

Heterogeneity of hepatitis C virus genotype 2 variants in West Central Africa (Guinea Conakry)

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An overall anti-hepatitis C virus (HCV) prevalence of 6·7% was found in a sero-epidemiological study carried out in the town of Conakry (Guinea Conakry, West Central Africa) on 1421 subjects who were either blood donors, pregnant women or in- and outpatients receiving treatment for conditions other than liver disease. Seven HCV isolates from a subsample of 73 sterile sera from this population were studied for genetic characterization and classification. The 5'NCR was analysed by the Line Probe Assay. This method assigned the isolates to genotype 2. Analysis of the 5'NCR sequences alone was unable to give a more accurate classification. Comparison of NS5b region sequences (nucleotides 7575–8196), from Guinea isolates and genotype 2 database sequences, showed evolutionary distances in the range 0·15–0·26. There was a high level of subtype heterogeneity among the genotype 2 Guinea HCV isolates. Four of the subtypes were possibly new.

Hepatitis C virus (HCV) (Choo *et al.*, 1991), a distant relative of pestiviruses and flaviviruses, is the major aetiological agent of non-A, non-B hepatitis. Differences have been found among HCV strains in geographical distribution (McOmish *et al.*, 1994), antigenicity (McOmish *et al.*, 1993), level of viraemia and severity of disease (Tanaka, 1994). Extensive phylogenetic studies of genome coding regions, such as E1 and NS5b, have

provided evidence for the existence of at least 11 major genetic groups of HCV (Bukh *et al.*, 1993; Tokita *et al.*, 1996). Some of these are widely distributed around the world (genotypes 1, 2, 3) while others have been found in particular geographical regions. Genotypes 4 and 5 are regarded as African genotypes (Xu *et al.*, 1994; Davidson *et al.*, 1995) while genotypes 6 to 11 seem to be mostly present in Eastern and Asian countries (Tokita *et al.*, 1996).

This study was carried out in an urban area of the West Central African region of Guinea Conakry with the aim of defining the prevalence of HCV infection in this region, where data are scarce, and to investigate the genetic relatedness and classification of HCV isolates.

The study population consisted of 1421 individuals, aged 16–45, consecutively observed during 1993 at the Coronthie Service Ambulatory and at the Donkà Hospital in the city of Conakry (Guinea Conakry): 530 individuals were blood donors or pregnant women; 891 individuals were in- or outpatients receiving treatment in the departments of physiology, dermatology, gynaecology or neurology. All sera were tested for the presence of anti-HCV by second generation ELISA (Ortho Diagnostics). Anti-HCV ELISA reactive sera showing two or more bands by RIBA III supplemental assay (Ortho Diagnostics) were considered as positive.

The prevalence of anti-HCV in the different categories is shown in Table 1. A positive trend towards increasing

Table 1. Prevalence of anti-HCV in different categories of subjects in Guinea Conakry

Category	Total no.	Anti-HCV	
		No.	%
Blood donors	228	10	(4·4)
Pregnant women	302	8	(2·6)
In- and outpatients	891	77	(8·6)
Total	1421	95	(6·7)

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The nucleotide sequence data reported in this paper have been deposited in the GenBank and EMBL databases under the following accession numbers: X93316 (5'NCR DO12), X93317 (5'NCR MED007), X93318 (5'NCR MED017), X93319 (5'NCR MED011), X93320 (5'NCR PH33), X93321 (5'NCR PH48), X93322 (5'NCR PH52), X93323 (NS5 MED017), X93324 (NS5 DO12), X93325 (NS5 PH48), X93326 (NS5 PH52) and X93327 (NS5 MED007).

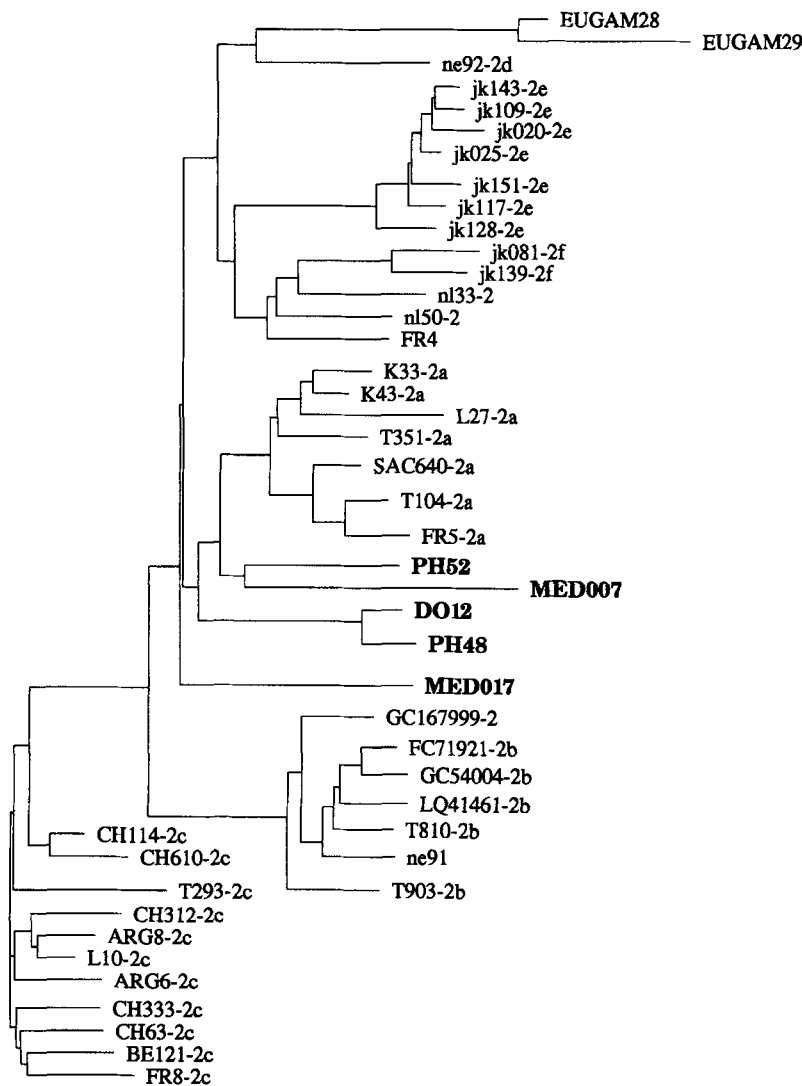


Fig. 1. Phylogenetic tree based on the NS5b region of HCV genotype 2 sequences (neighbour-joining method) of isolates PH52, MED007, DO12, PH48, MED017 and sequences from the GenBank database. The names are those indicated by the authors in the following publications: *Virus Research* **38**, 137–157, 1996; *Journal of General Virology* **74**, 2391–2399, 1993; *Journal of General Virology* **75**, 211–215, 1995; *Proceedings of the National Academy of Sciences, USA* **91**, 10134–10138, 1994; *Journal of Hepatology* **21**, 122–129, 1994; *Journal of General Virology* **77**, 243–301, 1996; *Journal of General Virology* **76**, 2493–2507, 1995). The analysis was based on the maximum likelihood method (program DNAML) and confirmed by neighbour-joining trees (NEIGHBOR); 1000 bootstrap replicates were performed and a consensus tree was drawn (CONSENSE). Clustering was accepted when the relevant branch was present in more than 95% of the trees.

prevalence by age was present rising from 4.6% and 7% to 12% among the age groups 16–25, 26–35 and 36–45, respectively.

Anti-HCV positive sera from a subsample of 73 sterile sera, collected for virological studies and stored at -80°C , were tested for the presence of HCV-RNA by a previously described RT-PCR method performed on the 5'NCR (Rapicetta *et al.*, 1992) and NS5b genome regions (Simmonds *et al.*, 1993). Seven HCV isolates were studied: one was from a blood donor (DO12) and the other six from in- and outpatients (MED007, MED011, MED017, PH33, PH48 and PH52). A preliminary characterization, performed by hybridization of 5'NCR RT-PCR products with the Line Probe Assay (INNO-LIPA HCV) (Stuyver *et al.*, 1993), classified all isolates as genotype 2.

HCV genomic regions corresponding to the 5'NCR, nucleotides –245–70, and to NS5b, nucleotides 7975–8196 (numbering according to Choo *et al.*, 1991), were amplified and cloned in T vectors (InVitrogen). Three clones were analysed for each amplified fragment. Sequencing, on both strands, was

performed by the modified dideoxynucleotide method with an ABI 373A automatic sequencer. Sequences were aligned by means of a pairwise comparison, using the Pileup program in the GCG package. The MSF format files were imported into the GDE (version 2.0) sequence analysis package. Phylogenetic studies were done with programs in the Phylip package (version 3.5) (Felsenstein, 1993).

Subtype classification based on the 5'NCR failed with all of the following methods: comparative sequence analysis, restriction analysis of sequence patterns (*RsaI*, *ScrFI*, *HaeIII*, *HinfI* and *ThaI* restriction enzymes) and secondary structure predictions (MAP and MFOLD GCG programs).

Analysis of nested PCR amplified products from NS5b was performed for five of the seven Guinea isolates. The lack of amplification with two samples (MED011 and PH33) may be ascribed to a low viral RNA content or to virus degradation. Alignments of the NS5b sequences of isolates DO12, MED007, MED017, PH48 and PH52 isolates, and comparison with the sequences of 45 isolates from databank representatives

Table 2. Average genetic distances among genotype 2 isolates

The average genetic distances, intra- and inter-subtype, were calculated for all the isolates reported in Fig. 1 (DNADIST program). ND, Not determined (because only a single isolate was clustered in the respective subtype).

	2a	2b	2c	2d	2e	2f	DO12- PH48	PH52	MED017	MED007
Intra-subtype	0.0813	0.0924	0.0966	ND	0.0390	0.0770	0.0450	ND	ND	ND
DO12-PH48	0.1762	0.2604	0.2225	0.2148	0.2548	0.2424	0.0450	0.1807	0.2058	0.2268
PH52	0.1574	0.2439	0.2007	0.2306	0.2694	0.2385	—	ND	0.1859	0.1835
MED017	0.2127	0.2242	0.1997	0.2127	0.2465	0.2648	—	—	ND	0.2229
MED007	0.2263	0.2664	0.2419	0.2263	0.2614	0.2614	—	—	—	ND

of genotype 2, showed the presence of single mutations characteristic of Guinea Conakry isolates (data not shown).

Fig. 1 is a phylogenetic tree of our HCV isolates in comparison with databank sequences of isolates belonging to genotype 2. Isolates MED007, PH52, DO12, PH48 and MED017 are clustered in branches separate from subtypes 2a, 2b, 2c, 2d, 2e and 2f and from the isolates (EUGAM28 and EUGAM29) studied by Mellor *et al.* (1995). Furthermore, as shown in Table 2, the evolutionary distance values clearly differentiated isolates MED017 and MED007 from all the other subtypes (0.19–0.26). Isolates DO12 and PH48 belong to the same subgroup. The lower values of phylogenetic distance observed for PH52–DO12, PH48 in respect of the 2a subtype may be ascribed to the short region analysed. Overall, these results indicate a considerable heterogeneity of HCV genotype 2 isolates from Guinea.

Little is known about distribution in Africa of HCV genotypes which are highly prevalent in Western and Asian countries, such as genotypes 1, 2 and 3. However, Mellor *et al.* (1995) showed the presence of genotype 2 in Africa. In the present study we found a high prevalence of anti-HCV in the region of Guinea Conakry in Central West Africa. All the isolates characterized were of genotype 2.

Sequence and phylogenetic analysis of the NS5b region revealed that the virus strains circulating in the studied population, homogeneous for geographical location and time-frame, also exhibited homogeneity in genotype distribution, but heterogeneity in subtype clustering. Only two out of five isolates (PH48 and DO12) could be classified as belonging to the same subgroup. The values found both from evaluation of genetic distances and from phylogenetic analysis show that Guinea Conakry isolates can be classified outside the previously described subtypes. We thus propose the possible existence of four new subtypes.

In conclusion, the importance of studying HCV variability in several countries, such as African countries, should be stressed, together with variations in deduced amino acid sequences of viral proteins and the consequent influence on the development of serological assays and future vaccines. This

work has further shown that we have to be cautious in interpreting the results of classification of HCV isolates from African populations, as novel variants could be present that could produce, by rapid classification methods, patterns resembling those of known genotypes or subtypes.

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