

# Hepatitis C virus genotypes in the Islamic Republic of Iran: a preliminary study

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الأنماط الجينية لفيروس التهاب الكبد C في جمهورية إيران الإسلامية: دراسة ميدانية  
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خلاصة: نظراً للتوزيع الجغرافي الواسع الانتشار لفيروس التهاب الكبد C، ولما كان لهذا الفيروس ستة أنماط جينية رئيسية وأكثر من تسعين نمطاً فرعياً، فقد قررنا تحديد معدل انتشار الأنماط الجينية الرئيسية للفيروس في جمهورية إيران الإسلامية. فتم مبدئياً اختبار عينات مصلية أخذت من واحد وعشرين شخصاً يحملون عدوى فيروس التهاب الكبد C، وذلك بالجيل الثاني من مقايمة الممتز المناعي الإنزيمي، وبطريقة التفاعل السلسلي للبوليميراز PCR ذات المرحلتين. ومن أجل تعيين الأشكال الفيروسية الخمسة الأكثر انتشاراً تم استخدام الجيل الثاني من طرائق تمييز الأنماط الجينية. وتبين أن انتشار الأنماط الجينية النوعية في خمس عشرة عينة كان على النحو التالي: النمط الأول/1 في سبع حالات، والنمط الثاني/1ب في ثلاث حالات، والنمط الخامس/3أ في أربعة مرضى. وظهر النمط الرابع في عينة واحدة.

**ABSTRACT** According to the divergent geographical distribution of the hepatitis C virus (HCV) and the fact that the virus possesses six major genotypes and more than 90 subtypes, we decided to determine the prevalence of the major HCV genotypes in the Islamic Republic of Iran. Serum samples from 21 HCV infected individuals were tested primarily by second generation of enzyme immunosorbent assay and the two-stage PCR method. For determining the five most common variants, second generation methods for genotype specification were used. The prevalence of specific genotypes in 15 samples is as follows: Type I/1a in seven cases, Type II/1b in three cases and Type V/3a in four patients. One sample disclosed Type 4.

## Les géotypes du virus de l'hépatite C en République islamique d'Iran: étude préliminaire

**RESUME** Etant donné la distribution géographique divergente du virus de l'hépatite C (VHC) et du fait que le virus possède six géotypes principaux et plus de 90 sous-types, nous avons décidé de déterminer la prévalence des principaux géotypes du VHC en République islamique d'Iran. Des échantillons de sérum prélevés sur 21 personnes infectées par le VHC ont été testés principalement avec la seconde génération des titrages immuno-enzymatiques et la méthode de la PCR nichée. Afin de déterminer les cinq variantes les plus courantes, on a utilisé les méthodes de la deuxième génération pour la spécification du géotype. La prévalence des géotypes spécifiques dans 15 échantillons était la suivante: type I/1a dans sept cas, type II/1b dans trois cas et type V/3a chez quatre patients. Un échantillon a mis en évidence le type 4.

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## Introduction

Hepatitis C virus (HCV) infection is found in 0.5% to 8.0% of blood donors worldwide [1]. Mainly because of the high rate of chronicity seen in more than 60% of infected individuals, it is one of the most important economic and public health problems [2]. According to comparative studies of viral RNA, HCV belongs to the family of flaviviridae and pestiviridae [1,3,4]. Because of high genetic heterogeneity, HCV is classified into various genotypes [2,5,6]. The viral genomic RNA is a single-stranded plus sense RNA, approximately 9379 nucleotides long (Figure 1) and is divided into the core, envelope and at least four non-structural (NS) proteins. This property of the virus particularly indicates its relationship to flaviviridae. Structural proteins code by the 5'-end, and nonstructural proteins code by the 3'-end of RNA [4]. The gene product is a viral polyprotein precursor of 3011 amino acids, which undergoes

proteolytic post-translational cleavage. The structural proteins are derived from the 5'-end of the genome and the nonstructural proteins from the 3'-end [2,3]. The diversity of this virus has led to its classification into genotypes that differ substantially in nucleotide sequence. Classification of HCV is based on genetic relatedness since no well defined culture system or classification based on disease manifestations has yet been identified for these variants. The variability of HCV is such as to suggest a two-tier classification [2,5,7,8,9]. Therefore, Simmonds et al. have proposed a nomenclature of "types" (corresponding to the major branches of a phylogenetic tree of sequences from genomic or subgenomic regions of the genome), and subtypes (corresponding to the more closely related sequences within some of the major groups) [10]. The known types are numbered from 1 and the subtypes are a, b and c in order of discovery. The current system of nomenclature includes six major genetic groups and

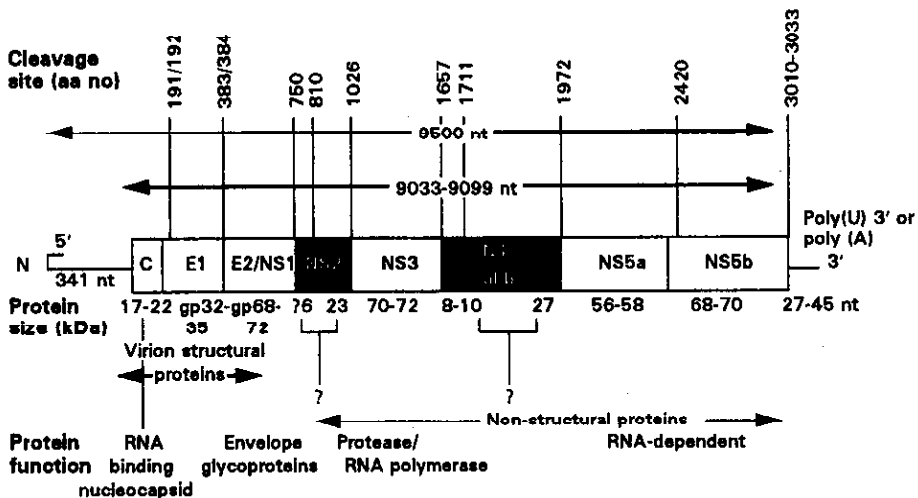


Figure 1 Schematic structure of hepatitis C virus genome and encoded proteins aa no. = amino acid number; nt = nucleotide; C = core; E = envelope; NS = non-structural; ? = uncertain

several recognized subtypes, the number of which varies according to typing system, but up to 40 different subtypes have been identified [11].

There have been numerous studies concerning the possible effect of HCV genotypes on pathogenesis, prognosis and therapeutic response. Therefore, we proposed a preliminary study to determine the most prevalent genotypes in the Islamic Republic of Iran. This is in continuum of a study of GBV-C infection in patients with HCV in the Islamic Republic of Iran.

## Patients and methods

The serum samples were taken from 21 HCV-infected individuals who were referred by internists, gastroenterologists, urologists and nephrologists from one private and two teaching hospitals in Tehran, Islamic Republic of Iran. The presence of HCV infection was initially detected by a serological test (ELISA II) and was also confirmed by two-stage polymerase chain reaction (PCR). Four patients had chronic liver disease, six had undergone renal transplant and three were under haemodialysis. Eight individuals were selected randomly

from HCV-positive sera taken from apparently healthy blood donors.

The five common HCV genotypes designated by the mixed nomenclature (I/1a, II/1b, III/2a, IV/2b and V/3a) were determined by second-generation genotyping using PCR with type-specific primers deduced from the HCV core gene [12]. HCV RNA samples that could not be classified into the five common genotypes were amplified with HCV core primers specific for subtype 2c [13] or type 4 [14]. Genotypes other than the five common ones were deduced by an NS5b sequence of 329 base pairs (nt 8279–8607) by the method reported previously [14].

## Results

Two-stage PCR confirmed the presence of viral RNA in 15 (72%) of the ELISA II-positive serum samples. Fourteen cases were grouped into five genotypes as follows: type I/1a in seven patients (47%), type II/1b in three patients (20%) and type V/3a found in four patients (27%). No patients were found to be III/2b or IV/2b. One patient (6%) was found to have type 4 (Table 1).

Table 1 Distribution of hepatitis C virus genotypes in different patient groups

Patient group	I/1a		II/1b		V/3a		4		Interferon treatment	
	No.	%	No.	%	No.	%	No.	%	No.	%
Blood donors	5	62.5	1	12.5	2	25.0	–	–	0	0
Haemodialysis patients	–	–	1	50.0	1	50.0	–	–	2	100.0
Chronic liver disease patients	1	25.0	1	25.0	1	25.0	1	25.0	4	100.0
Renal transplant patients	1	100.0	–	–	–	–	–	–	1	100.0

## Discussion

Understanding of the geographic distribution of common HCV genotypes requires detailed knowledge about the routes of viral transmission, prevalence of HCV in the general population and in various high-risk groups, and also phylogenetic evolution of types and subtypes over the decades and centuries. Unfortunately, there is little information on these topics, limiting the reliable interpretation of current data. There are also additional limitations in the precise geographic distribution in many areas of the world. Only a few countries have detailed information about the epidemiology of HCV [5].

In Western Europe and North America, blood donors and those with chronic hepatitis are repeatedly affected by types 1a, 1b, 2a, 2b and 3a, whereas in southern and eastern Europe type 1b is more common [15,16]. There is also a considerable difference between south-eastern Europe and Turkey (mainly type 1b) and the Middle East and Central Africa. For example, in Egypt, where HCV is highly prevalent (20%–30%), nearly all cases are affected by type 4a [17]. The major genotype in the Republic of Yemen, Kuwait, Iraq and Saudi Arabia (Middle East), Zaire, Burundi and Gabon (Central Africa) is type 4 [18]. Although the major strain reported in the Middle East and Central Africa is generally type 4, it should be noted that this comprises numerous subtypes [18–20]. For example, sequencing the E1 gene in six infected patients in Zaire resulted in the isolation of four different subtypes completely discrete from known strains in the Middle East and Gabon [5]. Genotype 5a was frequently separated from between 30% and 50% of infected blood donors in South Africa. In contrast to type 4, this genotype has less heterogeneity of nucleotide sequences in strains and thus most of them

conform to a single subtype. This is a highly specific strain in this region and is rarely found in Europe and other areas [5].

The distribution of genotypes in the Far East is complex. In Japan, Taiwan and probably China, the most prevalent ones are type 1b, 2a and 2b [21,22]. It seems that type 1a is restricted to haemophiliacs in Japan. Type 3 HCV has a more distinctive distribution. It is rarely isolated in Japan and is uncommon in Taiwan, Hong Kong and Macau, but has a prominent prevalence in more western regions, such as Singapore and Thailand [23]. A very limited evaluation has shown that it is the dominant strain in Bangladesh and eastern India. HCV genotype 3 has also considerable genetic polymorphism as does type 4. At least six different subtypes (designated 3a through 3f) have been isolated from affected patients in Nepal [24] and three additional subtypes in Bangladesh (D. Multimer, unpublished data, 1994) and Thailand [23] have been identified, totalling nine subtypes.

The genotype 6a has a distinctive distribution. This was first recognized in Hong Kong. It was identified according to its specific sequence in NS-5b and E1 genome [18,19]. Approximately 30% of HCV-positive blood donors in Hong Kong and Macau are infected by this strain [5]. Unfortunately, the information about the epidemiology of this type in South-East Asian countries is incomplete [5].

In most European countries, the geographic distribution of HCV genotypes correlates with the age of the patients [5]. This points to rapidly changing genotypes in an area with the passing of time.

Our study showed the isolation of types I/1a, V/3a and II/1b respectively in the Islamic Republic of Iran. As previously described, type 4 is the most common genotype reported in the Middle East,

which was isolated only from one case (6%) in our sample. No relation was found in each group between genotypes and route of viral transmission. Because of the small number of patients in our study and wide age distribution, no definite conclusions can be drawn about the correlation between viral types and age of the patients. Also, the interrelation between genotypes

and natural history of infection could not be evaluated because most of the patients had received interferon therapy before or during the study, and our knowledge of their history was incomplete [25,26]. We hope that future controlled, broad-based studies will delineate these problems and the prevalence of genotypes more precisely.

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