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ORIGINAL RESEARCH

Increased Mitochondrial Genetic Diversity in Persons Infected With Hepatitis C Virus

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SUMMARY

Hepatitis C virus infection strongly affects mitochondrial DNA genetic diversity, which can be used to discriminate recent from past infections.

BACKGROUND & AIMS: The host genetic environment contributes significantly to the outcomes of hepatitis C virus (HCV) infection and therapy response, but little is known about any effects of HCV infection on the host beyond any changes related to adaptive immune responses. HCV persistence is associated strongly with mitochondrial dysfunction, with liver mitochondrial DNA (mtDNA) genetic diversity linked to disease progression.

METHODS: We evaluated the genetic diversity of 2 mtDNA genomic regions (hypervariable segments 1 and 2) obtained from sera of 116 persons using next-generation sequencing.

RESULTS: Results were as follows: (1) the average diversity among cases with seronegative acute HCV infection was 4.2 times higher than among uninfected controls; (2) the diversity level among cases with chronic HCV infection was 96.1 times higher than among uninfected controls; and (3) the diversity was 23.1 times higher among chronic than acute cases. In 2 patients who were followed up during combined interferon and ribavirin therapy, mtDNA nucleotide diversity decreased dramatically after the completion of therapy in both patients: by 100% in patient A after 54 days and by 70.51% in patient B after 76 days.

CONCLUSIONS: HCV infection strongly affects mtDNA genetic diversity. A rapid decrease in mtDNA genetic diversity observed after therapy-induced HCV clearance suggests that the effect is reversible, emphasizing dynamic genetic relationships between HCV and mitochondria. The level of mtDNA nucleotide diversity can be used to discriminate recent from past infections, which should facilitate the detection of recent transmission events and thus help identify modes of transmission. *(Cell Mol Gastroenterol Hepatol 2016;2:676–684; http://dx.doi.org/10.1016/j.jcmgh.2016.05.012)*

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Hepatitis C virus (HCV) is a major public health problem in the United States and worldwide. HCV infects at least 3.5 million persons in the United States and 180 million persons worldwide.[1] HCV infection is the most common chronic blood-borne infection and the leading cause for liver transplantation.[2] Since 2007, HCV has surpassed human immunodeficiency virus (HIV) as a cause of death in the United States.[3] Approximately 80% of patients who become infected with HCV develop chronic hepatitis and are at risk for advanced liver disease, with 15%–30% of these patients progressing to liver fibrosis and cirrhosis and up to 5% dying from liver failure or hepatocellular carcinoma (HCC).[2] The outcome of HCV infection is determined by a complex interplay between host genetic, immunologic, and viral factors.[4] The host innate and adaptive immune responses are key determinants of viral clearance and persistence. Several host genetic factors have been associated with efficiency of immune response against HCV and with antiviral therapy outcome. The most well-known host genetic factors affecting HCV infection are ethnic background, interleukin 28B polymorphisms, and alleles of the inhibitory natural killer cell receptor and HLA classes I and II.[5] Recent genome-wide association studies identified several new factors influencing treatment efficacy or clinical course of HCV infection such as variants of interferon (IFN)L3-IFNL4, IRAK2, DEPDC5, MICA, RNF7, TULP1, and MERTK.[6,7]

It is well established that viruses, which are simple biological entities with high mutation rates, adapt rapidly to the host genetic environment, producing distinct genetic variants. In human beings, viral infections are not generally

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Abbreviations used in this paper: **AUC, area under the curve; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HVS, hypervariable segment; IFN, interferon; mtDNA, mitochondrial DNA; NGS, next-generation sequencing; PCR, polymerase chain reaction; pegIFN, peginterferon; ROC, receiver operating characteristic.**

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considered to be able to induce genetic changes in the host beyond those related to adaptive immune responses or rare mutations associated with cancer,[8] owing to the stability of nuclear DNA. Mitochondrial DNA (mtDNA), however, varies in copy number and has a high mutation rate.[9]

Chronic HCV infection has been associated strongly with prominent mitochondrial injury of the liver.[10–13] These effects include mitochondria swelling, loss of mitochondrial cristae, reduction in number of mitochondria, calcium-mediated mitochondrial depolarization and dysfunction, and reactive oxygen species generation. Under normal circumstances, these impaired mitochondria would be eliminated. However, in infected cells, HCV attenuates apoptosis by cleaving mitochondrial antiviral signaling protein[14] and promoting mitochondrial fission and mitophagy of the functionally compromised organelles,[12,13] which contribute to persistence of infection. These results are consistent with the low mtDNA copy number found in the blood of persons with chronic HCV infection.[15] Oxidative stress and reactive oxygen species generated by distressed mitochondria now are recognized as a major procarcinogenic cofactor in chronic HCV infection.[10] In addition, mtDNA mutations were found in the liver of patients with HCC[16–22] and were linked to outcomes of antiviral therapy.[23]

In this study, we used next-generation sequencing (NGS) to evaluate the intrahost mtDNA variants from HCV-uninfected persons and cases with seronegative acute and seropositive chronic HCV infection. We show that the mtDNA genetic diversity is higher in HCV-infected persons and decreases after completion of therapy.

Materials and Methods

Human Subjects

A total of 130 serum samples from 116 individuals belonged to 4 groups. Groups 1 and 2 were obtained from commercially available seroconversion panels (Seracare, Milford, MA), and groups 3 and 4 were obtained through clinical studies with Institutional Review Board approval, with all patients providing written consent to participate. The protocol was approved by Atlanta Medical Center's Institutional Review Board and the Icahn School of Medicine at Mount Sinai (study ID: 06-0974 ME).

Group 1 comprised 50 HCV-uninfected (anti-HCV-seronegative) plasma donors (control group). The samples also were negative for anti-HIV and anti–hepatitis B virus. No demographic or clinical information was available for this group.

Group 2 comprised 18 plasma donors with seronegative, polymerase chain reaction (PCR)-positive, acute HCV infection. In 12 cases, we analyzed 2 specimens obtained an average of 10.2 days apart, both before seroconversion. No demographic or clinical information was available for this group.

Group 3 comprised 46 treatment-naive patients with chronic HCV infection (anti-HCV-seropositive with PCR-positive HCV infection for $>$ 6 months). African American (n = 19), Caucasian (n = 27); female (n = 23), male (n = 23); noncirrhotic (n = 28), cirrhotic (n = 10); age at time of specimen (n = 45; range, 28–70 y; median, 53 y); steatosis absent (n = 24), steatosis present (n = 13); genotype 1a (n = 28), genotype 1b (n = 9), genotype 2 (n = 7), genotype 3 (n = 2); and HCV viral load quantified (n = 39).

Group 4 comprised 2 HIV-infected men (designated patient A and patient B) with a recent HCV infection who underwent successful therapy with peginterferon (pegIFN) plus ribavirin. Two samples from each man were evaluated, the first from treatment baseline and the second from the last HCV-positive sample during treatment. Patient A had not seroconverted at baseline and patient B was anti-HCV-positive at baseline, having seroconverted 0–7 days before.

Nucleic Acid Extraction

Blood was incubated in an upright position at room temperature for 30–45 minutes to allow clotting. Then the sample was centrifuged for 15 minutes at 2000 relative centrifugal force to obtain serum. Total nucleic acid was extracted from serum samples using the automated Roche MagNA Pure LC robot and the MagNA Pure LC Total Nucleic Acid Isolation kit (Roche Diagnostics, Indianapolis, IN), and eluted with 50 μL of elution buffer according to the manufacturer's instructions. Complementary DNA was generated using the high-temperature capability SuperScript VILO complementary DNA Synthesis Kit (Invitrogen, Life Technologies, Carlsbad, CA) on an ABI PRISM19700 PCR system (Thermo Fisher Scientific, Waltham, MA).

mtDNA Amplification

Hypervariable segment (HVS)1 and HVS2 in the D-loop domain of mtDNA were analyzed. Two sets of primers (external and nested) were used to amplify the HVS1 and HVS2 regions of the mitochondrial D-loop: (1) for HVS1: HVS1-H16401 (TGATTTCACGGAGGATGGTG),[24] HVS1-L 15900 (TAAACTAATACACCAGTCTTGTAAACC),[25] HVS1-H16391 (GAGGATGGTGGTCAAGGGAC), and HVS1-L15997 (CACCATTAGCACCCAAAGCT)[26]; (2) for HVS2: HVS2-F-16449 (CGCTCCGGGCCCATAACACTT), HVS2-R-722 (GAAC TCACTGGAACGGGGATGCT), HVS2-F-44 (CATGCATTTGG TATTTTCGTCTGG), and HVS2-R-638 (GGTGATGTGAGCC CGTCTAA). For external primers, the following cycling conditions were used: 95°C for 150 seconds, 40 cycles of 94°C for 1 minute, 55°C for 30 seconds, and 72°C for 1 minute. For the nested amplicons, PCR conditions were as follows: 95°C for 9 minutes, 36 cycles of 95°C for 10 seconds, 60°C for 30 seconds, and 72°C for 30 seconds (HVS1) and 95°C for 9 minutes, 36 cycles of 95°C for 10 seconds, 52°C for 30 seconds, and 72°C for 30 seconds (HVS2). For separation of PCR products, E-gel imager and E-gel size select gel (2%) (cat# G661002; Invitrogen) were used. After gel purification, samples were run in a bioanalyzer (Agilent 2200 tape station, Agilent Techonologies, Santa Clara, CA) to prepare for emulsion PCR.

NGS

PCR products were pooled and subjected to pyrosequencing using the GS FLX Titanium Sequencing Kit (454 Life Sciences, Roche Applied Sciences, Indiana, IN). Low-quality reads were removed using the GS Run Processor