Sexual Transmission of Hepatitis C Virus Among Monogamous Heterosexual Couples: The HCV Partners Study

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The efficiency of hepatitis C virus (HCV) transmission by sexual activity remains controversial. We conducted a cross-sectional study of HCV-positive subjects and their partners to estimate the risk for HCV infection among monogamous heterosexual couples. A total of 500 anti–HCV-positive, human immunodeficiency virus–negative index subjects and their long-term heterosexual partners were studied. Couples were interviewed separately for lifetime risk factors for HCV infection, within-couple sexual practices, and sharing of personal grooming items. Blood samples were tested for anti-HCV, HCV RNA, and HCV genotype and serotype. Sequencing and phylogenetic analysis determined the relatedness of virus isolates among genotype-concordant couples. The majority of HCV-positive index subjects were non-Hispanic white, with a median age of 49 years (range, 26-79 years) and median of 15 years (range, 2-52 years) of sexual activity with their partners. Overall, HCV prevalence among partners was 4% (n = 20), and nine couples had concordant genotype/serotype. Viral isolates in three couples (0.6%) were highly related, consistent with transmission of virus within the couple. Based on 8,377 person-years of follow-up, the maximum incidence rate of HCV transmission by sex was 0.07% per year (95% confidence interval, 0.01-0.13) or approximately one per 190,000 sexual contacts. No specific sexual practices were related to HCV positivity among couples. Conclusion: The results of this study provide quantifiable risk information for counseling long-term monogamous heterosexual couples in which one partner has chronic HCV infection. In addition to the extremely low estimated risk for HCV infection in sexual partners, the lack of association with specific sexual practices provides unambiguous and reassuring counseling messages.

(C)Hronic hepatitis C virus (HCV) infection affects 3 to 4 million people in the United States, most of whom are sexually active adults.1 The primary means of transmission of HCV is direct percutaneous exposure to infectious blood, and there are clearly defined counseling messages for infected persons to prevent spread from such exposures.2 The accumulated epidemiological evidence indicates that HCV can be transmitted by sex with an infected partner, presumably by mucosal exposure to infectious blood or serum-derived fluids. However, sexual activity is much less efficient for transmitting HCV than for other blood-borne, sexually transmitted viruses such as hepatitis B virus (HBV) and human immunodeficiency virus (HIV).3

The association between sexual activity and HCV infection was first demonstrated by case-control studies of subjects with acute hepatitis C.4 The few prospective cohort studies of monogamous heterosexual couples have reported incidence rates of HCV infection of
organ transplantation, or was currently using antiviral
medication. Partners had to report knowing or being aware
of HIV or HBV infection, or if one partner had prior
infection in the preceding 6 months. Couples were excluded
if either partner had known HIV or HBV infection, had prior
organ transplantation, or was currently using antiviral
medication. Researchers contacted index subjects for study
enrollment, and if eligible based on prescreening, contacted
their sexual partner. Criteria for study participation by
each couple included a heterosexual relationship for a
minimum of 36 months, monogamy for the duration
of the couple’s relationship reported by both partners, and a
minimum of three sexual contacts by the couple in the
preceding 6 months. Couples were excluded if either
partner had known HIV or HBV infection, had prior
organ transplantation, or was currently using antiviral
medication.

Subjects and Methods

Study Population. The recruitment phase of the study
was conducted in Northern California sites between January 2000 and May 2003. Recruitment
began by first identifying a known HCV-positive subject (referred to as the index subject) from multiple
sources, including liver clinics at the University of California at San Francisco, members of Kaiser
Permanente Medical Care Plan in Northern California, California Pacific Medical Center and affiliated clinics,
other community-based practices in the greater San Francisco Bay Area, and blood donors from Blood Cen-
ters of the Pacific, California Pacific Medical Center, Kaiser Permanente Northern California,
St. Louis University, and the Centers for Disease Control and Prevention. Researchers contacted index subjects for study enrollment, and if eligible based on prescreening, contacted
their sexual partner. Criteria for study participation by each couple included a heterosexual relationship for a
minimum of 36 months, monogamy for the duration
of the relationship reported by both partners, and a
minimum of three sexual contacts by the couple in the
preceding 6 months. Couples were excluded if either
partner had known HIV or HBV infection, had prior
organ transplantation, or was currently using antiviral
medication, or if both partners reported a history of injection drug use (IDU).

Partners of each couple were interviewed independently by phone (76%) or in person (24%) by trained
interviewers, with no difference in completing a questionnaire by interview type. Detailed information was
obtained on sexual history with the study partner (Supporting Information), nonsexual household expo-
sures (sharing of personal items, including nail grooming tools, razors, and toothbrushes), and all other
known risk factors for HCV acquisition. The risk period for sexual transmission was defined using a uni-
form method to capture sexual activities over the entire
duration of the couple’s relationship. Sexual histories
were collected in discrete time intervals defined by events in each participant’s sexual history and beginning from
the time of first sexual contact with the current partner up to the time of interview. Each participant identified
life events such as pregnancy, childbirth, medical illness,
and absences that significantly changed sexual activities
with their study partner and the corresponding year and
age for each life event. Sexual practices, including type
and frequency of sexual contact and use of protective
barriers, were obtained during each of these defined
intervals. When responses to questions about sexual or
personal grooming practices were discordant between
partners, responses were recoded for presence rather than
absence of the practice.

The study was approved by the Institutional Review
Boards of the University of California at San Fran-
cisco, Blood Centers of the Pacific, California Pacific
Medical Center, Kaiser Permanente Northern Califor-
nia, St. Louis University, and the Centers for Disease
Control and Prevention.

Serologic Testing. Serum samples from index sub-
jects were tested for anti-HCV via enzyme immunoas-
say (EIA 2.0) (Abbott Laboratories, Abbott Park, IL)
and for HCV RNA via qualitative polymerase chain
reaction (PCR) with detection limit ≤50 IU/mL
(Roche Amplicor, Roche Molecular Diagnostics, Pleas-
anton, CA) (if not documented in medical records in
prior 6 months). Serum samples from partners were
tested for anti-HCV via EIA and positive results con-
firmed via recombinant immunoblot assay (RIBA 3.0,
Chiron Corporation, Emeryville, CA). RIBA-positive
samples were tested for HCV RNA via qualitative
PCR. Serotyping of the antibody based on RIBA
methodology was used in anti–HCV-positive concord-
ant couples with HCV RNA–negative partners. Geno-
type was determined in samples from anti–HCV-
positive, HCV RNA–positive concordant couples using
the InnoLipa assay (Innogenetics, Ghent, Belgium).
Sequencing. HCV RNA–positive specimens from genotype-concordant couples were amplified via reverse-transcription nested PCR, and the HCV consensus sequences were determined by directly sequencing uncloned PCR products from the 897-nucleotide-long NS5B region for genotype 1a and 1b samples and from a 944-nucleotide-long NS5B region for the 2b samples employing ABI dye-termination techniques.11 The 1a and 1b sequences correspond to H77 positions 7479 to 8375 (with genotype 1b sequences missing three nucleotides relative to the 1a sequences, resulting in a gap corresponding to 7566 to 7568 in the H77 sequence). These 1a/1b alignments correspond to the H77 sequence 8326-9269, encoding NS5B from amino acid 242 to 556. To evaluate the relatedness between isolates from genotype-concordant partners, the consensus sequences from their isolates were compared with corresponding regions from reference sequences of the same subtype downloaded from the Broad Institute or from the National Center for Biotechnology Information; this included 99 genotype 1a and 97 genotype 1b sequences. The sequences were imported into the MEGA 4 sequence analysis package, and the pairwise distances and number of differences were calculated for each pair. These nucleotide sequences have been submitted to GenBank under accession numbers HQ022864-HQ022879.

Phylogenetic Analyses and Estimation of Minimal Divergence Time. The pairwise distance and number of nucleotide differences of sequences between partners in each couple were compared with the average pairwise distance and average number of nucleotide differences between the subtype-specific reference sequences using SPSS software. The values for the reference sequences had normal distributions in all cases. The concordance between isolates was measured using two different methods (genetic distance and number of differences) to ensure that there was no unexpected bias or skewing of the population average by one method. For the pairwise distance calculation, all positions were used, the Maximum Composite Likelihood substitution model was used, and all sites were assumed to have the same rate of variation. The cutoffs used to establish statistical significance were 1.65 SD below the mean (P = 0.05) and 2.3 SD below the mean (P = 0.01) in a single-tailed analysis. A single-tailed (left side) analysis was employed because we wished to determine whether the partner sequences were significantly more related to each other than to random HCV sequences.

We estimated the minimal divergence time needed to achieve the interpair nucleotide differences. These calculations are based on a nucleotide fixation rate for NS5 of 1.45 × 10⁻⁵ mutations per site per year.12,13 When this rate was applied to both viruses from the partners, the divergence rate was 2.6 nucleotide positions per year for the target region analyzed. This rate provided minimal time estimates and was used only to establish a plausibility window for transmission.

Projected Sample Size and Other Statistical Analyses. Prior to study initiation, widths of confidence intervals (CIs) around a prevalence estimate were calculated for a range of sample sizes. Given a 2%-5% prevalence of HCV infection in sexual partners, the widths of CIs would be between 1% and 2% for a projected sample size of 1,000 couples and between 4% and 7% for a projected sample size of 300 couples.

Demographic characteristics and risk factors for HCV infection were summarized with frequency distributions (categorical variables) and medians and ranges (continuous variables) separately for anti–HCV-positive index subjects and their partners. Data on sexual practices between partners are presented at the couple level. Duration of the sexual relationship was defined as the number of years between first sexual contact with the study partner and study enrollment, minus time intervals (in years) where sexual contact was absent within the couple. For example, a couple reporting a relationship start year of 1991, study enrollment in 2001, and no sexual activity between 1998 and 1999 would be assigned 9 years for the duration of the sexual relationship. To estimate the total number of sexual contacts for each relationship, the number of sexual contacts per month for each discrete time interval in their sexual history was multiplied by the duration of the time interval and summed over all intervals in the relationship. Changes in sexual activities over time were determined by comparing the types and frequencies of practices in the first year of the relationship relative to the year immediately prior to study enrollment (current year of sexual activity). Each partner independently reported time periods of sexual activity and the number of contacts per month during that time period. Since the time periods reported by each partner might not match perfectly, we calculated the number of contacts per time period per partner and summed the estimated number of contacts per each partner over the duration of the relationship. The average of the total number of contacts reported by partner 1 and partner 2 was used as the total number of contacts for the couple.
Prevalence of anti-HCV positivity and 95% CIs were calculated for the partners of index subjects. Incidence of sexually acquired HCV infection was estimated per number of sexual contacts (vaginal intercourse with and without menses and anal intercourse). Incidence density of HCV infection was calculated as the number of potential transmission events per total person-years of sexual relationship reported among partners. Duration of the sexual relationship was summed among the 500 partners to determine the total person-years of observation.

Results

Eligible and Enrolled Couples. Of the 2,077 couples screened for study inclusion, 672 (32%) were eligible. Reasons for study exclusion occurring in ≥5% of the 1,405 ineligible couples included lack of sexual activity (31%), prior organ transplant (12%), refused study participation (11%), doctor refused (8%), HIV or HBV coinfection (8%), partnership less than 3 years or nonmonogamous (6%), and history of IDU in both partners (6%). Of the 672 eligible couples, 500 (74%) enrolled and completed all the study requirements, at which time study enrollment was halted. The primary reasons for failure to participate among the remaining 172 eligible couples were nonresponse (54%) or refusal (29%). Of the 500 enrolled couples, 43% were referred from tertiary referral practices, 34% from community sources, and 21% were blood donors.

Characteristics of Participating Couples. The 500 couples were predominantly non-Hispanic white, educated, employed, and born in the United States (Table 1). The median duration of the couples’ sexual relationships was 15 years (range, 2-52 years). The most frequently reported risk factors for HCV infection among index subjects were IDU (53.8%) and blood transfusion before 1992 (31.6%); these risks were infrequently reported by partners. Twenty or more lifetime sex partners prior to the current relationship were reported by 46.2% of index subjects and 26.8% of partners.

The median number of sexual contacts per month was highest for vaginal intercourse during the first year of the relationship (12 contacts per month) (Table 2). The frequency of sexual contacts decreased over time for all types of sexual activity. Vaginal intercourse during menses and anal intercourse (≥1 occasion) were reported by 65.2% and 30.4% of couples, respectively. Condom use during vaginal intercourse was reported by 29.9% of couples and condom use decreased over time for vaginal and anal intercourse.

HCV Sequencing and Phylogenetic Analyses of HCV Strains. Among the 500 partners of anti-HCV-positive index subjects, 20 were confirmed anti-HCV-positive and 13 of the 20 partners were HCV RNA-positive. HCV genotyping/subtyping and HCV serotyping confirmed nine couples to be concordant, eight couples to be discordant, and three couples to be of indeterminant status (Table 3).

Of the nine genotype-concordant couples, both partners of six couples were viremic, allowing phylogenetic analyses; three had strong evidence that the partners were infected with the same HCV isolate, and three were consistent with infection by different HCV strains (Table 4). Couple 15 had HCV 1a strains that
were more similar to each other than 99% of random pairings of HCV sequences of subtype 1a. Both partners of couple 17 were infected with both HCV 1a and 1b strains, and their 1b strains were more similar to each other than 99% of random pairings of HCV 1b sequences; however, their 1a strains were no more closely related than random HCV isolates in the population. Both partners of couple 14 were infected with HCV strains 2b and 1a. The 2b strains were highly similar, with only a 1.8% difference in base pairs over a 944-bp region analyzed, whereas their 1a strains were no more closely related than random pairs of 1a sequences in the population. The HCV isolates in couples 9, 11, and 13 were no more similar to each other than random HCV isolates of the same subtype in the population.

Among the partners with highly-related strains (couples 14, 15, and 17), the estimated minimum divergence time was 6.5 years for couple 14, whose sexual relationship duration was 18 years; 14.6 years for couple 15, whose sexual relationship duration was 28 years; and 6.2 years for couple 17, whose sexual relationship duration was 10 years. The risk factor profiles of couple 14 revealed that the female partner had a

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### Table 2. Type and Frequency of Sexual Practices Among Couples According to Year of Relationship

<table>
<thead>
<tr>
<th>Type and Frequency of Sexual Practice</th>
<th>Ever</th>
<th>First Year of Relationship</th>
<th>Current Year of Relationship</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaginal intercourse, no. (%)</td>
<td>499 (99.8)</td>
<td>499 (99.8)</td>
<td>474 (94.8)</td>
</tr>
<tr>
<td>Contacts per month, median (range)*</td>
<td>12 (0-300)</td>
<td>4 (0-80)</td>
<td>6 (0-300)</td>
</tr>
<tr>
<td>Ever used condom, no. (%)</td>
<td>149 (29.9)</td>
<td>109 (23.0)</td>
<td>109 (23.0)</td>
</tr>
<tr>
<td>Frequent condom use, no. (%)</td>
<td>64 (16.8)</td>
<td>67 (13.4)</td>
<td>67 (13.4)</td>
</tr>
<tr>
<td>Vaginal intercourse during menses, no. (%)</td>
<td>326 (65.2)</td>
<td>297 (59.4)</td>
<td>297 (59.4)</td>
</tr>
<tr>
<td>Contacts per month, median (range)*</td>
<td>0 (0-10)</td>
<td>0 (0-4)</td>
<td>0 (0-4)</td>
</tr>
<tr>
<td>Frequent condom use, no. (%)</td>
<td>45 (15.2)</td>
<td>21 (15.7)</td>
<td>21 (15.7)</td>
</tr>
<tr>
<td>Anal intercourse, no. (%)</td>
<td>152 (30.4)</td>
<td>107 (21.4)</td>
<td>68 (13.6)</td>
</tr>
<tr>
<td>Contacts per month, median (range)*</td>
<td>0 (0-20)</td>
<td>0 (0-15)</td>
<td>0 (0-15)</td>
</tr>
<tr>
<td>Frequent condom use, no. (%)</td>
<td>14 (13.1)</td>
<td>6 (1.4)</td>
<td>7 (1.9)</td>
</tr>
<tr>
<td>Male receptive oral sex, no. (%)</td>
<td>456 (91.2)</td>
<td>428 (85.6)</td>
<td>370 (74.0)</td>
</tr>
<tr>
<td>Contacts per month, median (range)*</td>
<td>3 (0-100)</td>
<td>3 (0-100)</td>
<td>1 (&lt;1)</td>
</tr>
<tr>
<td>Frequent condom use, no. (%)</td>
<td>6 (1.4)</td>
<td>7 (1.9)</td>
<td>7 (1.9)</td>
</tr>
<tr>
<td>Female receptive oral sex, no. (%)</td>
<td>462 (92.4)</td>
<td>435 (87.0)</td>
<td>374 (74.8)</td>
</tr>
<tr>
<td>Contacts per month, median (range)*</td>
<td>3 (0-100)</td>
<td>3 (0-100)</td>
<td>1 (&lt;1)</td>
</tr>
<tr>
<td>Frequent barrier (dental dam) use, no. (%)</td>
<td>2 (0.5)</td>
<td>2 (0.3)</td>
<td>2 (0.3)</td>
</tr>
</tbody>
</table>

*Summation of individual level data.

### Table 3. Serologic and Virologic Results in Confirmed Anti–HCV-Positive Couples

<table>
<thead>
<tr>
<th>Couple ID</th>
<th>Index Subject Genotype</th>
<th>Index Gender</th>
<th>Partner Genotype</th>
<th>Partner Gender</th>
<th>Partner Serotype</th>
<th>Designation Based on Genotype* or Serotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4a</td>
<td>F</td>
<td>Indeterminate</td>
<td>M</td>
<td>1</td>
<td>Discordant</td>
</tr>
<tr>
<td>2</td>
<td>2a</td>
<td>F</td>
<td>1b</td>
<td>M</td>
<td>M</td>
<td>Discordant</td>
</tr>
<tr>
<td>3</td>
<td>1a</td>
<td>F</td>
<td>2b</td>
<td>M</td>
<td>M</td>
<td>Discordant</td>
</tr>
<tr>
<td>4</td>
<td>1b</td>
<td>F</td>
<td>1a</td>
<td>M</td>
<td>M</td>
<td>Discordant</td>
</tr>
<tr>
<td>5</td>
<td>1b</td>
<td>F</td>
<td>1a</td>
<td>M</td>
<td>M</td>
<td>Discordant</td>
</tr>
<tr>
<td>6</td>
<td>1a</td>
<td>F</td>
<td>1b</td>
<td>M</td>
<td>M</td>
<td>Discordant</td>
</tr>
<tr>
<td>7</td>
<td>3a</td>
<td>M</td>
<td>HCV RNA-negative</td>
<td>F</td>
<td>1</td>
<td>Discordant</td>
</tr>
<tr>
<td>8</td>
<td>1a</td>
<td>F</td>
<td>HCV RNA-negative</td>
<td>M</td>
<td>1</td>
<td>Concordant</td>
</tr>
<tr>
<td>9</td>
<td>1a</td>
<td>M</td>
<td>1a</td>
<td>F</td>
<td>F</td>
<td>Concordant</td>
</tr>
<tr>
<td>10</td>
<td>1a</td>
<td>M</td>
<td>HCV RNA-negative</td>
<td>F</td>
<td>F</td>
<td>Concordant</td>
</tr>
<tr>
<td>11</td>
<td>1b</td>
<td>F</td>
<td>1b</td>
<td>M</td>
<td>M</td>
<td>Concordant</td>
</tr>
<tr>
<td>12</td>
<td>1a</td>
<td>M</td>
<td>HCV RNA-negative</td>
<td>F</td>
<td>F</td>
<td>Concordant</td>
</tr>
<tr>
<td>13</td>
<td>1b</td>
<td>F</td>
<td>1b</td>
<td>M</td>
<td>M</td>
<td>Concordant</td>
</tr>
<tr>
<td>14</td>
<td>2b/1a</td>
<td>F</td>
<td>2b/1a</td>
<td>M</td>
<td>M</td>
<td>Concordant</td>
</tr>
<tr>
<td>15</td>
<td>1a</td>
<td>F</td>
<td>1a</td>
<td>M</td>
<td>M</td>
<td>Concordant</td>
</tr>
<tr>
<td>16</td>
<td>1a</td>
<td>M</td>
<td>1a</td>
<td>F</td>
<td>F</td>
<td>Concordant</td>
</tr>
<tr>
<td>17</td>
<td>1a/1b</td>
<td>F</td>
<td>1a/1b</td>
<td>M</td>
<td>M</td>
<td>Concordant</td>
</tr>
<tr>
<td>18</td>
<td>Unavailable</td>
<td>M</td>
<td>HCV RNA-negative</td>
<td>F</td>
<td>1</td>
<td>Unknown</td>
</tr>
<tr>
<td>19</td>
<td>1a</td>
<td>F</td>
<td>HCV RNA-negative</td>
<td>M</td>
<td>Indeterminate</td>
<td>Unknown</td>
</tr>
<tr>
<td>20</td>
<td>1a</td>
<td>M</td>
<td>HCV RNA-negative</td>
<td>F</td>
<td>1 or 3</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

*Couples that were genotype-concordant and viremic, allowing for phylogenetic analyses (Table 4), are indicated in bold.
*Genotyping was performed via InnoLipa assay with confirmation by sequencing for all genotype 1 samples.
Table 4. Phylogenetic Evaluation of Viremic, Genotype-Concordant Couples (n = 6)

<table>
<thead>
<tr>
<th>Couple ID (Genotype)</th>
<th>Pairwise Distance Between Sequences*</th>
<th>Between Partners†</th>
<th>Estimated Minimal Divergence Time‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Couple 9 (1a)</td>
<td>0.059</td>
<td>&gt;0.05</td>
<td>19.2</td>
</tr>
<tr>
<td>Couple 11 (1b)</td>
<td>0.071</td>
<td>&gt;0.05</td>
<td>22.3</td>
</tr>
<tr>
<td>Couple 13 (1b)</td>
<td>0.069</td>
<td>&gt;0.05</td>
<td>22.3</td>
</tr>
<tr>
<td>Couple 14 (1a)</td>
<td>0.062</td>
<td>&gt;0.05</td>
<td>20.4</td>
</tr>
<tr>
<td>Couple 14§ (2b)</td>
<td>0.018</td>
<td>&lt;0.01</td>
<td>6.5</td>
</tr>
<tr>
<td>Couple 15 (1a)</td>
<td>0.044</td>
<td>&lt;0.01</td>
<td>14.6</td>
</tr>
<tr>
<td>Couple 17 (1a)</td>
<td>0.076</td>
<td>&gt;0.05</td>
<td>24.6</td>
</tr>
<tr>
<td>Couple 17 (1b)</td>
<td>0.018</td>
<td>&lt;0.01</td>
<td>6.2</td>
</tr>
</tbody>
</table>

Three of the six couples had strong evidence of infection with the same HCV isolate (in bold).

*In the reference sets for subtypes 1a and 1b, the mean pairwise distance was 0.618 ± 0.0006 (SD) (<1.65 SD = 0.052) for subtype 1a and 0.071 ± 0.007 (<1.54 SD = 0.059) for subtype 1b.
†In the reference sets for subtypes 1a and 1b, the mean number of base pair differences was 52.33 ± 5.33 (<1.65 SD = 43.5) for subtype 1a and 59.53 ± 5.79 (<1.65 SD = 46.2) for subtype 1b sequences.
‡Minimal divergence time based on estimated divergence rate of 2.6 nucleotide positions per year for the target region analyzed.
§Significance was calculated relative to the subtype 1a distribution because an adequate background distribution could not be established with the publicly available subtype 2b sequences. These values are also significant when compared with the subtype 1a distribution.

Discussion

Sexual transmission of HCV among monogamous heterosexual couples is an extremely infrequent event. The maximum prevalence of HCV infection among sexual partners of subjects with chronic HCV infection was only 1.2%, and the maximum incidence of HCV transmission by sex was 0.07% per year or approximately one per 190,000 sexual contacts. Condom use was infrequent among the study participants and decreased over the duration of the sexual relationship, indicating that the very low rate of sexual transmission in our study population was not due to use of barrier methods during sexual activity.

This estimate includes couples who were antibody-concordant by serotyping assays but without confirmation of HCV strain relatedness by phylogenetic analysis because at least one of the partners was HCV RNA-negative. By including these couples, we minimized selection bias, but because couples with the same genotype/serotypes may not be infected with the same strain of HCV, we provided maximum (including aviremic serotype concordant couples) and minimum (based on
viremic couples only) estimates of HCV prevalence and incidence. The minimum estimate of prevalence of HCV infection among viremic couples was 0.6% (95% CI, 0.0%-1.3%) and the incidence was 0.04% per year.

Sexual transmission of HCV presumably occurs when infected serum-derived body fluids are exchanged across mucosal surfaces. Potential factors that may influence this exchange include the titer of virus, the integrity of the mucosal surfaces, and the presence of other genital infections (viral or bacterial). Studies to detect HCV RNA in semen (seminal fluid and cells), vaginal secretions, cervical smears, and saliva have yielded mixed results. Failure to detect HCV RNA in body secretions from chronically infected subjects may be due to technical factors (e.g., specimen collection and storage) and the inability to exclude cellular components and to overcome the presence of polymerase inhibitors in body fluids. Even in studies employing optimal methods to detect HCV RNA, the minority of samples were positive for HCV RNA, and all positive samples were of low titer ($\leq 10^2$ IU/mL). A low titer of virus in genital secretions may be one reason that HCV is transmitted less efficiently than HBV or HIV. Additionally, transmission of infection by sex may require a specific genital tract environment such as disrupted mucosal integrity or the presence of viral or bacterial coinfections. These factors may explain the recent reports of HCV transmission by sex in HIV-infected men who have sex with men.

Epidemiologically, specific factors that facilitate sexual transmission of HCV have not been identified, although most studies were not large enough to do so. Our study is the largest conducted in the United States and the first to include a rigorous assessment of sexual practices, none of which were associated with concordant HCV positivity in couples. Although a considerably larger sample size might yield different results, the very low estimated overall transmission risk indicates that any risk for infection from engaging in specific high-risk practices would be very low. Thus, this study supports the current recommendations that persons with HCV infection in long-term monogamous relationships need not change their sexual practices. Prospective studies from other countries of monogamous couples provide additional support for this recommendation. An Italian study of 775 HCV-negative partners followed for an average of 10 years identified new HCV infection in three partners, but none of these partners had viral strains related to those in the HCV-infected partner, indicating an outside source of infection rather than possible sexual transmission. However, this study excluded 33 partners who were infected at baseline, introducing a potential bias into the study. It is possible that the risk period of HCV acquisition by sexual contact early in the relationship and exclusion of infected partners in long-term relationships excludes those partners at greatest risk. In contrast to the Italian study, we chose to include all anti–HCV-positive partners and rely about the phylogenetic analysis and detailed risk histories to estimate likelihood of sexual transmission. The ideal prospective study to assess risk of HCV transmission among monogamous couples would target HCV-negative partners initiating a sexual relationship with an HCV-infected individual, but such a study would be extremely difficult to execute.

Interestingly, in two couples (couples 14 and 17), each of the partners had evidence of HCV superinfection with only one of the strains phylogenetically similar in both partners. In couple 14, it seems likely that the related strain was transmitted from the partner with a history of IDU to the partner who reported no risk factors for HCV infection other than contact with the infected partner. However, the origin of the unrelated HCV strain in the partner with no other HCV-related risk factors is unexplained. In couple 17, the index subject and partner both had different risk factors for HCV, which could explain why each member of the couple was infected with different HCV strains while they also shared a similar strain that was likely transmitted from one partner to the other. These cases highlight the complexity of using phylogenetic analysis to determine the direction or mode of transmission in individual situations when events occurred at unknown times in the past.

Among the 12 couples that had concordant (or indeterminant) HCV genotypes or serotypes, 50% were HCV RNA-negative. This rate of spontaneous clearance is similar to that observed among subjects infected at younger (<30 years) ages (by transfusion of whole blood, receipt of contaminated Rh immune globulin, IDU, or accidental needlestick injuries), and prospectively followed for 20 years. Although a younger age at infection might explain the high proportion of anti–HCV-positive, HCV RNA-negative partners in our study, one might speculate that repeated exposures to small “doses” of HCV resulted in an immunization-like effect or facilitated viral clearance once infection occurred.

We acknowledge that we have not genetically proven transmission among the phylogenetically linked partners, but rather have presented strong evidence for such a transmission. The method we used is much
more effective for excluding possible transmission than it is for confirming it. The consensus sequence of the virus is heavily dominated by a handful of dominant quasispecies, and it drifts relatively slowly. If the genetic distance is not significantly more similar between the pairs than to the rest of the population, then there is no realistic chance the dominant strains came from the same source. Proving (or providing strong evidence for) infection with HCV from a common source is difficult for several reasons. First, HCV passes a bottleneck upon infection (it has been estimated that only a dozen to <100 infectious particles initiate an infection, and these may not be randomly sampled from the donor quasispecies). Therefore, it is possible even with deep sequencing that finding identical quasispecies variants shortly after infection may not be possible. Second, HCV rapidly adapts to a new host over the first 1-2 months of infection, leading to a burst of diversity and genetic drift. During the rapid expansion in a new host, there is little constraining adaptive immunity, and consequently novel variants are not selected out as rapidly as in an established infection, and immune escape variants that were selected in the donor often revert to a more-fit sequence. Third, HCV’s mutation rate is far higher than its fixation rate (i.e., the number apparent from population sequencing as we did). Therefore, at a quasispecies level, the viral sequence is essentially “shimmering” from the combined effects of random mutation and its opponent, negative selection. This mandates a rather careful genetic analysis to prove common-source infection, and this problem rapidly increases with time since infection. Finally, deep sequencing is quite error-prone, and consequently rather extensive statistical treatment of the data is required to be sure that rare variants actually exist in a sample. This means that even if identical reads are reported in two paired samples, one or both of them could easily be a sequencing error. The integration of these issues is that it would be perfectly possible by a careful quasispecies analysis or a deep sequencing analysis to prove an identical source for two infections shortly after transmission, but the ability to prove a common source decays relatively quickly with time and is difficult in situations where the transmission occurred many years in the past.

A limitation of this study include its cross-sectional nature. A prospective cohort would be the ideal study design to determine incident HCV infections among uninfected partners, but the logistics and cost of undertaking such a longitudinal study are daunting given the low incidence of infection. Unlike prior studies, we sought to overcome the limitations of the cross-sectional design by obtaining a detailed relationship history of sexual practices using techniques similar to those used to obtain lifetime alcohol use histories. Because the partner’s HCV status was unknown in the majority of cases prior to history-taking, there would be minimal effect of differential bias in recall of sexual or other shared practices. Regardless, some participants may have unacknowledged histories of IDU or other sensitive risk factors, a limitation we tried to minimize by screening each participant on multiple occasions. Recall bias is a potential limitation with any cross-sectional study, but we found no difference in completeness of the sexual histories among HCV-positive versus HCV-negative couples. Another potential limitation was the sample size and the small number of positive partners for stratified analysis. Finally, the study population may not be representative. While index subjects were similar in age and gender distribution to HCV-positive adults identified in the general population,1 the study population was predominantly non-Hispanic white, and the majority had an education level beyond high school.

In conclusion, HCV transmission by sex from chronically infected persons to their heterosexual partners in a long-term monogamous relationship likely occurs, but is a rare event. Our results provide a basis for specific counseling messages that clinicians can use with their patients. These messages should be qualified given the limitations of the sample size, but they support the current national recommendations that couples not change their sexual practices if they are in a monogamous heterosexual relationship.

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