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## Abstract

Hepatitis C virus (HCV)-RNA in the blood was measured by polymerase chain reaction (PCR) in 37 subjects from eight families in which 2 or more persons tested seropositive for antibodies against C100-3 or CP9. HCV-RNA was positive in 17 of 37 subjects. Two or more HCV-RNA-positive subjects were observed in six of the families. Intrafamilial HCV infection was studied by determining the HCV-RNA type (I, II, III or IV) by PCR using type-specific primers. In two families, all of the subjects showed type III infection, and in three other families, all of the subjects showed type II infection, with different types of HCV infections being observed in only one family. The HCV type was uniform in all but one. These findings suggest a possibility of intrafamilial infection between husbands and wives and between members of the same household.

**KEYWORDS:** HCV, intrafamilial transmission, HCV-RNA genotype

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# Intrafamilial Clustering of Genotypes of Hepatitis C Virus RNA

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Hepatitis C virus (HCV)-RNA in the blood was measured by polymerase chain reaction (PCR) in 37 subjects from eight families in which 2 or more persons tested seropositive for antibodies against C100-3 or CP9. HCV-RNA was positive in 17 of 37 subjects. Two or more HCV-RNA-positive subjects were observed in six of the families. Intrafamilial HCV infection was studied by determining the HCV-RNA type (I, II, III or IV) by PCR using type-specific primers. In two families, all of the subjects showed type III infection, and in three other families, all of the subjects showed type II infection, with different types of HCV infections being observed in only one family. The HCV type was uniform in all but one. These findings suggest a possibility of intrafamilial infection between husbands and wives and between members of the same household.

**Key words:** HCV, intrafamilial transmission, HCV-RNA genotype,

We have reported that in families of patients with chronic hepatitis C, the positive rate for hepatitis C virus (HCV)-related antibodies is statistically significantly higher than the general blood donor population (1), suggesting the possibility of infection through sexual or household contacts between husbands and wives and between parents and children. Nevertheless, there have also been numerous reports that HCV infection by sexual transmission is extremely rare (2, 3), and infection by medically-related injection has not been ruled out. For this reason, we investigated HCV-RNA in families in which more than one person was positive for anti-HCV antibodies (anti-HCV), evaluated whether or not the current infection was persistent, and identified the type of HCV-RNA in positive subjects.

## Subjects and Methods

We measured anti-C100-3 (4) and anti-CP9 by enzyme immunoassay (EIA) (5), in the serum of 110 family members (7 parents, 9 siblings, 55 children, and 39 spouses) of 45 index cases diagnosed with hepatitis C. These index cases had anti-HCV or HCV-RNA in the blood and were diagnosed by liver biopsies with chronic hepatitis (CH) or liver cirrhosis (LC).

HCV-RNA in whole blood samples was measured by nested polymerase chain reaction (PCR) (6). Next, the genotype of each HCV-RNA sample (I, II, III or IV) was determined using a method based on PCR amplification with primers deduced from the putative core gene that were characteristic for each of the four types (7). PCR was carried out in two stages to increase the sensitivity of typing. The first PCR was performed on HCV cDNA with universal primers which generated fragments of 272 bp in the putative core gene of HCV. The second PCR was carried out with a universal, sense primer and a mixture of four type-specific, antisense primers that were designed to generate products of different sizes. The PCR products expected for HCV types I, II, III and IV were 57, 144, 174 and 123 bp, respectively.

The positive rate of anti-C100-3 in volunteer blood donors (total number, 27,567) in our district, who had no blood transfusion (1.06 %) was used as a control group.

Results were compared using the chi-square test, and $P < 0.05$ was taken as the level of significance.

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## Results

The prevalence of HCV antibodies in the sera of 110 family members of 45 probands was studied. Anti-C100-3 was detected in six family members (5.45 %) (5.45 % *vs.* 1.06 %, $P < 0.01$). Anti-C100-3 and/or anti-CP9

**Table I** Hepatitis C virus (HCV)-RNA and anti-HCV in 29 family members and eight probands

| | | Age | Sex | ALT[a] (IU/l) | Liver[b] | Anti-HCV CP9 | Anti-HCV C100-3 | HCV-RNA PCR | HCV-RNA typing | Known risk factors other than familial contacts |
|---|---|---|---|---|---|---|---|---|---|---|
| ① | Husband | 52 | M | 126 | CH | H | H | + | III | F2 and F7 |
| ☆ | Wife | 49 | F | NL | LC | H | H | + | III | F7 |
| | Child-1 | 29 | F | NL | ND | − | − | − | | F7 |
| | Child-2 | 27 | F | NL | ND | − | − | − | | F7 |
| | Child-3 | 24 | M | 47 | CH | H | − | + | III | F7 |
| ② | Husband | 63 | M | NL | ND | H | H | + | III | None |
| ☆ | Wife | 61 | F | 175 | CH | H | H | + | III | F1 and F2 |
| | Child-1 | 39 | M | NL | ND | − | − | − | | None |
| ③ | Husband | 62 | M | NL | CH | H | H | + | II | F1 and F2 |
| ☆ | Wife | 61 | F | 165 | CH | H | H | + | II | F1 and F2 |
| | Child-1 | 36 | M | NL | ND | − | − | − | | None |
| ④ ☆ | Husband | 65 | M | 251 | CH | L | H | + | II | None |
| | Wife | 61 | F | NL | ND | L | − | − | | F1 |
| | Child-1 | 35 | F | NL | ND | − | − | − | | None |
| | Child-2 | 33 | F | NL | ND | − | − | − | | None |
| ⑤ | Husband | 60 | M | NL | ND | L | − | − | | None |
| ☆ | Wife | 57 | F | 112 | LC | H | H | + | II | F1 |
| ⑥ | Husband | 60 | M | NL | ND | − | − | − | | None |
| ☆ | Wife | 60 | F | 121 | LC | H | H | + | II | F1 and F2 |
| | Child-1 | 35 | M | NL | ND | − | − | − | | None |
| | Wife | 34 | F | NL | ND | − | − | − | | None |
| | Child-2 | 32 | M | 241 | CH | H | H | + | II | None |
| | Wife | 29 | F | NL | ND | − | − | − | | None |
| | Child-1 | 5 | M | NL | ND | − | − | − | | None |
| | Child-2 | 3 | M | NL | ND | − | − | − | | None |
| | Child-3 | 29 | M | 374 | CH | H | H | + | II | None |
| | Wife | 28 | F | NL | ND | L | − | − | | None |
| | Child-1 | 5 | M | NL | ND | − | − | − | | None |
| | Child-2 | 2 | F | NL | ND | − | − | + | II | None |
| ⑦ | Husband | 82 | M | NL | ND | H | − | + | IV | None |
| | Wife (died of LC) | | | | | | | | | |
| ☆ | Child-1 | 54 | M | 107 | CH | H | H | + | III | None |
| | Wife | 52 | F | NL | ND | − | − | − | | None |
| | Child-1 | 22 | F | NL | ND | L | − | − | | F2 |
| | Child-2 | 14 | F | NL | ND | − | − | − | | None |
| ⑧ | Husband (died of LC) | | | | | | | | | |
| | Wife | 49 | F | NL | ND | H | H | + | II | None |
| ☆ | Child-1 | 26 | M | 75 | LC | H | H | + | II | None |
| | Child-2 | 24 | F | NL | ND | L | − | − | | None |

*a*, alanine aminotransferase (ALT) value on liver biopsy; *b*, histological diagnosis; ☆, proband; PCR, polymerase chain reaction; NL, normal liver function tests; CH, chronic hepatitis; LC, liver cirrhosis; ND, not done; H, 2 ≦ cut-off index; L, 1 ≦ cut-off index < 2; −, negative; F1, blood transfusion; F2, surgery; F3, acupuncture; F4, tattooing; F5 parenteral drug use; F6, occupational exposure; F7, living in epidemic area

were detected in 12 family members (two parents, one sibling, four children and five spouses) (10.9 %) of eight probands. The positive rate of anti-HCV in children whose fathers were patients was 2.5 %; on the other hand, it was 20.0 % in children whose mothers were patients. The latter was significantly higher than the former ($P < 0.05$). The positive rate of anti-HCV among spouses was 12.8 %. Additional family members were sought in these eight families and 29 individuals were studied (Table 1). HCV-RNA was measured in 37 subjects (eight probands and 29 family members) from these eight families. All eight probands were seropositive for HCV-RNA, and 9 of 29 family members were seropositive for HCV-RNA (Table 1). Eight of these 29 individuals who showed high anti-CP9 and/or anti-C100-3 seropositivity (cut-off index of 2 or above) were also seropositive for HCV-RNA: 5 of these had elevated levels of serum alanine aminotransferase (ALT) and CH on liver biopsy, but the other 3 had normal serum ALT. Five of the 29 family members were negative for anti-C100-3 and low seropositivity for anti-CP9; HCV-RNA was negative in these 5 cases, and serum ALT was normal. Sixteen of the 29 family members were negative for anti-HCV and serum HCV-RNA was observed in only 1 case, the daughter (2 years old) of the third child of family #6. In these families there were ten living married couples. Six of these 10 couples were positive for anti-HCV but only three were positive for HCV-RNA. Thus, in these eight families 17 of the 37 individuals were seropositive for HCV-RNA and 13 had evidence of chronic HCV infection.

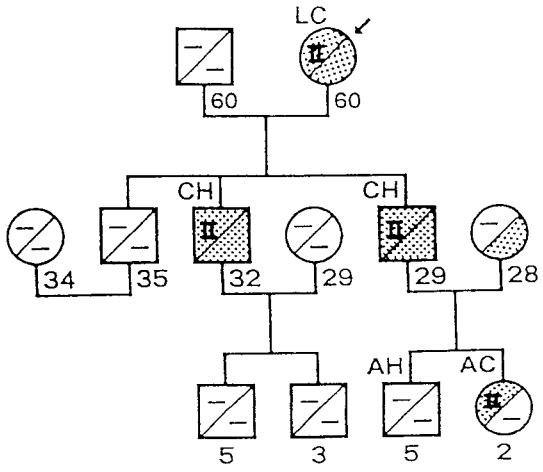


**Fig. 1** A family whose third-generation subjects had positive HCV-RNA (family #6 in Table 1).
□, men; ○, women; ▨,positive HCV-RNA; ▨, positive anti-HCV. Arrow indicates the proband. AC (asymptomatic carrier) means an individual positive for HCV-RNA whose liver function test is normal. Arabic numerals indicate age, and Roman numerals genotype.

In family #6 (Table 1, Fig. 1), HCV-RNA was detected in the sera of four individuals spanning three generations. The proband was the mother who received multiple blood transfusions just after the birth of her third son and developed posttransfusion hepatitis. She has active cirrhosis on liver biopsy with positive anti-HCV and HCV-RNA. Her second and third sons were both found to have developed chronic active hepatitis and had positive anti-HCV and HCV-RNA. The wife of the third son had low seropositivity for anti-CP9 but was HCV-RNA negative. The 5-year-old boy of the third son had a history of acute hepatitis (non-A, non-B) 2 years ago, and he had neither anti-HCV nor HCV-RNA. The 2-year-old daughter of the third son was negative for anti-HCV, but positive for HCV-RNA. Her serum ALT and other liver function tests were normal.

HCV-RNA genotyping was performed in the six families in which multiple RNA-positive individuals were observed (Table 1). In five of these families, both the RNA-positive married couples and the parents and children showed the same genotype, with a discrepancy being observed in only 1 family (family #7), where the father was type IV and the child was type III. In family #6, all 4 subjects: the mother, her second son, her third son, and the second daughter of the third son, were confirmed to be type II HCV carriers.

## Discussion

Approximately 50 % of chronic hepatitis C patients have a history of parenteral exposure, such as blood transfusion, drug abuse, tattooing, and needlestick injury (8-10). Intrafamilial transmission of non-A, non-B hepatitis has been reported to be infrequent compared to hepatitis B. However, in recent studies it has been suggested that perinatal infection with HCV is one of the causes of the silent disease process or chronic carrier state (11, 12). Furthermore, there have been reports of HCV detection in saliva (13) and semen (14). Accordingly, the causes of intrafamilial clustering of HCV antibody-positive subjects are assumed to include vertical transmission and horizontal transmission through sexual or household contacts, infection by parenteral exposure at health care

institutions, and infection through private acupuncture treatment (15).

In the present study, HCV infection was noted in 13 antibody-positive relatives of eight probands. By HCV-RNA measurement, at least 8 of 13 anti-HCV-positive family members were HCV carriers. There was also one family member who had positive HCV-RNA despite being anti-HCV-negative. In younger children (< 2 years) antibody may not have developed and therefore HCV-RNA measurement may be necessary in these cases to exclude infection.

Based on the full-length nucleotide sequence of the HCV genomes (16), at least the 4 genotypes (I-IV) were clearly identified. Studies of chronic hepatitis C patients in Japan have shown that type II is the most common, accounting for over half of the cases (7). In 68 patients, 57% had type II, 22% type III, 12% type IV, 6% type I and 3% mixed type. In this study, in five of the six families in which two or more subjects were positive for HCV-RNA, the individuals within a family all showed the same HCV genotype: two families had type III and three families had type II. This result is a finding which suggests that the families share the same HCV. However, types II and III are prevalent in Japan, so most infected individuals would be expected to have these genotypes. Then, to confirm the same virus, further examinations, *i.e.*, analysis of nucleotide sequences in the HCV genome would be required. That members of a given family all have the same virus does not mean that they gave it to each other through intimate contacts. For example, when medically related injections are done using the same needle without appropriate disinfection, HCV would be transmitted. These family members had neither medical injection at the same time nor acupuncture, so the above findings would suggest the possibility that HCV-positive family members got HCV through familial contacts. However, retrospective studies are rarely conclusive, then prospective studies are required in order to elucidate the transmission route.

Members of family #1 live in an area where hepatitis C was prevalent 40 years ago, but they moved into the area 30 years ago after the epidemic was over. In family #3, because both the wife and the husband had blood transfusion, it was possible that they both contracted hepatitis C separately by blood transfusion. The positive rate of anti-HCV between spouses, exclusive of family #3, was high, as compared with that in father-child pairs. It is hypothesized that sexual transmission is one of the main HCV infection routes. Positive rate of anti-HCV was significantly greater in mother-child pairs than others. In the case of mother to child transmission of hepatitis B virus, perinatal transmission is significant. However, in family #6, the mother had transfusion after she had given birth to her third son, and developed hepatitis C. Because members of family #6, except the mother, had no other known risk factors than familial contacts, it is suspected that infection routes are horizontal infection through intimate contacts between mother and child. Therefore, we need further examination in relation to causes of transmission of HCV.

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