G, A/A vs. G/G and A/G vs. G/G genotypes ( $P = NS$ ).  
 AAH, severe acute alcoholic hepatitis; TNFR1, tumour necrosis factor receptor type 1; TNFR2, tumour necrosis factor receptor type 2.

Within the cirrhosis group, the various TNFR1 36 A/A, A/G and G/G genotypes did not differ significantly in terms of serum levels of sTNFR1 ( $2.35 \pm 0.57$ ,  $2.55 \pm 1.7$  and  $3.85 \pm 2.07$  ng/ml, respectively) or sTNFR2 ( $2.53 \pm 1.94$ ,  $2.80 \pm 1.42$  and  $3.75 \pm 1.21$  ng/ml, respectively). Similarly, the TNFR2 676 T/T, T/G and G/G genotypes did not display significantly different serum levels of sTNFR2 ( $2.82 \pm 1.8$ ,  $2.98 \pm 1.6$  and  $1.76 \pm 1.6$  pg/ml, respectively) or sTNFR1 ( $2.7 \pm 1.2$ ,  $3.17 \pm 2.6$  and  $1.9 \pm 0.33$  ng/ml, respectively). Within the healthy control group, the TNFR1 36 A/A, A/G and G/G genotypes were not associated with significantly differing serum levels of sTNFR1 ( $1.14 \pm 0.26$ ,  $1.2 \pm 0.26$  and  $1.03 \pm 0.26$  ng/ml, respectively) or sTNFR2 ( $1.87 \pm 0.61$ ,

**Table 5 TNFR2 T676C polymorphism and serum levels of sTNFR1 and sTNFR2**

Group	TNFR2 T676C genotypes	n (%)	sTNFR1 (ng/ml)	sTNFR2 (ng/ml)
AAH	T/T	36 (62)	3.3 ± 1.3	3.7 ± 1.14
	T/G	18 (31)	2.9 ± 0.8	3.35 ± 0.75
	G/G	4 (7)	2.14 ± 0.25	2.64 ± 0.64
Cirrhosis, no AAH	T/T	30 (68)	2.7 ± 1.2	2.82 ± 1.8
	T/G	11 (25)	3.17 ± 2.6	2.98 ± 1.6
	G/G	3 (7)	1.9 ± 0.33	1.76 ± 1.6
Healthy controls	T/T	59 (66)	1.19 ± 0.27	1.99 ± 0.63
	T/G	26 (29)	1.09 ± 0.23	1.72 ± 0.41
	G/G	5 (6)	1.01 ± 0.22	1.59 ± 0.33

sTNFR1 and sTNFR2 according to T/T vs. T/G, T/T vs. G/G and T/G vs. G/G genotypes ( $P=NS$ ).

AAH, severe acute alcoholic hepatitis; TNFR1, tumour necrosis factor receptor type 1; TNFR2, tumour necrosis factor receptor type 2.

1.97 ± 0.58 and 1.62 ± 0.36 ng/ml, respectively). Similarly, the TNFR2 676 T/T, T/G and G/G genotypes did not display significantly differing serum levels of sTNFR2 (1.99 ± 0.63, 1.72 ± 0.41 and 1.59 ± 0.33 ng/ml, respectively) or sTNFR1 (1.19 ± 0.27, 1.09 ± 0.23 and 1.01 ± 0.22 ng/ml, respectively).

When comparing the wild-type haplotypes (TNFR1 36 A/A and TNFR2 676 T/T) with the mutated haplotypes, the sTNFR1 levels were 3.8 ± 1.4 and 3 ± 1.1 ng/ml, respectively ( $P=0.21$ ), and the sTNFR2 levels were 3.12 ± 2.8 and 3.1 ± 1.4 ng/ml, respectively ( $P=0.97$ ).

#### TNFR1 36 and TNFR2 676 polymorphisms and patient survival in the AAH group

Within the AAH group, there were no significant differences between the TNFR1 36 A/A, A/G and G/G genotypes in terms of 6-month mortality [with 5 of 16 (31.25%), 9 of 30 (30%) and 5 of 12 (41.6%) deaths, respectively;  $P=NS$ ]. The same was true for the TNFR2 676 T/T, T/G and G/G genotypes [with 10 of 36 (27.8%), 7 of 18 (39%) and 2 of 4 (50%) deaths, respectively;  $P=NS$ ]. Similarly, mortality was similar in the wild-type haplotype group (TNFR1 36 A/A and TNFR2 676 T/T) and the mutant haplotype group [with 6-month mortality rates of 1 of 6 (16%) and 18 of 52 (34.62%), respectively;  $P=0.66$ ].

Conversely, for the 19 deceased patients in the AAH group, the respective frequencies of the TNFR1 36 A/A, A/G and G/G genotypes did not differ statistically [with 5 (26.3%), 9 (47.4%) and 5 (26.3%) deaths, respectively]. The same was true of the TNFR2 676 T/T, T/G and G/G genotypes [with 10 (52.6%), 7 (36.8%) and 2 (10.5%) deaths, respectively].

#### TNFR1 36 and TNFR2 676 polymorphisms and the response to corticotherapy in AAH patients

In the AAH patient group, the response to corticosteroid treatment (evaluated as a drop in total bilirubin at day 7)

was not influenced by the TNFR1 36 A/A, A/G and G/G genotypes [with 13 of 16 (81.3%), 26 of 30 (86.7%) and 8 of 12 (66.7%) cases of lowered bilirubin, respectively] or the TNFR2 676 T/T, T/G and G/G genotypes [with 29 of 36 (80.56%), 15 of 18 (83.3%) and 3 of 4 (75%) cases, respectively].

Likewise, for the wild-type haplotypes (TNFR1 36 A/A and TNFR2 676 T/T) versus all the mutant haplotypes, the responses to corticosteroid treatment were similar [with respectively 5 of 6 (83.3%) and 42 of 52 (80.8%) cases of lowered bilirubin at day 7;  $P=1$ ].

#### Power analysis

For TNFR1 + 36 A/G, power analyses were at 0.068 and 0.140 when AAH group was compared with cirrhose and control groups, respectively. For TNFR2 + 676 T/G, power analyses were at 0.084 and 0.065 when AAH group was compared with cirrhose and control groups, respectively.

#### Discussion

Several studies have described high levels of TNF- $\alpha$  in patients with severe AAH [1–3]. The action of this strongly proinflammatory cytokine is exerted through the TNFR1 and TNFR2 hepatocyte membrane receptors, whose extracellular domains can be cleaved to generate soluble receptors, sTNFR1 and sTNFR2. The role of these soluble receptors is subject to debate, but may reflect activation of the membrane receptors [10] and modulation of circulating TNF- $\alpha$  levels [28]. In this study, we observed elevated serum levels of sTNFR1 and sTNFR2, with the highest concentrations in patients with histologically confirmed AAH, relative to non-AAH cirrhosis patients and healthy controls. These results confirm the findings of Naveau *et al.* [10], who reported higher sTNFR1 and sTNFR2 levels in 24 severe AAH patients than in patients with less severe ALD. In similar patients, Spahr *et al.* [13] also described elevated sTNFR1 levels. Finally, Auguet *et al.* [12] recently reported increased sTNFR1 and sTNFR2 levels in non-AAH cirrhotic patients, relative to noncirrhotic individuals and healthy controls. The increases in sTNFR1 observed in this study were similar to those reported in the literature for both AAH [10] and non-AAH cirrhotic patients [10,13]. In contrast, our results differ in two respects: first, sTNFR2 was significantly elevated, but less so (for both AAH and non-AAH cirrhotic patients) than in an earlier series [10]. In fact, in this latter study, sTNFR2 levels were higher than sTNFR1 levels, with a significantly lower sTNFR2/sTNFR1 ratio in AAH patients than in all the control groups, whereas we observed (in a larger study population) similar sTNFR2/sTNFR1 ratios in the AAH group and the two control groups (data not shown). Our findings do not support the use of this ratio as a diagnostic

criterion for AAH in alcoholic cirrhosis patients, as has been suggested [10]. Second, our series featured similar serum levels of sTNFR1 and sTNFR2 for both deceased patients and survivors in the AAH group – a result which does not confirm the prognostic nature of high sTNFR1 for 3-month mortality reported in one study [13].

The objective of this study was to test the involvement of TNFR1 and TNFR2 gene polymorphisms in the biochemical and clinical features of patients with severe AAH. The triggering factors for severe AAH are not well known because with similar durations and quantities of alcohol intake, not all patients will develop the disease. The hypothesis of genetic susceptibility has already been advanced for less severe forms of ALD [29], with studies having shown a higher frequency of late-stage ALD in monozygotic twins than in dizygotic twins [30]. In an earlier publication, we reported for the first time the influence of gene polymorphism in the –308 position of the TNF- $\alpha$  promoter region, with a lower frequency of the A allele in patients with severe AAH [14]. This polymorphism did not affect serum TNF- $\alpha$  levels, possibly a representative marker of susceptibility to AAH. In contrast, this study did not show any difference between the AAH and the two control groups in terms of the frequencies of the TNFR1 A36G and TNFR2 T676G genotypes. The allelic frequencies for A and G (TNFR1) and T and G (TNFR2) were similar to literature values for the UK [21] and Canada [24]. For the TNFR1 A36G and TNFR2 T676G genotypes, we found a few minor biochemical differences (PT, albumin and alkaline phosphatases) in AAH patients, but did not note any significant differences in terms of the clinical signs and the other biochemical results, notably serum levels of sTNFR1 and sTNFR2. Overall, the observed genotypes did not have any influence on the prognostic disease scores (such as the Maddrey and Child–Pugh scores), the response to corticotherapy and 6-month survival. We are aware of a lack of statistical power because of the size effect of our population. Therefore, we cannot rule out the fact that the lack of association might be because of the small cohort and subsequently to the increase of type II error. We are not aware of comparable results in the literature, as the TNFR1 A36G and TNFR2 T676G gene polymorphisms have hardly been studied in patients with severe AAH. Only Machado *et al.* [26] have reported the lack of effect of another SNP, TNFR2 T578G in noncomparable patients with less severe ALD without AAH.

In conclusion, our study did not support an association between the TNFR1 A36G and TNFR2 T676G gene polymorphisms and severe AAH.

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