

DIVERSITY AND NS5B NATURAL POLYMORPHISMS IN HCV VIRUSES DRIVING THE CURRENT EPIDEMIC IN PORTUGAL

Palladino C¹; Ezeonwumelu IJ¹; Marcelino R²; Briz V³; Moranguinho I¹; Serejo F⁵; Velosa JF⁵; Marinho RT⁵; Borrego P^{1,4}; Taveira N^{1,6}

1) Research Institute for Medicines (iMed.Ulisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; 2) Global Health and Tropical Medicine (GHTM), Instituto de Higiene e Medicina Tropical (IHMT), Universidade Nova de Lisboa, Lisbon, Portugal; 3) Laboratory of Viral Hepatitis, National Center for Microbiology, Institute of Health Carlos III, Majadahonda, Madrid, Spain; 4) Centro de Administração e Políticas Públicas (CAPP), Instituto Superior de Ciências Sociais e Políticas, Universidade de Lisboa, Lisbon, Portugal; 5) Department of Gastroenterology and Hepatology, Santa Maria Hospital, Universidade de Lisboa, Lisbon, Portugal; 6) Centro de Investigação Interdisciplinar Egas Moniz, Instituto Universitário Egas Moniz, Caparica, Portugal.

INTRODUÇÃO

Hepatitis C virus (HCV) infection continues to be a major public health problem globally despite the introduction of new treatment modalities based on combination of direct antiviral agents (DAAs). In 2015 alone there were about 71.1 (62.5-79.4) million viraemic infections, corresponding to a prevalence of 1% (0.8-1.1) ¹. HCV is classified into seven genotypes and numerous subtypes². Worldwide there are significant differences in epidemic history among the HCV subtypes which may differ in response to treatment and to antibody neutralization^{1,3-5}. Any successful HCV vaccination or control strategy requires an understanding of the epidemic behavior among subtypes. In Portugal, there is scarce epidemiological research on HCV and prevalence data are limited. A recent nationwide cross-sectional survey reported a low HCV prevalence (0.54%; 0.2-0.9)⁶ but prevalence in high in people who inject drugs (PWID) (83.5%)⁷ and in the prison population (10.7%)⁸. In this study we aimed to make the first characterization of the origin, epidemic history, transmission dynamics and diversity of HCV genotypes in Portugal.

MATERIAL/MÉTODOS

Direct sequencing of HCV nonstructural protein 5B (NS5B) was performed on plasma samples collected from a cross-section of 230 DAAs-naïve patients attending the Hospital Santa Maria in Lisbon, Portugal. Phylogenetic analysis was used for subtyping and transmission cluster identification. Time-scaled phylogeny (Bayesian estimation) was performed to date the origin of the different subtypes and delineate the epidemic history of the main HCV subtypes. NS5B sequences were analysed for polymorphisms and resistance associated substitutions (RASs).

RESULTADOS

A total of 230 patients were included. 59.1% (n=136) of subjects were men and had a median age of 41 years (IQR: 49-36). The majority of HCV strains were GT1 (62.6%), followed by GT3 (18.3%) and GT4 (16.1%). Among GT1, the most frequent subtype was 1a (75.5%) followed by 1b (24.5%). All GT3 were subtype 3a. Among GT4, the most frequent subtypes were 4a (10.4%) and 4d (4.3%) (Fig.1). Except for 12 HCV lineages segregating into six transmission clusters, polyphyletic patterns were found suggesting multiple and old introductions of the different HCV subtypes in this population. Four distinct epidemics caused by different HCV subtypes were identified over time in Portugal (Tab. 1, Fig 2-3). The first was caused exclusively by GT1b, occurred during 1930s and 1960s and was likely associated with contaminated blood transfusions. The second and third epidemics were likely associated with widespread use of intravenous drug use and were caused by GT3a in the 1960s and GT1a in the 1980s. The most recent HCV epidemic in Portugal was caused by GT4a and seems to be associated with the resurgence of opioid use. The majority of patients (93.9%; n=216) harbored viruses with baseline NS5B polymorphisms. There were no RAS to sofosbuvir in our patients but C316N that confers low-level resistance to dasabuvir was found in 31.4% of 1b-infected patients. In addition, amino acid substitutions on scored position for sofosbuvir were observed in one patient with GT1a clade I (V321I), one with GT1a clade II (V321I/V) and one with GT3a (V321S/L).

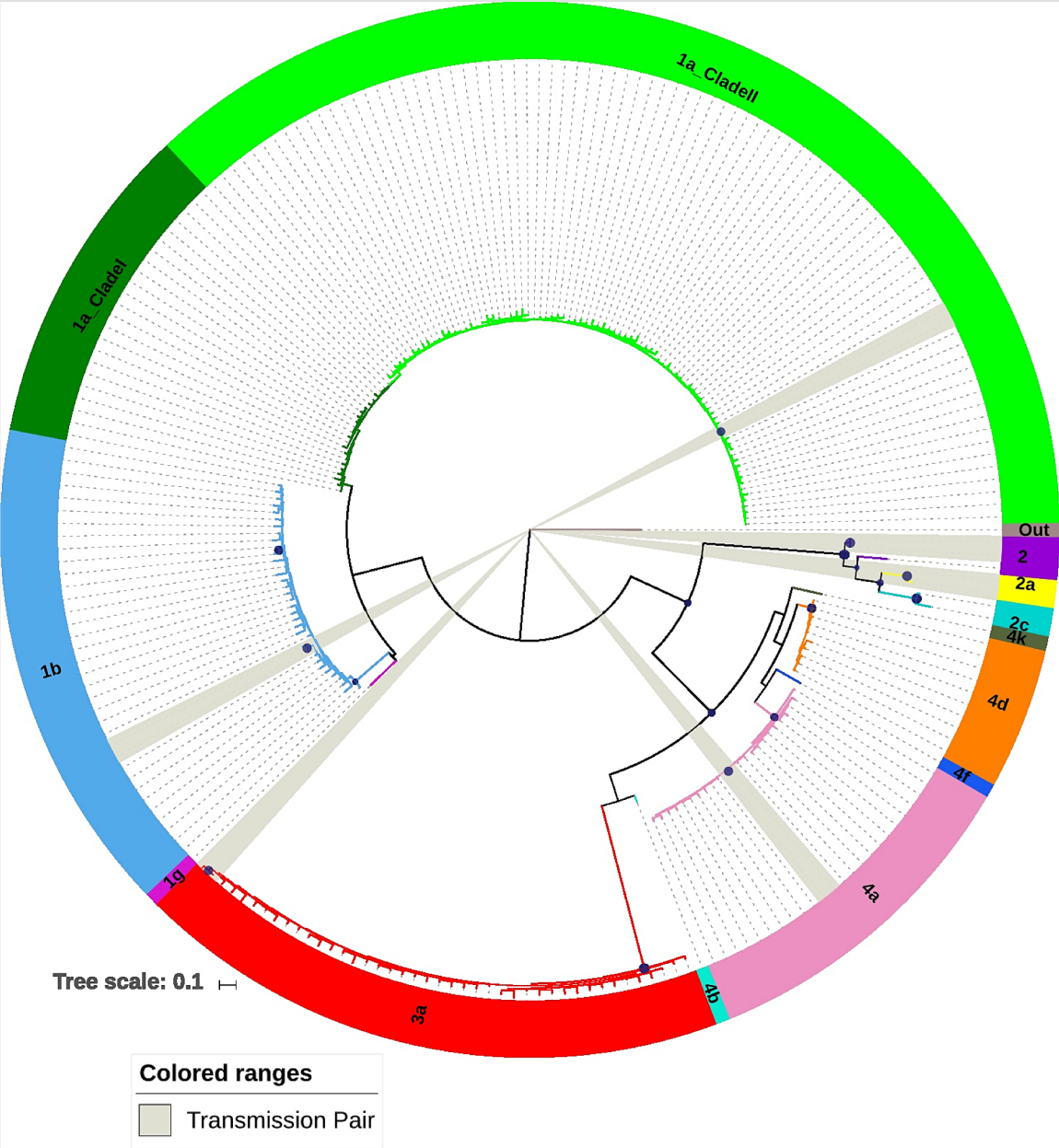


Fig 1. Phylogenetic representation of HCV subtypes identified in this study. ML tree was reconstructed under a GTR + Γ nucleotide substitution model with 1000 bootstrap replicates.

Tab 1. Estimated dates of MRCAs (mean estimates of Most Recent Common Ancestor) dates in calendar years for HCV subtypes identified in this study.

Subtype	Dates of MRCA* (95% HPD interval)
1a (n = 108)	1950 (1922, 1973)
1b (n = 35)	1946 (1847, 1976)
3a (n = 42)	1963 (1947, 1977)
4a (n = 24)	1988 (1980, 1995)
4d (n = 10)	1799 (1473, 2000)

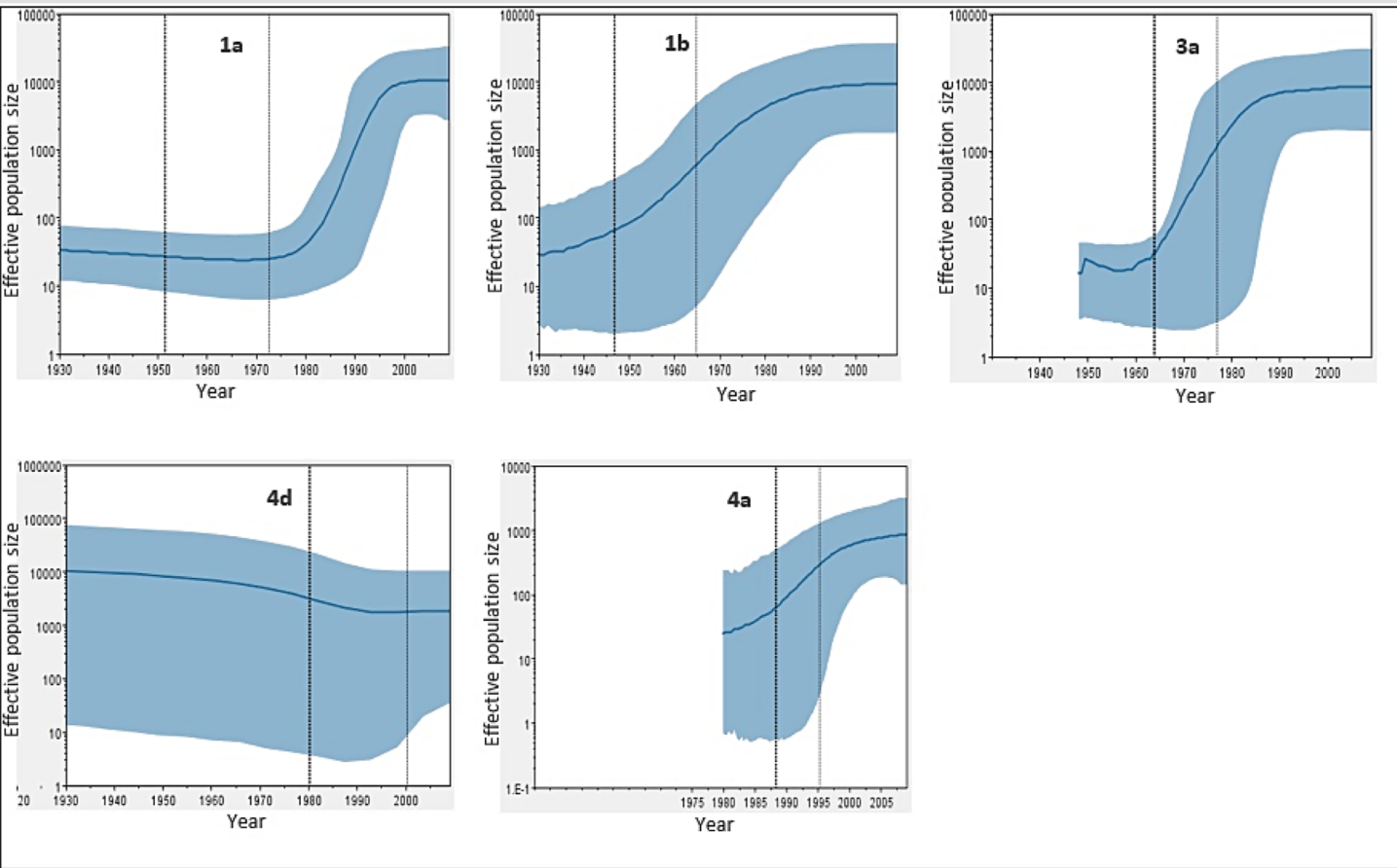


Fig 2. Bayesian skyline plot (BSP) showing the epidemic history of HCV subtypes 1a, 1b, 3a, 4a and 4d identified in this study. The solid blue line represents the changes in the mean effective population size through time on a log₁₀ scale, with the blue shaded area corresponding to the 95 % highest posterior density (95% HPD) interval. The bold dotted and faint dashed black vertical lines represent the median and upper boundaries of the time to the MRCA, respectively.

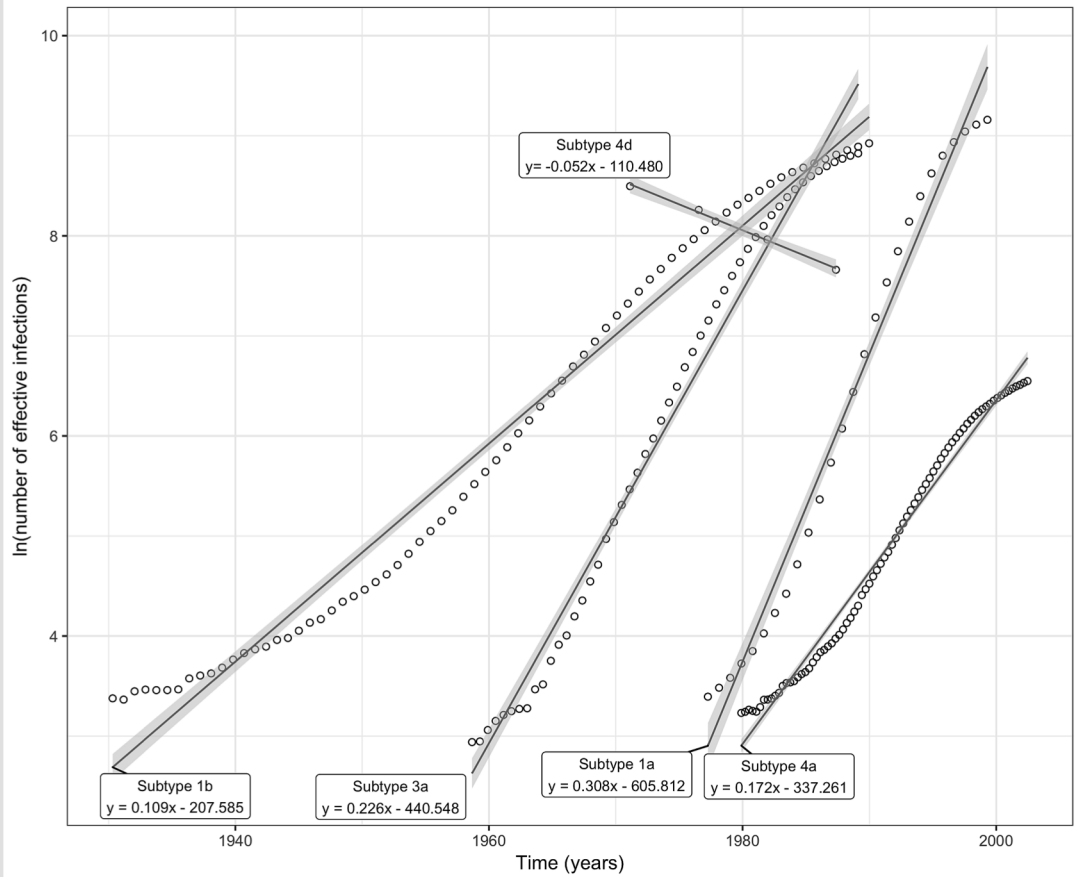


Fig 3. Exponential mean growth rates for the most prevalent HCV subtypes (1a, 1b, 3a, 4a and 4d) identified in this study. Linear regression equations were derived from the mean growth rates within the exponential phase of the Bayesian skyline plots (BSP) of each subtype as shown in Fig 2.

CONCLUSÕES

Close surveillance of patients bearing RASs and undergoing dasabuvir-based regimens will be important to determine its impact in treatment outcome.

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