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Research paper

HIV infection and hepatitis C virus genotype 1a are associated with phylogenetic clustering among people with recently acquired hepatitis C virus infection

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ABSTRACT

The aim of this study was to identify factors associated with phylogenetic clustering among people with recently acquired hepatitis C virus (HCV) infection. Participants with available sample at time of HCV detection were selected from three studies; the Australian Trial in Acute Hepatitis C, the Hepatitis C Incidence and Transmission Study — Prison and Community. HCV RNA was extracted and Core to E2 region of HCV sequenced. Clusters were identified from maximum likelihood trees with 1000 bootstrap replicates using 90% bootstrap and 5% genetic distance threshold. Among 225 participants with available Core-E2 sequence (ATAHC, n = 113; HITS-p, n = 90; and HITS-c, n = 22), HCV genotype prevalence was: G1a: 38% (n = 86), G1b: 5% (n = 12), G2a: 1% (n = 2), G2b: 5% (n = 11), G3a: 48% (n = 109), G6a: 1% (n = 2) and G6l 1% (n = 3). Of participants included in phylogenetic trees, 22% of participants were in a pair/cluster (G1a-35%, 30/85, mean maximum genetic distance = 0.031; G3a-11%, 12/106, mean maximum genetic distance = 0.021; other genotypes-21%, 6/28, mean maximum genetic distance = 0.023). Among HCV/HIV co-infected participants, 50% (18/36) were in a pair/cluster, compared to 16% (30/183) with HCV mono-infection (P = <0.001). Factors independently associated with phylogenetic clustering were HIV co-infection [vs. HCV mono-infection; adjusted odds ratio (AOR) 4.24; 95%CI 1.91, 9.39], and HCV G1a infection (vs. other HCV genotypes; AOR 3.33, 95%CI 0.14, 0.61).HCV treatment and prevention strategies, including enhanced antiviral therapy, should be optimised. The impact of targeting of HCV treatment as prevention to populations with higher phylogenetic clustering, such as those with HIV co-infection, could be explored through mathematical modelling.

1. Introduction

The burden of hepatitis C virus (HCV) infection continues to grow, despite targeted public health strategies to prevent transmission (Sacks-Davis et al., 2012b). There is a high incidence of HCV infection among people who inject drugs (PWID) (Maher et al., 2007, Page et al., 2009) and an increasing incidence of HCV infection has been observed among human immunodeficiency virus (HIV) positive gay and bisexual men (Danta et al., 2007). Ongoing HCV transmission in these groups suggests a clear need for further characterisation of factors influencing HCV transmission. This need is particularly pertinent due to the development of new therapies for the treatment of HCV infection, which while being highly curative (>90% sustained virological response), well tolerated and likely to have a short treatment duration (8–12 weeks) (Grebely et al., 2013), also carry considerable financial burden. More detailed characterisation of the transmission of HCV infection, in particular among those with acute and recently acquired infection, is needed to guide HCV prevention strategies, including treatment as prevention (Martin et al., 2013, Grebely and Dore 2014).

Characterising acute HCV transmission has historically been difficult as it is often asymptomatic and there is limited public health surveillance infrastructure to monitor populations at risk of infection, who are often marginalised and burdened by stigma (Treloar et al., 2014). Traditional epidemiological studies of acute infection tend to measure factors associated with acquisition rather than transmission, and are often complicated by multiple risk factors and overlapping modes of acquisition (Matthews et al., 2011, Mahony et al., 2013). However, novel molecular epidemiological methods used to study HIV transmission (Pillay et al., 2007, Lewis et al., 2008) have provided unique

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insights into the groups most at risk of transmission and are now beginning to shed light on the transmission dynamics of HCV (Pybus et al., 2005). It has been demonstrated that phylogenetic clustering of HCV is associated with social-injecting networks (Sacks-Davis et al., 2012a), sexual networks (Bradshaw et al., 2014), HIV co-infection (van de Laar et al., 2009, Matthews et al., 2011), HCV seroconversion and recent receptive syringe borrowing (Jacka et al., 2014, Cunningham et al., 2015). Although behavioural risk factors linked to transmission of HCV in HIV positive gay and bisexual men have been identified (Danta et al., 2007, van de Laar et al., 2009, Matthews et al., 2011), epidemiological factors associated with transmission clusters of acute and recently acquired HCV infection have not been well characterised.

The aim of this study was to investigate phylogenetic clustering of HCV and associated factors among individuals with acute or recently acquired HCV infection in Australia.

2. Methods

2.1. Study population and design

Data and specimens from three studies of recently acquired HCV in Australia were used for this study. The Australian Trial in Acute Hepatitis C (ATAHC) was a multicentre, prospective study of recent HCV recruited between 2004 and 2007 (Dore et al., 2010). The Hepatitis C Incidence and Transmission Study - prison (HITS-p) was a study of prison inmates at-risk of HCV infection in correctional centres recruited between 2005 and 2014 (Teutsch et al., 2010). The Hepatitis C Incidence and Transmission Study — community (HITS-c) was a study of community-based people who inject drugs (PWID) at risk of HCV infection, which recruited between 2008 and 2014 (White et al., 2014).

For inclusion, participants from these cohorts had to have acute or recently acquired HCV defined by an initial positive anti-HCV antibody test and either (1) a negative anti-HCV antibody test within 2 years prior to the initial positive anti-HCV test or (2) acute clinical hepatitis (either jaundice or alanine aminotransferase [ALT] >400 IU/mL) within 12 months of the initial positive anti-HCV result. Participants also had to have a HCV RNA positive plasma sample, with the first available sample following the detection of acute HCV selected. All participants provided written informed consent and protocols were approved by appropriate Human Research Ethics Committees.

The estimated date of infection was calculated for subjects who presented with acute clinical hepatitis as six weeks prior to onset of symptoms. For subjects identified by recent positive HCV antibody test with a negative test in the prior two years, the estimated date of infection was calculated as the midpoint the between the first positive test and the last negative test.

2.2. Detection and quantification of HCV RNA

Qualitative HCV RNA testing was performed using the Versant TMA assay (Bayer, Australia; <10 IU/mL; ATAHC) or COBAS AmpliPrep/COBAS TaqMan HCV assay (Roche, Branchburg, NJ; <15 IU/mL; HITS-p, HITS-c). Quantitative HCV RNA testing was performed using the Versant HCV RNA 3.0 (Bayer, Australia; <615 IU/mL; ATAHC) or COBAS AmpliPrep/COBAS TaqMan HCV assay (Roche; <15 IU/mL; HITS-p). HCV genotyping (Versant LiPa1 or LiPa2, Bayer, Australia) was performed on all participants with detectable HCV RNA at first HCV detection.

2.3. HCV RNA sequencing

HCV RNA was extracted from EDTA plasma using QIAamp viral extraction mini kit (#52906, QIAGEN, Limburg, NL). Reverse transcription and polymerase chain reaction (PCR) amplification of a region of the HCV genome encoding Core, Envelope-1 (E1) and the beginning of Envelope-2 (E2) was performed to generate a 1404 base pair (bp) amplicon (nucleotides 347–1750 in H77 reference sequence [GenBank ascension no. NC_004102]) using a method previously described (Lamoury et al., 2015). PCR amplicons were sequenced by Sanger sequencing and sequence chromatograms were processed using RECall: a fully automated sequence analysis pipeline (Woods et al., 2012). Subtypes were determined by constructing a subtyping tree using the panel of reference sequences classified by Smith et al. (Smith et al., 2014) (Supplementary Fig. 1).

2.4. Phylogenetics

Phylogenetic trees of the Core-E2 fragment were inferred separately for major subtypes and minor genotype groups (1a, 1b, 2a/c, 3a and 6a/l) using maximum-likelihood analysis implemented in RAxML (Stamatakis et al., 2005) through the CIPRES Science Gateway (Miller et al., 2010) under the General Time Reversible model of nucleotide substitution with a gamma shaped distribution of rate variation across sites (GTR + G). JModelTest (Guindon and Gascuel 2003, Darriba et al., 2012) was used to determine the most appropriate model of nucleotide substitution. Reference sequences obtained from the Los Alamos National Laboratory HCV database (Kuiken et al., 2004) and from previous sequencing studies (Jacka et al., 2014, Cunningham et al., 2015) were included to support identification of "local" clusters (Hué et al., 2005). All sequences were aligned using pair-wise alignment in ClustalX prior to phylogenetic analysis (Larkin et al., 2007).

The final fragment analysed was 1104 bp long following the removal of the hypervariable region one (HVR1) of E2 and gaps created by alignment. HVR1 was removed based on a previous finding that inclusion of this region leads to decreased ability to identify pairs and clusters due to extreme genetic variation seen between individuals in this region (Lamoury et al., 2015). The robustness of the resulting tree was assessed using a rapid bootstrap algorithm with 1000 replicates, and clusters were identified using ClusterPicker software (Ragonnet-Cronin et al., 2013). A sensitivity analysis was performed by varying the genetic distance threshold between 1.5–5% in ClusterPicker, with and without 90% bootstrap threshold, to determine the effect this had on the identification of factors associated with clustering (Supplementary Tables 1 and 2).

2.5. Study outcome

The primary study outcome was phylogenetic clustering of HCV infections, as defined by two or more participants with HCV genome sequence within the bootstrap and genetic distance threshold cut off. A pair was defined as two participants with HCV genome sequence within the bootstrap and genetic distance threshold cut off and a cluster was defined by three or more participants with HCV genome sequence within the bootstrap and genetic distance threshold cut off.

2.6. Statistical analyses

Unadjusted logistic regression analysis was used to identify factors associated with being in a pair/cluster. Factors hypothesised to be associated with HCV pairing or clustering that were assessed included: age (Page et al., 2013), female sex (vs. male sex) (Dore et al., 2003), HIV infection (Danta et al., 2007, Urbanus et al., 2009, van de Laar et al., 2009, Matthews et al., 2011), recent injection drug use (defined as injecting in the last 3–6 months) (Maher et al., 2007, Aitken et al., 2008, Sacks-Davis et al., 2012a), incarceration ever (Hellard et al., 2004) and current incarceration (Hellard et al., 2004). All variables with $P < 0.20$ in the unadjusted analysis were considered in the adjusted logistic regression model, using a backwards stepwise approach with factors sequentially eliminated according to the result of a likelihood ratio test. To account for potential unmeasured confounding introduced by the different