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ORIGINAL ARTICLE

# Intrafamilial transmission of hepatitis C virus in patients with severe haemophilia A

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**Summary.** The history behind the production of clotting factor concentrates produced differences in the prevalence of Hepatitis C Virus (HCV) and other blood-borne infections in haemophilic patients. Prevalence rates of HCV infection up to 100% were reported in patients treated with concentrates before 1985. Conversely, nowadays, viral inactivation and recombinant technologies have effectively prevented transfusion-transmitted viral pathogens. Recently, new HCV infections in three young brothers were observed. In the absence of any other risk of transmission, their HIV/HCV coinfected uncle, who was living in the same house, was subject to study. Plasma samples of the four relatives were investigated in order to test whether the infections have a common source. A phylogenetic approach using the most variable (E2) viral sequences was carried out using samples from the four family members. The HCV sequences from the study resulted highly related, being those obtained from the uncle the most ancestral ones. Because of the chronological order in which the infections occurred and the relatedness of the sequences, an infection from the uncle to his nephews is the most likely explanation. Special cares must be applied in the case of household contact among members of a family with inherited bleeding disorders.

**Keywords**: hepatitis C virus, intrafamiliar transmission, phylogenetic analysis

## Introduction

The early use of non-inactivated clotting factor concentrates resulted in human immunodeficiency virus (HIV) and hepatitis C virus (HCV) epidemics in the haemophilic population [1,2]. However, the introduction of heat inactivation, donor screening against blood-borne pathogens, and use of recombinant clotting factors have reduced the risk of transmission of HCV in this vulnerable population [2]. Thus, young patients with haemophilia infected with HCV are scarce but virtually all adults who received clotting factor concentrates manufactured before 1985 were exposed to HCV.

The phylogenetic analysis allows assessing the level of relatedness between viral sequences and has already proved to be the tool of choice in the study of nosocomial [3] and occupational transmission of HCV [4].

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### *Aim*

To carry out phylogenetic analyses of the viral sequences obtained from an adult haemophilic patient and his three young haemophilic nephews, who were infected with HCV, in order to determine a putative common origin of infection and the HCV transmission mechanisms.

## Materials and methods

### *Ethical statement*

This research study was approved by the local ethic committee. Plasma samples were taken under written informed consent of the patients or their legal tutor.

### *Patients*

Four male patients, members of the same family, with severe haemophilia A were studied. The four patients were periodically screened for blood-borne infections due to their inherited bleeding disorder.

The uncle (Patient 1), born in 1983, co-infected with HCV and HIV, died in 2010. Virtually, all haemophiliacs

who received clotting factor concentrates prior to the implementation of viral inactivation techniques, became infected with hepatitis C virus at the time of the first infusion [5] and this was probably the case of Patient 1. The nephews (Patients 2, 3 and 4) who are currently 15, 11 and 8 years old, respectively, have never been exposed to first-generation clotting factors. Unexpectedly, Patients 2, 3 and 4 seroconverted for HCV antibody tests. Although it is difficult to ascertain the exact date, HCV infection seems to have occurred between April 2009 and June 2010 in Patient 2 and between April 2008 and April 2009 in Patient 3. Patient 4 was HCV negative when tested in 2002 (nine months after his birth) but showed HCV antibody positive results in April 2009. All of them remained negative for HIV antibody tests.

### *Genotyping and sequences*

All plasma samples were subject to RNA extraction with the QIAmp viral RNA minikit (Qiagen, Gmbh, Hilden, Germany). Then the cDNA was prepared by retrotranscription with M-MLV RT (Promega, Madison, WI, USA) using random hexamer primers. The cDNA was used as template for nested PCR reactions targeted to 5′UTR, NS5B and E2 regions using the Go-Taq (Promega) *Taq* polymerase. The list of primers and PCR protocols employed are available in Data S1 (Primers and Protocols). Purified PCR products were sequenced in an automated sequencer by Sanger's method.

The HCV genotype was determined by restriction fragment length polymorphism (RFLP) of the 5′UTR region (length = 251 bp, nucleotides 63–313 in the AF009606 'H77' reference sequence) [6] and then it was confirmed and subtypified by phylogenetic analysis of NS5B sequences (length = 367 bp, nucleotides 8259–8625 in AF009606) [7] with 44 reference sequences: 34 from the NCBI Viral Genotyping Tool and 10 from unrelated complete genomes (five HCV-1b and five HCV-1a).

In order to assess the relationships between the HCV sampled from the patients studied, phylogenetic analysis was carried out using the E2 sequences (length = 661 bp, nucleotides 1376–2036 in AF009606) with the best 20 matches of BLASTN ('nr' sequence database), which represented the most closely related sequences to the analyzed dataset. It is worth noting that there are no sequences of this region from haemophilic patients belonging to the local community. It is important to notice that clotting factor concentrates used for Argentinean haemophilic patients treatments were not manufactured in the country but brought from the US or Europe.

HCV genotypes in individuals with haemophilia always reflect the geographic distribution of genotypes from the blood donor population where the commercial products were manufactured [8].

### *Phylogentic analysis*

The sequences were visualized and aligned using the programs BioEdit [9] v7.0.5.3 and ClustalX [10] v2.12 respectively. Then, for each dataset, the model of nucleotide substitution was selected according to the results of the Akaike Information Criterion analysis carried out with the jModelTest [11] software.

The phylogenetic trees were constructed with the Maximum Likelihood methodology, using the software PhyML [12] v3.0 (for Linux) and the branch support was assessed by non-parametric bootstrapping (1000 pseudoreplica).

## Results

### *Genotyping*

The RFLP pattern of all samples was compatible with genotype 1 (not shown). The sequence analysis of NS5B region showed that Patients 1, 2 and 4 were infected with HCV-1a (Fig. 1). The samples from patient 3 failed to amplify any region other than 5′UTR and were further analysed.

### *Phylogenetic analysis*

As the result of the BLAST searches with the E2 sequences from the Patients 1, 2 and 4, 42 sequences were retrieved after discarding duplicates and identical ones. Details about the retrieved sequences are available in Data S2 (Retrieved Sequences). The model of nucleotide substitution for the E2 dataset was TIM2 + Γ+I (Transversion-Inversion Model 2 with site rate heterogeneity modelled as gamma distribution and a proportion of invariant sites [11]). The best tree showed that Patients 1, 2 and 4 formed a monophyletic group with a support value of 100% (Fig. 2). The topology of the best tree obtained for E2 region showed that the sequence from Patient 1 was very close to the putative ancestor of the above mentioned monophyletic group. Similar results were obtained when the analyses were carried out with NS5B region (Data S2).

## Discussion

The phylogenetic data shows that the HCV viral sequences from the uncle and his nephews form a monophyletic group, suggesting an intrafamiliar transmission. However, its detailed chain cannot be resolved by this tool. At least two of the nephews (Patients 2 and 4) may have acquired the HCV infection directly from their uncle (Patient 1) or, alternatively, one of them could have been infected first and then transmitted the infection to the others. An infection from a common source for the uncle and his nephews is very unlikely

 

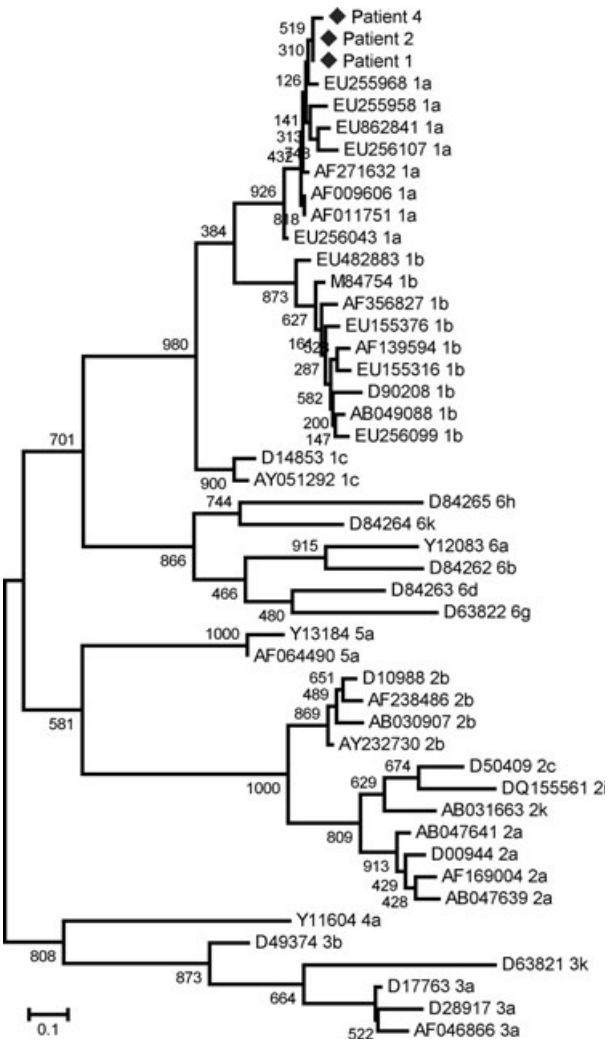


**Fig. 1.** Phylogenetic genotyping of HCV in Patients 1, 2 and 4 by sequence analysis of the NS5B region. Maximum likelihood tree for the NS5B region. Model of nucleotide substitution: GTR+Γ+I. Patients 1, 2 and 4 are highlighted with a grey diamond. Reference sequences are named after their GenBank's accession number and subtype. Numbers in the branches represent the bootstrap support value (over 1000 pseudo-replica).

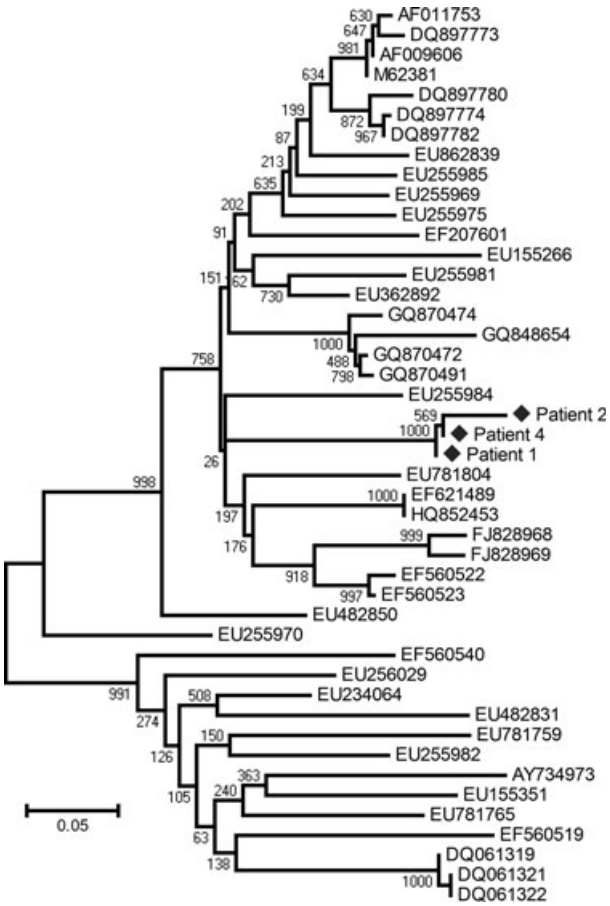


**Fig. 2.** Maximum likelihood tree for the E2 region. Model of nucleotide substitution: TIM2 + Γ+I. Patients 1, 2 and 4: sequences from the present study. The 20 most closely related sequences downloaded from GenBank were named after their GenBank's accession number. Numbers in the branches represent the bootstrap support value (1000 pseudoreplica).

since the uncle had already been infected before the nephews were born and at least Patients 2 and 3 showed HCV negative results in 2008. In addition, the mother of the boys claimed that needles have never been shared between them and the needle manipulation was always performed with extreme care since the whole family is aware of the risk involved in these situations. In the absence of any other risk behaviour, the most likely hypothesis about the route of transmission is the household contact between blood-contaminated utensils. Frequent bleedings in all four members of the family probably favoured HCV transmission. It is important to point out that, even when the uncle was also co-infected with HIV, none of the nephews were infected with this virus. This observation is compatible with the haematological route of infection since it is documented that the transmission by blood-contaminated elements is easier for HCV than for HIV [13]. The difference in environmental survival capabilities of HCV compared with HIV may be one of the explanations for the more efficient transmission of HCV infection [14].

Finally, the phylogenetic analysis showed that the viral strains infecting the family were highly related, indicating a common origin of the infections. This information allowed the inclusion of the rest of the circumstantial information (seroconversion times and needle manipulation behaviour) in order to build a hypothesis about the possible mechanism of the intrafamilial transmission. Taking preventive measures would contribute to protect uninfected household members, particularly children who have HCV-infected relatives.

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## Authors' contributions

ACAC, PB and RHC designed the research study. NA, PB and ACAC performed the research. MC contributed with the serum samples and background information. ACAC analyzed the data. ACAC, RHC and PB wrote the paper.

## Disclosures

The authors stated that they had no interests which might be perceived as posing a conflict or bias.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Data S1.** Primers and Protocols.

**Data S2.** Reteieved Sequences.

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