Antibodies to hepatitis B virus surface antigen and interleukin 12 and interleukin 18 gene polymorphisms in hemodialysis patients

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Abstract

Background: The interleukin (IL)18 rs360719 CC genotype is associated with the development of antibodies to hepatitis B virus surface antigen (anti-HBs) in hemodialysis (HD) patients. IL18 shares biological properties with IL12 in promoting the T-hepler 1 (Th1) system. We studied whether polymorphisms in the IL12A 3' untranslated region (UTR) and IL12B 3'UTR may contribute to anti-HBs development (titre ≥ 10 IU/L) in HD patients either individually or jointly with the IL18 polymorphism.

Methods: In 518 HD patients and 240 controls the IL12A rs568408 3'UTR G > A polymorphism was genotyped by high-resolution melting curve analysis. Polymerase chain reaction restriction fragment length polymorphism was used to detect the IL12B rs3212227 3'UTR A > C and IL18 -1297 T > C rs360719 polymorphisms. The associations between the IL12A, IL12B and IL18 genotypes and the risk of impaired anti-HBs development were estimated by computing the odds ratios and their 95% confidence intervals using logistic regression analysis.

Results: In the logistic regression analysis, the higher frequency of rs360719 CC individually (2.9% in 207 patients without anti-HBs development vs 8.0% in 311 patients with anti-HBs development, p = 0.009) and of rs360719 CC combined with rs568408 GG (p = 0.048), rs568408 GA (p = 0.035), rs568408 GG/AA (p = 0.034) or rs3212227 AA (p = 0.046) was associated with an increased chance for the development of anti-HBs in HD patients. Patients bearing both rs568408 AA and rs360719 TT had a 10.9-fold or 8.9-fold lower chance, respectively, to develop anti-HBs compared with those carrying any other genotype (p = 0.005) or those who had both wild-type rs568408 GG and rs360719 TT (p = 0.011). Carriers of both rs3212227 CC and rs360719 TT had a 4.6-fold lower chance for anti-HBs development than carriers of any other genotype (p = 0.042).

Conclusion: Development of anti-HBs in HD patients is associated with gene polymorphisms of interleukins involved in the Th1 system.

Keywords: Antibodies to surface antigen of hepatitis B virus, Gene polymorphisms, Hemodialysis, Interleukin 12, Interleukin 18

Background

Chronic kidney disease patients on intermittent hemodialysis (HD) have been known to exhibit impaired immune system function with regards to the formation of antibodies against hepatitis B virus surface antigen (anti-HBs). Cytokines, among them interleukin (IL) 12 and IL18, play a key role in the regulation of hepatitis B virus (HBV) clearance and the immune response to HBV antigens during spontaneous natural infection [1-4] or planned vaccination [5-9].

IL-12 is a heterodimeric cytokine formed by a 35,000 dalton (Da) light chain (known as p35) and a 40,000 Da heavy chain (known as p40). The subunits p35 and p40 of IL12 are encoded by IL12A and IL12B, respectively, which are located on separate chromosomes (3p12-q13.2 and 5q31-33). IL-18 is an 18,300 Da cytokine. The human IL-18 gene is located on chromosome 11q22.2_q22.3. IL-12 shares biological properties with...
IL-18, known as an interferon (IFN) -gamma inducing factor [10-13]. In mice, IL-12 p40 and IL-18 acted in concert in a poxvirus infection [14]. In other studies using IL12 p40−/− or IL18−/− mice, only IL-12 p40 or IL-18 was important for defense against human viruses adapted to the mouse [15,16]. In HD patients, the IL18 -1297CC rs360719 genotype, attributed to increased IL-18 secretion [17], was recently connected with the development of anti-HBs [18]. It is not known whether the IL18 genotype is concomitantly associated with the IL18 genotype in the development of anti-HBs.

The aim of our study was to determine whether polymorphisms in IL12A and IL12B may individually or jointly with the IL18 polymorphism contribute to anti-HBs development in HD patients. We have demonstrated that rs360719 CC individually and rs360719 CC combined with rs568408 GG, rs568408 GA, rs568408 GG/AA or rs3212227 AA are associated with an increased chance of developing anti-HBs in HD patients, whereas combined rs568408 AA and rs360719 TT or combined rs3212227 CC and rs360719 TC are associated with a lower chance of anti-HBs development. In HD the patients development of anti-HBs has been shown to be associated with gene polymorphisms of IL-18 and IL-12B.

Methods

Patients and controls

Studies were carried out in HD patients treated in 20 dialysis centers of the Wielkopolska region of Poland between February 11, 2009 and August 01, 2011. All patients with negative HBV seromarkers were vaccinated against HBV according to the standard rules for HD patients (4 vaccine doses of 40 µg each given at 0–1–2–6 months) [19]. An anti-HBs titre was checked after 4–8 weeks from the last vaccine dose. An anti-HBs titre > 10 IU/L is assumed to be protective in vaccinated patients [20]. When an anti-HBs titre remained below 10 IU/L, vaccination against HBV was repeated. Due to fluctuations of anti-HBs in HD patients, blood testing for anti-HBs was repeated on a mandatory basis every 6 months to determine if vaccine booster doses were required.

Patients enrolled to the study had to fulfill the following criteria:

1. treatment with intermittent HD due to end-stage renal disease,
2. no signs and symptoms of acute infection with blood-borne viruses,
3. known anti-HBs titre (all available results of each patient were analyzed),
4. in patients without serological signs of HBV transmission, having an anti-HBs titre below 10 IU/L, two full vaccination series against HBV (4 doses of 40 µg each given at 0–1–2–6 months) had to be given or equivalent vaccine dosage had to be applied and the patients’ anti-HBs titre had to be determined 4–8 weeks from the last vaccine dose,
5. from patients who disclosed a genetic relationship only one person could participate in the study,
6. written consent to participate in the study.

A response to HBsAg after vaccination or natural HBV transmission was considered to be positive when an anti-HBs titre exceeded 10 IU/L.

The inclusion criteria were fulfilled by 518 HD patients. These patients were divided into two groups dependent on anti-HBs development. Responders developed anti-HBs, whereas non-responders did not develop anti-HBs. Responders were the reference group for non-responders.

Group I (HBsAg non-responders, n = 207) included HD patients who did not develop an anti-HBs titre > 10 IU/L in response to HBsAg from the HBV vaccine [patients with negative total antibodies to HBV core antigen (anti-HBc), n = 177] or in response to HBsAg transmitted during natural HBV infection (patients with total anti-HBc positive, n = 30). The available medical documents for these patients did not reveal any anti-HBs > 10 IU/L.

Group II (HBsAg responders, n = 311) consisted of HD patients who developed an anti-HBs titre > 10 IU/L as a result of vaccination (patients with total anti-HBc negative, n = 213) or as a result of HBV transmission (patients with total anti-HBc positive, n = 98). In some patients with a long course of renal disease, a history of vaccination effectiveness revealed periods with or without anti-HBs > 10 IU/L. If a patient had anti-HBs > 10 IU/L in the past but lost it during the course of renal disease, she/he was considered as constitutionally able to respond for HBsAg and was included into group II.

Registered blood donors from the Wielkopolska region of Poland (n = 240), qualified for blood donation according to the criteria of Polish Ministry of Health [21], served as controls for HD patients. The control persons had serum alanine aminotransferase activity not higher than 2 times the upper normal limit of the applied laboratory method. All controls showed negative blood testing for HBsAg and HBV DNA as well as for seromarkers of infection with the hepatitis C virus. Unfortunately, the vaccination rate against HBV and an anti-HBs titre were not known in these healthy individuals.

Genotype analysis for rs568408 3’UTR G>A in IL12A, rs3212227 3’UTR A>C in IL12B and -1297 C>T rs360719 in IL18 was done in all HD patients and controls.
Laboratory methods
HBsAg and anti-HBc were determined by Microparticle Enzyme Immunoassay (MEIA) technology (AxSYM, Abbott Laboratories, Abbott Park, USA). MEIA technology (ABBOTT, Wiesbaden, Germany) was also used for detection of anti-HBs and antibodies to hepatitis C virus (anti-HCV). HBV DNA was determined using a qualitative test COBAS AMPLICOR HBV MONITOR; HCV RNA was tested using COBAS AMPLICOR Hepatitis C Virus Test, version 2.0 (both Roche Diagnostics Ltd., Rotkreutz, Switzerland). Serum activities of liver enzymes were determined by routine laboratory methods.

IL12A, IL12B and IL18 genotyping
DNA was isolated from peripheral leukocytes using a standard salting out procedure. The IL12A 3’UTR G>A (rs568408) DNA fragments were amplified using primers 5’ ATGAGGAAAATTTGA TAGGATG 3’ and 5’TTCCTCCTCCTTAGCAATTCATTCC 3’. This polymorphism was then genotyped by high-resolution melting curve analysis (HRM) using the Light Cycler 480 system (Roche Diagnostics, Mannheim, Germany).

Identification of the IL12B 3’UTR A>C (rs3212227) and IL-18 -1297 T>C (rs360719) polymorphic variants was carried out by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). PCR for IL12B 3’UTR A>C (rs3212227) was conducted using the primer pair 5’ TTAAGACACAACCGGAATAGAC 3’and 5’ TGCTTTATCAACACCACCTCC 3’. The PCR-amplified fragments of IL12B that were 557 bp in length were isolated and digested with the endonuclease TaqI (T/CGA) (New England Biolabs, Ipswich, USA). The IL12B 3’UTR A allele remained uncut whereas the IL12B 3’UTR C allele was cleaved into 454 bp and 103 bp fragments. PCR for IL-18 -1297 T>C (rs360719) was conducted using the primer pair 5’ CAACAGT GATTACAAGGAAAGT 3’ and 5’ TAAATGGGTTAG GAATAAGTGA 3’. The PCR-amplified fragments of IL18 that were 474 bp in length were digested with endonuclease NlaIII (CATG/) (New England Biolabs, Ipswich, USA). The IL-18 C allele remained uncut, whereas the IL-18 T allele was cleaved into 295 bp and 179 bp fragments. DNA digestion products for the IL12B 3’UTR A>C and IL-18 -1297 T>C polymorphisms were separated by electrophoresis on 2% agarose gel and visualized by ethidium bromide staining. The PCR-RFLP analysis was repeated for all patient and control samples.

For quality control of the tested polymorphisms, approximately 10% of the randomly chosen samples were re-genotyped using commercial sequencing.

Statistical methods
Differences in the distributions of demographic characteristics and selected variables between the examined groups were analyzed. The normality of distribution of variables was checked by the Shapiro-Wilk test. Descriptive statistics are presented as percentage for categorical variables, as mean with one standard deviation for normally distributed continuous variables or as median with range for not normally distributed continuous variables. The prevalence of variables was assessed by the chi square ($\chi^2$) test or Yates’ test, as appropriate. Results were compared using Student’s t-test for non-paired data if distribution of variables was normal or the Mann–Whitney U-test for other than normal distributions.

Hardy-Weinberg equilibrium was tested by a goodness-of-fit m2 test to compare the observed genotype frequencies to the expected ones. Power analysis was conducted employing the Fisher exact test, which was available at an on-line internet service, http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowersampleSize.

The associations between the IL12A, IL12B and IL18 genotypes and risk of impaired anti-HBs development were estimated by computing the odds ratios (OR) and their 95% confidence intervals (95% CI) using logistic regression analysis. To address the possibility of a gene–gene interaction effect between analyzed polymorphisms, a Multifactor Dimensionality Reduction (MDR) approach (MDR version 2.0 beta 5) was used [22]. Values of $P < 0.05$ were judged to be significant.

Ethical issues
This study was approved by the Institutional Review Board of Poznań University of Medical Sciences, Poland.

Results
The selected demographic, clinical and laboratory data of groups I (non-responders) and II (responders) are shown in Table 1. All patients were Caucasian. The patients in group I were significantly older and had shorter duration of renal replacement therapy (RRT). Among the 4 main causes of end-stage renal disease in groups I and II diabetic nephropathy was the most frequent with the least frequent being chronic glomerulonephritis in group I and chronic tubulointerstitial nephritis in group II. Significant differences in HBV seromarkers between groups resulted from categorization of patients to groups I or II.

There was no significant deviation from Hardy-Weinberg equilibrium in the genotype frequencies in HD patients of groups I and II (Table 2), and in controls (Table 3).

The logistic regression analysis (Table 2), performed in HD patients with a titre of anti-HBs ≤ 10 UI/L (Group I) or > 10 UI/L (Group II), revealed that a lower frequency of the rs360719 CC genotype was individually associated with a significantly increased risk of immune non-responsiveness
to HBsAg. The rs360719 CC variant was associated with a
3.13-fold increased chance to develop anti-HBs in HD
patients \( (P = 0.009) \). The logistic regression analysis
(Table 3), performed in HD patients with a titre of anti-HBs
\( \leq 10 \) IU/L (Group I) and controls, also revealed that a lower
frequency of the rs360719 CC variant was associated with a
significantly \( (P = 0.006) \) increased risk of immune non-
responsiveness to HBsAg compared with the rs360719 TT
variant.

Selected combined or dichotomized effects of the IL12A
rs568408 3’UTR G > A, IL12B rs3212227 3’UTR A > C
and -1297 T > C (rs360719) IL-18 variants on the develop-
ment of anti-HBs in HD patients are shown in Tables 4
and 5, respectively. A higher frequency of rs360719 CC
combined with rs568408 GG, rs568408 GA or rs568408
GG/AA was associated in HD patients with a significantly
higher chance to develop anti-HBs \( (P = 0.048, P = 0.035
\) and \( P = 0.034, \) respectively) compared to HD patients hav-
ing both wild-type genotypes \( (rs568408 \text{ GG and rs360719}
\text{ TT}) \). A higher frequency of 360719 CC combined with
rs3212227 AA was also associated with anti-HBs develop-
ment in HD patients \( (P = 0.046) \) compared to HD patients
bearing both rs3212227 AA and rs360719 TT. Combined
rs568408 AA and rs360719 TT were associated with an
8.94-fold increased risk of non-responsiveness \( (anti-
HBs<10 \) IU/L \( (P = 0.011) \) compared to the combined
effects of rs568408 GG and rs360719 TT (Table 4) and
with a 10.85-fold elevated risk of non-responsiveness
\( (P = 0.005) \) compared to all other genotypes (Table 5).
Combined rs3212227 CC and rs360719 TC were
associated with a 4.61-fold increased risk of non-
responsiveness \( (P = 0.042) \) compared to all other geno-
types (Table 5). There were no significant effects of having
the combined rs568408 and rs3212227 variants.

MDR approach revealed a borderline gene-gene inter-
action effect between the analyzed polymorphic variants
of IL12A, IL12B and IL18 in HD patients of both groups
(testing balance accuracy = 0.556, \( p = 0.094 \)).

Discussion

Vaccination against HBV resulting in the formation of
an anti-HBs titre conferring protection (over 10 IU/L
[20]) was reported in only 54% – 86% of HD patients
using a recombinant vaccine [23-26]. HD patients that

| Parameter | All patients N = 518 | Group I n = 207 | Group II n = 311 | \( P \) value for analysis between groups I and II |
|-----------|---------------------|----------------|----------------|-----------------------------------|
| Men, n (% of all) | 290 (56.0) | 110 (53.1) | 180 (57.9) | 0.320 |
| Age, years | 62.5 ± 15.5 | 64.8 ± 14.9 | 60.9 ± 15.8 | 0.004 |
| RRT duration, years | 1.82 (0.002 – 26.1) | 1.24 (0.04 – 23.8) | 2.71 (0.002 – 26.1) | 0.001 |
| Diabetic nephropathy, n (% of all) | 141 (27.2) | 72 (34.8) | 69 (22.2) | 0.002 |
| Chronic glomerulonephritis, n (% of all) | 86 (16.6) | 22 (10.6) | 64 (20.6) | 0.003 |
| Hypertensive nephropathy, n (% of all) | 84 (16.2) | 34 (16.4) | 50 (16.1) | 1.000 |
| Chronic tubulointerstitial nephritis, n (% of all) | 60 (11.6) | 24 (11.6) | 36 (11.6) | 1.000 |
| History of acute hepatitis, n (% of all) | 23 (4.4) | 6 (2.9) | 17 (5.5) | 0.195 |
| Positive HBsAg, n (% of all) | 16 (3.1) | 13 (6.3) | 3 (1.0) | 0.001 |
| Positive HBV DNA, n (% of all HBsAg positive) | 16 (100.0)* | 13 (100.0) | 3 (100.0) | 1.000 |
| Positive anti-Hbc, n (% of all) | 128 (24.7) | 30 (14.5) | 98 (31.5) | < 0.0001 |
| Isolated positive anti-Hbc, n (% of all anti-Hbc positive) | 17 (13.3) | 17 (56.7) | 0 (0.0) | < 0.0001 |
| Full vaccination series against HBV with developed anti-Hbs titre > 10 IU/L in anti-Hbc negative patients, n (% of all anti-Hbc negative patients) | 213 (54.6) | 0 (0.0) | 213 (100.0) | < 0.0001 |
| Positive anti-HcV, n (% of all) | 55 (10.6) | 20 (9.7) | 35 (11.3) | 0.663 |
| Positive HCV RNA (n, % of all examined anti-HCV positive) | 32 (58.2) | 9 (45.0) | 23 (65.7) | 0.163 |
| ALT (U/L) | 13 (4 309) | 14 (4 621) | 13 (2 209) | 0.392 |
| AST (U/L) | 14 (4 177) | 14 (5 97) | 145 (4 177) | 0.570 |
| GGT (U/L) | 27 (0 – 498) | 28 (5 – 308) | 26 (0 – 498) | 0.757 |

* HBsAg was checked in two HBV DNA positive patients, being on the transplant waiting list, and the results were negative. HBV viral load was determined in three patients and varied from 4,210 to 1,19E + 09 copies/mL.

Data are expressed as mean ± standard deviation or median and range.

Significant results are indicated using bold font.

Abbreviations: ALT – alanine aminotransferase, anti-Hbc – antibodies to core antigen of hepatitis B virus, anti-HBs – antibodies to surface antigen of hepatitis B virus, anti-HCV – antibodies to hepatitis C virus, AST – aspartate aminotransferase, GGT – gamma-glutamyltranspeptidase, HBsAg – antigen e of hepatitis B virus, HBV – hepatitis B virus, HBV DNA – deoxyribonucleic acid of hepatitis B virus, HCV RNA – ribonucleic acid of hepatitis C virus, RRT – renal replacement therapy.
do not respond to vaccination are susceptible to HBV infection. Natural HBV transmission, if it does not lead to anti-HBs development, results in:

1. HBsAg carrier status, which is usually associated with persistent HBV replication (in this study HBV DNA was detected in all HBsAg positive patients) and infectivity to other persons,
2. occurrence of isolated anti-HBc positivity (anti-HBc positive persons are both HBsAg and anti-HBs negative), which is associated in approximately 8% of cases with HBV DNA detectable in the blood [27].

Moreover, an anti-HBs titre > 10 IU/L in HD patients is not always protective against HBV infection and sero-conversions to anti-HBc positivity, also without clinical signs of disease, may occur [28].

Prevalence of HBsAg carrier status or isolated anti-HBc positivity in HD patients varies between individual HD facilities. As shown in this study, HBsAg carriers amounted for 3.1% of all HD patients and isolated anti-HBc positivity occurred in 13.3% of the anti-HBc positive HD patients. As such frequencies are in the medium range [26,29-31], these results indicate thousands of affected HD people worldwide.

The reasons of non-responsiveness to HBsAg are not fully understood. It has been shown that effective seroconversion after vaccination of HD patients depends on age, body mass, serum albumin concentration, type of dialyzer, duration of RRT, and underlying kidney disease [18,32-36]. Such risk factors of non-responsiveness as older age, shorter RRT duration and diabetic nephropathy were also present in the examined non-responders compared to responders.

Genetic aspects of responsiveness to HBsAg were also taken into account, linking responsiveness with the human leukocyte antigen system [37,38]. More recently, IL genotypes (IL10, IL-18) were associated with anti-HBs development in response to HBsAg in HD patients [18,39].

IL12 and IL18 share biological properties through their synergism in the promotion of IFN-gamma production [11-13,40,41]. IL12, generated by macrophages, monocytes, dendritic cells, and B cells, is significantly elevated in HD patients [42-46], but despite this increase a constitutive IFN-gamma release by peripheral blood mononuclear cells (PBMCs) of HD patients may be undetectable [45]. Plasma levels of free IL18 are also increased in dialysis patients [47], but Th1 lymphocyte immunodeficiency was reported owing to the deficit of IFN-gamma [44,47]. Thereby, genes promoting expression of these IL may be helpful under specific clinical conditions. In experimental studies, mice immunized with an HBV DNA vaccine and the DNA fragments containing the p35 and p40 coding sequences of murine IL-12 demonstrated increased production of both immunoglobulin (Ig) M and IgG anti-HBs titers [5]. Mice vaccinated with a recombinant plasmid carrying the gene encoding HBsAg linked to a DNA segment encoding full-length murine IL18 revealed significant serum anti-HBs IgG response after two intramuscular injections [8].

These effects may be related to the indirect influence of

| Variable | Group I (n = 207) n (%) | Group II (n = 311) n (%) | OR (95 % CI) | P value | Genotype frequencies (n, %) expected by Hardy-Weinberg equilibrium, Group I; Group II |
|----------|------------------------|------------------------|-------------|--------|-----------------------------------------------|
| IL12A rs568408 | | | | | |
| GG | 157 (75.8) | 220 (70.7) | 1.00 | 152 (73.5); 222 (71.5) |
| GA | 41 (19.8) | 86 (27.7) | 0.67 (0.44 – 1.02) | 0.059 | 51 (24.4); 81 (26.1) |
| AA | 9 (4.4) | 5 (1.6) | 2.52 (0.83 – 7.70) | 0.094 | 4 (2.0); 2 (0.6) |
| GA/AA | 50 (24.2) | 91 (29.3) | 0.77 (0.51 – 1.15) | 0.199 | P = 0.213; P = 0.783 |
| IL12B rs3212227 | | | | | |
| AA | 129 (62.3) | 193 (62.1) | 1.00 | 130 (62.8); 198 (63.6) |
| AC | 70 (33.8) | 110 (35.4) | 0.95 (0.65 – 1.38) | 0.796 | 68 (32.9); 100 (32.3) |
| CC | 8 (3.9) | 8 (2.6) | 1.50 (0.55 – 4.10) | 0.433 | 9 (4.3); 3 (1.4) |
| AC/CC | 78 (37.7) | 118 (37.4) | 0.99 (0.69 – 1.42) | 0.952 | P = 0.783; P = 0.421 |
| IL18 rs360719 | | | | | |
| TT | 118 (57.0) | 160 (51.4) | 1.00 | 123 (59.4); 160 (51.4) |
| TC | 83 (40.1) | 126 (40.5) | 0.89 (0.62 – 1.29) | 0.544 | 73 (35.4); 126 (40.5) |
| CC | 6 (2.9) | 25 (8.0) | 0.32 (0.13 – 0.82) | 0.009 | 11 (5.4); 25 (8.0) |
| TC/CC | 89 (43.0) | 151 (48.6) | 0.80 (0.56 – 1.14) | 0.214 | P = 0.330; P = 1.000 |

A significant result (a sample power 72.7%) is indicated using bold font.
these cytokines on anti-HBs development, may be mediated through the observed increased INF-gamma production or both. In this study neither the IL12A rs568408 nor the IL12B rs3212227 polymorphic variants were individually associated with anti-HBs development in the examined HD patients as was shown for IL18 rs360719 CC. However, patients bearing the IL18 rs360719 CC genotype had a greater chance to develop anti-HBs also when occurring concomitantly with the IL12A rs568408 GG, IL12A rs568408 GA or rs3212227 AA polymorphic variants, but the IL12A rs568408 AA and IL12B rs3212227 CC variants occurring together with other than the CC variant of IL18 rs360719 were negatively associated with anti-HBs development.

Liu et al. [48] using http://pupasuite.bioinfo.cipf.es/, http://exon.cshl.edu/ESE/ and http://genes.mit.edu/burgelab/rescueese found that rs568408 may disrupt exonic splicing enhancers. They hypothesized that IL12 mRNA in individuals carrying the rs360719 C allele. As significantly elevated expression of IL12 in Epstein-Barr virus transformed human cell lines. Similar results were reported using peripheral lymphocytes: the expression of the 1159A allele was approximately 50% higher than that of the 1159 C allele [50]. On the other hand, Seegers et al. [51] correlated a TaqI polymorphism (C/C) in IL-12B p40 3’UTR with increased IL-12B p70 secretion by stimulated monocytes. Additionally, Yilmaz et al. [52] associated the 1188A/C polymorphism in the 3’UTR of the IL-12B gene with the expression of IL-12B mRNA and IL-12B secretion level from lipopolysaccharide (LPS) and purified protein derivative (PPD) stimulated PBMCs. Individuals +16974CC homozygous at the IL12B 3’UTR had significantly higher IL-12 secretion levels from LPS and PPD stimulated PBMCs than AC heterozygotes or AA homozygotes [52]. Sánchez et al. [17] found a significant increase in the relative expression of IL-18 mRNA in individuals carrying the rs360719 C allele. As

| Variable | Group I (n = 207) | OR (95 % CI) | P value |
|----------|------------------|--------------|---------|
| IL12A rs568408 | | | |
| GG | 157 (75.8) | 171 (71.3) | 1.00 |
| GA | 41 (19.8) | 63 (26.3) | 0.71 (0.45 – 1.11) | 0.131 |
| AA | 9 (4.4) | 6 (2.5) | 1.63 (0.57 – 4.71) | 0.357 |
| GA/AA | 50 (24.2) | 69 (28.8) | 0.79 (0.52 – 1.21) | 0.272 |

| Variable | Controls (n = 240) | OR (95 % CI) | P value |
|----------|------------------|--------------|---------|
| IL12B rs3212227 | | | |
| AA | 129 (62.3) | 151 (62.9) | 1.00 |
| AC | 70 (33.8) | 77 (32.1) | 1.06 (0.71 – 1.59) | 0.761 |
| CC | 8 (3.9) | 12 (5.0) | 0.78 (0.31 – 1.97) | 0.597 |
| AC/CC | 78 (37.7) | 89 (37.1) | 1.03 (0.70 – 1.51) | 0.896 |

| Variable | Controls (n = 240) | OR (95 % CI) | P value |
|----------|------------------|--------------|---------|
| IL18 rs360719 | | | |
| TT | 118 (57.0) | 121 (50.4) | 1.00 |
| TC | 83 (40.1) | 98 (40.8) | 0.87 (0.59 – 1.28) | 0.475 |
| CC | 6 (2.9) | 21 (8.8) | 0.29 (0.11 – 0.75) | 0.006 |
| TC/CC | 89 (43.0) | 119 (49.6) | 0.77 (0.53 – 1.11) | 0.163 |

A significant result (a sample power 78.1 %) is indicated using bold font.

Table 3. IL12 and IL18 polymorphisms in hemodialysis patients with a titre of antibodies to surface antigen of hepatitis B virus ≤ 10 IU/L (Group II) and controls

n (%)

| Genotype frequencies (n, %) expected by Hardy-Weinberg equilibrium |
|--------------------------|--------------------------|
| Controls | |
| n (%) | | | |
| n (%) | | | |
| n (%) | | | |
shown in this study, combined effects of IL-18 rs360719 CC and IL12B rs3212227 AA were positively associated with anti-HBs development. In this case, an elevated expression of IL18 could be accompanied by increased expression of IL12B. Thereby, our results confirm previous results indicating that IL12B rs3212227 AA is associated with elevated IL12 levels [49,50].

It has been discussed that genetic investigations could help in the development of new and improved vaccines against HBV and may eventually reduce the proportion of vaccine failures [53]. It has been shown that the use of exogenous IL12 as an adjuvant to augment anti-HBs development in response to vaccines against HBV [54,55] may help overcome at least some immunologic deficits of genetic origin. There are also experimental studies that take advantage of the recombinant plasmid carrying gene encoding the HBsAg linked to DNA segment encoding full-length murine IL18 [8]. We have suggested such a vaccine for non-responders bearing other IL18 polymorphic variants than −1297 CC rs360719 [18]. However, at present we are very careful in our conclusions, because associations that have been found between polymorphic variants of genes encoding cytokines may disturb the unique homeostasis between

Table 4 The selected combined effects of IL12 and IL18 polymorphisms in hemodialysis patients with a titre of antibodies to surface antigen of hepatitis B virus ≤ 10 UI/L (Group I) and > 10 UI/L (Group II)

| Variable | Group I (n = 207) n (%) | Group II (n = 311) n (%) | OR (95 % CI) | P value | Sample power (%) for significant differences |
|----------|------------------------|--------------------------|--------------|---------|----------------------------------------------|
| Combined effects of rs568408 and rs360719 | | | | | |
| rs568408 GG and rs360719 TT | 90 (43.5) | 115 (37.0) | 1.00 | | |
| rs568408 GG and rs360719 TC | 62 (30.0) | 88 (28.3) | 0.90 (0.59 – 1.38) | 0.629 | |
| rs568408 GG and rs360719 CC | 5 (2.4) | 17 (5.5) | 0.38 (0.13 – 1.06) | 0.048 | 45.7 |
| rs568408 GG and rs360719 TC/CC | 67 (32.4) | 105 (33.8) | 0.81 (0.54 – 1.23) | 0.331 | |
| rs568408 GA and rs360719 TT | 21 (10.1) | 44 (14.1) | 0.61 (0.34 – 1.10) | 0.095 | |
| rs568408 GA and rs360719 TC | 19 (9.2) | 34 (10.9) | 0.71 (0.38 – 1.34) | 0.287 | |
| rs568408 GA and rs360719 CC | 1 (0.48) | 8 (2.6) | 0.16 (0.02 – 1.32) | 0.035 | 45.3 |
| rs568408 GA and rs360719 TC/CC | 20 (9.7) | 42 (13.5) | 0.61 (0.33 – 1.11) | 0.099 | |
| rs568408 GA/AA and rs360719 TT | 28 (13.5) | 43 (13.8) | 0.83 (0.48 – 1.45) | 0.511 | |
| rs568408 GA/AA and rs360719 TC | 21 (10.1) | 38 (12.2) | 0.71 (0.39 – 1.29) | 0.252 | |
| rs568408 GA/AA and rs360719 CC | 1 (0.48) | 8 (2.6) | 0.16 (0.02 – 1.32) | 0.034 | 45.3 |
| Combined effects of rs3212227 and rs360719 | | | | | |
| rs3212227 AA and rs360719 TT | 7 (3.4) | 1 (0.32) | 8.94 (1.07 – 74.94) | 0.011 | 65.5 |
| rs3212227 AA and rs360719 TC | 2 (0.97) | 4 (1.3) | 0.64 (0.11 – 3.60) | 0.062 | |
| rs3212227 AA and rs360719 CC | 0 (0) | 0 (0) | - | - | |
| Significant results are indicated using bold font. | | | | | |
cytokines with opposing action. Thus, practical significance of the obtained results cannot yet be declared, although it does indicate a necessity and implications for further studies.

There are some limitations of our study which need to be addressed. The measurement of IL12A, IL12B, IL18 and INF-gamma serum concentrations, especially during vaccination or natural HBV transmission, was not possible due to a lack of patient material, although it could provide further information on mechanisms of anti-HBs formation in relation to the respective genotypes. An other limitation of our study is the moderate number of the examined patients, especially since genetic influences on responsiveness to HBsAg with anti-HBs development were shown in homozygotes carrying polymorphic variants of low frequency, which limits the statistical power of the study. Numerous analyses showed borderline significance and were not used to support our conclusion, as they may indicate the involvement of ILS of the Th1 pathway in the immune response to HBsAg. Therefore, large population-based studies are warranted to further elucidate the impact of the examined IL polymorphisms on anti-HBs development. Finally, we would like to stress that our results were obtained in Caucasian HD patients living in the Wielkopolska region of Poland. Prevalence of rare homozygotes of both IL12 and IL18 may vary in other ethnicities. The frequency of the IL12A rs568408 AA polymorphism in a Chinese control population was 1.2%, and 18.7% for IL12B rs3212227 CC [48], whereas in our Caucasian controls the respective frequencies were 2.5% and 5.0%. Prevalence of IL18 rs360719 CC was 5.7%, 5.5% and 6.1% in Spain, Italy and Argentina, respectively [17]. In controls from the South Moravia region (more proximal to Poland), the IL18 rs360719 CC frequency was 8.0% [56]; in our study this frequency was 8.8%. The ethnic differences in IL genotype prevalence may modulate the effect of ILS on the humoral and cellular immune response, but further investigations are needed for IL12 and IL18.

Conclusions

1. Polymorphisms in IL12A and IL12B may jointly with IL18 polymorphism contribute to anti-HBs development in HD patients.
2. In HD patients, the development of anti-HBs is associated with gene polymorphisms of ILS involved in the Th1 system.

Competing interests
The authors declare that they have no competing interests.

Table 5 Selected dichotomized effects of IL12A rs568408 and IL18 rs360719 in hemodialysis patients with a titre of antibodies to surface antigen of hepatitis B virus ≤ 10 UI/L (Group I) and > 10 UI/L (Group II)

| Variable | Group I (n = 207) n (%) | Group II (n = 311) n (%) | OR (95 % CI) | P value | Sample power (%) for significant differences |
|----------|-------------------------|-------------------------|--------------|---------|------------------------------------------|
| All other genotypes | 202 (97.6) | 294 (94.5) | 1.00 | | |
| rs568408 GG and rs360719 CC | 5 (2.4) | 17 (5.5) | 0.43 (0.15 – 1.18) | 0.080 | |
| All other genotypes | 185 (89.4) | 263 (84.6) | 1.00 | | |
| rs568408 GA/AA and rs360719 TC/CC | 22 (10.6) | 48 (15.4) | 0.65 (0.38 – 1.12) | 0.112 | |
| All other genotypes | 206 (99.5) | 303 (97.4) | 1.00 | | |
| rs568408 GA and rs360719 CC | 1 (0.48) | 8 (2.6) | 0.18 (0.02 – 1.49) | 0.052 | |
| rs568408 GA/AA and rs360719 CC | 1 (0.48) | 8 (2.6) | 0.18 (0.02 – 1.49) | 0.052 | |
| All other genotypes | 200 (96.6) | 310 (99.7) | 1.00 | | |
| rs568408 AA and rs360719 TT | 7 (3.4) | 1 (0.32) | 10.85 (1.32 – 89.30) | 0.005 | 75.2 |
| All other genotypes | 202 (97.6) | 293 (94.2) | 1.00 | | |
| rs3212227 AA and rs360719 CC | 5 (2.4) | 18 (5.8) | 0.40 (0.15 – 1.10) | 0.058 | |
| All other genotypes | 206 (99.5) | 304 (97.7) | 1.00 | | |
| rs3212227 AC and rs360719 CC | 1 (0.48) | 7 (2.3) | 0.21 (0.03 – 1.73) | 0.084 | |
| All other genotypes | 206 (99.5) | 304 (97.7) | 1.00 | | |
| rs3212227 AC/CC and rs360719 CC | 1 (0.48) | 7 (2.3) | 0.21 (0.03 – 1.73) | 0.084 | |
| All other genotypes | 201 (97.1) | 309 (99.4) | 1.00 | | |
| rs3212227 CC and rs360719 TC | 6 (2.9) | 2 (0.64) | 4.61 (0.92 – 23.16) | 0.042 | 52.6 |
| All other genotypes | 201 (97.1) | 309 (99.4) | 1.00 | | |
| rs3212227 CC and rs360719 TC/CC | 6 (2.9) | 2 (0.64) | 4.61 (0.92 – 23.16) | 0.042 | 52.6 |

Significant results are indicated using bold font.
Authors’ contributions
AEG gave a conception, participated in the design of the study, performed a clinical interpretation of the data and wrote the manuscript. PMW performed the statistical analysis and participated in its interpretation. AM performed MDR analysis and interpreted its results. PJP carried out the molecular genetic studies and participated in the study design. All authors read and approved the final manuscript.

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