Molecular epidemiology of GB type C virus among individuals exposed to hepatitis C virus in Cameroon

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Abstract

GB Virus Type C (GBV-C), a blood-borne flavivirus currently infects about one sixth of the world's population. Its transmission has been reported through parenteral, sexual and vertical routes. Unusually for RNA viruses, it exhibits a high degree of conservation of the polyprotein sequence. The geographical distribution of GBV-C suggests an African origin and a long-term co-evolution in the human population but without any known pathogenicity. The aim of this study was to describe the different sub-types of this virus in Southern Cameroon. We studied the genetic epidemiology of GBV-C among rural populations where many HIV-1 and HCV genotypes have been identified. Plasma samples of 345 subjects with evidence of HCV exposure were tested for GBV-C infection. To detect GBV-C RNA, reverse transcription followed by a nested PCR of 5'UTR were performed. Direct sequencing and phylogenetic studies using PHYLIP, PAUP* and SimPlot were carried out. In total, 31 GBV-C RNA-positive samples were detected giving a prevalence of 9.0% among HCV-exposed individuals. Phylogenetic analysis of the 5'UTR showed two distinct clusters: Genotype 1 and Genotype 2. Twenty-eight isolates (8.0%) clustered with Genotype 1 and 3 (1.0%) with Genotype 2. More than one genotype of GBV-C is prevalent in Cameroon of which GBV-C Genotype 1 is more common, confirming reports in the literature. Studying the near full-length genome sequences of GBV-C isolates from primates in this region may provide clues of viral recombination, evolution and origin.

Introduction

GB Viruses, designated GBV Type A, GBV Type B and GBV Type C have been classified in the *Flaviviridae* family and genus Pegivirus on the basis of their structure, genomic organization and sequences.[1] This flavivirus identified by two different groups, was designated GB Virus Type C or Hepatitis G virus (HGV) with no known pathogenicity,[2,3] although its infection is common and persistent in human populations. GBV-C which is closely related to Hepatitis C virus (HCV) (Figure 1),[4] has been identified in humans and chimpanzees,[2,3,5] and its infection correlates with HCV infection or HCV endemicity.[6] Of the six GBV-C Genotypes designated Genotypes 1 through 6 that have been identified,[7] three were reported from sub-Saharan Africa. In 1998, GBV-C Genotype 1 and Genotype 2 were reported in Cameroon and Congo in Central Africa,[7] after the first report from Ghana.[2] International travel, blood transfusion and life style may have contributed to the spread of GBV-C in Cameroon, a region of broad genetic diversity of HCV and HIV-1.

HIV (a lentivirus) and GBV-C (a flavivirus) are two RNA viruses that are transmissible through sexual activity, via blood, blood products, intravenous drug use, from mother-to-child through pregnancy or delivery,[8,9] and also show a propensity to recombine in natural populations. During co-infection of these viruses, progression to AIDS is delayed in some individuals,[10] mortality rate is reduced and longer survival rates observed once AIDS has developed.[11-14] However, other studies did not report these effects probably due to the different stages of AIDS in the study populations.[15-17] In addition, these viruses may have an African origin,[18] but show different degrees of fitness in primates. The six genotypes of GBV-C show marked geographical differences in distribution.[19] Thus far, Genotype 1, which shows greatest overall sequence diversity, is confined in sub-Saharan Africa.[7,20] Human isolates of GBV-C show low sequence diversity and are more distantly related to simian isolates. GBV-C Genotype 2b may be associated with better immunological response, *i.e.* lower HIV-1 RNA viral load and higher $CD4^+$ lymphocyte count.[10,21]

GBV-C shows no known pathogenicity in humans. The challenges in assessing the possible risks this virus may pose to safety of blood are many. Blood and blood product recipients are vulnerable to emerging and re-emerging infections, such as GBV-C. With several infections, and possibly in the presence of other pathogens and other selective pressures, an isolated epidemic may occur and go unnoticed. A virus closely related to GBV-C co-circulates among chimpanzees and provides evidence of the origin of GBV-C in Africa that pre-dates human migration out of Africa around 100,000 years ago.[18,22,23] If recombination plays a role in the genetic diversity pattern of GBV-C, the emerging recombinant strains may show different degrees of fitness, transmissibility and disease induction. In this study, we aimed to identify the genetic diversity of GBV-C among isolated human populations with HCV exposure living in the Cameroon rainforest.

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Key words: GBV-C, flavivirus, Cameroon, phylogeny, genotype.

Funding: this work has been supported by a Fellowship/Grant from the Fogarty International Center/US NIH: Grant # 2 D 43 TW000010-16-AITRP.

Conflict of interests: the authors declare no potential conflict of interests.

Received for publication: 1 October 2012.
Revision received: 28 May 2012.
Accepted for publication: 11 June 2012.

Microbiology Research 2013; 4:e1
doi:10.4081/mr.2013.e1

Materials and Methods

Ethical approval for this study was given by the Cameroon Ministry of Public Health and the Johns Hopkins School of Public Health, USA. On obtaining informed consent, blood specimens were collected from inhabitants of 11 rural communities in southern Cameroon (Figure 2).

Screening for anti-HCV antibodies in plasma of 345 subjects was performed by ELISA. RNA was extracted from each of the 345 plasma specimens with evidence of HCV exposure for GBV-C typing. Amplification of 5'UTR was performed by nested RT-PCR and direct sequencing of the amplified products to confirm the specificity of amplification as described by Stuart *et al.*[24] The query sequences of the 5'UTR were compared to a GBV-C/HGV reference sequences from Japan,

USA, China and Ghana. Phylogenetic analysis was performed by PHYLIP, PAUP* and SIMPLOT.

Results

Thirty-one (9.0%) of the 345 samples were positive for GBV-C RNA. The phylogenetic tree of 5'UTR indicated 2 clusters: Genotype 1 and Genotype 2. Twenty-eight 5'UTR sequences (8.0%) clustered with reference sequences from Ghana and USA as Genotype 1. Within the Genotype 1 cluster, 4 distinct clades were identified (Figure 3). Three of the 31 (1.0%) GBV-C sequences were detected from the same study site (Figure 2, white dot) and they clustered with Genotype 2 sequences from Japan, China, and USA. These 3 isolates formed a distinct clade in the Genotype 2 Cluster.

Discussion and Conclusions

Three hundred and forty-five HIV-negative individuals exposed to HCV living in rural locations in the rainforest of Cameroon were tested for GBV-C RNA. Our interest in carrying out this study was based on reports from several countries on the epidemiology, genomic heterogeneity and quasi-species composition of this RNA virus in HCV endemic areas. Furthermore, information on co-infection of HIV-1 and GBV-C raises questions on virus influence on HIV disease progression in particular, and correlation with reduction of HIV-1 viral burden while maintaining high CD4+ lymphocyte counts and better immunological response.

In Cameroon, HIV-1 and HCV show a high genetic diversity. Of the six GBV-C Genotypes identified, Genotypes 1 and 2 have been reported in Cameroon. Of the 345 HCV positive individuals studied, 3 (1.0%) were infected with GBV-C Genotype 2 and 28 (9.0%) with Genotype 1. In the absence of any commercially available serological kit for GBV-C, RNA detection is used, a technique that is not widely available because of cost and low access to molecular biology facilities in resource-limited settings such as Cameroon. Although GBV-C infection has not been associated to any liver disease, it is blood-borne and shares routes of transmission.[25] Furthermore, its epidemiology should be studied among different target human populations such as blood-donors, HIV-infected individuals, HCV-infected individuals and pregnant women. GBV-C may have an African origin but its prognosis has not been well studied.

GBV-C and HCV are endemic in Cameroon. There have been reports of GBV-C Genotype 1

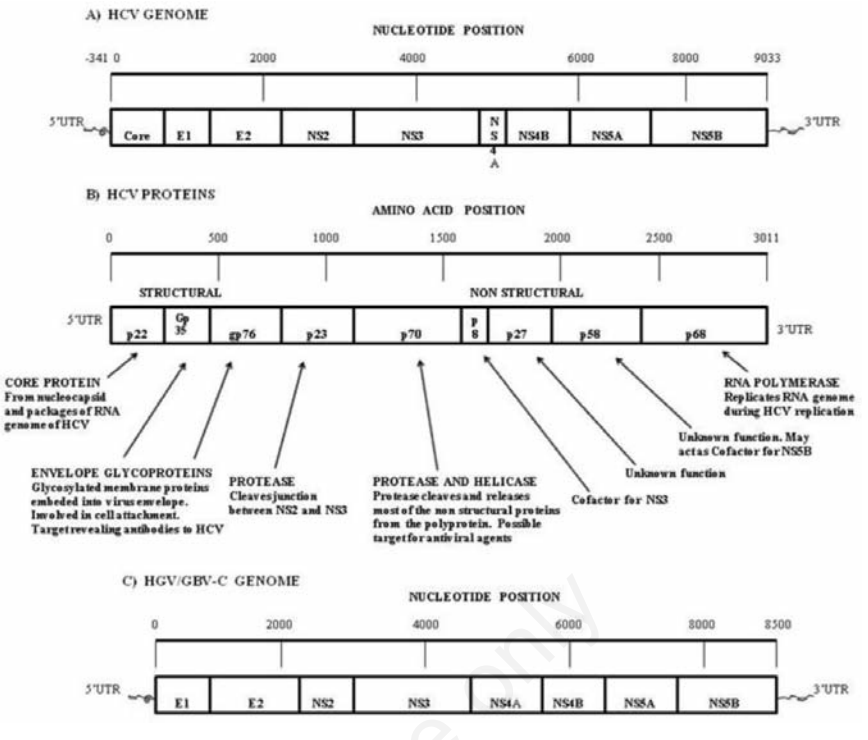

Figure 1. Genome Organization of HCV and HGV/GBV-C. (A) Organization of the HCV genome, showing the 5' and 3' untranslated regions, the single open reading frame and its cleavage sites. (B) The relative sizes of the resulting HCV proteins and what is currently understood of their functions. (C) Genome organization of HGV/GBV-C, with genes homologous to those of HCV indicated; note the lack of a protein corresponding to the capsid protein of HCV.

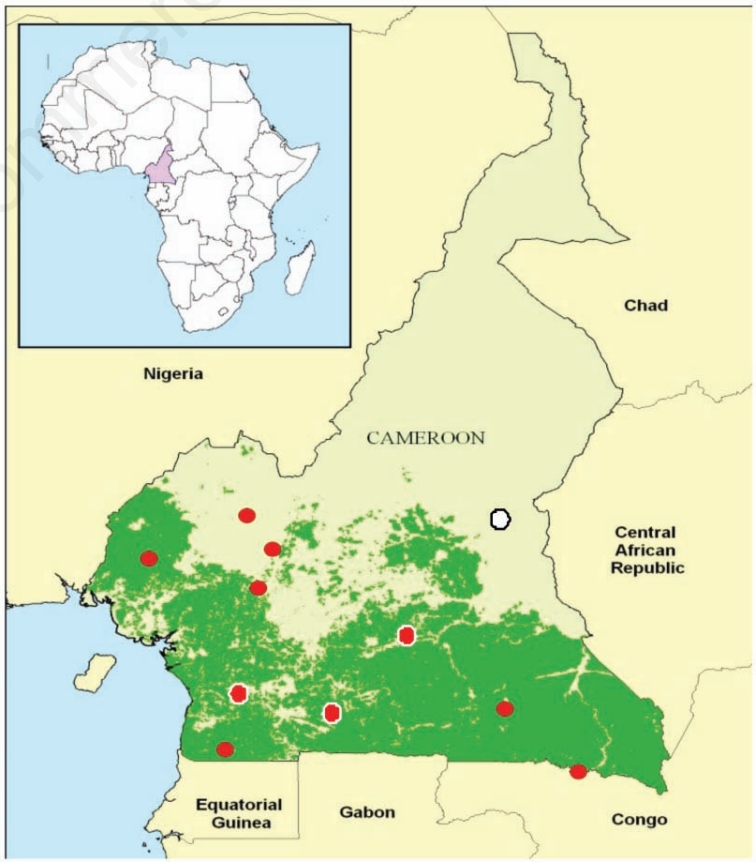

Figure 2. Map of Cameroon showing study sites (red and white dots).

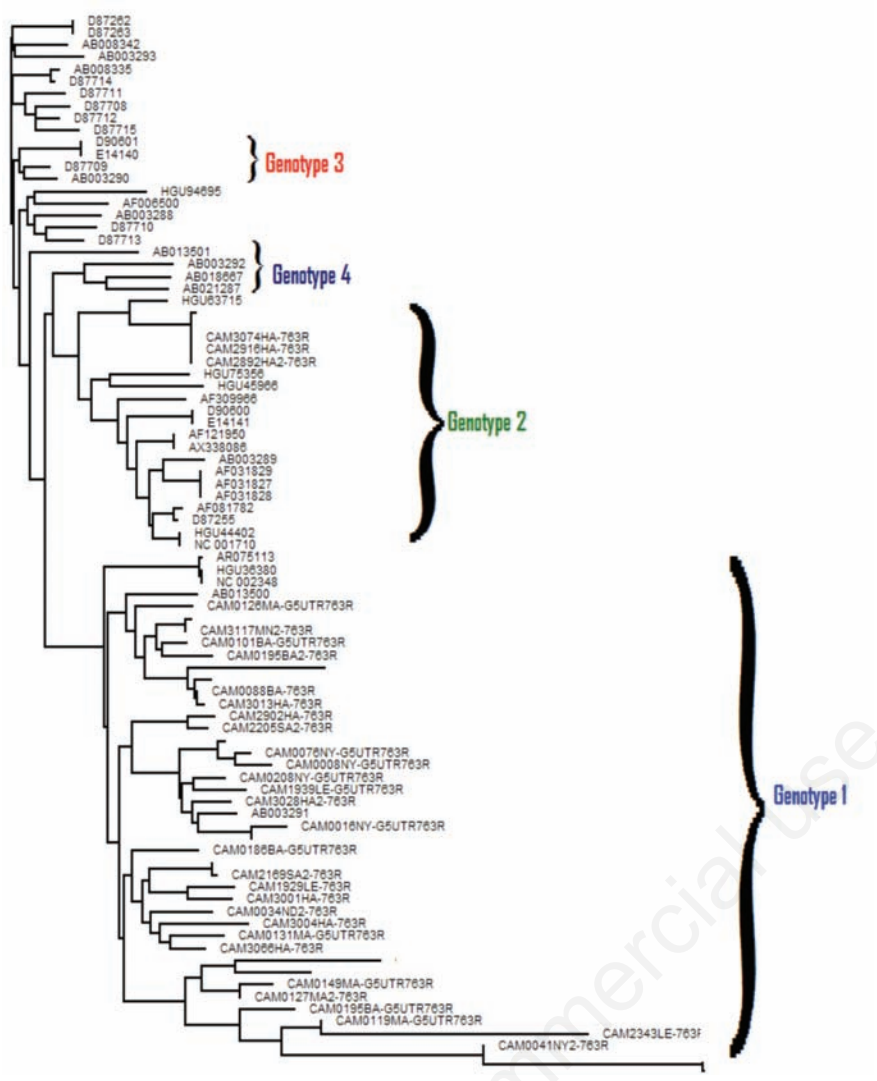

Figure 3. Phylogenetic tree of GBV-C 5'UTR sequences.

from Ghana in West Africa,[2] of GBV-C Genotype 1 and Genotype 2 from Cameroon and Congo in Central Africa,[7] and GBV-C Genotype 1, Genotype 2 and Genotype 5 from South Africa in Southern Africa.[19] We identified GBV-C Genotype 2 and Genotype 1 from our study with remarkable predominance of Genotype 1 showing four distinct clades. Ten years after the identification of GBV-C in Ghana, many isolates have been reported from around the world. Our results and those of others, provide evidence of the emergence of new GBV-C in human populations, which may show different biological properties. There is, therefore, a need to set-up prevention measures to reduce the spread of GBV-C. Our results may also provide clues about the development of serological assays for GBV-C E2 protein. If these assays are reliable, then screening of the populations to minimize its spread becomes an important initiative in virus surveillance in the geographical region where there is a broad diversity of other RNA viruses, such as HIV-1 and HCV. It is important to study GBV-C infection in a natural setting of people with mono-infection or co-infection with HIV or HCV in disease progression.

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