the Asia Pacific region. Historically, blood transfusions and IV injections appear to be the most prominent risk factors. Additional work is required to better understand the level of existing and new HCV infections in China.

## Egypt

The Egypt Demographic and Health Survey (EDHS) reported extensive epidemiological data for the country, including data on HCV knowledge, risk factors and prevalence. The most recent report in 2008 had a sample size of over 12 000 individuals aged 15–59 years, randomly selected throughout the country (59). This study did not distinguish between acute and chronic infections. The blood supply in the country is screened and HCV-infected blood donors are notified.

### *Risk factors*

The EDHS study estimated that 29.6% of anti-HCV antibody positives (25.3% of women and 31.5% of men) received injections to treat schistosomiasis. Additionally, blood transfusion was identified in 24.3% and needle reuse in 20.6% of the HCV-positive cases in this nationwide sample (59). Similar results were described by Frank *et al.* (60). The parenteral injections occurred from the late 1950s to 1980s as the result of a campaign to treat schistosomiasis. In 1969, during the height of this campaign, over 300 000 individuals received IV injections, with an average of eight injections per person in and around the Nile River (60).

Risk factor for new infections were described in a case–control study which found parenteral therapy of schistosomiasis and blood transfusions as risk factors accounting for 13.2 and 9%, respectively, of total infections (61). Invasive hospital procedures and frequent injections were also cited as risks for ongoing transmission. The presence of these factors was seen in over 90% of individuals studied. Nosocomial infection continued to be a risk factor as well. In a study among paediatric oncology patients HCV prevalence was 0.9% at diagnosis, 13.1% after 6 months and 39.6% after cessation of therapy (62). Additionally, familial clustering was noted by multiple authors, suggesting the possibility of household transmission (spouse, father-offspring, sibling transmission) (63–65). Public shaving and IDU were implicated as largely secondary routes of transmission, but these associations were not consistently reproduced (61, 65).

### *Prevalence*

The EDHS report estimated a prevalence of 14.9% for the sampled population of 11 126 aged 15–59 in 2008. Prevalence increased with age, with 55–59 year olds showing a rate of 39.4%. Overall prevalence was 17.4% in males and 12.2% in females (59).

There were a number of studies among blood donors (66–76), and the 2006–2007 studies suggested an overall prevalence in the range of 7.6–8% nationwide (66, 68). Males had a modestly higher infection rate. Prevalence increased with age; 50–59 year olds had the highest prevalence. Rural areas had a higher prevalence than urban (66).

There were a number of studies in subgroups (60, 61, 64, 65, 67, 68, 77–93) and many sampled highly endemic areas, which gave evidence for very high prevalence in select regions (60, 61, 65, 77, 81). A study investigating the differences along the Nile River and its relationship to historical antischistosomiasis treatment in 10–50 year olds found a prevalence of 21.9% (60). A recent estimate among children estimated prevalence of 2% for 1–9 year olds (67). Prevalence was higher in males (11.3%) compared with females (6.5%) in areas along the Nile River (61). Evidence existed that females clear HCV more often than males, which could have accounted for the difference (79).

### *Diagnosed/incidence*

In the EDHS study, 14.9% of the sampled population was anti-HCV-positive and 1.4% was tested positive before this study (59). This implied that 9.4% of the prevalent population had been previously diagnosed before 2008. In 2008, the Egyptian Health Ministry used a national probability sample and reported an incidence of 6.9/1000 persons per year based on regression modeling (94). This estimate is the current gold standard. Other reports calculated 5.2/1000 person years in a study of rural, pregnant women (95).

### *Genotype distribution*

Genotype 4 predominates in Egypt. There are a number of reports (96–98), and a study of 131 HCC and chronic hepatitis C patients found the following genotype distribution: 1 (6%), 3 (1%) and 4 (93% with 4a = 63%) (96).

### *Summary*

Egypt has one of the highest HCV prevalences in the world (nearly 15% of the population). This was caused by repeated IV injections to resolve the schistosomiasis epidemic and transmission through needle reuse. Consequently, the older generations have a higher HCV prevalence than younger ones. Geographically, areas near the Nile River continue to exhibit very high rates of infection. Recent modeling data have also revealed a continuing trend of high incidence rates despite better blood screening measures and better sanitization practices within hospitals. This is in part because of the large reservoir of infected individuals, which increases the potential for continued transmission. Genotype 4 comprises 93% of the total HCV infections, and other genotypes comprise only small proportions of the infected population.

## India

There is no national surveillance reporting system in place, and presently the epidemiology is described by isolated studies and blood bank data.

### *Risk factors*

In 2002, the National Blood Policy was created with the hope of creating a unified system to provide a safe and sufficient blood supply for the entire country (99). Despite these efforts, blood transfusion in India carries a higher risk of infection through use of replacement blood donors. While paid donation is illegal, many former paid donors pose as friends or family of patients needing blood (100). In addition, both private and government blood banks are poorly regulated and testing for HCV is viewed as unsatisfactory, in part because of extra costs (100).

A number of studies identified risk factors associated with HCV infection (101–104). Parenteral transmission was

identified as a key risk factor, primarily through exposure in a medical setting. The largest risk factor was blood transfusion, accounting for 38–75% of chronic HCV infections (101, 102, 105). Other risk factors included medical exposures such as the use of reusable glass and traditional syringes (101, 106, 107), which was practiced by as many as 18% of physicians (108). Additionally, haemodialysis and a history of surgery were listed as risk factors (105).

### *Prevalence*

The estimated HCV prevalence was 1–1.9%. A study of 2973 randomly selected individuals in West Bengal determined a prevalence of 0.87% (106). However, this study represented data from one region and blood donor studies showed significant variation between regions. The Northern part of the country had similar practices and risk factors as Pakistan, where prevalence was above 2%. A study of 8130 pregnant women reported a prevalence of 1.03% (109), which is likely to underestimate the prevalence in the general population.

The majority of the reported studies were among blood donors (103, 110–124), with rates ranging from 0.28 to 1.85%. The differences were attributed to different generations of the anti-HCV testing and differences in the populations and practices between different regions of the country (111–113, 117–119, 121). A study of 28 956 mainly male replacement blood donors (family and friends of the patient) in Delhi found a prevalence of 0.66%, which decreased with time from 1.01% in 2000 to 0.29% in 2005 (111). Many of the more recent blood donor studies report prevalence of $< 1.0\%$, indicating that increased screening and education of donors may be working, although testing for anti-HCV is poorly regulated and not always done (110). Replacement donors typically have higher HCV infection rates than voluntary donors (103, 112, 120). Overall, blood donors underestimated the true prevalence because of self-selection.

Studies in high-risk groups found varying prevalence rates. Among IDUs in Northern India, prevalence was 33.7% (125). Haemodialysis patients and thalassaemics receiving multiple blood transfusions showed a prevalence of 41.9 and 25.45% respectively (123). Attendees at a sexually transmitted disease clinic had a prevalence of 2.6% (125). As expected, there was a higher prevalence in those with chronic liver disease with a range of 14–43% (101, 121).

Studies of prevalence by age provided mixed results. One study reported an increased prevalence with age, from 0.31% in individuals $< 10$ years to 1.85% in those $> 60$ years, while another found a decrease in prevalence, with the highest rates in adults aged 20–29 years (106, 117). Among volunteer blood donors the highest rate was found in those aged 41–50 (112). Males made up the majority of the study populations in India, but most studies did include some data on females.

### *Diagnosed/incidence*

There were no reported numbers of individuals diagnosed with HCV infection or rate of new infections.

### *Genotype distribution*

The most prevalent genotype was 3, with estimates ranging between 61.8 and 80.2% (101, 102, 104–106, 126–135). A study of 2118 patients across the country found genotypes 1 (31.2% with 1a/b = 92.4%, 1c = 7.6%), 2 (0.5%), 3 (61.8% with 3a/b = 94.9%, 3g/k = 5.1%), 4a/d (4.5%) and 6 (1.9%) (126). They also reported that genotype 3 was most prevalent in the Northern and Eastern regions, while in Western and Southern India the distributions of genotypes 3 and 1 were more even, with genotype 3 between 43 and 52% and genotype 1 between 43 and 48%. A study of 398 patients from North and Central regions also showed genotype 3 as the most common (80.2%), followed by genotype 1 (13.1%) (102). The presence of genotypes 4 and 6 in these populations could indicate a spread from Eastern Asia, where these genotypes are more prevalent.

### *Summary*

There are no studies that measured the general population prevalence across all regions and there appear to be significant variations across the country. Parenteral transmission remains the most significant risk factor, due mainly to IDU and reuse of syringes. Blood transfusion, because of lack of standardized testing and use of replacement donors rather than voluntary donors, remains a potentially large risk factor, and could result in more HCV infections in the general population. Data on newly diagnosed patients are lacking, and more studies are warranted. Genotype 3 is the most common HCV genotype.

## Japan

In 2002, a national screening programme was implemented by the Japanese Ministry of Health, Labour and Welfare. This screening programme reports on HCV infection in both high-risk groups and the general population (136). Blood donations have also been screened since 1989 (137).

### *Risk factors*

A 2010 study built on the earlier work of Moriya and colleagues and Yoshizawa and colleagues and commented on the cohort effect evident from multiple sources in the literature (137–139). They identified IV stimulant drug (methamphetamine) abuse among the youth during and after World War II, blood transfusion from paid blood donors, and injections using contaminated syringes and needles, particularly for the treatment of *Schistosoma japonicum* infection, which was endemic in Japan before the introduction of IV antimony in 1921. It was difficult to assign estimates attributable to study designs. The study of 42 young chronic HCV-infected patients identified IDU and exposure during medical procedures as risk factors (137). In a study of pregnant women, 30% of infections were linked to blood transfusions. However, 53% of these individuals were not linked to any particular transmission type (140). An older community-based study of inhabitants suggested that age, blood transfusions and positivity for anti-HBc were all linked to HCV infection (141). Historically, 1/3 of all HCV-positive blood donors were linked to blood transfusions (142).

### *Prevalence*

The estimated HCV prevalence was 1–1.9%. Published estimates come from a large number of blood donor (138, 143–149) and subgroup-based studies throughout the country from 1991 to 2010 (140, 141, 145, 147, 150–184). A study of pregnant women

in 1990–1994 found a prevalence of 0.3% in women < 40, and 1.8% in women over 40, suggestive of the importance of historical risk factors (140). Others showed a 3-year downward trend from 3.9% in 2003 to 3.0% in 2005 in a community-based sample in Osaka, known to contain a high proportion of IDUs (150). In a hospital-based study, 7.1% were HCV positive (152). Further evidence of a large age gradient was reported by other studies as well, with increased prevalence among individuals above 50 and 60 years of age (154, 155).

Blood donor population studies date back to 1990, and all estimated prevalence under 1.1% (138, 143–149). A study of 3 485 648 individuals who donated blood between 1995 and 2000 from eight jurisdictions reported a prevalence of 0.49%, with males and females being almost equal. A Southwest to Northeast gradient of infection was seen, with the highest prevalence in regions located in the Southwest portion of the country. A strong age gradient was also seen: individuals over age 60 were at highest risk for being carriers of HCV (143). Blood donors represented a self-selected population, with the prevalence in the general population always being higher.

### *Diagnosed population*

Studies estimating incidence were scant, although they point to a low incidence. Tanaka *et al.* (185) reported an incidence rate of 1.86/100 000 among blood donors from Hiroshima. Another study reported an incidence range of 1.8–3.4/100 000 (186). On the high end, an incidence of 362/100 000 was reported in a highly prevalent region (156). Diagnosis rate was difficult to determine based on published work.

### *Genotype distribution*

There were a number of publications from 1993 to 2000, and almost all indicated genotype 1b as dominant (158, 159, 171, 187–189). In a 2000 study with 166 samples from an endemic area, the following genotypes were identified: 1b (63%), 2a (25%) and mixed/other (12%) (158). Other studies showed similar results, but further breakout of subtypes (187, 188). The only exception was a study by Kobayashi *et al.* (189), which showed no genotype 1, genotype 2 at 73.3% and genotype 3 at 18.2%. This study was limited to a single hospital located in Tokyo. Except for the latter study, all others showed very small percentages for genotypes 3 and 4.

### *Summary*

Japan can be characterized as a low HCV prevalence country with relatively low incidence numbers, despite a large burden of hepatocellular carcinoma in older populations. Age-specific prevalence rates indicate significant historical transmission routes using unsanitized needles. Regional differences, particularly a Southwest to Northeast prevalence gradient, have been observed and replicated in studies through time. Genotype 1b dominates.

## Korea

While a National Health and Nutrition Survey has been in place in South Korea since 1998, it does not yet report on HCV infections (190), and no other general population-based reporting systems have been mentioned in the literature. However, individuals over 40 are suggested to represent a significant portion of infected individuals. In response to this, the Ministry of Health and Welfare and the National Cancer Center initiated an anti-HCV screening programme in 2003 targeted at individuals over 40 (190).

### *Risk factors*

Among existing cases, blood transfusion was reported as the main risk factor (191). However, comparisons between blood transfusions before and after 1992 suggested that there was minimal risk for infection because of transfusion since the start of blood screening in 1991 (191). New infections were therefore arising from other routes of transmission. In a study of 178 infected patients, both previous blood transfusion and a history of endoscopy procedures were found to be associated with HCV infections for patients with genotypes 1b and 2a. Among patients infected with subtypes 1b and 2a, 45.7 and 39.7% were attributed to previous blood transfusions (192). Rural areas reported similar risk factors. In a study of 77 anti-HCV-positive individuals from a rural town in Southeast Korea, blood transfusion before 1992 was found to be significant. Acupuncture was also found to be at risk in this group, with 81.8% of anti-HCV-positive persons reporting this exposure (193).

Injection drug use, which is a key risk factor in other countries, was identified as a potential risk factor. However, this was not thoroughly studied, and therefore further investigation was needed to determine its contribution to incident cases (191).

### *Prevalence*

A prevalence rate of 1.29%, or 193 000 infected-persons aged 40 or older, was estimated in 1995–2000 (191) based on analysis of four large studies with a total sample size of 124 605, where prevalence increased from 0.57% among 40–49 years old to 2.16% among 60+ years old. An earlier study by the author pooled results from 15 reports with a total samples size of 146 561 yielding a prevalence of 1.68% in the general population over 40 years old in 1990–2000 (190). Other studies in 1992–2008 provided a range of 1.00–1.35% (194–196). A much higher prevalence of 5.52% was found among rural volunteers (197).

Prevalence among blood donors decreased since the start of donor blood screening in 1991 (190). In a study done in 1991 of 150 blood donors, 1.30% of individuals were found to be infected (198). More recent blood donor information shows a significant decrease. In 2002–2006, the overall prevalence among blood donors from the Korean Red Cross, hospitals, and for-profit donation centres remained below 0.25%. The decline in prevalence could be because of first generation assays that resulted in false positives, tighter guidelines for blood donation, or an actual decline in prevalence among donors. However, prevalence among blood donors was not representative of prevalence in the general population, as blood donors in Korea were typically young individuals such as students or military recruits (199).

### *Genotype distribution*

There were a few recent studies, with most reports dating back from the 1990s (192, 200–204). The Park *et al.* study (201)

reported the following genotypes: 1 [50.3% with 1a (3.0%) and 1b (47.3%)], 2 [45.0% with 2a (42.6%) and 2b (2.4%)] and mixed/other (4.7%). These findings were consistent with other studies of infected patients, which report subtypes 1b and 2a to be the most frequent (192, 202–204).

### *Summary*

Hepatitis C infection in Korea is a significant problem in older generations, likely because of blood transfusions before screening was implemented. The general population prevalence is about 1.29%, based mainly on investigations of older age groups (40+). In the absence of additional studies, it is unclear what percentage of Korean youth is infected, or if IDU is a significant risk factor for new infections. Genotype 1 is most commonly reported from the current literature.

## Pakistan

There is currently no general surveillance or reporting system in Pakistan to track trends in HCV. Owing to the estimated high number of infections, health authorities occasionally run educational campaigns to increase awareness throughout the country, but it is uncertain if these activities are causing a measurable decrease in infections (205). There is also no reporting system in place in transfusion services, therefore data on the safety of the blood supply throughout Pakistan are scarce (206). It is suggested that screening of blood donors for anti-HCV is still insufficient (207).

### *Risk factors*

Pakistan has one of the highest rates of injections by providers in the world. One analysis included 3351 individuals from across the country and identified the following risk factors: reuse of needles or syringes for injections (61.45%), surgeries and dental procedures (10.62%), blood transfusion or blood products (4.26%) and other causes including razor sharing and circumcision by barbers (3.9%) (208). A separate study by the same lead author reported reuse of syringes for antibiotics, vitamins and drugs as the factor most strongly associated with HCV infection in a large study ($n = 6817$) based in Punjab province (209). More than 50% of the cases were acquired in hospitals, pointing to nosocomial infections as the primary source of transmission. Additionally, there was the possibility that public shaving in the male population was a significant transmission route. A large proportion of cases were identified as sporadic, or because of unidentified sources of contamination (209).

A broad, qualitative risk factor assessment based on meta-analyses confirmed the above observations (206). The prevalent exposure pattern was associated with frequent injections for a variety of purposes: intramuscular injections, IV drips used in the summertime to cool down, and prevalent use of injection within the general practice medical setting. A study in the Punjab province indicated smallpox vaccination was associated with HCV transmission (210). Blood transfusions and surgery were also reported as risk factors. A recent case–control survey reassessed anti-HCV prevalence in a volunteer blood donor population, confirming hospital-based transmission through the reuse/multiple use of needles by unqualified providers (211).

### *Prevalence*

A meta-analysis which pooled data from 132 published studies from 1992 to 2008 found prevalence of 3% among blood donors and 4.7% in the general population (212). Similarly, another review of 84 publications using a variety of sampling strategies and subgroups estimated an overall prevalence of 3% in all adults, 2.8% in adult blood donors, 5.4% in adult non-blood donors and 2.1% in children (206). There were geographical differences, as studies from Punjab showed higher rates than the three other provinces, and males had a higher rate of infections than females. Extreme variances existed in Punjab, the largest province, with reported rates upward of 30% HCV positive, which suggested a higher prevalence rate in this region than the rest of the country (206, 209, 212). A 2008 review reported the countrywide prevalence estimate between 2.4 and 6.5% (213).

Blood donor studies typically underestimated the true prevalence because of exclusion of high-risk groups (205, 214–220); however, this was not necessarily the case in Pakistan, consistent with the identified risk factors. Prevalence among blood donors ranged from 0.5 to 8.9%. The largest blood donor study with a sample size of 103 858 was published in 2002 and showed an overall prevalence of 4% (218). Higher rates were observed among rural donors (215) and lower rates were seen among college students (219).

There were a number of studies in subgroups (206, 207, 209, 213, 219, 221–243). A prevalence of 4.57% was reported in 16 400 outpatients in 1998–2002 (232). Higher prevalence among older individuals was seen as consistent across studies of different methodologies and design (206, 208, 209) and higher rates were observed for males over females (232, 241).

### *Diagnosed/incidence*

The incidence and the number of diagnosed patients was largely unknown. From 2002 to 2004, an audit of a single teaching hospital documented an increase in the number of requests for possible HCV positivity. The number of cases, however, appeared to decrease in the same time period from 14.19 to 5.84% (244). This decrease should be taken with caution, as it was based on a single laboratory, and was not likely representative for the country.

### *Genotype distribution*

Genotype distribution information was derived from three studies, which agreed that genotype 3 is the most prevalent genotype (208, 225, 245). The largest study included 3351 individuals from across the county and found the following genotypes: 1 [11.5% with 1a (8.3%) and 1b (3.0%)], 2 [8.4% with 2a (7.5%) and 2b (0.8%)], 3 [67.5% with 3a (49.1%) and 3b (17.7%)] (208). The smaller studies estimate genotype 3 at higher rates of 81.0–86.7% (225, 245), potentially because of the sampling. Similarly, however, genotype 1 was the next most prevalent, showing near agreement among all studies.

### *Summary*

Pakistan has one of the highest HCV infection prevalence rates in the world. Recent work has revealed good estimates in the absence of broad central reporting or a unified data collection

system. The most recent prevalence is estimated at 3%. The Punjab province, in particular, may have a much higher prevalence than the rest of the country. New infections, however, are less certain. The predominance of genotype 3 and the overwhelming role IV injections play in society leaves open the possibility of continued transmission. However, more data on incidence rates are needed.

## Saudi Arabia

The HCV infection has been a reportable disease in Saudi Arabia since 1990, although compliance varies. Blood donors are screened and pre-marital testing for HCV has been mandatory since 2007. It is estimated that over one million individuals have already been screened.

### *Risk factors*

Few studies have reported the risk factors in Saudi Arabia. A history of schistosomiasis was found in 7.4% of anti-HCV-positive patients, and prior blood transfusion in 14.8% (246). Another study looked at intrafamilial transmission and found no risk for HCV infection (247). Currently, IDU and blood transfusion are uncommon, indicating other forms of transmission such as bloodletting, traditional tattooing and iatrogenic nosocomial transmission (248, 249).

### *Prevalence*

HCV prevalence was estimated at 1–1.9% among adults. The prevalence in the general population is uncertain given that most studies were conducted more than 10 years ago (246, 250–254). Two studies showed a relatively high prevalence of 5.87% in a cosmopolitan area, and 5.09% in an agricultural region, indicating little difference in urban/rural rates (250, 251).

Although more recent studies among blood donors were available, these studies may not accurately reflect the overall prevalence as they represent healthy adults consisting mostly of males (248, 255–262). Among 557 813 blood donors, a prevalence of 1.1% was reported (259), although two recent studies showed prevalence of 0.6% (255, 258). Older blood donor studies reported higher prevalence rates, likely because of less stringent donation guidelines and no prior testing for anti-HCV (248, 260, 262). However, this higher rate could also be because of a higher prevalence rate among expatriate donors (4.52%) compared with nationals (1.24%) (261).

There were a number of studies in subgroups (253, 254, 257, 259, 263–273). High-risk groups such as haemodialysis patients had a prevalence of 14.7–68% (257, 265, 267, 268, 271, 272). Varying rates were found in the healthy population (5.3%), individuals with a sexually transmitted disease (15.9%), haemodialysis patients (26.1%), thalassemics (33.3%) and haemophiliacs (78.6%), indicating the role of blood transfusion or other nosocomial transmission routes in high-risk groups (254).

Studies reported differences in prevalence with age. In children, the prevalence was reported between 0.1 and 0.9% (259, 273). A general increase in prevalence with age was observed: 4.49% in $< 15$, 2.05% in 15–24, 5.10% in 25–34, 8.64% in 35–44, 15% in 45–54 and 11.9% in $\geq 55$ years old in a cohort of outpatient attendees and admitted patients (250). Others reported that prevalence was highest in males aged $> 40$ years (6.2%) and in females 40–49 years (5.0%) (246). One study found that in male blood donors, the peak age was 30–39 (260), with similar results from a community-based sample (252). This could indicate that the primary source of transmission in the past was through blood transfusion.

Men made up the majority of the study populations in Saudi Arabia, and had more than twice the rate of women (9.6 vs. 4%) in an outpatient setting (250). However, in a community-based study with equal numbers of men and women, no gender differences were reported (246).

### *Diagnosed/incidence*

HCV infection has been a reportable disease in Saudi Arabia since 1990. Based on data from 2000 to 2005, 37.7/100 000 cases were reported, which included both chronic and acute cases (274). From 1995 to 2005, 24 948 were reported, while in 2007 alone there were 2776 reported cases. Incidence was higher in adults (202/100 000) as compared with children (12/100 000). There were also regional differences—16/100 000 in Jizan to 322/100 000 in Al Baha (275). A study of the population served by the National Guard Health Affairs (NGHA) from 2000 to 2007 found a rate of 78.4/100 000, which may be declining with time (276).

### *Genotype distribution*

Genotype 4 is the most prevalent genotype, followed by genotype 1 (277–282). Among 561 consecutive genotypes performed in a single centre (NGHA) in 2006–2010, the following genotypes were identified: 1 (23.4%), 2 (3.2%), 3 (3.4%), 4 (60.9%) and mixed genotypes, mostly genotypes 4 and 1 (8.7%). Genotype 5 was rare and genotype 6 was non-existent (I. Altraif *et al.*, unpublished data).

Other studies found varying genotypes, where genotype 4 was found in 74% and genotype 1 in 14% (283, 284). In haemodialysis and chronic renal failure patients, infection with genotypes 1 and 4 was almost equally distributed (283, 284). In IDUs, however, genotype 1 was more prevalent (48%), with the majority genotype 1b (39%), followed by genotype 4 (36%) (283).

### *Summary*

The prevalence of HCV infection in Saudi Arabia varies between 0.6 and 1% among blood donors. More recent prevalence studies in the general population across Saudi Arabia are needed in order to get an accurate picture of the current prevalence and risk factors, given that infection by blood transfusion is minimal. There is an increase in prevalence with age, possibly because of varying modes of transmission over time or different risk factor exposures in different age groups. Hepatitis C is a reportable disease in Saudi Arabia, with 37.7 newly diagnosed cases/100 000 inhabitants. In 2007, there were 2776 cases reported. The majority of chronic HCV infections are because of genotype 4, followed by genotype 1.

## Syria

There were no reported general population surveillance or screening systems in place in Syria, and epidemiology data were only available from isolated reports in specific populations.

### *Risk factors*

A study among 295 RNA-positive patients aged 2–80 from eight medical centres in 2004–2006 found the following risk factors: blood transfusions or haemodialysis (49%) and tattooing (44%) (285). IDU was identified as a risk factor in only one case (0.3%).

### *Prevalence*

The estimated HCV prevalence was 1–1.9%. While few prevalence studies were published, a study among 2100 predominantly male blood donors reported a prevalence of 0.95% (286). Other studies described much higher rates in specific subgroups. The percent of HCV infected IDUs was similar to other countries at 60.5% (286). Healthcare workers and haemodialysis workers were found to have an infection rate of 3 and 6% respectively (287, 288). However, blood donor studies usually underestimated the HCV prevalence.

### *Genotype distribution*

Genotype 4 was the most common genotype. From a sample of 636 patients from eight medical centres throughout the country, the following genotypes were identified: 1 (28.5%), 2 (0.8%), 3 (1.8%), 4 (59.0%) and 5 (10.1%) (285). On the other hand, a small single-centre study ($n=37$) found genotype 4 (30%) to be less common than genotype 1 (46%) (289).

### *Summary*

There are few published studies describing the current state of HCV in Syria. From the data available, the prevalence in the general population is likely between 1.0 and 1.9%. However, community studies are needed to investigate true prevalence rates. Healthcare associated parenteral routes such as transfusions and haemodialysis are responsible for about a fifth of infections. The most common genotype is 4.

## Taiwan

A national reporting system in Taiwan has not been reported in the literature. However, hepatocellular carcinoma because of HCV infection is a recognized problem in the country and the National Health Insurance programme has funded treatment for HCV since 2003 (290). Taiwan also has one of the most active patient education, awareness and screening programmes in the region funded by companies and individual donors. The Liver Disease Prevention and Treatment Research Foundation was founded in 1994 and initiated screening programmes in 1996. By 2005, approximately 160 000 screenings were performed (291).

### *Risk factors*

Historic high prevalence of hepatitis B in Taiwan has led to a rich literature for both HBV and HCV. One study suggested iatrogenic causes driven by a cultural desire for IV injections for minor conditions and inadequate equipment disinfection (291). Others observed that nosocomial sources were because of past rural medical care being mainly provided by unlicensed practitioners (290). Disposable needles and syringes were not in common use until 1980 (292).

In a study of 272 seropositive men aged 30–64, a negative correlation between anti-HCV seropositivity and education level, a positive correlation with age and a positive correlation with blood transfusions and medical IV injections were found (292). The negative correlation with education was supported by another study which suggested that lower education/lower income persons may delay in seeking medical care, leading to more intrusive procedures (293). A geographical analysis indicated that the age correlation applied only in endemic areas (292). Multivariate analysis was used to analyse risk factors in infected and control groups identifying odds ratio for the following risk factors: blood transfusion (8.6), medical injection (2.4) and acupuncture (2.4). There was no statistically significant correlation for tattooing and haemodialysis, because of too few infected subjects with these risk factors. However, in a study among adolescents, significantly higher rates of anti-HCV prevalence were found among those with a history of transfusion, surgical operation, tattooing, or ear lobe piercing (294). Sun and colleagues examined the HCV genotypes of spousal partners. Most had different genotypes, supporting the conclusions that sexual intercourse was infrequently, if ever, a transmission mechanism for HCV and infections were likely because of exposure to common extrafamilial sources (292).

### *Prevalence*

Using data from the Liver Disease Prevention and Treatment Research Foundation, the HCV prevalence was estimated at 4.4% (or 423 283 anti-HCV-positive carriers) in adults aged $\geq 20$ years (291). This study analysed 157 720 subjects in 1996–2005 and found similar infection rates among males and females, an increasing prevalence with age, and significant geographical variation. There were a number of other studies reporting prevalence across Taiwan with a range of 2.9–17.0% (290, 291, 293, 295–298). Geographical variation, with very high endemic regions, was reported by several authors (290–292, 296). For example, Tsai *et al.* (290) reported a prevalence of 2.6–30.9% in townships and 0–90.5% in villages of Tainan county. The prevalence among youth was considerably lower—0% in 3–6 year olds, 0.8% in 7–12 year olds and 1.9% in 13–15 year olds (297, 298). In line with other countries, prevalence among blood donors was considerably lower at 1.2% (299), while haemophiliacs and IDUs had higher rates of infection at 90 and 81% respectively (300).

### *Diagnosed/incidence*

Through active screening, over 300 000 individuals have been screened; however, that accounts for 1.3% of the population. There were no data published on the total diagnosed population. One study did report that in an endemic area, Tzukuan Township, anti-HCV incidence was 4.5% (297).

### *Genotype distribution*

A study of 418 chronic HCV patients at a tertiary referral hospital and another on 1164 patients from three hyperendemic areas found very similar genotype distribution with 1b and 2a being dominant: 1 [48% with 1a (2.6%), 1b (45.5%)], 2 [39.5% with 2a/c (30.9%), and 2b (6.9%)], 3a (1%), 4 (0.2%), 6 (0.5%) and mixed/other (10.0%) (301, 302). It was noted that genotype 1b increased with age, while genotype 2a decreased with age

(302). Other publications reported genotype distribution among students, blood donors and endemic populations (75, 294, 297, 298, 303, 304).

*Summary*

Taiwan has one of the highest HCV prevalence rates in Northeast Asia, with the highest rates reported in older age groups. This is most likely because of the common use of IV injections for minor conditions, including the inadequate sterilization and reuse of syringes. There is a large geographical variation in HCV prevalence, with certain areas reporting a prevalence of over 30%.

### Thailand

To date, there is no national HCV reporting system in Thailand, but blood donors are screened with questionnaires (305).

*Risk factors*

A study of 214 mostly male patients at a hospital in Northeastern Thailand in 1997–1998 found IDU as the most important risk factor reported in 46.7% of cases, followed by tattoos (32.2%) and blood transfusions (18.8%) (306). Others confirmed the rank order of risk factors in a study of 166 HCV-positive blood donors and found a statistically significant association with previous IDU and transfusion. They also reported an association with multiple sex partners, but this result should be taken with caution as this may be confounded by the presence of multiple risk factors (307).

*Prevalence*

A study of 5525 persons aged 2–60 across four provinces found a prevalence of 2.15% (aged 2–60) and 2.8% (aged 21–60) in 2004 (305). Prevalence increased with age–1.1% (aged 5–10) to 3.4% (aged 51–60). Interestingly, children aged 2–4 also showed a high prevalence, at nearly 2.1%, indicating the potential for vertical transmission. A separate study of 1534 persons across six provinces found significant geographical variation with a prevalence range of 0.41–2.03% in 2000–2002 (308). There was also evidence of high endemicity (3.8–7.5%) among tribes in Northern Thailand (309, 310). The prevalence among IDU was 86–95% (311–314), while blood donor population prevalence ranged from 0.31 to 3.54%, with higher infection rates among males than females (315, 316). The most recent blood donor study reported a prevalence of 1.37%, and was comprised of voluntary donors from five separate studies (317).

*Genotype distribution*

An analysis of 45 samples collected in 2004 from four separate regions of Thailand showed genotype 3 as dominant: 1 [33.3% with 1a (6.7%) and 1b (26.7%)], 2c (4.4%), 3 [53.3% with 3a (51.1%) and 3b (2.2%)] and 6 (8.9%) (305). Additional studies sampled blood donors and estimated genotype 3 at 44% of the infected population (318, 319). In contrast, a sample of 46 chronic liver disease patients found genotype 1 as most prevalent. Genotype 1 and its subtypes comprised 48% (320).

*Summary*

A clear description of HCV in Thailand is largely unavailable, with missing reported data for incidence and diagnosis. IDU appears to be a continuing problem. There are significant geographical variations with very high endemic pockets in the country.

### Vietnam

The epidemiology of HCV in Vietnam comes exclusively from isolated studies, as there is no general surveillance system in place. Blood donor screening is not mandatory in the country.

*Risk factors*

Blood transfusion remained the predominant risk factor, because a large portion of the blood donors in Vietnam were paid and HCV screening was not mandatory (321, 322). It was speculated that the high use of IDU among the donor population was contributing to the increased risk because of blood transfusion. Differences in the prevalence of the disease between North and South Vietnam were also attributed to the longer use of IV drugs by those in the South (323, 324). Tattoos, a history of hospitalization, and occupations other than farmer were also reported as risk factors (325).

*Prevalence*

HCV prevalence was estimated at 2.0–2.9% among adults. There were considerably different data reported in the literature, ranging from 0.8 to 21% (321–323, 325, 326). Two studies reported prevalence near 0.8% among blood donors in Hanoi and 21% among blood donors in Ho Chi Minh City (321, 323), with higher prevalence among males than females in Ho Chi Minh City. This suggested an increasing prevalence from North to South. A more recent study among 100 individuals in Ho Chi Minh City reported a prevalence of 2% (326). Studies in more rural areas reported a prevalence of 1% (322, 325).

*Genotype distribution*

The most common HCV genotypes in Vietnam were 1 and 6. There were a number of genotype studies (322, 322, 323, 326, 327). A study in 70 RNA-positive blood donors in Hanoi reported the following genotypes: 1 [47.1% with 1a (30.0%) and 1b (17.1%)], 3 [5.8% with 3a (2.9%) and 3b (2.9%)] and 6 [47.1% with 6a (37.1%), 6e (8.6%) and 6i (1.4%)] (328). Genotype 6 was reported to occur in South China as well as Vietnam, Laos, Thailand and Myanmar (328). In 79 HCV RNA-positive donors from Ho Chi Minh City and four HCV RNA-positive donors from Hanoi, genotype 1 was the predominant genotype (54.0%), composed of genotype 1a (27.0%), 1b (23.0%) and mixed genotype 1 (4%) (323). However, 41% of the genotyped samples were not classifiable into genotypes 1, 2 or 3, and further analysis indicated the majority of the unknown samples were genotype 6a (19.3%) (327). This suggested a geographical distribution of HCV genotypes in Vietnam. Smaller studies reported genotype 1 as the predominant genotype, ranging from 42.8 to 75.0% (322, 326), with the majority typed as genotype 1a (23.8–50.0%), followed by 1b (23.8–25.0%).


Liver International (2011)

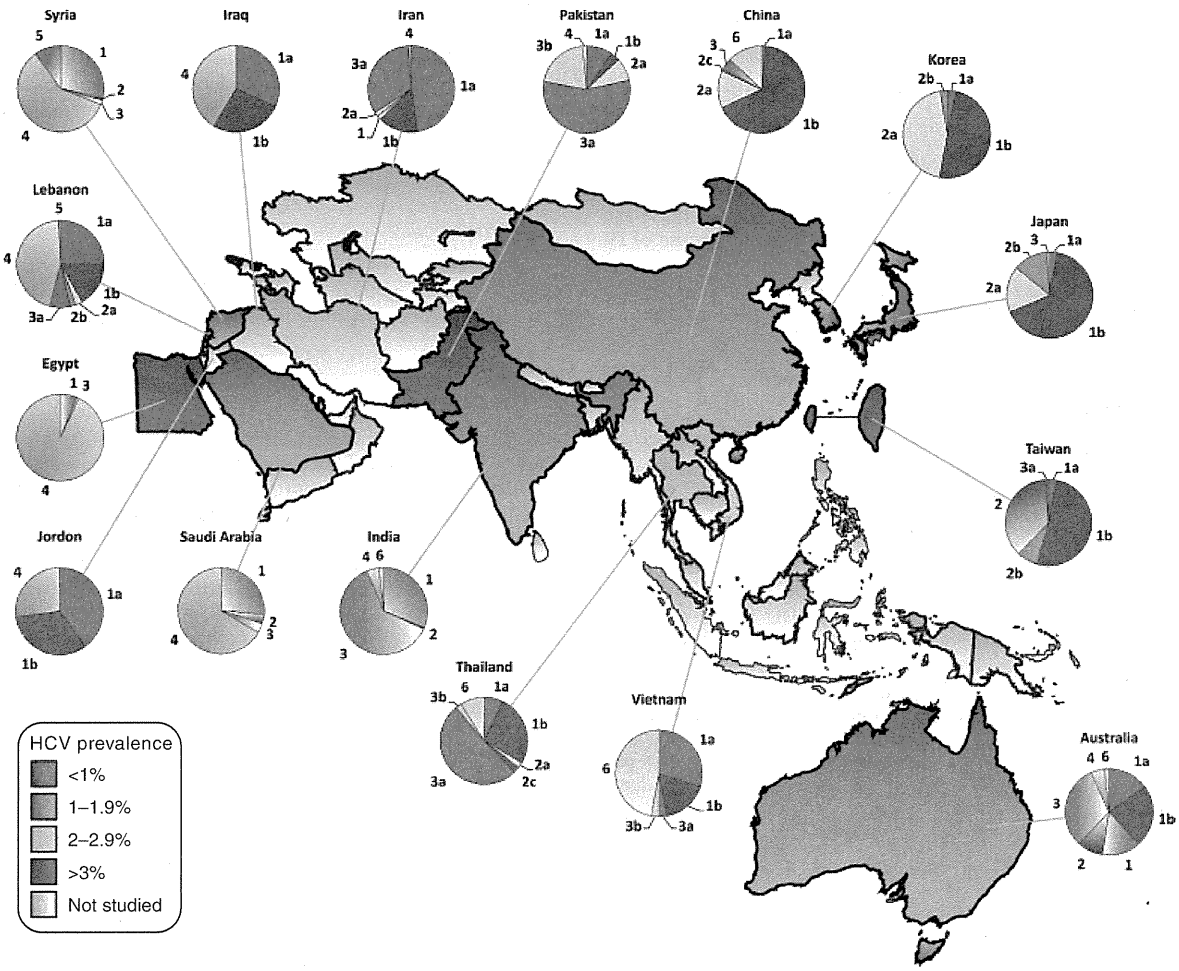


**Fig. 1.** Hepatitis C virus prevalence among adults and genotype distribution in Asia, Australia and Egypt.

*Summary*

The prevalence of HCV infection in Vietnam was estimated at 2–2.9%. Additional community studies from both the North and South are needed for a more accurate estimate. Lower prevalence rates are reported in the North, due mainly to less IDU. Blood transfusion remains the predominant risk factor in Vietnam, as almost all blood donors are paid. The most common genotypes are 1 and 6.

## Discussion

We estimate that 49.3–64.0 million adults in Asia, Australia and Egypt are anti-HCV positive. This region has the largest population of HCV infected persons with China alone having more HCV infections than all of Europe or the Americas. China (est. 13 million), India (est. 9.5 million) and Egypt (est. 6.5 million) have the highest number of HCV infected persons globally. As expected from such a large geographical and population distribution, there is considerable variability in HCV incidence, prevalence, genotype distribution (Fig. 1) and risk factors (Table 1). While most countries had prevalence rates from 1 to 2%, we documented several with relatively high prevalence rates, including Egypt (15%), Pakistan (4.7%) and Taiwan (4.4%). Even these high overall rates are dwarfed when regional differences within these countries are taken into account. For example, in some populations along the Nile in Egypt, prevalence rates approach 22% and in the Punjab, the most populous province in Pakistan, prevalence rates as high as 30% have been reported. This wide variability in prevalence likely reflects differences in risk factors for acquiring the infection, such as the previous common use of IV injections for the treatment of schistosomiasis in Egypt and the high rate of injections, with reuse of needles and syringes, given for a variety of treatments in Pakistan. Japan has a similar pattern, with nosocomial infection and blood transfusion (before screening) as the most common factors, resulting in the bulk of HCV infection in older patients. Some of these risks are now historical, given changes in immunization and blood transfusion practices. Nonetheless, unique factors in some countries suggest avenues for controlling the spread of infection, although cultural and educational barriers may exist. For example, in Pakistan, where public shaving of beards and other body hair is common and has been identified as a route of

**Table 1.** Summary of HCV epidemiology data by country

| | Top three risk factors | Prevalence rate in adult population | Diagnosis rate |
|---|---|---|---|
| Australia | 80% IDU<br>5–10% Blood transfusion | 1.3% | 85% |
| China | 75.4% IV use of glass syringes/needles<br>73.9% Blood transfusion<br>27.5% oesophageal balloon | 1–1.9% | – |
| Egypt | 29.6% Injections for schistosomiasis<br>24.3% Blood transfusion<br>20.6% Needle reuse | 14.9% | 9.4% as of 2008 |
| India | 38–75% Blood transfusion<br>Use of reusable glass syringes<br>Nosocomial | 1–1.9% | – |
| Japan | 30% Blood transfusion<br>Injections with contaminated syringes/needles<br>IDU among youth | 1–1.9% | – |
| Korea | 39.7–45.7% Blood transfusion<br>History of endoscopy<br>Acupuncture | 1.3% | – |
| Pakistan | 61.45% Syringe/needle reuse<br>10.62% Surgery/dental work<br>4.26% Blood transfusion | 4.7% | – |
| Saudi Arabia | 14.8% Blood transfusion<br>7.4% History of schistosomiasis<br>Bloodletting & traditional tattoos | 1–1.9% | – |
| Syria | 49% Blood transfusion or haemodialysis<br>44% Tattooing<br>0.3% IDU | 1–1.9% | – |
| Taiwan | 76.1% Medical injection<br>26.8% Blood transfusion<br>21%Acupuncture | 4.4% | 1.3% |
| Thailand | 46.7% IDU<br>32.2% Tattooing<br>18.8% Blood transfusion | 2.8% | – |
| Vietnam | Blood transfusion<br>IDU<br>Tattooing | 2–2.9% | – |

HCV, hepatitis C virus; IDU, injection drug use; IV, intravenous.

acquiring HCV and HBV infection, education programmes may help to reduce transmission in future (329). Tattooing in Asian countries is another risk factor which might be addressed via education of at-risk populations (325). Relatively little data are available from these countries on the recently described epidemics of sexually transmitted HCV infection among men who have sex with men compared with that available from Europe and North America; however, the overall prevalence rates appear to be low (330, 331). However, the addition of education programmes regarding the risk of sexually transmitted HCV infection to populations at risk of HIV infection would seem logical.

In addition to the unique risk factors noted above, commonly recognized factors such as IDU will be recognized as widespread in many countries, including Australia, where it is the most common route for acquiring HCV infection. Migration from countries of greater prevalence to those of lower prevalence is likely to result in a general admixture of many different risk factors. Thus, strategies to decrease the spread of HCV infection must include, in addition to the education programmes previously noted, screening of the blood supply before transfusion, avoidance of paid blood donors and, where politically feasible, needle-exchange programmes to decrease the incidence of new infections among IDU populations.

Similar to the large diversity of risk factors, this large geographical region has a great diversity of genotypes. Genotype 1 is common in Australia, China, Taiwan and most countries in North Asia, while genotype 6 is common in Vietnam and other Southeast Asian countries. Genotype 2 is found in substantial proportions, albeit lower than genotype 1, in Japan, Korea and Taiwan. In India and Pakistan genotype 3 predominates, which, because of the very large populations in these two countries, constitutes one of the largest concentrations of people infected with genotype 3 in the world. Middle Eastern countries such as Egypt, Saudi Arabia and Syria predominantly have genotype 4 infection, although genotype 3 can be found in other Middle Eastern countries such as Iraq and Iran, probably related to migration patterns in this area. Genotype 5 occurs in small numbers of patients in Syria but is rarely reported in Asia; similarly, genotype 6 is predominantly in Asia, with the greatest concentration in Vietnam. The clinical significance of this genotype distribution is based in the influence of genotype on response rates to combination therapy with interferon and ribavirin, being greater for genotypes 2 and 3 and lower for

**Table 2.** Availability and quality of hepatitis C virus (HCV) epidemiology data by country

| | Risk factors | Prevalence rate | Genotype distribution | Incidence rate | Diagnosis rate |
|---|---|---|---|---|---|
| Australia | ***** | ***† | **** | **** | ***** |
| China | ** | ** | *** | ** | |
| Egypt | ***** | ***** | *** | ***† | ***** |
| India | *** | ** | ***** | | |
| Japan | *** | ** | ** | ** | |
| Korea | *** | *** | *** | | |
| Pakistan | ***** | *** | ***** | | |
| Saudi Arabia | * | *** | *** | *** | |
| Syria | *** | ** | *** | | |
| Taiwan | ***** | ***** | *** | * | |
| Thailand | *** | *** | ** | | |
| Vietnam | * | ** | ** | | |

†Australia's prevalence and incidence data is based on a model rather than a general population study. Egypt's incidence is based on a robust model.

Prevalence, risk factors, genotype distribution, diagnosis rate, treatment rate:

*Estimate without a formal study

**Small study in select population (prevalence and diagnosed < 1000; genotype < 100, risk factors < 200) or blood donors study

***Large study in select population (prevalence and diagnosed > 1000; genotype > 100, risk factors > 200)

****Small study in the general population (prevalence and diagnosed < 1000; genotype < 100, risk factors < 200)

*****Large study in the general population (prevalence and diagnosed > 1000; genotype > 100, risk factors > 200)

Incidence:

*Estimate new infections without a formal study

**Incidence study

***Country wide registry-voluntary or with low level of participation

****Country wide registry-manditory or with high level of participation

*****Country wide registry-distinction between new and existing infections

genotypes 1 and 4. Response rates in genotypes 5 and 6 to combination therapy are less well characterized. Combination therapy with interferon and ribavirin is likely to remain the most commonly available treatment in many countries throughout Asia and the Middle East for the medium term, despite the greater availability of the new direct-acting antiviral agents in Europe and North America.

The studies reported here have several limitations, as shown in Table 2. Reliable reports on new infections are rarely available and often there are little data concerning the size of the diagnosed population. There is considerable variability in the type and quality of prevalence studies among the countries assessed. Countries like Australia, Egypt and Taiwan have completed large population studies or developed predictive models, while data from other countries, for example India and China, have relied on studies in subgroups. Over-representation among men in studies from Egypt, Saudi Arabia and Pakistan make estimates for women less certain, although the results of small studies, showing high prevalence rates, have been reported (332).

In summary, the vast population that resides in the area from the Middle East to Oceania presents an extraordinary and wide-ranging, but perhaps not unexpected, variability in the epidemiology of HCV infection. All of the known genotypes have been documented and a diverse range of risk factors for acquiring the infection is evident. The gaps in knowledge are clear and will depend on countries with large populations, such as India, Pakistan and China, continuing to develop high-quality surveillance and reporting programmes. The knowledge gained from such programmes can then be used to develop effective public health policy that may lead to the eventual curtailment of the spread of this pandemic infection.

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## References

1. Chiquete E, Panduro A. Low prevalence of anti-hepatitis C virus antibodies in Mexico: a systematic review. *Intervirology* 2007; **50**: 1–8.
2. Abdel-Hamid M, El-Daly M, El-Kafrawy S, *et al.* Comparison of second- and third-generation enzyme immunoassays for detecting antibodies to hepatitis C virus. *J Clin Microbiol* 2002; **40**: 1656–9.
3. Kershenobich D, Razavi HA, Cooper CL, *et al.* Applying a system approach to forecast the total HCV-infected population size-Model validation using US data. *Liver Int* 2011; **31**(Suppl. 2): 4–19.
4. Law MG. Modelling the hepatitis C virus epidemic in Australia. Hepatitis C Virus Projections Working Group. *J Gastroenterol Hepatol* 1999; **14**: 1100–7.
5. Dore GJ, Law M, MacDonald M, Kaldor JM. Epidemiology of hepatitis C virus infection in Australia. *J Clin Virol* 2003; **26**: 171–84.
6. Dore GJ, MacDonald M, Law MG, Kaldor JM. Epidemiology of hepatitis C virus infection in Australia. *Aust Fam Physician* 2003; **32**: 796–8.
7. Robotin MC, Copland J, Tallis G, *et al.* Surveillance for newly acquired hepatitis C in Australia. *J Gastroenterol Hepatol* 2004; **19**: 283–8.

8. Department of Health and Ageing. *National Hepatitis C Testing Policy.* Hepatitis C subcommittee of the Ministerial Advisory Committee on AIDS, Sexual Health and Hepatitis, Australia, 2007.
9. Razali K, Thein HH, Bell J, *et al.* Modelling the hepatitis C virus epidemic in Australia. *Drug Alcohol Depend* 2007; **91**: 228–35.
10. Razali K, Amin J, Dore GJ, Law MG, HCV Projections Working Group. Modelling and calibration of the hepatitis C epidemic in Australia. *Stat Methods Med Res* 2009; **18**: 253–70.
11. Amin J, Gidding H, Gilbert G, *et al.* Hepatitis C prevalence-a nationwide serosurvey. *Commun Dis Intell* 2004; **28**: 517–21.
12. Law MG, Dore GJ, Bath N, *et al.* Modelling hepatitis C virus incidence, prevalence and long-term sequelae in Australia, 2001. *Int J Epidemiol* 2003; **32**: 717–24.
13. Wasley A, Alter MJ. Epidemiology of hepatitis C: geographic differences and temporal trends. *Semin Liver Dis* 2000; **20**: 1–16.
14. Balogun MA, Ramsay ME, Hesketh LM, *et al.* The prevalence of hepatitis C in England and Wales. *J Infect* 2002; **45**: 219–26.
15. Alter MJ. Hepatitis C virus infection in the United States. *J Hepatol* 1999; **31**(Suppl. 1): 88–91.
16. Miller ER, Hellard ME, Bowden S, Bharadwaj M, Aitken CK. Markers and risk factors for HCV, HBV and HIV in a network of injecting drug users in Melbourne, Australia. *J Infect* 2009; **58**: 375–82.
17. Falster K, Kaldor JM, Maher L. Hepatitis C virus acquisition among injecting drug users: a cohort analysis of a national repeated cross-sectional survey of needle and syringe program attendees in Australia, 1995–2004. *J Urban Health* 2009; **86**: 106–18.
18. Bowden FJ, O'Keefe EJ, Primrose R, Currie MJ. Sexually transmitted infections, blood-borne viruses and risk behaviour in an Australian senior high school population-the SHLiRP study. *Sex Health* 2005; **2**: 229–36.
19. McDonald A. HIV infection, AIDS, hepatitis C, and sexually transmissible infections in Australia: national surveillance results to December 1998. *N S W Public Health Bull* 2000; **11**: 58–60.
20. Sfameni SF, Francis B, Wein P. Seroprevalence and assessment of risk factors for hepatitis C virus infection in pregnancy. *Aust N Z J Obstet Gynaecol* 2000; **40**: 263–7.
21. Mison LM, Young IF, O'Donoghue M, *et al.* Prevalence of hepatitis C virus and genotype distribution in an Australian volunteer blood donor population. *Transfusion* 1997; **37**: 73–8.
22. Archer GT, Buring ML, Clark B, *et al.* Prevalence of hepatitis C virus antibodies in Sydney blood donors. *Med J Aust* 1992; **157**: 225–7.
23. Allain JP, Coghlan PJ, Kenrick KG, *et al.* Prediction of hepatitis C virus infectivity in seropositive Australian blood donors by supplemental immunoassays and detection of viral RNA. *Blood* 1991; **78**: 2462–8.
24. National notifiable diseases surveillance system. Australian Government, Department of Health and Aging, 2009. Available at http://www9.health.gov.au/cda/source/Rpt_5_sel.cfm (accessed 10 October 2010).
25. Chen J, McGuinness PH, Koorey DJ, *et al.* Hepatitis C virus genotypes in a cohort of Australian blood donors and haemophiliac and liver transplant patients. *J Gastroenterol Hepatol* 1997; **12**: 182–7.
26. McCaw R, Moaven L, Locarnini SA, Bowden DS. Hepatitis C virus genotypes in Australia. *J Viral Hepat* 1997; **4**: 351–7.
27. Kaba S, Dutta U, Byth K, *et al.* Molecular epidemiology of hepatitis C in Australia. *J Gastroenterol Hepatol* 1998; **13**: 914–20.
28. Mak I, Tse S, Ho CF, Tse I, Wong KH, eds. *Surveillance of Viral Hepatitis in Hong Kong – 2008*, Update 2008 edn. Hong Kong: Viral Hepatitis Preventive Service, Department of Health, 2009.
29. Zhao SM, Jiang TL, Li RQ, *et al.* HCV infection in voluntary donors and its influence on recruitment of donors in Chongqing area. *Zhongguo Shi Yan Xue Ye Xue Za Zhi* 2008; **16**: 676–80.
30. Liu F, Chen K, He Z, *et al.* Hepatitis C seroprevalence and associated risk factors, Anyang, China. *Emerg Infect Dis* 2009; **15**: 1819–22.
31. Qiu Y, Shi L, Wang Y, *et al.* Risk factors for hepatitis C virus infection among blood donors in Beijing and implications for improving the pretesting donor screening process. *Transfusion* 2008; **48**: 1207–12.
32. Zhang M, Sun XD, Mark SD, *et al.* Hepatitis C virus infection, Linxian, China. *Emerg Infect Dis* 2005; **11**: 17–21.
33. Wei D, Zhang Y. Detection of HCV antibody in 1 280 inpatients admitted not for infectious diseases. *Di Yi Jun Yi Da Xue Xue Bao* 2004; **24**: 1125, 1129.
34. Chen YD, Liu MY, Yu WL, *et al.* Hepatitis C virus infections and genotypes in China. *Hepatobiliary Pancreat Dis Int* 2002; **1**: 194–201.
35. Qu JB, Zhang ZW, Shimbo S, *et al.* Urban–rural comparison of HBV and HCV infection prevalence in eastern China. *Biomed Environ Sci* 2000; **13**: 243–53.
36. Zhang ZW, Shimbo S, Qu JB, *et al.* Hepatitis B and C virus infection among adult women in Jilin Province, China: an urban-rural comparison in prevalence of infection markers. *Southeast Asian J Trop Med Public Health* 2000; **31**: 530–6.
37. Chen M, Xia S. A prevalence study on hepatitis C infection in 4,055 healthy children of Beijing. *Zhonghua Yu Fang Yi Xue Za Zhi* 1999; **33**: 158–9.
38. Huang F, Dong Y, Wang Z. Study on HCV infection and the distribution of HCV genotypes in different populations in Shenyang area. *Zhonghua Liu Xing Bing Xue Za Zhi* 1998; **19**: 134–7.
39. Shimbo S, Zhang ZW, Gao WP, *et al.* Prevalence of hepatitis B and C infection markers among adult women in urban and rural areas in Shaanxi Province, China. *Southeast Asian J Trop Med Public Health* 1998; **29**: 263–8.
40. Suzuki K, Mizokami M, Cao K, *et al.* Prevalence of hepatitis C virus infection in Nanjing, southern China. *Eur J Epidemiol* 1997; **13**: 511–5.
41. Fang F, Dong YS, Zhang M. Hepatitis C virus infection in different groups of children in Wuhan area. *J Tongji Med Univ* 1993; **13**: 239–43.
42. Sherlock CH, Zhuo L, Meng XL, Lin XY, Stiver HG. Seroepidemiology of hepatitis C virus in Beijing, China. *Clin Diagn Virol* 1993; **1**: 17–22.
43. Chan GC, Lim W, Yeoh EK. Prevalence of hepatitis C infection in Hong Kong. *J Gastroenterol Hepatol* 1992; **7**: 117–20.
44. Tao QM, Wang Y, Wang H, *et al.* Preliminary report on seroepidemiology of HCV and HBV infection in northern China. *Chin Med J* 1992; **105**: 209–11.
45. Leung N, Chu C, Tam JS. Viral hepatitis C in Hong Kong. *Intervirology* 2006; **49**: 23–7.
46. Ding X, Gu H, Zhong ZH, *et al.* Molecular epidemiology of hepatitis viruses and genotypic distribution of hepatitis B and C viruses in Harbin, China. *Jpn J Infect Dis* 2003; **56**: 19–22.
47. Sun Y, Zhao Y, Liu X. Anti-hepatitis C virus screening to prevent hepatitis C virus infection in blood donors. *Zhonghua Nei Ke Za Zhi* 1995; **34**: 696–9.
48. Wu RR, Mizokami M, Lau JY, *et al.* Seroprevalence of hepatitis C virus infection and its genotype in Lanzhou, western China. *J Med Virol* 1995; **45**: 174–8.
49. Ito S, Yao DF, Nii C, *et al.* Incidence of hepatitis C virus (HCV) antibodies and HCV-RNA in blood donors and patients with liver diseases in the inshore area of the Yangtze River. *J Gastroenterol Hepatol* 1994; **9**: 245–9.
50. Wang Y, Tao QM, Zhao HY, *et al.* Hepatitis C virus RNA and antibodies among blood donors in Beijing. *J Hepatol* 1994; **21**: 634–40.
51. Tang S. Seroepidemiological study on hepatitis C virus infection among blood donors from various regions in China. *Zhonghua Liu Xing Bing Xue Za Zhi* 1993; **14**: 271–4.
52. Wu X. Investigation on anti-HCV antibody in Shashi District. *Zhonghua Liu Xing Bing Xue Za Zhi* 1993; **14**: 331–3.
53. Zhang YY, Guo LS, Hao LJ, *et al.* Antibodies to hepatitis C virus and hepatitis C virus RNA in Chinese blood donors determined by ELISA, recombinant immunoblot assay and polymerase chain reaction. *Chin Med J* 1993; **106**: 171–4.
54. Shan H, Ren FR, Zhao HY, *et al.* A multi-Chinese blood center study testing serologic-negative donor samples for hepatitis C virus and human immunodeficiency virus with nucleic acid testing. *Transfusion* 2007; **47**: 2011–6.
55. Zhou DX, Tang JW, Chu IM, *et al.* Hepatitis C virus genotype distribution among intravenous drug user and the general population in Hong Kong. *J Med Virol* 2006; **78**: 574–81.
56. Lu L, Nakano T, He Y, *et al.* Hepatitis C virus genotype distribution in China: predominance of closely related subtype 1b isolates and existence of new genotype 6 variants. *J Med Virol* 2005; **75**: 538–49.
57. Xie Y, Zhao H, Ou WN, *et al.* The difference in distribution of HCV genotypes between patients infected with HCV by transfusion and non-transfusion routes. *Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi* 2004; **18**: 247–50.
58. Xu B, Lu P, Zhu M. Epidemiological aspects of the genotype of hepatitis C virus. *Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi* 2000; **14**: 148–50.
59. El-Zanaty F, Way A. *Egypt Demographic and Health Survey, 2008.* Cairo, Egypt: Ministry of Health and Population, 2009.
60. Frank C, Mohamed MK, Strickland GT, *et al.* The role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt. *Lancet* 2000; **355**: 887–91.

61. Medhat A, Shehata M, Magder LS, *et al.* Hepatitis c in a community in Upper Egypt: risk factors for infection. *Am J Trop Med Hyg* 2002; **66**: 633–8.
62. Mostafa A, Ebeid E, Mansour T, *et al.* Seroprevalence of hepatitis B and C in pediatric malignancies. *J Egypt Natl Canc Inst* 2003; **15**: 33–42.
63. Magder LS, Fix AD, Mikhail NN, *et al.* Estimation of the risk of transmission of hepatitis C between spouses in Egypt based on seroprevalence data. *Int J Epidemiol* 2005; **34**: 160–5.
64. Plancoulaine S, Mohamed MK, Arafa N, *et al.* Dissection of familial correlations in hepatitis C virus (HCV) seroprevalence suggests intrafamilial viral transmission and genetic predisposition to infection. *Gut* 2008; **57**: 1268–74.
65. Arafa N, El Hoseiny M, Rekacewicz C, *et al.* Changing pattern of hepatitis C virus spread in rural areas of Egypt. *J Hepatol* 2005; **43**: 418–24.
66. El Damaty SI, Hassan SK, Mohamed MK, *et al.* Surveillance system for HCV infection: testing a model based on blood banks. *J Egypt Public Health Assoc* 2007; **82**: 451–71.
67. El-Raziky MS, El-Hawary M, Esmat G, *et al.* Prevalence and risk factors of asymptomatic hepatitis C virus infection in Egyptian children. *World J Gastroenterol* 2007; **13**: 1828–32.
68. Agha S, El-Mashad N, El-Malky M, *et al.* Prevalence of low positive anti-HCV antibodies in blood donors: schistosoma mansoni co-infection and possible role of autoantibodies. *Microbiol Immunol* 2006; **50**: 447–52.
69. El-Gilany AH, El-Fedawy S. Bloodborne infections among student voluntary blood donors in Mansoura University, Egypt. *East Mediterr Health J* 2006; **12**: 742–8.
70. Tanaka Y, Agha S, Saudy N, *et al.* Exponential spread of hepatitis C virus genotype 4a in Egypt. *J Mol Evol* 2004; **58**: 191–5.
71. Arthur RR, Hassan NF, Abdallah MY, *et al.* Hepatitis C antibody prevalence in blood donors in different governorates in Egypt. *Trans R Soc Trop Med Hyg* 1997; **91**: 271–4.
72. Bassily S, Hyams KC, Fouad RA, Samaan MD, Hibbs RG. A high risk of hepatitis C infection among Egyptian blood donors: the role of parenteral drug abuse. *Am J Trop Med Hyg* 1995; **52**: 503–5.
73. Darwish NM, Abbas MO, Hady SI, Mohammed TA. Study of the high prevalence of HCV in Egypt. *J Egypt Public Health Assoc* 1995; **70**: 397–414.
74. el Gohary A, Hassan A, Nooman Z, *et al.* High prevalence of hepatitis C virus among urban and rural population groups in Egypt. *Acta Trop* 1995; **59**: 155–61.
75. McOmish F, Yap PL, Dow BC, *et al.* Geographical distribution of hepatitis C virus genotypes in blood donors: an international collaborative survey. *J Clin Microbiol* 1994; **32**: 884–92.
76. Darwish NM, Abbas MO, Abdelfattah FM, Darwish MA. Hepatitis C virus infection in blood donors in Egypt. *J Egypt Public Health Assoc* 1992; **67**: 223–36.
77. Eassa S, Eissa M, Sharaf SM, Ibrahim MH, Hassanein OM. Prevalence of hepatitis C virus infection and evaluation of a health education program in el-ghar village in zagazig, egypt. *J Egypt Public Health Assoc* 2007; **82**: 379–404.
78. Marzouk D, Sass J, Bakr I, *et al.* Metabolic and cardiovascular risk profiles and hepatitis C virus infection in rural Egypt. *Gut* 2007; **56**: 1105–10.
79. Bakr I, Rekacewicz C, El Hosseiny M, *et al.* Higher clearance of hepatitis C virus infection in females compared with males. *Gut* 2006; **55**: 1183–7.
80. Mohamed MK, Bakr I, El-Hoseiny M, *et al.* HCV-related morbidity in a rural community of Egypt. *J Med Virol* 2006; **78**: 1185–9.
81. el-Sadawy M, Ragab H, el-Toukhy H, *et al.* Hepatitis C virus infection at Sharkia Governorate, Egypt: seroprevalence and associated risk factors. *J Egypt Soc Parasitol* 2004; **34**: 367–84.
82. Pybus OG, Drummond AJ, Nakano T, Robertson BH, Rambaut A. The epidemiology and iatrogenic transmission of hepatitis C virus in Egypt: a Bayesian coalescent approach. *Mol Biol Evol* 2003; **20**: 381–7.
83. Strickland GT, Elhefni H, Salman T, *et al.* Role of hepatitis C infection in chronic liver disease in Egypt. *Am J Trop Med Hyg* 2002; **67**: 436–42.
84. Nafeh MA, Medhat A, Shehata M, *et al.* Hepatitis C in a community in Upper Egypt: I. Cross-sectional survey. *Am J Trop Med Hyg* 2000; **63**: 236–41.
85. Abdel-Aziz F, Habib M, Mohamed MK, *et al.* Hepatitis C virus (HCV) infection in a community in the Nile Delta: population description and HCV prevalence. *Hepatology* 2000; **32**: 111–5.
86. Kumar RM, Frossad PM, Hughes PF. Seroprevalence and mother-to-infant transmission of hepatitis C in asymptomatic Egyptian women. *Eur J Obstet Gynecol Reprod Biol* 1997; **75**: 177–82.
87. Darwish MA, Faris R, Clemens JD, Rao MR, Edelman R. High seroprevalence of hepatitis A, B, C, and E viruses in residents in an Egyptian village in The Nile Delta: a pilot study. *Am J Trop Med Hyg* 1996; **54**: 554–8.
88. el-Sayed NM, Gomatos PJ, Rodier GR, *et al.* Seroprevalence survey of Egyptian tourism workers for hepatitis B virus, hepatitis C virus, human immunodeficiency virus, and Treponema pallidum infections: association of hepatitis C virus infections with specific regions of Egypt. *Am J Trop Med Hyg* 1996; **55**: 179–84.
89. el-Nanawy AA, el Azzouni OF, Soliman AT, *et al.* Prevalence of hepatitis-C antibody seropositivity in healthy Egyptian children and four high risk groups. *J Trop Pediatr* 1995; **41**: 341–3.
90. Quinti I, Renganathan E, El Ghazzawi E, *et al.* Seroprevalence of HIV and HCV infections in Alexandria, Egypt. *Zentralbl Bakteriol* 1995; **283**: 239–44.
91. Abdel-Wahab MF, Zakaria S, Kamel M, *et al.* High seroprevalence of hepatitis C infection among risk groups in Egypt. *Am J Trop Med Hyg* 1994; **51**: 563–7.
92. Kamel MA, Miller FD, el Masry AG, *et al.* The epidemiology of Schistosoma mansoni, hepatitis B and hepatitis C infection in Egypt. *Ann Trop Med Parasitol* 1994; **88**: 501–9.
93. Farghaly AG, Barakat RM. Prevalence, impact and risk factors of hepatitis C infection. *J Egypt Public Health Assoc* 1993; **68**: 63–79.
94. Miller FD, Abu-Raddad LJ. Evidence of intense ongoing endemic transmission of hepatitis C virus in Egypt. *Proc Natl Acad Sci USA* 2010; **107**: 14757–62.
95. Saleh DA, Shebl F, Abdel-Hamid M, *et al.* Incidence and risk factors for hepatitis C infection in a cohort of women in rural Egypt. *Trans R Soc Trop Med Hyg* 2008; **102**: 921–8.
96. Abdel-Hamid M, El-Daly M, Molnegren V, *et al.* Genetic diversity in hepatitis C virus in Egypt and possible association with hepatocellular carcinoma. *J Gen Virol* 2007; **88**: 1526–31.
97. Ray SC, Arthur RR, Carella A, Bukh J, Thomas DL. Genetic epidemiology of hepatitis C virus throughout egypt. *J Infect Dis* 2000; **182**: 698–707.
98. El-Zayadi A, Simmonds P, Dabbous H, Selim O. Hepatitis C virus genotypes among HCV-chronic liver disease patients in Egypt: a leading trial. *J Egypt Public Health Assoc* 1994; **69**: 327–34.
99. National AIDS Control Organization (NACO). An action plan for blood safety. National AIDS Control Organisation, Ministry of Health & Family Welfare, Govt. of India, 2003.
100. Sharma R. South East Asia faces severe shortage of safe blood. *BMJ* 2000; **320**: 1026.
101. Saravanan S, Velu V, Kumarasamy N, *et al.* The prevalence of hepatitis B virus and hepatitis C virus infection among patients with chronic liver disease in South India. *Int J Infect Dis* 2008; **12**: 513–8.
102. Hissar SS, Goyal A, Kumar M, *et al.* Hepatitis C virus genotype 3 predominates in North and Central India and is associated with significant histopathologic liver disease. *J Med Virol* 2006; **78**: 452–8.
103. Thakral B, Marwaha N, Chawla YK, *et al.* Prevalence & significance of hepatitis C virus (HCV) seropositivity in blood donors. *Indian J Med Res* 2006; **124**: 431–8.
104. Amarapurkar D. Natural history of hepatitis C virus infection. *J Gastroenterol Hepatol* 2000; **15**(Suppl.): E105–10.
105. Raghuraman S, Shaji RV, Sridharan G, *et al.* Distribution of the different genotypes of HCV among patients attending a tertiary care hospital in south India. *J Clin Virol* 2003; **26**: 61–9.
106. Chowdhury A, Santra A, Chaudhuri S, *et al.* Hepatitis C virus infection in the general population: a community-based study in West Bengal, India. *Hepatology* 2003; **37**: 802–9.
107. Sood A, Midha V, Sood N, *et al.* Chronic hepatitis C in northern India-the pathological and clinical spectrum. *J Assoc Physicians India* 2004; **52**: 380–4.
108. Sood A, Midha V, Awasthi G. Hepatitis C – knowledge & practices among the family physicians. *Trop Gastroenterol* 2002; **23**: 198–201.
109. Kumar A, Sharma KA, Gupta RK, Kar P, Chakravarti A. Prevalence & risk factors for hepatitis C virus among pregnant women. *Indian J Med Res* 2007; **126**: 211–5.
110. Makroo RN, Choudhury N, Jagannathan L, *et al.* Multicenter evaluation of individual donor nucleic acid testing (NAT) for simultaneous detection of

human immunodeficiency virus -1 & hepatitis B & C viruses in Indian blood donors. *Indian J Med Res* 2008; **127**: 140–7.
111. Pahuja S, Sharma M, Baitha B, Jain M. Prevalence and trends of markers of hepatitis C virus, hepatitis B virus and human immunodeficiency virus in Delhi blood donors: a hospital based study. *Jpn J Infect Dis* 2007; **60**: 389–91.
112. Bagga PK, Singh SP. Seroprevalence of hepatitis C antibodies in healthy blood donors-a prospective study. *Indian J Pathol Microbiol* 2007; **50**: 429–32.
113. Bhattacharya P, Chandra PK, Datta S, *et al.* Significant increase in HBV, HCV, HIV and syphilis infections among blood donors in West Bengal, Eastern India 2004–2005: exploratory screening reveals high frequency of occult HBV infection. *World J Gastroenterol* 2007; **13**: 3730–3.
114. Gupta N, Kumar V, Kaur A. Seroprevalence of HIV, HBV, HCV and syphilis in voluntary blood donors. *Indian J Med Sci* 2004; **58**: 255–7.
115. Singh B, Kataria SP, Gupta R. Infectious markers in blood donors of East Delhi: prevalence and trends. *Indian J Pathol Microbiol* 2004; **47**: 477–9.
116. Patel Y. Seroprevalence of HIV, HBV, HCV and syphilis in blood donors. *Indian J Med Sci* 2004; **58**: 306–7.
117. Jain A, Rana SS, Chakravarty P, *et al.* The prevalence of hepatitis C virus antibodies among the voluntary blood donors of New Delhi, India. *Eur J Epidemiol* 2003; **18**: 695–7.
118. Garg S, Mathur DR, Garg DK. Comparison of seropositivity of HIV, HBV, HCV and syphilis in replacement and voluntary blood donors in western India. *Indian J Pathol Microbiol* 2001; **44**: 409–12.
119. Chandrasekaran S, Palaniappan N, Krishnan V, Mohan G, Chandrasekaran N. Relative prevalence of hepatitis B viral markers and hepatitis C virus antibodies (anti HCV) in Madurai, south India. *Indian J Med Sci* 2000; **54**: 270–3.
120. Das PK, Harris VK, Sitaram U, *et al.* Hepatitis C virus prevalence among blood donors from South India. *Vox Sang* 2000; **78**: 254–5.
121. Panigrahi AK, Panda SK, Dixit RK, *et al.* Magnitude of hepatitis C virus infection in India: prevalence in healthy blood donors, acute and chronic liver diseases. *J Med Virol* 1997; **51**: 167–74.
122. Kumar S, Agnihotri SK. Antibodies to hepatitis C virus in Chandigarh blood donors. *Vox Sang* 1997; **73**: 258–9.
123. Jaiswal SP, Chitnis DS, Naik G, *et al.* Prevalence of anti-HCV antibodies in central India. *Indian J Med Res* 1996; **104**: 177–81.
124. Arankalle VA, Chadha MS, Jha J, Amrapurkar DN, Banerjee K. Prevalence of anti-HCV antibodies in western India. *Indian J Med Res* 1995; **101**: 91–3.
125. Jindal N, Arora U, Singh K. Prevalence of human immunodeficiency virus (HIV), hepatitis B virus, and hepatitis C virus in three groups of populations at high risk of HIV infection in Amritsar (Punjab), Northern India. *Jpn J Infect Dis* 2008; **61**: 79–81.
126. Narahari S, Juwle A, Basak S, Saranath D. Prevalence and geographic distribution of Hepatitis C Virus genotypes in Indian patient cohort. *Infect Genet Evol* 2009; **9**: 643–5.
127. Abraham R, Ramakrishna B, Balekuduru A, *et al.* Clinicopathological features and genotype distribution in patients with hepatitis C virus chronic liver disease. *Indian J Gastroenterol* 2009; **28**: 53–8.
128. Verma V, Chakravarti A, Kar P. Genotypic characterization of hepatitis C virus and its significance in patients with chronic liver disease from Northern India. *Diagn Microbiol Infect Dis* 2008; **61**: 408–14.
129. Chandra M, Thippavuzzula R, Ramachandra Rao VV, *et al.* Genotyping of Hepatitis C virus (HCV) in infected patients from South India. *Infect Genet Evol* 2007; **7**: 724–30.
130. Raghuraman S, Abraham P, Sridharan G, *et al.* HCV genotype 4 – an emerging threat as a cause of chronic liver disease in Indian (south) patients. *J Clin Virol* 2004; **31**: 253–8.
131. Singh S, Malhotra V, Sarin SK. Distribution of hepatitis C virus genotypes in patients with chronic hepatitis C infection in India. *Indian J Med Res* 2004; **119**: 145–8.
132. Das BR, Kundu B, Khandapkar R, Sahni S. Geographical distribution of hepatitis C virus genotypes in India. *Indian J Pathol Microbiol* 2002; **45**: 323–8.
133. Amarapurkar D, Dhorda M, Kirpalani A, Amarapurkar A, Kankonkar S. Prevalence of hepatitis C genotypes in Indian patients and their clinical significance. *J Assoc Physicians India* 2001; **49**: 983–5.
134. Panigrahi AK, Roca J, Acharya SK, Jameel S, Panda SK. Genotype determination of hepatitis C virus from northern India: identification of a new subtype. *J Med Virol* 1996; **48**: 191–8.
135. Valliammai T, Thyagarajan SP, Zuckerman AJ, Harrison TJ. Diversity of genotypes of hepatitis C virus in southern India. *J Gen Virol* 1995; **76**(Part 3): 711–6.
136. Nakamura J, Terajima K, Aoyagi Y, Akazawa K. Cost-effectiveness of the national screening program for hepatitis C virus in the general population and the high-risk groups. *Tohoku J Exp Med* 2008; **215**: 33–42.
137. Chung H, Ueda T, Kudo M. Changing trends in hepatitis C infection over the past 50 years in Japan. *Intervirology* 2010; **53**: 39–43.
138. Moriya T, Koyama T, Tanaka J, Mishiro S, Yoshizawa H. Epidemiology of hepatitis C virus in japan. *Intervirology* 1999; **42**: 153–8.
139. Yoshizawa H, Tanaka J, Miyakawa Y. National prevention of hepatocellular carcinoma in Japan based on epidemiology of hepatitis C virus infection in the general population. *Intervirology* 2006; **49**: 7–17.
140. Ohto H, Ishii T, Kitazawa J, *et al.* Declining hepatitis C virus (HCV) prevalence in pregnant women: impact of anti-HCV screening of donated blood. *Transfusion* 2010; 693–700.
141. Sata M, Nakano H, Suzuki H, *et al.* Sero-epidemiologic study of hepatitis C virus infection in Fukuoka, Japan. *J Gastroenterol* 1998; **33**: 218–22.
142. Shimoyama R, Sekiguchi S, Suga M, Sakamoto S, Yachi A. The epidemiology and infection route of asymptomatic HCV carriers detected through blood donations. *Gastroenterol Jpn* 1993; **28**(Suppl. 5): 1–5.
143. Tanaka J, Kumagai J, Katayama K, *et al.* Sex- and age-specific carriers of hepatitis B and C viruses in Japan estimated by the prevalence in the 3,485,648 first-time blood donors during 1995–2000. *Intervirology* 2004; **47**: 32–40.
144. Fujiyama S, Tanaka M. Hepatitis C: epidemiology and therapy – with special reference to long-term prognosis after IFN therapy. *Rinsho Byori* 2000; **48**: 5–13.
145. Nakashima K, Kashiwagi S, Hayashi J, *et al.* Prevalence of hepatitis C virus infection among female prostitutes in Fukuoka, Japan. *J Gastroenterol* 1996; **31**: 664–8.
146. Yamaguchi K, Kiyokawa H, Machida J, *et al.* Seroepidemiology of hepatitis C virus infection in Japan and HCV infection in haemodialysis patients. *FEMS Microbiol Rev* 1994; **14**: 253–8.
147. Kihara M, Imai M, Kondoh M, *et al.* Prevalence of hepatitis C virus and human immunodeficiency virus infection among Japanese female prostitutes. *Nippon Koshu Eisei Zasshi* 1993; **40**: 387–91.
148. Khan M, Husain M, Yano M, *et al.* Comparison of seroepidemiology of hepatitis C in blood donors between Bangladesh and Japan. *Gastroenterol Jpn* 1993; **28**(Suppl. 5): 28–31.
149. Watanabe S, Kano U, Ito T, *et al.* Prevalence and heterogeneity of serum DNA polymerase activity in patients with non-A, non-B hepatitis and HBsAg-negative blood donors with elevated SGPT. *Hepatogastroenterology* 1990; **37**(Suppl. 2): 126–9.
150. Matsumoto K, Takahashi M, Tamori A, Nishiguchi S. Present status of community-based HCV screening in Osaka City and evaluation of the utility of follow-up programs on hepatitis]. *Nippon Koshu Eisei Zasshi* 2008; **55**: 75–82.
151. Narai R, Oyama T, Ogawa M, *et al.* HBV- and HCV- infected workers in the Japanese workplace. *J Occup Health* 2007; **49**: 9–16.
152. Taguchi S, Nishioka K, Kawaguchi R, *et al.* Epidemiological study of hepatitis B and C in 34,336 patients operated at Hiroshima Prefectural Hospital during the period from 1993 to 2000. *Masui* 2004; **53**: 696–700.
153. Nakamura Y, Koh M, Miyoshi E, *et al.* High prevalence of the hepatitis C virus infection among the inpatients of schizophrenia and psychoactive substance abuse in Japan. *Prog Neuropsychopharmacol Biol Psychiatry* 2004; **28**: 591–7.
154. Yoshida K, Nakano H, Yoshitomi F, Oshika T. Prevalence of seropositivity for hepatitis C virus in cataract patients and the general population. *J Cataract Refract Surg* 2002; **28**: 1789–92.
155. Wada I, Hara T, Kajihara S, *et al.* Population-based study of hepatitis C virus infection and hepatocellular carcinoma in western Japan. *Hepatol Res* 2002; **23**: 18–24.
156. Okayama A, Stuver SO, Tabor E, *et al.* Incident hepatitis C virus infection in a community-based population in Japan. *J Viral Hepat* 2002; **9**: 43–51.
157. Ishi K, Suzuku F, Saito A, Yoshimoto S, Kubota T. Prevalence of human immunodeficiency virus, hepatitis B and hepatitis C virus antibodies and hepatitis B antigen among commercial sex workers in Japan. *Infect Dis Obstet Gynecol* 2001; **9**: 215–9.

158. Inoue Y, Sulaiman HA, Matsubayashi K, *et al.* Genotypic analysis of hepatitis C virus in blood donors in Indonesia. *Am J Trop Med Hyg* 2000; **62**: 92–8.
159. Yoshii E, Shinzawa H, Saito T, *et al.* Molecular epidemiology of hepatitis C virus infection in an area endemic for community-acquired acute hepatitis C. *Tohoku J Exp Med* 1999; **188**: 311–6.
160. Kuboki M, Shinzawa H, Shao L, *et al.* A cohort study of hepatitis C virus (HCV) infection in an HCV epidemic area of Japan: age and sex-related seroprevalence of anti-HCV antibody, frequency of viremia, biochemical abnormality and histological changes. *Liver* 1999; **19**: 88–96.
161. Kayaba K, Igarashi M, Okamoto H, Tsuda F. Prevalence of anti-hepatitis C antibodies in a rural community without high mortality from liver disease in Niigata prefecture. *J Epidemiol* 1998; **8**: 250–5.
162. Miyajima I, Sata M, Murashima S, *et al.* Prevalence of hepatitis C antibodies in health care personnel. *Kansenshogaku Zasshi* 1997; **71**: 103–7.
163. Fukuizumi K, Sata M, Suzuki H, Nakano H, Tanikawa K. Hepatitis C virus seroconversion rate in a hyperendemic area of HCV in Japan: a prospective study. *Scand J Infect Dis* 1997; **29**: 345–7.
164. Noguchi S, Sata M, Suzuki H, Mizokami M, Tanikawa K. Routes of transmission of hepatitis C virus in an endemic rural area of Japan. Molecular epidemiologic study of hepatitis C virus infection. *Scand J Infect Dis* 1997; **29**: 23–8.
165. Watanabe Y, Machida K, Sato A, Ota S, Kiyosawa K. Survey for hepatitis in an isolated endemic area. *Nippon Koshu Eisei Zasshi* 1996; **43**: 989–96.
166. Yamakawa Y, Sata M, Suzuki H, Noguchi S, Tanikawa K. Higher elimination rate of hepatitis C virus among women. *J Viral Hepat* 1996; **3**: 317–21.
167. Ishibashi M, Shinzawa H, Kuboki M, Tsuchida H, Takahashi T. Prevalence of inhabitants with anti-hepatitis C virus antibody in an area following an acute hepatitis C epidemic: age-and area-related features. *J Epidemiol* 1996; **6**: 1–7.
168. Ichimura H, Kurimura O, Tamura I, *et al.* Prevalence of blood-borne viruses among intravenous drug users and alcoholics in Hiroshima, Japan. *Int J STD AIDS* 1995; **6**: 441–3.
169. Yanaga K, Wakiyama S, Soejima Y, *et al.* Hepatitis C virus infection among Japanese general surgical patients. *World J Surg* 1995; **19**: 694–6.
170. Hayashi J, Kishihara Y, Yamaji K, *et al.* Transmission of hepatitis C virus by health care workers in a rural area of Japan. *Am J Gastroenterol* 1995; **90**: 794–9.
171. Tawaraya H, Ohkoshi S, Kuwana K, *et al.* Epidemiologic survey and genetic analysis of endemic hepatitis C virus infection in a Japanese town with a high prevalence of hepatitis B virus carriers. *J Med Virol* 1995; **45**: 367–72.
172. Fukui M. Natural history of HCV seropositive cases found in health screening. *Hokkaido Igaku Zasshi* 1995; **70**: 69–82.
173. Kiyosawa K, Tanaka E, Sodeyama T, *et al.* Transmission of hepatitis C in an isolated area in Japan: community-acquired infection. The South Kiso Hepatitis Study Group. *Gastroenterology* 1994; **106**: 1596–602.
174. Hayashi J, Yoshimura E, Nabeshima A, *et al.* Seroepidemiology of hepatitis C virus infection in hemodialysis patients and the general population in Fukuoka and Okinawa, Japan. *J Gastroenterol* 1994; **29**: 276–81.
175. Sato Y, Matsunami M, Maruoka H, *et al.* A seroepidemiological study of hepatitis C virus (HCV) in an area with a high prevalence of chronic liver disease in the Kyushu district of Japan. *Kurume Med J* 1994; **41**: 41–50.
176. Goto T, Misumi J, Shimaoka A, *et al.* Seroepidemiological study on hepatitis C virus infection in an endemic area of hepatitis C virus. *Kansenshogaku Zasshi* 1993; **67**: 635–41.
177. Nakashima K, Kashiwagi S, Hayashi J, *et al.* Low prevalence of hepatitis C virus infection among hospital staff and acupuncturists in Kyushu, Japan. *J Infect* 1993; **26**: 17–25.
178. Nakashima K, Kashiwagi S, Hayashi J, *et al.* Sexual transmission of hepatitis C virus among female prostitutes and patients with sexually transmitted diseases in Fukuoka, Kyushu, Japan. *Am J Epidemiol* 1992; **136**: 1132–7.
179. Suou T, Ikuta Y, Hasegawa M, Kawasaki H. Prevalence of HCV antibodies in Yatsuka town of Simane prefecture, Japan. *Nippon Shokakibyo Gakkai Zasshi* 1992; **89**: 1173–8.
180. Fujiyama S, Kawano S, Sato S, *et al.* Prevalence of hepatitis C virus antibodies in hemodialysis patients and dialysis staff. *Hepatogastroenterology* 1992; **39**: 161–5.
181. Tanaka E, Kiyosawa K, Sodeyama T, *et al.* Prevalence of antibody to hepatitis C virus in Japanese schoolchildren: comparison with adult blood donors. *Am J Trop Med Hyg* 1992; **46**: 460–4.
182. Ochi S, Onji M, Shiraishi K, *et al.* Prevalence of hepatitis C virus antibody in an area endemic for hepatitis B virus and human T cell leukaemia virus. *J Gastroenterol Hepatol* 1991; **6**: 599–602.
183. Michitaka K, Horiike N, Ohta Y. An epidemiological study of hepatitis C virus infection in a local district in Japan. *Rinsho Byori* 1991; **39**: 586–91.
184. Ito S, Ito M, Cho MJ, Shimotohno K, Tajima K. Massive sero-epidemiological survey of hepatitis C virus: clustering of carriers on the southwest coast of Tsushima, Japan. *Jpn J Cancer Res* 1991; **82**: 1–3.
185. Tanaka J, Mizui M, Nagakami H, *et al.* Incidence rates of hepatitis B and C virus infections among blood donors in Hiroshima, Japan, during 10 years from 1994 to 2004. *Intervirology* 2008; **51**: 33–41.
186. Katayama K, Tanaka J, Yoshizawa H. Past trends in hepatitis C virus infection and route of transmission in Japan. *Rinsho Byori* 2001; **49**: 741–6.
187. Ohno O, Mizokami M, Wu RR, *et al.* New hepatitis C virus (HCV) genotyping system that allows for identification of HCV genotypes 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a, and 6a. *J Clin Microbiol* 1997; **35**: 201–7.
188. Ohno T, Mizokami M, Yamauchi M, *et al.* Genotype distribution in Nagoya and new genotype (genotype 3a) in Japanese patients with hepatitis C virus. *J Gastroenterol* 1995; **30**: 209–14.
189. Kobayashi M, Kumada H, Chayama K, *et al.* Prevalence of HCV genotype among patients with chronic liver diseases in the Tokyo metropolitan area. *J Gastroenterol* 1994; **29**: 583–7.
190. Shin HR, Hwang SY, Nam CM. The prevalence of hepatitis C virus infection in Korea: pooled analysis. *J Korean Med Sci* 2005; **20**: 985–8.
191. Shin HR. Epidemiology of hepatitis C virus in Korea. *Intervirology* 2006; **49**: 18–22.
192. Kim YS, Ahn YO, Lee HS. Risk factors for hepatitis C virus infection among Koreans according to the hepatitis C virus genotype. *J Korean Med Sci* 2002; **17**: 187–92.
193. Shin HR, Kim JY, Kim JI, *et al.* Hepatitis B and C virus prevalence in a rural area of South Korea: the role of acupuncture. *Br J Cancer* 2002; **87**: 314–8.
194. Suh DJ, Jeong SH. Current status of hepatitis C virus infection in Korea. *Intervirology* 2006; **49**: 70–5.
195. Kwon JH, Bae SH. Current status and clinical course of hepatitis C virus in Korea. *Korean J Gastroenterol* 2008; **51**: 360–7.
196. Kim YS, Pai CH, Chi HS, *et al.* Prevalence of hepatitis C virus antibody among Korean adults. *J Korean Med Sci* 1992; **7**: 333–6.
197. Shin HR, Kim JY, Ohno T, *et al.* Prevalence and risk factors of hepatitis C virus infection among Koreans in rural area of Korea. *Hepatol Res* 2000; **17**: 185–96.
198. Park YM, Kim IS, Lee CD, Kim BS. Seroprevalence of antibody against hepatitis C virus (anti-HCV) in various groups of individuals in Korea. *Gastroenterol Jpn* 1991; **26**(Suppl. 3): 159–63.
199. Baek EJ, Kim HO, Kim S, Park Q, Oh D. The trends for nationwide blood collection and the supply of blood in Korea during 2002–2006. *Korean J Blood Transfus* 2008; **19**: 83–90.
200. Lee H, Cho YK, Kim HU, *et al.* Distribution of hepatitis C virus genotypes in Jeju Island. *Korean J Hepatol* 2008; **14**: 28–35.
201. Park YS, Lee KO, Oh MJ, Chai YG. Distribution of genotypes in the 5′ untranslated region of hepatitis C virus in Korea. *J Med Microbiol* 1998; **47**: 643–7.
202. Han CJ, Lee HS, Kim HS, Choe JH, Kim CY. Hepatitis C virus genotypes in Korea and their relationship to clinical outcome in type C chronic liver diseases. *Korean J Intern Med* 1997; **12**: 21–7.
203. Lee DS, Sung YC, Whang YS. Distribution of HCV genotypes among blood donors, patients with chronic liver disease, hepatocellular carcinoma, and patients on maintenance hemodialysis in Korea. *J Med Virol* 1996; **49**: 55–60.
204. Kim CJ, Shin KS, Kim WY, *et al.* Genotype distribution and comparison of the putative envelope region of hepatitis C virus from Korean patients. *J Med Virol* 1995; **46**: 380–6.
205. Akhtar S, Rozi S. An autoregressive integrated moving average model for short-term prediction of hepatitis C virus seropositivity among male volunteer blood donors in Karachi, Pakistan. *World J Gastroenterol* 2009; **15**: 1607–12.
206. Ali SA, Donahue RM, Qureshi H, Vermund SH. Hepatitis B and hepatitis C in Pakistan: prevalence and risk factors. *Int J Infect Dis* 2009; **13**: 9–19.
207. Raja NS, Janjua KA. Epidemiology of hepatitis C virus infection in Pakistan. *J Microbiol Immunol Infect* 2008; **41**: 4–8.

208. Idrees M, Riazuddin S. Frequency distribution of hepatitis C virus genotypes in different geographical regions of Pakistan and their possible routes of transmission. *BMC Infect Dis* 2008; **8**: 69.
209. Idrees M, Lal A, Naseem M, Khalid M. High prevalence of hepatitis C virus infection in the largest province of Pakistan. *J Dig Dis* 2008; **9**: 95–103.
210. Aslam M, Aslam J, Mitchell BD, Munir KM. Association between smallpox vaccination and hepatitis C antibody positive serology in Pakistani volunteers. *J Clin Gastroenterol* 2005; **39**: 243–6.
211. Younus M, Siddiqi AE, Akhtar S. Reassessment of selected healthcare associated risk factors for HBV and HCV infections among volunteer blood donors, Karachi, Pakistan. *Cent Eur J Public Health* 2009; **17**: 31–5.
212. Umar M, Busra MT, Ahmed M, *et al.* Hepatitis C in Pakistan: a review of available data. *Hepatitis Monthly* 2010; **10**: 205–14.
213. Jafri W, Subhan A. Hepatitis C in Pakistan: magnitude, genotype, disease characteristics and therapeutic response. *Trop Gastroenterol* 2008; **29**: 194–201.
214. Khattak MN, Akhtar S, Mahmud S, Roshan TM. Factors influencing Hepatitis C virus sero-prevalence among blood donors in north west Pakistan. *J Public Health Policy* 2008; **29**: 207–25.
215. Mujeeb SA, Pearce MS. Temporal trends in hepatitis B and C infection in family blood donors from interior Sindh, Pakistan. *BMC Infect Dis* 2008; **8**: 43.
216. Abdul Mujeeb S, Nanan D, Sabir S, Altaf A, Kadir M. Hepatitis B and C infection in first-time blood donors in Karachi-a possible subgroup for sentinel surveillance. *East Mediterr Health J* 2006; **12**: 735–41.
217. Akhtar S, Younus M, Adil S, Jafri SH, Hassan F. Hepatitis C virus infection in asymptomatic male volunteer blood donors in Karachi, Pakistan. *J Viral Hepat* 2004; **11**: 527–35.
218. Khattak MF, Salamat N, Bhatti FA, Qureshi TZ. Seroprevalence of hepatitis B, C and HIV in blood donors in northern Pakistan. *J Pak Med Assoc* 2002; **52**: 398–402.
219. Abdul Mujeeb S, Aamir K, Mehmood K. Seroprevalence of HBV, HCV and HIV infections among college going first time voluntary blood donors. *J Pak Med Assoc* 2000; **50**: 269–70.
220. Kakepoto GN, Bhally HS, Khaliq G, *et al.* Epidemiology of blood-borne viruses: a study of healthy blood donors in Southern Pakistan. *Southeast Asian J Trop Med Public Health* 1996; **27**: 703–6.
221. Sami S, Korejo R, Bhutta SZ. Prevalence of hepatitis B and C: a Jinnah Postgraduate Medical Centre experience. *J Obstet Gynaecol Res* 2009; **35**: 533–8.
222. Abbas Z, Jeswani NL, Kakepoto GN, *et al.* Prevalence and mode of spread of hepatitis B and C in rural Sindh, Pakistan. *Trop Gastroenterol* 2008; **29**: 210–6.
223. Butt T, Amin MS. Seroprevalence of hepatitis B and C infections among young adult males in Pakistan. *East Mediterr Health J* 2008; **14**: 791–7.
224. Khan S, Rai MA, Khan A, *et al.* Prevalence of HCV and HIV infections in 2005-Earthquake-affected areas of Pakistan. *BMC Infect Dis* 2008; **8**: 147.
225. Ahmad N, Asgher M, Shafique M, Qureshi JA. An evidence of high prevalence of Hepatitis C virus in Faisalabad, Pakistan. *Saudi Med J* 2007; **28**: 390–5.
226. Aziz S, Muzaffar R, Hafiz S, *et al.* Helicobacter pylori, hepatitis viruses A, C, E, antibodies and HBSAG-prevalence and associated risk factors in pediatric communities of Karachi. *J Coll Physicians Surg Pak* 2007; **17**: 195–8.
227. Mirza IA, Kazmi SMH, Janjua AN. Frequency of Hepatitis B surface antigen and anti-HCV in young adults-experience in southern Punjab. *J Coll Physicians Surg Pak* 2007; **17**: 114–5.
228. Alam M, Zaman Tariq W, Akram S, Zia Qureshi T. Frequency of hepatitis B and C in central Punjab. *Pak J Pathol* 2006; **17**: 140–1.
229. Jafri W, Jafri N, Yakoob J, *et al.* Hepatitis B and C: prevalence and risk factors associated with seropositivity among children in Karachi, Pakistan. *BMC Infect Dis* 2006; **6**: 101.
230. Mirza IA, Mirza SH, Irfan S, *et al.* Seroprevalence of Hepatitis B and C in young adults seeking recruitment in armed forces. *Pak Armed Forces Med J* 2006; **56**: 192–7.
231. Sherif TB, Tariq WZ. Seroprevalence of Hepatitis B and C in healthy adult male recruits. *Pak J Pathol* 2006; **17**: 147–50.
232. Muhammad N, Jan MA. Frequency of hepatitis "C" in Buner, NWFP. *J Coll Physicians Surg Pak* 2005; **15**: 11–4.
233. Waseem R, Kashif MA, Khan M, Qureshi AW. Seroprevalence of HbsAg and anti-HCV in Ghurki Teaching Hospital, Lahore. *Ann King Edward Med Coll* 2005; **11**: 232–4.
234. Khokhar N, Gill ML, Malik GJ. General seroprevalence of hepatitis C and hepatitis B virus infections in population. *J Coll Physicians Surg Pak* 2004; **14**: 534–6.
235. Rizvi TJ, Fatima H. Frequency of hepatitis C in obstetric cases. *J Coll Physicians Surg Pak* 2003; **13**: 688–90.
236. Shaikh MA, Shaikh WM, Solangi GA, Abro H. Frequency and transmission mode of hepatitis C virus in northern Sindh. *J Coll Physicians Surg Pak* 2003; **13**: 691–3.
237. Aziz S, Memon A, Tily HI, *et al.* Prevalence of HIV, hepatitis B and C amongst health workers of Civil Hospital Karachi. *J Pak Med Assoc* 2002; **52**: 92–4.
238. Aslam M, Aslam J. Seroprevalence of the antibody to hepatitis C in select groups in the Punjab region of Pakistan. *J Clin Gastroenterol* 2001; **33**: 407–11.
239. Khan AJ, Luby SP, Fikree F, *et al.* Unsafe injections and the transmission of hepatitis B and C in a periurban community in Pakistan. *Bull World Health Organ* 2000; **78**: 956–63.
240. Tanwani AK, Ahmad N. Prevalence of Hepatitis B surface antigen and Anti-Hepatitis C Virus in laboratory based data at Islamabad. *J Surg* 2000; **19–20**: 25–9.
241. Tariq WU, Hussain AB, Karamat KA, *et al.* Demographic aspects of hepatitis C in northern Pakistan. *J Pak Med Assoc* 1999; **49**: 198–201.
242. Luby SP, Qamruddin K, Shah AA, *et al.* The relationship between therapeutic injections and high prevalence of hepatitis C infection in Hafizabad, Pakistan. *Epidemiol Infect* 1997; **119**: 349–56.
243. Agboatwalla M, Isomura S, Miyake K, *et al.* Hepatitis A, B and C seroprevalence in Pakistan. *Indian J Pediatr* 1994; **61**: 545–9.
244. Ally SH, Hanif R, Ahmed A. HBsAg and HCV: increasing test requests and decreasing frequency of positive tests at clinical laboratory of Ayub Teaching Hospital, Abbottabad. *J Ayub Med Coll Abbottabad* 2005; **17**: 81–4.
245. Shah HA, Jafri W, Malik I, Prescott L, Simmonds P. Hepatitis C virus (HCV) genotypes and chronic liver disease in Pakistan. *J Gastroenterol Hepatol* 1997; **12**: 758–61.
246. Al-Faleh FZ, Ramia S, Arif M, *et al.* Profile of hepatitis C virus and the possible modes of transmission of the virus in the Gizan area of Saudi Arabia: a community-based study. *Ann Trop Med Parasitol* 1995; **89**: 431–7.
247. Arif M, al-Swayeh M, Al-Faleh FZ, Ramia S. Risk of hepatitis C virus infection among household contacts of Saudi patients with chronic liver disease. *J Viral Hepat* 1996; **3**: 97–101.
248. Ahmad MS, Mahtab AM, Abdullatif AS, *et al.* Prevalence of antibodies against the hepatitis C virus among voluntary blood donors at a makkah hospital. *Saudi J Kidney Dis Transpl* 1995; **6**: 122–4.
249. Altraif I. Hepatitis C acquisition in Saudi Arabia: are the majority of cases without risk factor? *Ann Saudi Med* 1995; **15**: 428–30.
250. Fakeeh M, Zaki AM. Hepatitis C: prevalence and common genotypes among ethnic groups in Jeddah, Saudi Arabia. *Am J Trop Med Hyg* 1999; **61**: 889–92.
251. Mahaba H, el-Tayeb A, Elbaz H. The prevalence of antibodies to hepatitis C virus in Hail region, Saudi Arabia. *J Egypt Public Health Assoc* 1999; **74**: 69–80.
252. al Nasser MN. Intrafamilial transmission of hepatitis C virus (HCV): a major mode of spread in the Saudi Arabia population. *Ann Trop Paediatr* 1992; **12**: 211–5.
253. Ayoola EA, al-Mofleh IA, Al-Faleh FZ, *et al.* Prevalence of antibodies to hepatitis C virus among Saudi patients with chronic liver diseases. *Hepatogastroenterology* 1992; **39**: 337–9.
254. Bahakim H, Bakir TM, Arif M, Ramia S. Hepatitis C virus antibodies in high-risk Saudi groups. *Vox Sang* 1991; **60**: 162–4.
255. Bashawri LA, Fawaz NA, Ahmad MS, Qadi AA, Almawi WY. Prevalence of seromarkers of HBV and HCV among blood donors in eastern Saudi Arabia, 1998–2001. *Clin Lab Haematol* 2004; **26**: 225–8.
256. El-Hazmi MM. Prevalence of HBV, HCV, HIV-1, 2 and HTLV-I/II infections among blood donors in a teaching hospital in the Central region of Saudi Arabia. *Saudi Med J* 2004; **25**: 26–33.
257. Qadi AA, Tamim H, Ameen G, *et al.* Hepatitis B and hepatitis C virus prevalence among dialysis patients in Bahrain and Saudi Arabia: a survey by serologic and molecular methods. *Am J Infect Control* 2004; **32**: 493–5.

258. Tamimi WG, Altraif IM, Auumah A, *et al.* Impact of new AABB guidelines on hepatitis B and C testing among Saudi blood donors. *Br J Biomed Sci* 2004; **61**: 215–7.
259. Shobokshi OA, Serebour FE, Al-Drees AZ, *et al.* Hepatitis C virus seroprevalence rate among Saudis. *Saudi Med J* 2003; **24**: S81–6.
260. Abdelaal M, Rowbottom D, Zawawi T, Scott T, Gilpin C. Epidemiology of hepatitis C virus: a study of male blood donors in Saudi Arabia. *Transfusion* 1994; **34**: 135–7.
261. Al-Mofarreh M, Fakunle YM, El-Karamany WM, *et al.* Prevalence of antibodies to hepatitis C virus in blood donors in Riyadh. *Ann Saudi Med* 1991; **11**: 501–3.
262. Bernvil SS, Andrews VJ, Kariem AA. Hepatitis C antibody prevalence in Saudi Arabian blood donor population. *Ann Saudi Med* 1991; **11**: 563–7.
263. Alzahrani AJ. Simultaneous detection of hepatitis C virus core antigen and antibodies in Saudi drug users using a novel assay. *J Med Virol* 2008; **80**: 603–6.
264. Njoh J, Zimmo S. Prevalence of antibodies to hepatitis C virus in drug-dependent patients in Jeddah, Saudi Arabia. *East Afr Med J* 1997; **74**: 89–91.
265. Saxena AK, Panhotra BR, Naguib M, *et al.* Prevalence of hepatitis C antibodies among hemodialysis patients in Al-hasa region of saudi arabia. *Saudi J Kidney Dis Transpl* 2001; **12**: 562–5.
266. Harakati MS, Abualkhair OA, Al-Knawy BA. Hepatitis C Virus infection in Saudi Arab patients with B-cell non-Hodgkin's lymphoma. *Saudi Med J* 2000; **21**: 755–8.
267. Soyannwo MA, Khan N, Kommajosyula S, *et al.* Hepatitis C antibodies in haemodialysis and pattern of end-stage renal failure in Gassim, Saudi Arabia. *Afr J Med Med Sci* 1996; **25**: 13–22.
268. Huraib S, al-Rashed R, Aldrees A, *et al.* High prevalence of and risk factors for hepatitis C in haemodialysis patients in Saudi Arabia: a need for new dialysis strategies. *Nephrol Dial Transplant* 1995; **10**: 470–4.
269. al Karawi MA, Shariq S, el Shiekh Mohamed AR, Saeed AA, Ahmed AM. Hepatitis C virus infection in chronic liver disease and hepatocellular carcinoma in Saudi Arabia. *J Gastroenterol Hepatol* 1992; **7**: 237–9.
270. Jamjoom GA, Quli SK. Serodiagnosis of hepatitis C in acute and chronic liver disease in southwestern Saudi Arabia. *J Trop Med Hyg* 1992; **95**: 428–31.
271. Mitwalli A, al-Mohaya S, al Wakeel J, *et al.* Hepatitis C in chronic renal failure patients. *Am J Nephrol* 1992; **12**: 288–91.
272. Ayoola EA, Huraib S, Arif M, *et al.* Prevalence and significance of antibodies to hepatitis C virus among Saudi haemodialysis patients. *J Med Virol* 1991; **35**: 155–9.
273. Al-Faleh FZ, Ayoola EA, al-Jeffry M, *et al.* Prevalence of antibody to hepatitis C virus among Saudi Arabian children: a community-based study. *Hepatology* 1991; **14**: 215–8.
274. Al-Tawfiq JA, Anani A. Profile of viral hepatitis A, B, and C in a Saudi Arabian hospital. *Med Sci Monit* 2008; **14**: CR52–6.
275. Madani TA. Hepatitis C virus infections reported over 11 years of surveillance in Saudi Arabia. *Trans R Soc Trop Med Hyg* 2009; **103**: 132–6.
276. Memish ZA, Knawy BA, El-Saed A. Incidence trends of viral hepatitis A, B, and C seropositivity over eight years of surveillance in Saudi Arabia. *Int J Infect Dis* 2010; **14**: e115–20.
277. Al-Traif I, Handoo FA, Al-Jumah A, al Nasser MN. Chronic hepatitis C. Genotypes and response to anti-viral therapy among Saudi patients. *Saudi Med J* 2004; **25**: 1935–8.
278. Shobokshi OA, Serebour FE, Skakni LI. Hepatitis C genotypes/subtypes among chronic hepatitis patients in Saudi Arabia. *Saudi Med J* 2003; **24**: S87–91.
279. Osoba AO, Ibrahim M, Abdelaal MA, *et al.* Hepatitis C virus genotyping by polymerase chain reaction and DNA enzyme immunoassay among Saudi patients in the Western Province, Saudi Arabia. *Ann Saudi Med* 2000; **20**: 394–7.
280. Al-Ahdal MN, Rezeig MA, Kessie G. Genotyping of hepatitis C virus isolates from Saudi patients by analysis of sequences from PCR-amplified core region of the virus genome. *Ann Saudi Med* 1997; **17**: 601–4.
281. Al-Knawy B, Okamoto H, Ahmed El-Mekki A, *et al.* Distribution of hepatitis C genotype and co-infection rate with hepatitis G in Saudi Arabia. *Hepatol Res* 2002; **24**: 95.
282. Bosmans JL, Nouwen EJ, Behets G, *et al.* Prevalence and clinical expression of HCV-genotypes in haemodialysis-patients of two geographically remote countries: Belgium and Saudi-Arabia. *Clin Nephrol* 1997; **47**: 256–62.
283. Shobokshi OA, Serebour FE, Skakni L, Al-Saffy YH, Ahdal MN. Hepatitis C genotypes and subtypes in Saudi Arabia. *J Med Virol* 1999; **58**: 44–8.
284. Al-Faleh FZ, Huraib S, Sbeih F, *et al.* Hepatitis C virus genotypes in patients with chronic liver disease and haemodialysis patients from Saudi Arabia. *J Viral Hepat* 1995; **2**: 293–6.
285. Antaki N, Haddad M, Kebbewar K, *et al.* The unexpected discovery of a focus of hepatitis C virus genotype 5 in a Syrian province. *Epidemiol Infect* 2009; **137**: 79–84.
286. Othman BM, Monem FS. Prevalence of hepatitis C virus antibodies among intravenous drug abusers and prostitutes in Damascus, Syria. *Saudi Med J* 2002; **23**: 393–5.
287. Othman B, Monem F. Prevalence of antibodies to hepatitis C virus among hemodialysis patients in Damascus, Syria. *Infection* 2001; **29**: 262–5.
288. Othman BM, Monem FS. Prevalence of hepatitis C virus antibodies among health care workers in Damascus, Syria. *Saudi Med J* 2001; **22**: 603–5.
289. Abdulkarim AS, Zein NN, Germer JJ, *et al.* Hepatitis C virus genotypes and hepatitis G virus in hemodialysis patients from Syria: identification of two novel hepatitis C virus subtypes. *Am J Trop Med Hyg* 1998; **59**: 571–6.
290. Tsai MC, Kee KM, Chen YD, *et al.* Excess mortality of hepatocellular carcinoma and morbidity of liver cirrhosis and hepatitis in HCV-endemic areas in an HBV-endemic country: geographic variations among 502 villages in southern Taiwan. *J Gastroenterol Hepatol* 2007; **22**: 92–8.
291. Chen CH, Yang PM, Huang GT, *et al.* Estimation of seroprevalence of hepatitis B virus and hepatitis C virus in Taiwan from a large-scale survey of free hepatitis screening participants. *J Formos Med Assoc* 2007; **106**: 148–55.
292. Sun CA, Chen HC, Lu CF, *et al.* Transmission of hepatitis C virus in Taiwan: prevalence and risk factors based on a nationwide survey. *J Med Virol* 1999; **59**: 290–6.
293. Wang CS, Chang TT, Yao WJ, Chou P. Comparison of hepatitis B virus and hepatitis C virus prevalence and risk factors in a community-based study. *Am J Trop Med Hyg* 2002; **66**: 389–93.
294. Lee PL, Wang JH, Tung HD, Lee CM, Lu SN. A higher than expected recovery rate from hepatitis C infection amongst adolescents: a community study in a hepatitis C-endemic township in Taiwan. *Trans R Soc Trop Med Hyg* 2004; **98**: 367–72.
295. Dai CY, Huang JF, Hsieh MY, *et al.* The role of gender on clearance of hepatitis C virus: a different story in an area endemic for hepatitis B and C. *Gut* 2007; **56**: 737–8.
296. Sun CA, Chen HC, Lu SN, *et al.* Persistent hyperendemicity of hepatitis C virus infection in Taiwan: the important role of iatrogenic risk factors. *J Med Virol* 2001; **65**: 30–4.
297. Huang JF, Lu SN, Chue PY, *et al.* Hepatitis C virus infection among teenagers in an endemic township in Taiwan: epidemiological and clinical follow-up studies. *Epidemiol Infect* 2001; **127**: 485–92.
298. Lu SN, Chen HC, Tang CM, *et al.* Prevalence and manifestations of hepatitis C seropositivity in children in an endemic area. *Pediatr Infect Dis J* 1998; **17**: 142–5.
299. Lai MY. Combined interferon and ribavirin therapy for chronic hepatitis C in Taiwan. *Intervirology* 2006; **49**: 91–5.
300. Chen DS, Kuo GC, Sung JL, *et al.* Hepatitis C virus infection in an area hyperendemic for hepatitis B and chronic liver disease: the Taiwan experience. *J Infect Dis* 1990; **162**: 817–22.
301. Lee CM, Lu SN, Hung CH, *et al.* Hepatitis C virus genotypes in southern Taiwan: prevalence and clinical implications. *Trans R Soc Trop Med Hyg* 2006; **100**: 767–74.
302. Yu ML, Chuang WL, Chen SC, *et al.* Changing prevalence of hepatitis C virus genotypes: molecular epidemiology and clinical implications in the hepatitis C virus hyperendemic areas and a tertiary referral center in Taiwan. *J Med Virol* 2001; **65**: 58–65.
303. Liou YW, Chou P, Chen YM. Genotypic analysis of HCV infection in kinmen. *J Microbiol Immunol Infect* 2000; **33**: 149–53.
304. Kao JH, Chen PJ, Lai MY, *et al.* Genotypes of hepatitis C virus in Taiwan and the progression of liver disease. *J Clin Gastroenterol* 1995; **21**: 233–7.
305. Sunanchaikarn S, Theamboonlers A, Chongsrisawat V, *et al.* Seroepidemiology and genotypes of hepatitis C virus in Thailand. *Asian Pac J Allergy Immunol* 2007; **25**: 175–82.
306. Chunlertrith K, Sukeepaisarnjaroen W, Mairiang P, *et al.* Clinico-epidemiology of hepatitis C viral infection in northeastern Thailand. *Southeast Asian J Trop Med Public Health* 2000; **31**: 273–6.

307. Thaikruea L, Thongsawat S, Maneekarn N, *et al.* Risk factors for hepatitis C virus infection among blood donors in northern Thailand. *Transfusion* 2004; **44**: 1433–40.
308. Ratanasuwan W, Sonji A, Tiengrim S, Techasathit W, Suwanagool S. Serological survey of viral hepatitis A, B, and C at Thai Central Region and Bangkok: a population base study. *Southeast Asian J Trop Med Public Health* 2004; **35**: 416–20.
309. Ishida T, Takao S, Settheetham-Ishida W, Tiwawech D. Prevalence of hepatitis B and C virus infection in rural ethnic populations of Northern Thailand. *J Clin Virol* 2002; **24**: 31–5.
310. Wiwanitkit V. A note in the high prevalence of anti-HCV seropositivity among hilltribers in Mae Jam District, Northern Thailand. *Viral Immunol* 2002; **15**: 645–6.
311. Jittiwutikarn J, Thongsawat S, Suriyanon V, *et al.* Hepatitis C infection among drug users in northern Thailand. *Am J Trop Med Hyg* 2006; **74**: 1111–6.
312. Hansurabhanon T, Jiraphongsa C, Tunsakun P, *et al.* Infection with hepatitis C virus among intravenous-drug users: prevalence, genotypes and risk-factor-associated behaviour patterns in Thailand. *Ann Trop Med Parasitol* 2002; **96**: 615–25.
313. Apichartpiyakul C, Apichartpiyakul N, Urwijitaroon Y, *et al.* Seroprevalence and subtype distribution of hepatitis C virus among blood donors and intravenous drug users in northern/northeastern Thailand. *Jpn J Infect Dis* 1999; **52**: 121–3.
314. Luksamijarulkul P, Plucktaweesak S. High hepatitis C seroprevalence in Thai intravenous drug abusers and qualitative risk analysis. *Southeast Asian J Trop Med Public Health* 1996; **27**: 654–8.
315. Luksamijarulkul P, Thammata N, Sujirarat D, Tiloklurs M. Hepatitis C virus infection among Thai blood donors: antibody prevalence, risk factors and development of risk screening form. *Southeast Asian J Trop Med Public Health* 2004; **35**: 147–54.
316. Nantachit N, Robison V, Wongthanee A, *et al.* Temporal trends in the prevalence of HIV and other transfusion-transmissible infections among blood donors in northern Thailand, 1990 through 2001. *Transfusion* 2003; **43**: 730–5.
317. Wiwanitkit V. Anti HCV seroprevalence among the voluntary blood donors in Thailand. *Hematology* 2005; **10**: 431–3.
318. Theamboonlers A, Chinchai T, Bedi K, *et al.* Molecular characterization of Hepatitis C virus (HCV) core region in HCV-infected Thai blood donors. *Acta Virol* 2002; **46**: 169–73.
319. Kanistanon D, Neelamek M, Dharakul T, Songsivilai S. Genotypic distribution of hepatitis C virus in different regions of Thailand. *J Clin Microbiol* 1997; **35**: 1772–6.
320. Doi H, Apichartpiyakul C, Ohba KI, Mizokami M, Hotta H. Hepatitis C virus (HCV) subtype prevalence in Chiang Mai, Thailand, and identification of novel subtypes of HCV major type 6. *J Clin Microbiol* 1996; **34**: 569–74.
321. Nakata S, Song P, Duc DD, *et al.* Hepatitis C and B virus infections in populations at low or high risk in Ho Chi Minh and Hanoi, Vietnam. *J Gastroenterol Hepatol* 1994; **9**: 416–9.
322. Kakumu S, Sato K, Morishita T, *et al.* Prevalence of hepatitis B, hepatitis C, and GB virus C/hepatitis G virus infections in liver disease patients and inhabitants in Ho Chi Minh, Vietnam. *J Med Virol* 1998; **54**: 243–8.
323. Song P, Duc DD, Hien B, *et al.* Markers of hepatitis C and B virus infections among blood donors in Ho Chi Minh City and Hanoi, Vietnam. *Clin Diagn Lab Immunol* 1994; **1**: 413–8.
324. Nakano T, Lu L, Liu P, Pybus OG. Viral gene sequences reveal the variable history of hepatitis C virus infection among countries. *J Infect Dis* 2004; **190**: 1098–108.
325. Nguyen VT, McLaws ML, Dore GJ. Prevalence and risk factors for hepatitis C infection in rural north Vietnam. *Hepatol Int* 2007; **1**: 387–93.
326. Tran HT, Ushijima H, Quang VX, *et al.* Prevalence of hepatitis virus types B through E and genotypic distribution of HBV and HCV in Ho Chi Minh City, Vietnam. *Hepatol Res* 2003; **26**: 275–80.
327. Tokita H, Okamoto H, Tsuda F, *et al.* Hepatitis C virus variants from Vietnam are classifiable into the seventh, eighth, and ninth major genetic groups. *Proc Natl Acad Sci USA* 1994; **91**: 11022–6.
328. Pham DA, Leuangwutiwong P, Jittmittraphap A, *et al.* High prevalence of Hepatitis C virus genotype 6 in Vietnam. *Asian Pac J Allergy Immunol* 2009; **27**: 153–60.
329. Jokhio AH, Bhatti TA, Memon S. Knowledge, attitudes and practices of barbers about hepatitis B and C transmission in Hyderabad, Pakistan. *East Mediterr Health J* 2010; **16**: 1079–84.
330. Ruan Y, Jia Y, Zhang X, *et al.* Incidence of HIV-1, syphilis, hepatitis B, and hepatitis C virus infections and predictors associated with retention in a 12-month follow-up study among men who have sex with men in Beijing, China. *J Acquir Immune Defic Syndr* 2009; **52**: 604–10.
331. Hao C, Yan H, Yang H, *et al.* The incidence of syphilis, HIV and HCV and associated factors in a cohort of men who have sex with men in Nanjing, China. *Sex Transm Infect* 2011; **87**: 199–201.
332. Qazi HA, Saleem K, Mujtaba I, Hashmi A, Soomro JA. Prevalence and factors associated with HCV (hepatitis C virus) seropositivity in Islamabad, Pakistan. *Acta Med Iran* 2010; **48**: 394–8.

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# Dysfunction of Autophagy Participates in Vacuole Formation and Cell Death in Cells Replicating Hepatitis C Virus

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# Dysfunction of Autophagy Participates in Vacuole Formation and Cell Death in Cells Replicating Hepatitis C Virus▽§

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**Hepatitis C virus (HCV) is a major cause of chronic liver diseases. A high risk of chronicity is the major concern of HCV infection, since chronic HCV infection often leads to liver cirrhosis and hepatocellular carcinoma. Infection with the HCV genotype 1 in particular is considered a clinical risk factor for the development of hepatocellular carcinoma, although the molecular mechanisms of the pathogenesis are largely unknown. Autophagy is involved in the degradation of cellular organelles and the elimination of invasive microorganisms. In addition, disruption of autophagy often leads to several protein deposition diseases. Although recent reports suggest that HCV exploits the autophagy pathway for viral propagation, the biological significance of the autophagy to the life cycle of HCV is still uncertain. Here, we show that replication of HCV RNA induces autophagy to inhibit cell death. Cells harboring an HCV replicon RNA of genotype 1b strain Con1 but not of genotype 2a strain JFH1 exhibited an incomplete acidification of the autolysosome due to a lysosomal defect, leading to the enhanced secretion of immature cathepsin B. The suppression of autophagy in the Con1 HCV replicon cells induced severe cytoplasmic vacuolation and cell death. These results suggest that HCV harnesses autophagy to circumvent the harmful vacuole formation and to maintain a persistent infection. These findings reveal a unique survival strategy of HCV and provide new insights into the genotype-specific pathogenicity of HCV.**

Hepatitis C virus (HCV) is a major causative agent of bloodborne hepatitis and currently infects at least 180 million people worldwide (58). The majority of individuals infected with HCV develop chronic hepatitis, which eventually leads to liver cirrhosis and hepatocellular carcinoma (25, 48). In addition, HCV infection is known to induce extrahepatic diseases such as type 2 diabetes and malignant lymphoma (20). It is believed that the frequency of development of these diseases varies among viral genotypes (14, 51). However, the precise mechanism of the genotype-dependent outcome of HCV-related diseases has not yet been elucidated. Despite HCV's status as a major public health problem, the current therapy with pegylated interferon and ribavirin is effective in only around 50% of patients with genotype 1, which is the most common genotype worldwide, and no effective vaccines for HCV are available (35, 52). Although recently approved protease inhibitors for HCV exhibited a potent antiviral efficacy in patients with genotype 1 (36, 43), the emergence of drug-resistant mutants is a growing problem (16). Therefore, it is important to clarify the life cycle and pathogenesis of HCV for the development of more potent remedies for chronic hepatitis C.

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HCV belongs to the genus *Hepacivirus* of the family *Flaviviridae* and possesses a single positive-stranded RNA genome with a nucleotide length of 9.6 kb, which encodes a single polyprotein consisting of approximately 3,000 amino acids (40). The precursor polyprotein is processed by host and viral proteases into structural and nonstructural (NS) proteins (34). Not only viral proteins but also several host factors are required for efficient replication of the HCV genome, where NS5A is known to recruit various host proteins and to form replication complexes with other NS proteins (39). In the HCV-propagating cell, host intracellular membranes are reconstructed for the viral niche known as the membranous web, where it is thought that progeny viral RNA and proteins are concentrated for efficient replication and are protected from defensive degradation, as are the host protease and nucleases (38).

Autophagy is a bulk degradation process, wherein portions of cytoplasm and organelles are enclosed by a unique membrane structure called an autophagosome, which subsequently fuses with the lysosome for degradation (37, 60). Autophagy occurs not only in order to recycle amino acids during starvation but also to clear away deteriorated proteins or organelles irrespective of nutritional stress. In fact, the deficiency of autophagy leads to the accumulation of disordered proteins that can ultimately cause a diverse range of diseases, including neurodegeneration and liver injury (12, 29, 30), and often to type 2 diabetes and malignant lymphoma (9, 32).

Recently, it has been shown that autophagy is provoked upon replication of several RNA viruses and is closely related to their propagation and/or pathogenesis. Coxsackievirus B3

utilizes autophagic membrane as a site of genome replication, whereas influenza virus attenuates apoptosis through the induction of autophagy (10, 59). Moreover, several groups have reported that HCV induces autophagy for infection or replication (5, 49); however, the role(s) of autophagy in the propagation of HCV is still controversial and the involvement of autophagy in the pathogenesis of HCV has not yet been clarified. In this study, we examined the biological significance of the autophagy observed in cells in which the HCV genome replicates.

## MATERIALS AND METHODS

**Plasmids.** The plasmids pmStrawberry-C1, pmStrawberry-Atg4B$^{C74A}$, pmRFP-GFP-LC3, pEGFP-LC3, and pEGFP-Atg16L were described previously (7, 8, 24). The plasmids pFGR-JFH1 and pSGR-JFH1 were kind gifts from T. Wakita.

**Cell culture.** All cell lines were cultured at 37°C under a humidified atmosphere with 5% $CO_2$. Huh7 cells were cultivated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), nonessential amino acids, 100 U/ml penicillin, and 100 mg/ml streptomycin. For the starvation, the cells were cultivated with Earle's balanced salt solution (EBSS) (Sigma) for 6 h. HCV replicon cells were established as described previously (53). The plasmid pairs pFK-I$_{389}$ neo/NS3-3′/NK5.1 and pFK-I$_{389}$ neo/FGR/NK5.1 and pFGR-JFH1 and pSGR-JFH1 were linearized with ScaI or XbaI. The plasmids pFGR-JFH1 and pSGR-JFH1 were treated with mung bean exonuclease. The linearized DNA was transcribed *in vitro* by using the MEGAscript T7 kit (Applied Biosystems) according to the manufacturer's protocol. The transcribed RNA was electroporated into cells under conditions of 270 V and 960 mF using a Gene Pulser (Bio-Rad). All HCV replicon cells were maintained in DMEM containing 10% FBS, nonessential amino acids, and 1 mg/ml G418 (Nacalai).

**Reagents and antibodies.** Concanamycin A and bafilomycin A1 were purchased from Sigma and Fluka, respectively. E64D and pepstatin A were from Peptide Institute Inc. Rabbit anti-HCV NS5A polyclonal antibody was described previously (45). Mouse monoclonal anti-JEV NS3 antibody was prepared by immunization using the recombinant protein spanning amino acid residues 171 to 619 of JEV NS3. Rabbit polyclonal anti-LC3 (PM036), mouse monoclonal anti-RFP (8D6), and anti-62/SQSTM1 (5F2) antibodies were purchased from Medical & Biological Laboratories. Rabbit polyclonal anti-cathepsin B (FL-339) and mouse monoclonal anti-LAMP1 (H4A3) antibodies were from Santa Cruz Biotechnology. Mouse monoclonal anti-HCV NS5A (HCM-131-5), rabbit polyclonal anti-β-actin, and mouse monoclonal anti-Golgin97 (CDF4) antibodies were from Austral Biologicals, Sigma, and Invitrogen, respectively. Mouse monoclonal and rabbit polyclonal anti-cathepsin B antibodies were from Calbiochem. Mouse monoclonal anti-p62/SQSTM1 (5F2) and anti-ATP6V0D1 (ab56441) antibodies were from Abcam. Rabbit polyclonal anti-Atg4B antibody was from Sigma. Mouse anti-double-stranded RNA (dsRNA) IgG2a (J2 and K1) antibodies were from Biocenter Ltd. (Szirak, Hungary).

**Transfection, infection, and immunoblotting.** Transfection and infection were carried out as described previously (53). Each lysosome-enriched fraction was isolated by using the Lysosome Enrichment Kit for Tissue and Cultured Cells (Pierce) according to the manufacturer's protocol. Samples were subjected to 12.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The proteins were transferred to polyvinylidene difluoride membranes (Millipore) and were reacted with the appropriate antibodies. The immune complexes were visualized with Super Signal West Femto substrate (Pierce) and detected by an LAS-3000 image analyzer system (Fujifilm). The protein bands of LC3 and β-actin were quantified by Multi Gauge software (Fujifilm), and the values of LC3 were normalized to those of β-actin.

**Fluorescence microscopy.** Cells were cultured on glass slides and then fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) at room temperature for 30 min. After being washed twice with PBS, the cells were permeabilized at room temperature for 20 min with PBS containing 0.25% saponin and then blocked with PBS containing 0.2% gelatin (gelatin-PBS) for 60 min at room temperature. The cells were incubated with gelatin-PBS containing appropriate antibodies at 37°C for 60 min and washed three times with PBS containing 1% Tween 20 (PBST). The resulting cells were incubated with gelatin-PBS containing corresponding fluorescent-conjugated secondary antibodies at 37°C for 60 min and then washed three times with PBST. The stained cells were covered with Vectashield mounting medium containing DAPI (4′,6-diamidino-2-phenylindole) (Vector Laboratories Inc.) and observed with a FluoView FV1000 laser scanning confocal microscope (Olympus). Time-lapse video microscopy was performed at 37°C with a DeltaVision microscope system (Applied Precision Inc.) equipped with a ΔTC3 culture dish system (Bioptechs) for temperature control.

**Quantification of pro-cathepsin B.** Each cell line was seeded on 12-well type I collagen-coated dishes (IWAKI) and cultured for 48 h. The supernatant and the cells were harvested and subjected to quantification of pro-cathepsin B by using Quantikine human pro-cathepsin B immunoassay (R&D Systems) according to the manufacturer's protocol.

**Statistical analysis.** Estimated values were represented as the means ± standard deviations. The significance of differences in the means was determined by Student's *t* test.

## RESULTS

**Autophagy is induced in the HCV replicating cell in a strain-dependent manner.** To determine whether autophagy is induced during the replication of HCV, we investigated the phosphoethanolamine (PE) conjugation of LC3 in HCV replicon cells in which HCV RNA was autonomously replicating. As shown in Fig. 1A, the amounts of PE-conjugated LC-3 (LC3-II), a conventional marker for an autophagosomal membrane, in Huh7 cells were slightly increased by starvation, in conjunction with a reduction of the unmodified LC-3 (LC3-I). In contrast, the amount of LC3-II was significantly increased in the subgenomic and full genomic HCV replicon cells of the genotype 1b strain Con1 (SGR$^{Con1}$ and FGR$^{Con1}$), whereas a small amount of LC3-II was detected in the full genomic replicon cells of the genotype 2a strain JFH1 (FGR$^{JFH1}$). We also examined the subcellular localization of LC3 by using confocal microscopy. Although LC3 was diffusely detected in the cytoplasm of naïve Huh7 cells, small foci of the accumulated LC3 appeared after starvation (Fig. 1B), whereas many LC3 foci that were larger in size than those in the starved cells appeared in the cytoplasm, particularly near the nucleus, in both SGR$^{Con1}$ and FGR$^{Con1}$ cells. However, a low level of LC3 focus formation comparable to that in the starved cells was observed in the FGR$^{JFH1}$ cells. Most of the LC3 foci were not colocalized with NS5A, an HCV protein of the viral replication complex, in the HCV replicon cells, as reported previously (49). Elimination of HCV RNA from the SGR$^{Con1}$ cells by treatment with alpha interferon (SGR$^{cured}$) abrogated the lipidation and accumulation of LC3 (Fig. 1C and D). Interestingly, overexpression of the HCV polyprotein of genotype 1b by an expression plasmid induced no autophagy (data not shown), suggesting that replication of viral RNA is required for induction of autophagy. Furthermore, neither lipidation nor accumulation of LC3 was observed in SGR$^{JEV}$ cells harboring subgenomic replicon RNA cells of Japanese encephalitis virus (JEV), which is also a member of the family *Flaviviridae* (Fig. 1C and D). These results suggest that replication of HCV but not that of JEV induces autophagy.

**The autophagy flux is impaired in the replicon cells of HCV strain Con1 after a step of autophagosome formation.** To further examine the autophagy induced in the HCV replicon cells in more detail, Huh7 and SGR$^{Con1}$ cells were treated with pepstatin A and E64D, inhibitors of aspartic protease and cysteine protease, respectively. In this assay, treatment of intact cells capable of inducing autophagy with the inhibitors increases the amount of LC3-II, whereas no increase is observed in cells impaired in the autophagic degradation. The amount of LC3-II was significantly increased in the naïve Huh7