INTRODUCTION

Reverse transcriptase polymerase chain reaction (RT-PCR) is used to confirm true hepatitis C virus (HCV) infection in clinical practice (1). The HCV genotype is useful for identifying the transmission of HCV (2, 3) and for understanding the natural history of HCV infection (4). Several studies have reported that the distribution of the HCV genotype differs between countries; moreover, response to interferon therapy depends upon HCV genotype (5, 6). It would be helpful for physicians in their decision-making process to have knowledge about the distribution of HCV genotype and the genotypic factors associated with HCV infection. In addition, it has been suggested that the distribution of HCV genotype in Koreans differs substantially from those of the Japanese and western people (7). Therefore, we undertook this study to identify the HCV genotype profile and possible risk factors by genotype in Korean patients.

MATERIALS AND METHODS

Case selection

The subjects were anti-HCV ELISA-positive cases who had undergone a periodic health examination between September 1994 and December 1998 at the Asan Medical Center in Seoul, Korea. This Medical Center is a private university-based tertiary medical institution primarily serving residents of Seoul. By November of 1999, 390 (57.4%) of 680 subjects, mailed an invitation to participate in the study, had volunteered to take part. Using a structured questionnaire, participants were asked about their past medical history including surgical and dental procedures, blood transfusions, needle sharing, jaundice, acupuncture, endoscopy, tattoos, and ear piercing. Testing included alanine aminotransferase (ALT) measurement and an abdominal sonogram, and RT-PCR was performed to confirm the status of HCV infection. Of the 390 patients, 242 (62.1%) were found to be infected with HCV by RT-PCR (PCR-positive). The HCV genotype was identified in 178 (73.6%) out of 242 patients, who tested positive for RT-PCR by repeated examination at the Liver Research Institute, Seoul National University. All 178 cases were evaluated for liver disease by ALT and received an ultrasound examination; 83 had elevated ALT, 18 cirrhosis, and in 77 the liver function was normal.

Control selection

The spouses of 242 PCR-positive patients were invited by letter to participate as controls. Of these, 130 responded and tested for anti-HCV antibody. 125 spouses negative for anti-HCV ELISA were recruited from spouses of HCV-PCR-positive patients and the other 101 from hospital visitors (hospital control). HCV genotyping was performed by PCR, and epidemiological data were obtained from all participants. The distribution of HCV genotypes was as follows - 1a (0.6%), 1b (39.9%), 2a (38.2%), 2b (0%), 3 (1.1%), and unclassified (20.2%). By multivariate analysis, blood transfusion (OR 2.90) and endoscopy (OR 2.80) were found to be risk factors for HCV genotype 1b versus the community control. Similarly, blood transfusion (OR 3.17) was found to be risk factors for HCV genotype 1b versus the hospital control. Blood transfusion (OR 2.75) and endoscopy (OR 3.57) were risk factors for HCV genotype 2a versus the community control, and blood transfusion (OR 4.55) and endoscopy (OR 2.16) were those versus the hospital control. Our results suggest that the risk factors for HCV infection are similar among the different genotypes. Blood transfusion and endoscopy were found to be associated with HCV infection.
HCV ELISA were enrolled as community controls. The proportion of women in the community controls was higher than that of the cases (Table 1).

All 104 subjects who underwent a periodic health examination during the same period and revisited Asan Medical Center were tested for anti-HCV ELISA. Of the 104 subjects, 101 who were negative for anti-HCV ELISA were enrolled as hospital controls (Fig. 1). A higher number of women and younger subjects were enrolled in the hospital controls than those in the cases (Table 1). All control subjects were interviewed using the same questionnaire.

### Laboratory tests

All the participants were screened with a third generation anti-HCV ELISA (Abbott Laboratories). Those who tested positive for anti-HCV antibody and RT-PCR were defined as HCV infection and those who showed elevated ALT levels exceeding the upper limit of normal for our laboratories (ALT > 40 IU/L) as elevated ALT. To detect hepatitis C viral sequences, serum RNA was extracted, reverse transcribed, and amplified by nested PCR (1) at the Liver Research Institute, Seoul National University. We were able to determine five genotypes of HCV, 1a, 1b, 2a, 2b and 3, using this method (Table 2).

### Statistical analysis

The association between possible risk factors with HCV infection for the PCR-positive cases and controls was assessed using odds ratios. The $\chi^2$ test and Fisher’s exact test were used as appropriate to determine whether the associations were statistically significant. Factors that were significant by univariate analyses ($p<0.05$) were examined after stratifying by possible confounders using the Mantel Haenszel method. Multiple logistic regression analysis was used to determine whether the observed associations were independent of other risk factors.

### RESULTS

#### Distribution of HCV genotypes

Of the 178 HCV RNA-positive subjects, genotyping results were obtained in 142. The distribution of HCV genotypes is detailed in Table 2. The most common genotypes were type 1b (39.9%) and type 2a (38.2%), while type 3 (1.1%) and type 1a (0.6%) were less common. None of the patients studied had genotype 2b. In 77 carriers of HCV

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**Fig. 1. Schematic presentation of cases and controls selection.**
RNA, genotype 2a was most common (40.3%), followed by genotype 1b (33.8%) and genotype 3 (1.3%). In 83 cases of chronic hepatitis, genotype 1b was most common (50.6%), followed by genotype 2a (32.5%) and genotype 1a and 3 (12.2%). In 18 patients with liver cirrhosis, genotype 2a was most common (55.6%), followed by genotype 1b (16.7%).

**Risk factors for HCV genotype 1b**

By univariate analysis, blood transfusion and endoscopy were associated with the risk of the HCV genotype 1b versus the community controls, and surgical procedure, blood transfusion, endoscopy and needle sharing were associated with the risk of HCV genotype 1b versus the hospital controls (Table 3). Other possible risk factors, such as a history of sexually transmitted disease (STD), were also evaluated.

**Table 2. Clinical diagnoses and genotypes of 178 hepatitis C virus PCR-positive patients**

| Characteristics       | PCR-positive cases | Community controls | Hospital controls |
|-----------------------|--------------------|--------------------|------------------|
|                       | No. | %   | No. | %   | No. | %   | No. | %   |
| Diagnosis             |     |     |     |     |     |     |     |     |
| Normal ALT*           | 77  | 43.3| 125 | 100.0| 101 | 100.0|
| Elevated ALT          | 83  | 46.6| 125 | 100.0| 101 | 100.0|
| Cirrhosis             | 18  | 10.1| 125 | 100.0| 101 | 100.0|
| Genotype              |     |     |     |     |     |     |     |     |
| 1a                    | 1   | 0.6 | 125 | 100.0| 101 | 100.0|
| 1b                    | 71  | 39.9| 88  | 72.5 | 88  | 87.3|
| 2a                    | 68  | 38.2| 57  | 45.6 | 57  | 63.0|
| 2b                    | 0   | 0.0 | 88  | 72.5 | 88  | 87.3|
| 3                     | 2   | 1.1 | 88  | 72.5 | 88  | 87.3|
| unclassified           | 36  | 20.2| 101 | 83.5 | 101 | 100.0|

**Table 3. Univariate analysis of potential risk factors for hepatitis C virus genotype 1b**

| Characteristics       | Genotype 1b cases | Community controls | OR* (95% CI) | Hospital controls | OR* (95% CI) |
|-----------------------|-------------------|--------------------|--------------|------------------|--------------|
|                       | No. | %   | No. | %   | No. | %   | No. | %   | No. | %   | No. | %   |
| Total                 | 71   | 100.0| 125 | 100.0| 101 | 100.0|     |     |     |     |     |     |
| Surgical procedure    | Yes  | 43  | 60.6| 62  | 50.8| 1.57 (0.84-2.95) | 37  | 37.0| 2.67 (1.36-5.24) |
|                       | No   | 29  | 39.4| 60  | 49.2| 63  | 63.0| 4.85 (2.26-10.42) |
| Blood transfusion     | Yes  | 32  | 45.7| 23  | 19.8| 3.35 (1.72-6.51) | 81  | 83.5| 11.38 (1.44-89.97) |
|                       | No   | 38  | 54.3| 93  | 80.2| 16  | 16.5| 4.85 (2.26-10.42) |
| Needle sharing        | Yes  | 4   | 5.8 | 2   | 1.8 | 4.05 (0.87-18.86) | 88  | 1.1 | 11.38 (1.44-89.97) |
|                       | No   | 65  | 94.2| 110 | 98.2|     |     |      |     |     |     |     |
| Endoscopy             | Yes  | 49  | 70.0| 56  | 46.3| 2.87 (1.47-5.60) | 61  | 60.4| 1.62 (0.80-3.28) |
|                       | No   | 46  | 29.0| 65  | 53.7| 65  | 68.4| 0.90 (0.44-1.83) |
| Dental procedure      | Yes  | 46  | 67.6| 78  | 67.2| 0.86 (0.43-1.74) | 65  | 68.4| 0.90 (0.44-1.83) |
|                       | No   | 22  | 32.4| 38  | 32.8| 30  | 31.6| 0.90 (0.44-1.83) |
| Acupuncture           | Yes  | 58  | 81.7| 95  | 78.5| 1.03 (0.47-2.78) | 74  | 77.1| 1.10 (0.49-2.47) |
|                       | No   | 13  | 18.3| 26  | 21.5| 22  | 22.9| 1.10 (0.49-2.47) |
| Tattooing             | Yes  | 13  | 18.8| 20  | 16.9| 1.67 (0.72-3.92) | 28  | 29.2| 0.88 (0.36-2.12) |
|                       | No   | 56  | 81.2| 98  | 83.1| 68  | 70.8| 0.88 (0.36-2.12) |
| Ear piercing          | Yes  | 17  | 24.3| 38  | 31.9| 1.61 (0.66-3.93) | 38  | 39.2| 0.94 (0.37-2.42) |
|                       | No   | 53  | 75.7| 81  | 68.1| 59  | 60.8| 0.94 (0.37-2.42) |
| History of STD*       | Yes  | 18  | 26.5| 22  | 18.6| 1.22 (0.56-2.66) | 13  | 13.5| 1.69 (0.72-3.94) |
|                       | No   | 50  | 73.5| 96  | 81.4| 83  | 86.5| 1.69 (0.72-3.94) |
| Family history of hepatitis | Yes | 19  | 28.8| 59  | 51.8| 0.42 (0.21-0.84) | 24  | 25.8| 1.20 (0.57-2.54) |
|                       | No   | 47  | 71.2| 55  | 48.2| 69  | 74.2| 1.20 (0.57-2.54) |

* Sexually transmitted disease; * Mantel-Haenszel estimates adjusted for age and sex. OR: Odds ratio.
of dental procedure, acupuncture, tattooing, ear piercing, sexually transmitted disease and family history of hepatitis were not associated with an increased risk of HCV genotype 1b (Table 3).

By multivariate analysis, the risk factors for HCV genotype 1b were age higher than 10 yr (OR 1.75, 95% CI 1.12-2.74), blood transfusion (OR 2.90, 95% CI 1.24-6.77) and endoscopy (OR 2.80, 95% CI 1.34-5.84) versus the community controls, and age higher than 10 yr (OR 2.10, 95% CI 1.34-3.30) and blood transfusion (OR 3.17, 95% CI 1.29-7.83) versus the hospital controls (Table 4).

Risk factors for HCV genotype 2a

Blood transfusion and endoscopy were associated with the risk of HCV genotype 2a versus the community controls, and blood transfusion was associated with the risk of HCV genotype 2a versus the hospital controls (Table 5). Other possible risk factors, such as a surgical procedure, needle sharing, history of dental procedure, acupuncture, tattooing, ear piercing, sexually transmitted disease and family history of hepatitis were not associated with an increased risk of HCV genotype 2a (Table 5).

By multivariate analysis, the risk factors for HCV genotype 2a were blood transfusion (OR 2.75, 95% CI 1.28-5.91) and endoscopy (OR 3.57, 95% CI 1.69-7.55) versus the community controls, and blood transfusion (OR 4.55, 95% CI 2.00-10.38) and endoscopy (OR 2.16, 95% CI 1.00-4.66) versus the hospital controls (Table 6).

DISCUSSION

This study shows that the most frequent genotypes among

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**Table 4. Multivariate analysis of risk factors for hepatitis C virus genotype 1b**

| Risk factors          | Community controls | Hospital controls |
|-----------------------|--------------------|-------------------|
|                       | Adjusted OR   | 95% CI           | Adjusted OR   | 95% CI           |
| Female                | 0.39           | 0.19-0.81        | 0.37           | 0.17-0.80        |
| Age (10 yr)           | 1.75           | 1.12-2.74        | 2.10           | 1.34-3.30        |
| Surgical procedure    | 0.86           | 0.39-1.90        | 1.63           | 0.74-3.60        |
| Blood transfusion     | 2.90           | 1.24-6.77        | 3.17           | 1.29-7.83        |
| Endoscopy             | 2.80           | 1.34-5.84        | 2.06           | 0.94-4.51        |
| Needle sharing        | 9.73           | 0.83-114.09      | 4.08           | 0.40-41.23       |

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**Table 5. Univariate analysis of potential risk factors for hepatitis C virus genotype 2a**

| Characteristics      | Genotype 2a cases | Community controls | Hospital controls |
|----------------------|-------------------|--------------------|-------------------|
|                      | No. | %       | OR* (95% CI)       | No. | %       | OR* (95% CI)       |
| Surgical procedure   | Yes  | 32 | 47.8 | 50.8 | 1.47 (0.59-2.20) | 37 | 37.0 | 1.93 (0.98-3.83) | No  | 35 | 52.2 | 60 | 49.2 | 3.02 (1.55-5.88) | 63 | 63.0 |
| Blood transfusion    | Yes  | 26 | 39.7 | 23 | 19.8 | 2.48 (0.28-21.66) | 1 | 1.1 | 4.86 (0.33-72.2) | No  | 38 | 60.3 | 93 | 80.2 | 81 | 83.5 |
| Needle sharing       | Yes  | 3 | 4.8 | 2 | 1.8 | 2.87 (1.47-5.60) | 61 | 60.4 | 2.00 (0.98-4.07) | No  | 59 | 95.2 | 110 | 98.2 | 88 | 98.9 |
| Endoscopy            | Yes  | 49 | 70.0 | 56 | 46.3 | 0.83 (0.42-8.44) | 65 | 68.4 | 0.82 (0.39-1.70) | No  | 21 | 30.0 | 65 | 53.7 | 40 | 39.6 |
| Dental procedure     | Yes  | 41 | 67.2 | 78.7 | 1.27 (0.56-2.88) | 74 | 77.1 | 1.01 (0.45-2.26) | No  | 20 | 32.8 | 38 | 32.8 | 30 | 31.6 |
| Acupuncture          | Yes  | 53 | 80.3 | 95 | 78.5 | 1.64 (0.63-4.29) | 28 | 29.2 | 0.69 (0.29-1.64) | No  | 13 | 19.7 | 26 | 21.5 | 22 | 22.9 |
| Tattooing            | Yes  | 11 | 16.9 | 20 | 16.9 | 1.40 (0.69-2.81) | 13 | 13.5 | 2.07 (0.93-4.63) | No  | 54 | 83.1 | 98 | 83.1 | 83 | 87.0 |
| Ear piercing         | Yes  | 10 | 15.4 | 38 | 31.9 | 0.79 (0.32-1.98) | 38 | 39.2 | 0.42 (0.17-1.03) | No  | 55 | 84.6 | 81 | 68.1 | 59 | 60.8 |
| History of hemodialysis | Yes | 3 | 10.0 | 0 | 0.0 | – | 0 | 0.0 | – | No  | 27 | 90.0 | 54 | 100.0 | 80 | 100.0 |
| History of STD*      | Yes  | 18 | 28.6 | 22 | 18.6 | 1.40 (0.69-2.81) | 13 | 13.5 | 2.07 (0.93-4.63) | No  | 45 | 71.4 | 96 | 81.4 | 83 | 86.5 |
| Family history of hepatitis | Yes | 20 | 31.7 | 59 | 51.8 | 0.51 (0.26-0.98) | 24 | 25.8 | 1.46 (0.72-2.98) | No  | 43 | 68.3 | 55 | 48.2 | 69 | 74.2 |
| Total                |        | 68 | 100.0 | 125 | 100.0 | 101 | 100.0 |

*: Sexually transmitted disease; *: Mantel-Haenszel estimates adjusted for age and sex.
Korean patients with HCV infection are 1b and 2a. It also demonstrates that the risk factors for HCV infection are previous transfusion, a history of jaundice and endoscopy, which are not different between genotypes. To our knowledge, this is the first case-control study to examine the risk factors for HCV infection according to genotypes in Korea. Our results thus provide an interesting contrast to investigations of hepatitis C in other countries.

By selecting the cases and hospital controls from a group of health examinees during a defined period, and who were assigned the same hospital, we designed a hospital-based case-control study. Although a higher proportion of women and younger subjects were enrolled in the hospital controls than were present in the cases, no significant differences exist between the two groups with respect to demographic and other general characteristics. We also enrolled the spouses of the case group as community controls and compared their results with those of hospital-based controls. Moreover, despite the higher representation of women in the community controls than in the cases, no significant differences were found between the two groups. In addition, the risk factors for HCV infection, such as past medical history, were not affected by the spouse. As such, these groups make valid comparisons with respect to risk factor evaluation.

In this study, genotype 1b and genotype 2a were the most frequent genotypes, which is consistent with some studies in Korea (7) and Europe (8), but not all studies (2, 6). In patients with liver cirrhosis, genotype 2a was more common genotype (55.6%) than in those with HCV RNA carrier and hepatitis (33-40%). Genotypic associations with more severe forms of liver disease is an issue that deserves separate study. Several studies have suggested that genotype 1b is associated with the risk for cirrhosis (8) and the development of hepatocellular carcinoma (9), whilst others found no association between genotypes and the progression to cirrhosis (10).

This study showed that transfusion, the classic form of parenteral transmission, was related significantly to HCV infection, and this is consistent with other studies (11, 12). Endoscopy was found to be associated with an increased risk of HCV infection, again this is consistent with other studies, which suggested that HCV could be transmitted during endoscopic examination (13, 14). The odds ratio of endoscopy as a risk of HCV infection, as determined by logistic regression, was significant in the community controls and inconsistent in the hospital controls. The difference may be due to more experience of endoscopy in the hospital controls. Our previous hospital-based case-control study also showed that endoscopy was not associated with HCV infection (15). We did not fully evaluate intravenous drug use (needle sharing) as a risk factor due to the low prevalence of drug users amongst the cases and the controls. However, our previous study showed that needle sharing is not a major risk factor for HCV infection among Koreans (16). Previous surgery was found not to be a risk factor for HCV infection in this study, which is consistent with some (17, 18), but not all studies (3, 19). Even though the prevalence of acupuncture was high in the study population, it was not found to be a risk factor for HCV infection. This finding conflicts with the results of another study in Taiwan (3) that found acupuncture to be a route of HCV transmission. Other risk factors of parenteral exposure, such as tattooing, dental procedures and ear piercing, were not found to be associated with HCV infection, but this is also controversial (3, 12, 19, 20).

Some limitations of our study are; first, there may be some volunteer effect in the selection of cases and controls from the health examinees (21). Second, our study population was not large enough to evaluate uncommon risk factors. Even with these limitations, we are able to conclude that the most frequent genotypes among Korean patients with HCV infection are 1b and 2a, and that the risk factors for HCV infection are previous transfusion and endoscopy, none of which showed genotypic differences.

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