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CB2-63 polymorphism and immune-mediated diseases associated with HCV chronic infection

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ABSTRACT

Aims: To evaluate whether CB2 variants are associated with the presence of immune-mediated disorders (IMDs) in patients with chronic HCV infection.
Methods: One hundred and sixty-eight anti-HCV/HCV-RNA-positive patients were enrolled, 81 with signs of IMDs and 87 without. In the IMDs group, 22 (27.2%) showed ANA positivity (titers ≥1:160), 3 (3.7%) SMA positivity (titers ≥1:160), 24 (29.6%) had cryoglobulinemia, 25 (30.9%) autoimmune thyroiditis, 4 (4.9%) psoriasis, 2 (2.5%) B-cell non-Hodgkin lymphoma and 1 (1.2%) autoimmune hemolytic anemia. All patients were screened for the CNR2 rs35761398 single nucleotide polymorphism using a TaqMan Assay.
Results: Compared with the 87 patients without IMDs, the 81 with IMDs were more frequently females (65% vs. 45%, $p = 0.01$), but no significant difference was found in the initial demographic, epidemiological, serological, biochemical or virological data. Instead, the prevalence of patients with the CB2-63 RR variant was significantly higher in the IMD than in the non-IMD group (49.4% vs. 24.1%, $p = 0.001$). A logistic regression analysis including the CB2-63 receptor (RR vs. QR or QQ), age and sex identified the CB2-63 RR as the only independent predictor of IMDs ($p = 0.005$).
Conclusions: The data suggest a significant, previously unknown, independent association between the CB2-63 RR variant and IMDs in anti-HCV-positive patients.
The study was approved by the Ethics Committee of the Azienda Ospedaliera Universitaria of the Second University of Naples (n° 214/2012).

1. Introduction

Hepatitis C virus (HCV) affects more than 170 million people worldwide and is a leading cause of liver cirrhosis and hepatocellular carcinoma [1,2]. The severity of chronic hepatitis C (CHC) varies among patients and over time in single patients, with a benign indolent course in the majority of cases, but in some cases with a rapid transition to the more severe stages of the illness, severe liver fibrosis, cirrhosis and hepatocellular carcinoma (HCC) [3–6].

Besides its role of etiologic agent of acute and chronic hepatitis C, HCV is a lymphotropic virus responsible for a polyclonal B-lymphocyte expansion leading to the development of extrahepatic manifestations [8–11] such as type II cryoglobulinemia [8,12,13] and some types of B-cell non-Hodgkin lymphoma such as lymphoplasmacytic lymphoma/immunocytoma or marginal-zone lymphomas [12,14–18]. In addition, chronic HCV infection is considered a trigger for immune-mediated disorders through a crossover immune response to self-antigens due to sequence similarities between viral proteins and self-proteins (molecular mimicry theory) or through the activation of autoreactive T-cells due to viral-induced local inflammation (bystander activation theory) [7]. In fact, chronic HCV infection has been associated

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with autoimmune diseases such as psoriasis, lichen planus, Sjogren's syndrome [14,19,20], autoimmune thyroiditis and with the presence of organ-specific circulating autoantibodies such as anti-thyroperoxidase and anti-thyroglobulin and non-organ-specific autoantibodies such as high-titer antinuclear antibody (ANA), anti-smooth muscle antibody (ASMA) and anti-liver/kidney microsome (anti-LKM) antibody [8–11].

The Cannabinoid (CB) receptors are seven-transmembrane-domain receptors of the G protein-coupled receptor superfamily that are activated by endogenous (endo) or exogenous (phyto and synthetic) cannabinoids. Two types of CB receptors have been described to date, type 1 (CB1) and type 2 (CB2): CB1 is predominantly expressed in the central nervous system but is also present in the lung, liver and kidney, and CB2 is expressed in the immune and immune-derived cells (T and B lymphocytes natural- killer cells, monocytes) and at high levels in Kupffer cells. A polymorphism at codon 63 of the Cannabinoid Receptor 2 gene (CNR2) leads to the substitution of glutamine, Gln (Q), with arginine, Arg (R), causing different polarizations of the protein. The CB2 variants affect differently the ability of the CB2 receptor to exert its inhibitory function on T lymphocytes. Specifically, *in-vitro* T lymphocytes from CB2-63 RR homozygotes showed an approximately two-fold reduction in the endocannabinoid-induced inhibition of proliferation compared to cells from CB2-63 QQ homozygotes.

Recent studies have shown an association between the rs35761398 polymorphism of CB2 and the clinical presentation of chronic hepatitis due to HCV or hepatitis B virus (HBV) infection [21–23].

The present paper analyzes the association of CB2 variants with immune-mediated disorders in 168 patients with chronic HCV infection.

2. Patients and methods

2.1. Patients

Four Liver Units in the Campania region (southern Italy) carried out the present study. These four centers have cooperated in several investigations using the same clinical approach and the same laboratory methods [24–26]. The senior investigators from the participating centers planned the study during a preliminary consensus meeting as prospective with progressive enrolment. Considering that 20–30% of anti-HCV-positive patients show signs of immune-mediated disorders (IMDs) [27], the period of enrolment lasted 12 months for patients with IMDs and 4 months for those without. All patients had been anti-HCV/HCV-RNA-positive for 18–36 months and were naïve for antiviral treatment at the time of enrolment, from September 2013 to August 2014 for those with IMDs and from September 2013 to December 2013 for those without. An anti-HCV/HCV-RNA-positive patient was considered to have an IMD if he/she showed at least one of the following: ANA positivity at ≥1:160 dilution with a homogenous pattern, ASMA positivity at ≥1:160 dilution, anti-LKM1 positivity ≥1:160, a cryocrit > 2% or a cryocrit > 1% with clinical evidence of cryoglobulinemia, autoimmune thyroiditis, B-cell non-Hodgkin lymphoma, autoimmune hemolytic anemia, lichen planus, psoriasis or Sjogren's syndrome. These IMDs were chosen because they are the most frequent IMDs associated to HCV infection in literature and clinical practice [14,27]. At the end of the enrolment periods, 168 consecutive Caucasian patients were recruited, 81 with signs of IMD and 87 without. In all patients the diagnosis of IMD was made after HCV diagnosis

A pre-coded questionnaire containing demographic and epidemiological information was filled out for each patient at the time of enrolment. A consumption of alcohol exceeding 30 g per day for females and 40 g per day for males over the last 6 months or more was considered ongoing alcohol abuse, information corroborated by family members in uncertain cases. All patients received a physical examination, an upper abdomen US scan and the determination of HCV, HBV, hepatitis delta virus (HDV) and human immunodeficiency virus (HIV) serum markers, of thyroid-specific and non-organ specific autoantibodies, liver biochemistry and routine analyses.

All patients were anti-HIV, anti-HBsAg and anti-HDV-negative and none declared ongoing intravenous drug addiction, information corroborated by family members in uncertain cases. No patients had hepatocellular carcinoma.

In accordance with the Italian guidelines [28], a transcutaneous US-assisted liver biopsy was requested by the physicians in care for clinical purposes and performed in 45 (55.5%) of the 81 patients with IMDs and in 69 (79.3%) of the 87 without. The Ishak scoring system was used to grade necroinflammation and fibrosis [29]; a home-made scoring system was used to assess liver steatosis [26].

Samples of serum and whole blood were obtained from each patient at the time of enrolment and stored at −80 °C until used for this investigation.

All procedures used were in accordance with the international guidelines [29] and with the Helsinki Declaration of 1975 and revised in 1983. The Ethics Committee of the Azienda Ospedaliera Universitaria of the Second University of Naples approved the study. All patients signed an informed consent for liver biopsy, the collection and storage of biological samples and for the anonymous use of their data for research purposes.

2.2. Methods

HBV and HDV serum markers were sought using commercial immunoenzymatic assays (Abbott Laboratories, North Chicago, IL, USA, for HBsAg, anti-HBs and anti-HBc, and DiaSorin, Saluggia, VC, Italy, for anti-HDV). The anti-HCV antibody was sought using a 3rd generation commercial immunoenzymatic assay (Ortho Diagnostic Systems, Neckargemund, Germany). Antibodies to HIV 1 and 2 were sought using a commercial ELISA (Abbott Lab., North Chicago, IL, USA). Liver biochemistry and routine analyses were performed by routine methods in a CobasModular 6000 automated analyzer using c501 biochemistry modules (Roche Diagnostics Ltd, Rotkreuz, Switzerland).

The diagnosis of B-cell non-Hodgkin lymphoma was based on the World Health Organization classification [30]. The diagnosis of autoimmune thyroiditis was based on a marked increase in titers of organ-specific autoantibodies, abnormalities in thyroid hormones, the presence of US/CT abnormalities and clinical symptoms.

ANA, ASMA and anti-LKM were detected by indirect immunofluorescence on rat liver, kidney and stomach sections and on Hep2-G cells using commercial reagents (Diamedix IS, Miami, FL, USA) at an initial serum dilution of 1:40. Positive sera were titrated testing doubled serum dilutions.

Serum levels of thyroid-stimulating hormone, free-triiodothyronine, free-thyroxine, anti-peroxidase (anti-TPO) and anti-thyroglobulin antibodies were determined in fresh serum samples using standard assays (Bayer Diagnostics, Tarrytown, NY).

The presence of cryoglobulins was assessed according our local laboratory standard, based on reference methodologies [31], and confirmed by at least two positive tests at ≥12-week intervals.

The diagnosis of autoimmune hemolytic anemia was confirmed by a positive Coombs test in conjunction with a direct antiglobulin test [32].

Psoriasis and lichen planus were sought on a clinical basis with the dermatologist's help: sharply demarcated, erythematous plaques with mica-like scales for psoriasis; purple polygonal papules with severe pruritus and lacy white markings often