Research Article

FAS and FASL Gene Polymorphisms Are Not Associated with Hepatitis B Virus Infection Based on a Case-Control Study in a Brazilian Population

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Objective. This study investigated the association of the single nucleotide polymorphisms (SNPs) in the FAS and FASL genes with the outcome of hepatitis B virus (HBV) infection. Methods. Blood samples were collected from 116 HBV-infected patients at the Hospital of the Santa Casa de Misericórdia Foundation (Belém, PA, Brazil). Seronegative individuals were used as controls. DNA samples were extracted from the leukocytes and assayed using the polymerase chain reaction (PCR) followed by RFLP analysis with restriction endonucleases. Results. The frequencies of the mutant genotypes for -670 FAS (GG), Ivs2nt-124 FASL (GG), Ivs3nt-169 FASL (ΔT/ΔT), and -844 FASL (TT) were higher in the HBV patients, and the FAS -1377 AA genotype was more frequent in the control group; however, the differences between the allele and genotype frequencies were not statistically significant. When the HBV patient population was divided into two groups (inactive carriers and active chronic hepatitis patients), the mutant genotypes were found to be more prevalent in the active chronic hepatitis group with respect to the FAS gene polymorphisms; however, this difference was not statistically significant. Conclusions. The results suggest that the polymorphisms in FAS and FASL genes are not associated with HBV infection or even with the natural history of the infection in the Brazilian Amazon region.

1. Introduction

The hepatitis B virus (HBV) is a member of the Hepadnaviridae family and the Orthohepadnavirus genus and shares structural and functional characteristics with other family members, such as tropism for hepatic cells, enveloped viral particles, an incomplete double-stranded DNA genome, and viral replication via reverse transcription [1].

In approximately 3–8% of adults infected with HBV, the immune defense system cannot destroy the infected hepatocytes and the inflammation process (hepatitis) persists. When the virus persists for more than 6 months, the infection is defined as chronic hepatitis and the chance of spontaneous healing is very low. When the HBV infection becomes chronic, it is a significant cause of hepatic cirrhosis and hepatocellular carcinoma (HCC) [2, 3].

In the mid-1990s, Suda and colleagues identified a molecule that initiates the process of programmed cell death (apoptosis). This new molecule was reported to bind to a cell membrane receptor (Fas) encoded by the FAS gene and was termed the Fas ligand (FasL) [4, 5].

The FAS gene is located on human chromosome 10q24.1, contains 9 exons and 8 introns, and encodes a 334-amino acid protein. The Fas receptor is expressed on the surface of several types of cells, such as lymphocytes, fibroblasts, epithelial cells, and some endothelial cells, and the peptide is responsible for triggering apoptosis in these cells [6].

The FASL gene is located on chromosome 1q23, is composed of 4 exons, and encodes a 281-amino acid protein. FasL was first described as a cytotoxic protein that is only expressed in activated T cells. However, it is now known that FasL is present in many other cell types in various...
organs, such as the brain, eyes, placenta and testicles. This protein has been associated with the mechanism of “immune privilege,” protecting the organs from inflammation through the activation of apoptosis [7, 8]. Furthermore, this ligand is a major mediator of the cytolytic action of CD8+ T lymphocytes and natural killer cells [9].

The association of the FAS and FASL gene polymorphisms with many types of carcinomas (prostate cancer, breast cancer, nonsmall cell lung cancer, renal cell carcinoma and epithelial ovarian cancer) has been reported over the last 5 years [10–20]. Additionally, these polymorphisms are associated with various other pathologies, including infectious diseases [21–24]. This study investigated whether the single nucleotide polymorphisms (SNPs) in the FAS gene are associated with HBV infection in various populations in the Brazilian Amazon region.

2. Materials and Methods

2.1. Study Population and Sample Collection. Following approval by the research ethics committee of the Santa Casa de Misericórdia do Pará Foundation, 116 hepatitis B–infected patients under treatment at a renowned hepatology clinic in Belém (PA, Brazil) were briefed about the project and selected for this study. The patients were divided into the following two groups: (A) inactive carriers, defined as HBeAg-negative and exhibiting a viral load lower than 2,000 IU/mL and normal levels of alanine aminotransferase (10 to 49 U/L); and (B) patients with chronic active hepatitis with or without cirrhosis, defined as HBeAg-negative or -positive and exhibiting a high viral load, a liver biopsy with inflammatory activity or fibrosis greater than or equal to 2 on the METAVIR scale, and high levels of alanine aminotransferase (>50 U/L). The control group consisted of 235 seronegative subjects. After the laboratory and clinical diagnoses, the individuals signed an informed consent form, the blood samples were collected in vacutainer tubes containing the anticoagulant K3-EDTA, and plasma and leukocyte samples were prepared. The samples were sent to the Virology Laboratory at the Institute of Biological Sciences of the Federal University of Para (Belém, Para, Brazil) and were stored at −20°C before use.

2.2. Polymorphism Analysis. Genomic DNA was extracted from the leukocytes using the Puregene kit (Gentra Systems, Inc., USA). The FAS and FASL gene polymorphisms were identified using PCR. The amplification was performed in a final volume of 30 μL containing 500 ng total DNA, 0.2 μM of each dNTP, 5 pmol/μL of each primer, 2.0 mM MgCl2, 50 mM KCl, 10 mM Tris-HCl (pH 8.3) and 1.0 U Taq DNA polymerase.

The primers used for the amplification of the FAS and FASLG promoter regions were as follows: 5′-CTA CCT AAG AGC TAT CTA CCG TCT-3′ (forward) and 5′-GGC TGT CCA TGT TGT GGC TGC-3′ (reverse) for the FAS-670 A>G polymorphism; 5′-TGT GTG CAC AAG GCT TGC GC-3′ (forward) and 5′-TGC ATC TGT CAT CAC ACT TAC CAC CA-3′ (reverse) for the FAS-1377 G>A polymorphism; 5′-GCA GTT CAG ACC TAC ATG ATG AT-3′ (forward) and 5′-CCA ATT CTC ACC TGT ACC TTC-3′ (reverse) for FASLG IVS2nt -124 A>G; 5′-AGG AAA GGA CTT CAA AGC CTA-3′ (forward) and 5′-TTG ATG CAT CAC AGA ATT TCG TC-3′ (reverse) for FASLG IVS3nt -169 T>A; and 5′-CAA TGA AAA TGA ACA CAT TG-3′ (forward) and 5′-CCC ACT TTA GAA ATT AGA TC-3′ (reverse) for the FASLG -844 C>T polymorphism. The amplification reactions were performed following previously described protocols [25, 26].

The PCR products were digested as described previously using five different restriction endonucleases, MvaI, BstUI, FokI, HincII, and DraIII, to distinguish the FAS-670 G, FAS-1377 G>A, FASLG IVS2nt-124 A>G, FASLG IVS3nt-169 T>A, and FASLG -844 C>T polymorphisms, respectively. The digestion products included three fragments (184, 47 and 101 bp) for the -670 G allele, two fragments (104 and 18 bp) for the -1377 G allele, two fragments (210, 29 bp) for the -124 G allele, two fragments (162 and 23 bp) for the -169 T allele and two fragments (66 and 19 bp) for the -844 T allele.

Both the PCR and RFLP products were visualized following electrophoresis (100 V/45 min) on 4% agarose gels containing 5 μL SYBR Safe DNA gel stain in 1x TAE buffer (the 40x TAE stock buffer contained 1.6 M Tris base, 0.8 M sodium acetate and 40 mM EDTA Na2 per 1L of deionized water) via transilluminlation using ultraviolet light.

2.3. Statistical Methods. The allele and genotype frequencies were obtained through direct counts, and a comparative analysis of the frequencies between the studied groups was performed using the G test. The association of the polymorphisms with the risk of HBV infection or chronic infection was estimated using multiple logistic regression. BioEstat v5.3 software was used to determine the significance level of P = 0.05 (5%) for all of the analyses [27].

3. Results

The genotype and allele distributions of the FAS and FASL gene polymorphisms are shown in Table 1. The genotype frequencies of the polymorphisms were consistent with Hardy-Weinberg equilibrium (patients P = 0.6424 and control P = 0.9074 for -670 FAS; patients P = 0.4476 and control P = 0.0978 for -1377 FAS; patients P = 0.6061 and control P = 0.7434 for -124 FASLG; patients P = 0.2084 and control P = 0.2838 for -169 FASLG; and patients P = 0.2026 and control P = 0.3474 for -844 FASLG). Comparison of the FAS and FASL gene polymorphism frequencies in the HBV patients and healthy controls did not reveal statistically significant differences.

The patient group was divided into two subgroups: (1) inactive carriers (IC), who demonstrated seroreactivity but no viral replication and (2) patients with chronic active hepatitis (CAH), including the clinical forms, who demonstrated liver cirrhosis and HCC. The genotypic frequencies of the patients according to their clinical profiles are presented in
Table 1: Comparison of genotype frequencies of the FAS and FASL polymorphisms among the HBV patients and healthy controls.

| Genotype profile | HBV patients (n = 116) n (%) | Healthy controls (n = 235) n (%) | Adjusted OR* | 95% CI | P value† |
|------------------|-----------------------------|---------------------------------|--------------|--------|---------|
| FAS-670 A>G      |                             |                                 |              |        |         |
| AA               | 25 (21.55)                  | 51 (21.70)                      |              |        |         |
| AG               | 55 (47.41)                  | 116 (49.36)                     |              |        |         |
| GG               | 36 (31.04)                  | 68 (28.94)                      |              |        |         |
| AG + GG          | 91 (78.45)                  | 184 (78.30)                     | 1.0427       | 0.60–1.81 | 0.9154 |
| FAS-1377 G>A     |                             |                                 |              |        |         |
| GG               | 94 (81.04)                  | 185 (78.73)                     |              |        |         |
| GA               | 20 (17.24)                  | 44 (18.72)                      |              |        |         |
| AA               | 02 (01.72)                  | 06 (02.55)                      |              |        |         |
| GA + AA          | 22 (18.96)                  | 50 (21.27)                      | 0.8623       | 0.49–1.53 | 0.8283 |
| Ivs2ntFASLG-124 A>G |                     |                                 |              |        |         |
| AA               | 100 (86.21)                 | 198 (84.26)                     |              |        |         |
| AG               | 15 (12.93)                  | 35 (14.89)                      |              |        |         |
| GG               | 01 (00.86)                  | 02 (00.85)                      |              |        |         |
| AG + GG          | 16 (13.79)                  | 37 (15.74)                      | 0.8724       | 0.45–1.68 | 0.8936 |
| Ivs3ntFASLG-169 T>ΔT |                     |                                 |              |        |         |
| TT               | 87 (75.00)                  | 177 (75.32)                     |              |        |         |
| T/ΔT             | 25 (21.55)                  | 56 (23.83)                      |              |        |         |
| ΔT/ΔT            | 04 (03.45)                  | 02 (00.85)                      |              |        |         |
| T/ΔT + ΔT/ΔT     | 29 (25.00)                  | 58 (24.68)                      | 0.9987       | 0.59–1.69 | 0.2403 |
| FASLG-844 C>T    |                             |                                 |              |        |         |
| CC               | 51 (43.96)                  | 99 (42.13)                      |              |        |         |
| CT               | 47 (40.52)                  | 112 (47.66)                     |              |        |         |
| TT               | 18 (15.52)                  | 24 (10.21)                      |              |        |         |
| CT + TT          | 65 (56.04)                  | 136 (57.87)                     | 0.9527       | 0.60–1.52 | 0.2513 |

* Multiple logistic regression; † Chi-squared test; ‡ G test.

Table 2. Both the IC and CAH patients demonstrated Hardy-Weinberg equilibrium. As shown in Table 2, the genotypic differences between the groups were not statistically significant for the FAS or FASL gene polymorphisms.

Finally, the frequency of combined genotypes of the FAS and FASL genes did not differ between the HBV patients and healthy controls (Tables 3 and 4).

4. Discussion

The occurrence of genetic variabilities, especially in genes encoding proteins involved in the immune system, is thought to play an important role in the regulation of the host immunity by regulating gene expression. We recently demonstrated a strong association of the FAS-670 A/G single nucleotide polymorphism with HTLV-1 infection as well as with the clinical evolution to TSP/HAM [26]. Therefore, considering that the physiopathology of hepatitis caused by HBV involves the apoptosis of the HBV-infected cells, we have investigated whether the SNPs in the FAS and FASL genes are associated with the risk for HBV infection and the status of the infected individual as an IC or CAH patient.

Previous studies have described an association between the FAS and FASL gene polymorphisms and infection with HTLV-1 and HSV-2 [26, 28, 29]. However, the data from other studies on HPV and Helicobacter pylori infection [28, 30, 31] are consistent with our data in that there was no evidence of an association between the allele and genotype frequencies of the FAS and FASL genes and the risk for HBV infection or the status of the infected individual as an IC or CAH patient (with or without cirrhosis), even when the combined genotype frequencies were estimated.

The FAS-670 A>G and FAS-1377 G>A polymorphisms occur in the physical location where the STAT1 and SPI transcriptional factors bind, respectively [32]. The FAS-670 GG and FAS-1377 AA genotypes reduce the binding of the transcription factors STAT1 and SPI and downregulate the expression of Fas [25, 33]. Our data suggests that the assumed low expression of Fas due to the presence of the FAS-670 GG and FAS-1377 AA genotypes may not be an important risk factor for HBV infection or the status of the infected individual as an IC or CAH patient because both genotypes demonstrated similar frequencies among the patients and healthy controls. However, our results do not correlate with
Table 2: Comparison of genotype frequencies of the FAS and FASL polymorphisms among the active chronic hepatitis patients and inactive carriers.

| Genotype profile | Active chronic (n = 89) n (%) | Inactive carrier (n = 27) n (%) | Adjusted OR* | 95% CI | P value‡ |
|------------------|-------------------------------|---------------------------------|--------------|-------|---------|
| FAS-670 A>G      |                               |                                 |              |       |         |
| AA               | 19 (21.35)                    | 06 (22.22)                      |              |       |         |
| AG               | 41 (46.07)                    | 14 (51.85)                      |              |       |         |
| GG               | 29 (32.58)                    | 07 (25.93)                      |              |       |         |
| AG + GG          | 70 (78.65)                    | 21 (77.78)                      | 0.9666       | 0.32–2.89 | 0.8003  |
| FAS-1377 G>A     |                               |                                 |              |       |         |
| GG               | 72 (80.90)                    | 22 (81.48)                      |              |       |         |
| GA               | 15 (16.85)                    | 05 (18.52)                      |              |       |         |
| AA               | 02 (02.25)                    | 0 (00.00)                       |              |       |         |
| GA + AA          | 17 (19.10)                    | 05 (18.52)                      | 1.0990       | 0.34–3.54 | 0.6368  |
| Ivs2ntFASLG-124 A>G |                           |                                 |              |       |         |
| AA               | 78 (87.64)                    | 22 (81.48)                      |              |       |         |
| AG               | 11 (12.36)                    | 04 (14.82)                      |              |       |         |
| GG               | 0 (00.00)                     | 01 (03.70)                      |              |       |         |
| AG + GG          | 11 (12.36)                    | 05 (18.52)                      | 0.5348       | 0.16–1.78 | 0.3328  |
| Ivs3ntFASLG-169 T>ΔT |                           |                                 |              |       |         |
| TT               | 69 (77.53)                    | 18 (66.67)                      |              |       |         |
| T/ΔT             | 17 (19.10)                    | 08 (29.63)                      |              |       |         |
| ΔT/ΔT            | 03 (03.70)                    | 01 (03.70)                      |              |       |         |
| T/ΔT + ΔT/ΔT     | 20 (22.47)                    | 09 (33.33)                      | 0.5594       | 0.21–1.49 | 0.5499  |
| FASLG-844 C>T   |                               |                                 |              |       |         |
| CC               | 38 (42.70)                    | 13 (48.15)                      |              |       |         |
| CT               | 39 (43.82)                    | 08 (29.63)                      |              |       |         |
| TT               | 12 (13.48)                    | 06 (22.22)                      |              |       |         |
| CT + TT          | 51 (57.70)                    | 14 (51.85)                      | 1.2228       | 0.49–3.03 | 0.3484  |

* Multiple logistic regression; ‡ G test.

Table 3: Combined genotype frequencies of the FAS polymorphisms among the HBV patients and healthy controls.

| Genotype profile | HBV patients (n = 116) n (%) | Healthy controls (n = 235) n (%) | P value‡ |
|------------------|-----------------------------|---------------------------------|---------|
| FAS-670 A>G      |                             |                                 |         |
| AA               | 25 (21.55)                  | 48 (20.42)                      | 0.5033  |
| AA               | 0 (00.00)                   | 03 (01.28)                      |         |
| AG               | 69 (59.48)                  | 137 (58.30)                     |         |
| AG + GG          | 22 (18.97)                  | 47 (20.00)                      |         |

‡ G test.

The data reported by Jung et al. [34], which demonstrate a protective effect of the FAS-1377 A > G polymorphism against HCC in HBV-infected patients due to a reduction in the rate of cell death induced by the Fas-Fasl system. In this study, only a small number of patients exhibited cirrhosis or hepatocellular carcinoma, invalidating any comparative analysis regarding this issue. Furthermore, the sample size and the difference in the ethnicity of the two populations in this study are important bias factors.

The FASLG-844 C>T polymorphism is located in the promoter region of the FASLG gene, and the basal expression of this gene is higher in cells carrying the C allele than in cells carrying the T allele [35]. Similar to the observations for the FAS gene polymorphisms, our results demonstrate that the presence of genotypes carrying the C or T alleles was not significantly different between the patients and controls, and there was no evidence that this polymorphism affects HBV infection. Similar results were observed for
the FASLG IVS2nt-124 A>G and FASLG IVS3nt-169 T/ΔT polymorphisms, and it is unknown if the presence of these polymorphisms affects FASL expression [17, 22, 36]. In conclusion, we believe that further studies are required, enrolling other ethnic human population groups as well as in vitro assays to reveal the apoptosis pathway, to determine the role of the FAS and FALG gene polymorphisms in HBV infection.

Conflict of Interests

The authors declare that they have no conflict of interests.

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Table 4: Combined genotype frequencies of the FASLG polymorphisms among the HBV patients and healthy controls.

| IVS2ntFASLG-124 A>G | Genotype profile | FASLG-844 C>T | HBV patients (n = 116) n (%) | Healthy controls (n = 235) n (%) | P value\(^a\) |
|---------------------|-----------------|-------------|--------------------------|-------------------------------|------------|
| AA                  | TT              | CC          | 31 (26.73)                | 60 (25.53)                    | 0.8477     |
| AA                  | TT              | CT + TT     | 42 (36.21)                | 84 (35.74)                    |            |
| AA                  | T/ΔT + ΔT/ΔT    | CC          | 16 (13.79)                | 35 (14.89)                    |            |
| AA                  | T/ΔT + ΔT/ΔT    | CT + TT     | 11 (09.48)                | 19 (08.08)                    |            |
| AG + GG             | TT              | CC          | 03 (02.59)                | 04 (01.71)                    |            |
| AG + GG             | TT              | CT + TT     | 11 (09.48)                | 29 (12.34)                    |            |
| AG + GG             | T/ΔT + ΔT/ΔT    | CC          | 01 (00.86)                | 0 (00.00)                     |            |
| AG + GG             | T/ΔT + ΔT/ΔT    | CT + TT     | 01 (00.86)                | 04 (01.71)                    |            |

\(^a\)G test.
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