Poster 163

ORIGIN, DIVERSITY AND TRANSMISSION DYNAMICS OF HEPATITIS C VIRUS GENOTYPES IN PORTUGAL

Marinho RT[1]; Palladino C[2]; Ezeonwumelu IJ[2]; Marcelino R[3]; Briz V[4]; Moranguinho I[2]; Serejo F[1]; Velosa JF[1]; Borrego P[2,5]; Taveira N[2,6]

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

INTRODUCTION

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 subtypes[2]. 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 behaviour 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.

MATERIALS & METHODS

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).

RESULTS

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%; n=108) followed by 1b (24.5%; n=35). All GT3 were subtype 3a. Among GT4 (16.1%), the most frequent subtypes were 4a (10.4%; n=24) and 4d (4.3%; n=10). One isolate of GT4f and one of GT4k were also identified (Fig.1). The GT1a clade II was more prevalent than clade I (78.7%; n = 85/108 vs. 21.3%; n = 23/108; P < 0.0001). 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. Five 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 epidemics were caused by GT4a and GT4d and seem to be associated with the resurgence of opioid use. The majority of patients (93.9%; n=216) harboured 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).

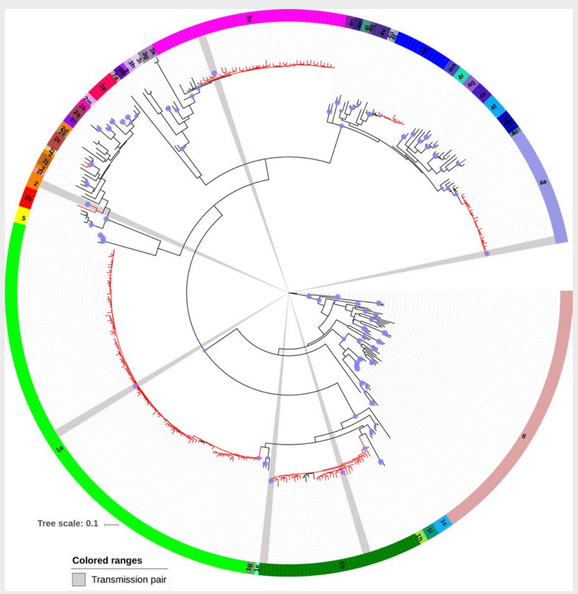

Fig 1. Phylogenetic representation of HCV subtypes identified in this study. ML tree was reconstructed under a GTR + Γ nucleotide substitution model with 1000 bootstrap replicates.

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

**Contains additional GT4d sequences (n = 14) from a previous study in Portugal.

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 = 24)*	1982 (1964, 1995)

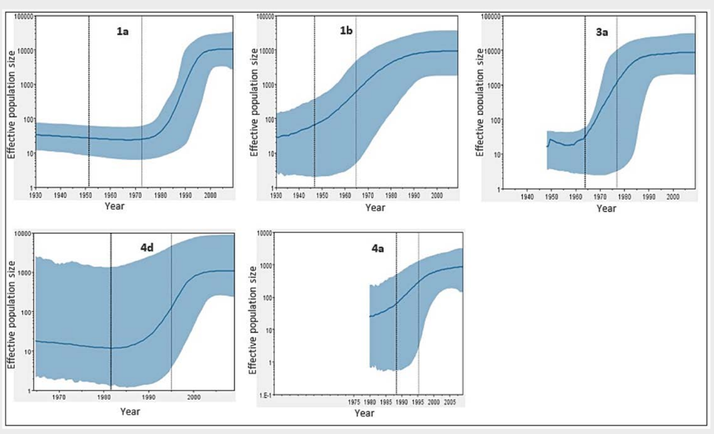

Fig 2. Bayesian skyline plot (BSP) showing the epidemic history of HCV subtypes 1a, 1b, 3a, 4a and 4d identified in this study. The solid blue line represents the changes in the mean effective population size through time on a $\log_{10}$ scale, with the blue shaded area corresponding to the 95 % highest 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.

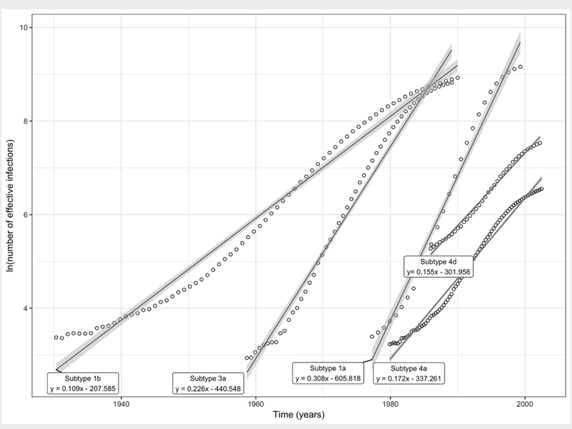

Fig 3. Exponential mean growth rates for the most prevalent HCV subtypes (1a, 1b, 3a, 4a and 4d) identified in this study. Linear regression equations were derived from the mean growth rates within the exponential phase of the Bayesian skyline plots (BSP) of each subtype as shown in Fig 2.

CONCLUSIONS

The fastest growing HCV epidemic in Portugal occurred during the 1980s and 1990s and was caused by GT1a. A relatively high prevalence of GT4 was observed, that is about three-fold higher than the estimated prevalence for Western Europe[9,10]. Importantly, there are indications that GT4 is becoming increasingly prevalent in Europe, mainly among people who inject drugs[11-13]. To the extent of our knowledge, this is the first report of GT4f and GT4k in Portugal. Close surveillance of patients bearing RASs is important to determine their impact on treatment outcome.

REFERENCIES

1. Blach S, *et al. Lancet Gastroenterol Hepatol* 2017; 2. Smith DB, *et al. Hepatology* 2014; 3.Timm J & Roggendorf M. *World J Gastroenterol* 2007; 4. Pawlotsky JM. *Gastroenterology* 2014; 5. Prentoe J, *et al. Hepatology* 2016; 6. Anjo J *et al. Gastrenterologia* 2014; 7. EMCDDA *Statistical Bulletin* 2017; 8. Fundação Francisco Manuel dos Santos. PORDATA, Base de Dados de Portugal Contemporâneo; 9. Kartashev V, *et al. J Clin Virol* 2016; 10. Petruzziello A, *et al. World J Gastroenterol* 2016; 11. Calado RA et al. J Med Virol 2011; 12. De Bruijne J, *et al. J Clin Microbiol* 2009; 13. Wiessing L, *et al.* PLoS One 2014.

The first two authors (R.T.M. and C.P.) contributed equally to this work.
The last two authors (P.B. and N.T.) contributed equally to this work.

Correspondence to N. Taveira, ntaveira@ff.ulisboa.pt,
and C. Palladino,palladino.claudia@gmail.com