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Phenotypic and genotypic differences between a child with vertically acquired severe hepatitis C liver disease and his mother

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Introduction

Liver transplantation for end stage liver disease secondary to CHC in children is rare. According to the United Network for Organ Sharing 133 subjects less than 22 years of age have undergone liver transplantation for CHC in the US from 1/1988 – 11/2009 (1); survival rates are lower than rates for other conditions, primarily because of recurrent CHC (2). Host and viral factors responsible for recurrent CHC after orthotopic liver transplant are not well studied in children. We report a case of a pediatric patient with vertically-transmitted CHC who presented with end stage liver disease and underwent a living related liver transplant from his HCV-infected mother with mild disease. This case presented a unique opportunity to characterize phenotypic and genotypic differences in HCV between the mother and her child.

Case report

A five and a half year old male Caucasian child presented to the clinic with liver failure and portal hypertension secondary to CHC, having failed antiviral therapy with alpha-interferon. Extensive work-up failed to reveal any co-morbid conditions. The mother was a 40 year old Caucasian female with bipolar affective disorder who was in stable condition under psychiatric care. The mother had lost her only other child from causes unrelated to HCV (severe congenital heart disease) and was highly motivated to donate her liver. At approximately age 14 years, she had acquired HCV (Genotype 1) from injection drug use, but drug testing had been repeatedly negative for the last 17 years. She had no clinical or biochemical stigmata of liver disease, despite having been infected with HCV for approximately 26 years. Given the unusual nature of the request the case was extensively reviewed by both our adult and pediatric liver transplant teams as well as the Ethics Committee of the hospital. Our consensus was that the mother's HCV virus had apparently been tolerated by her for 26 years without clinical evidence of liver disease. We presumed that the child (like almost all recipients undergoing liver transplantation for HCV) would

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experience universal recurrence of the infection that he had acquired from his mother (whether he received her liver or a cadaveric HCV negative liver) so we had no precedent for believing that his course would have been any worse with utilization of the mother's liver.

The child underwent living related liver transplantation from his mother at age five years. A biopsy of her liver at the time of donation was consistent with mild CHC (Figure 1, panels A, B). The child's liver explant showed established cirrhosis (Figure 1, panel C). HCV RNA was detected in the child's plasma 15 months post operative and he was then treated with alpha interferon and acyclovir as was a common practice at the time (3). Liver biopsy at 10 and 13 months post transplant showed mild chronic hepatitis consistent with CHC (Figure 1, panels D and E). The child was admitted for acute hepatic decompensation secondary to recurrent CHC at seven years of age, a year and a half after his first transplant, and received a second liver transplant (cadaveric, orthotopic). The liver explant showed established cirrhosis with mild to moderate ongoing portal and septal chronic inflammation. The post transplant period was complicated by recurrent CHC and liver failure necessitating a third orthotopic liver transplant at eight years of age. The liver explant showed early cirrhosis (Figure 1, panel F). The child expired from hemorrhagic shock secondary to gastrointestinal bleeding two months after his third transplant, less than three years after his initial transplant.

Serum samples

These were obtained from the child eighteen months after the initial liver transplant when he was experiencing severe recurrent CHC, and from the mother fifteen months after the initial liver transplant after she provided written informed consent for serum samples to be obtained via a protocol approved by the Johns Hopkins Institutional Review Board. Samples were analyzed by RT-PCR, sequencing, and quasispecies in the Hypervariable Region 1 (HVR1) as previously described (4)

Analyses of quasispecies—No nucleotide or amino acid variations were found in the 18 clones of the HVR1 region of HCV present in the serum of the child (online-only Supplemental Figures 1 [<http://links.lww.com/MPG/A62>] and 2 [<http://links.lww.com/MPG/A63>] and Table). On the other hand, three quasispecies at the nucleotide level were found in the 19 clones of the HVR1 region of HCV in the mother's serum (Table). However, these three variants represented synonymous nucleotide mutations that did not change the amino acid sequence of HVR1. In HVR1, there were no identical clones at the nucleotide or amino acid level observed between child and mother (Supplemental Figures 1 and 2.)

Analyses of amino acid R groups of HVR1—There are three basic residues, H, R, K, in the consensus sequence of HVR1. Based on the reference patterns of basic residues in HVR1 previously reported for HCV genotypes (5), the sequence for the child had an “F” pattern (basic residues in position 3, 11, 14, 25) (Supplemental Figure 1). Interestingly, the sequence of HVR1 in the circulating virus of the mother had basic residues in position 3, 14, 25, which is different from the most commonly reported pattern of HVR1. The quasispecies analysis suggests a divergence of the child's virus from the mother. While evolution of quasispecies is known to progress in children who acquire HCV from their mother, the few studies which address this question suggest that usually some sequences remain identical between mother and child. For example, in the report of two HCV-infected mother-infant pairs by Ishii et al (6), the sequence identity with the mothers at 7 to 10 years of age was 69.3–90.7% for nucleotides. The significance of the divergence of our patient's HVR1 sequence from that of his mother is unknown but it is possible that the child's quasispecies

was more virulent, possibly because of the basic amino acids (5) and/or that the innate immunologic systems in mother and child were strikingly different. Due to the relatively small number of clones sequenced, the use of a pair of samples from a single time point and the relatively low viral loads detected in these samples, it is highly likely that minor quasispecies have been missed in our analysis. However the low HCV genetic diversity in our patient seems to be consistent with observations of others reporting much less viral diversity in immunosuppressed liver transplant recipients compared to patients with CHC (7 - 11).

We present this report in the hope that it will stimulate further investigations of the role of viral evolution in the severity of vertically transmitted HCV. For example, the application of more sensitive sequencing strategies such as 454 deep sequencing may be required to shed further light on the evolution of HCV in the setting of vertical transmission (12).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Panel A (H&E, original magnification of 64×): The donor liver from the mother showed mild to moderate portal chronic inflammation at frozen section. A portal tract with moderate portal chronic inflammation is shown. Panel B (H&E, original magnification of 64×): The donor liver from the mother showed mild lobular chronic inflammation. Panel C (H&E, original magnification of 40×): The native liver of the child showed established cirrhosis with mild ongoing portal and septal chronic inflammation. Panel D (H&E, original magnification of 100×): A liver biopsy at 13 months post transplant showed generally mild to focally moderate portal chronic inflammation. Panel E (H&E, original magnification of 100×): The 13 month post transplant liver biopsy also showed mild diffuse lobular hepatitis.

Panel F (Masson Trichrome stain, original magnification of 20×): The second explanted liver showed early cirrhosis. An area with marked bridging fibrosis and developing nodularity is shown.

Table 1
Quasispecies in the hypervariable region of E2

		HVR1		
		Child	Mother	Total diversity
Number of clones		18	19	
Number Of quasispecies* (frequency)	nt	1(18)	3(5/11/3)	4(37)
	aa	1(18)	1(19)	2(37)

* No identical quasispecies found between child and mother.

Abbreviations: nt, nucleotide; aa, amino acid