Future VIROLOGY

Editorial

Comparative host genomics: how has human evolution affected our immune defence against hepatitis C virus?

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> "Intriguingly, while permissive for chronic HCV infection, rates of chronicity and associated disease differ in chimpanzees and treeshrews when compared with humans."

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Pathogens are undeniably a critical environmental pressure influencing the evolution of humans and other animals; the resulting genetic changes can significantly affect the outcome of an infection [1]. Fossil and genetic evidence suggests that anatomically modern humans (*Homo sapiens*) arose in Africa approximately 200,000 years ago, following approximately 6 million years of divergence from the shared great ape ancestors of *Homo* and chimpanzees (*Pan troglodytes*) [2]. Unlike chimpanzees, who remained confined to their home continent, approximately 60,000 years ago a single human population migrated out of Africa ultimately colonizing Eurasia, the Americas and Oceania. The long period since human–chimpanzee divergence combined with our subsequent worldwide spread has provided ample opportunity for evolution to differentially adapt the two species to novel environments.

Hepatitis C virus (HCV), an enveloped positive-sense RNA virus in the family Flaviviridae (genus *Hepacivirus*), is a blood-borne pathogen that is seemingly restricted to humans [3]. HCV currently infects an estimated 71 million people worldwide (~1% of the human population) resulting in 399,000 deaths annually due its efficient ability to establish a chronic – typically life long – infection in the liver that can lead to fatal disease decades after primary infection in the absence of treatment. HCV is highly diverse with at least eight distinct 'genotypes', which can be over 30% different genetically from each other [4].

While HCV likely originated in the 'Old World' – with genotypes endemic only in sub-Saharan Africa and Asia – a precise origin for the virus has not been identified [5]. Estimated timings for the expansion in HCV genomic diversity suggest an origin between hundreds of thousands to thousands of years ago. Thus, current evidence fails to distinguish between whether HCV has a 'recent' beginning in humans reflecting independent origins across Africa and Asia or a single more ancient one, with HCV leaving Africa at the time of human migration. This pattern of high genetic diversity in a single host species could be interpreted as evidence of either long-term coexistence of HCV with humans, and/or relatively efficient spillover into humans from an as yet unknown animal reservoir present in Africa and Asia; both scenarios indicate an intrinsic high level of HCV adaptation to humans. Of note, hepaciviral sequences have been found across a very diverse selection of mammals [6] with the greatest diversity found in bats and rodents, yet the closest relative to HCV is one found in horses and dogs but not primates.

To explore the virus' host tropism, HCV has only been inoculated into species belonging to the Euarchontoglires (rodents, rabbits, colugos, treeshrews and primates), for example: mice and woodchucks, treeshrews, and primates (e.g., apes, Old and New World monkeys) [7–10]. Only chimpanzees and, to a much lesser degree, treeshrews are permissive for HCV infection. Using *in vivo* and *in vitro* studies the lack of permissivity and susceptibility in lab mice has been mapped to blocked viral entry and immune evasion [7]. Intriguingly, while permissive for chronic HCV infection, rates of chronicity and associated disease differ in chimpanzees and treeshrews when compared with humans. Most notably, HCV-infected chimpanzees have reduced rates of chronicity [11], disease [8], treatment responses [12] and viral glycoprotein gene mutation rates indicative of enhanced immune pressure [13].

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By exploiting variation of HCV infection outcomes, combined with comparisons of whole genome sequences or intraspecies genomic variation, research has started uncovering the specific host molecules that define species and individual differences in HCV chronicity and pathogenesis [14]. Downstream experimental analysis can provide additional functional evidence to support statistical associations. Initial studies demonstrated a significant role of interindividual genetic differences in humans in the cellular immune response, including the T cell and natural killer cell responses. More recently, genome-wide association studies in humans for HCV clearance revealed the impact of innate immune signaling genes. This work identified a common loss-of-function frameshift variant in the antiviral signaling protein gene *IFNL4*, associated with HCV clearance [15]. Somewhat counterintuitively for an antiviral protein, production of IFNL4 is associated with reduced clearance of acute HCV infection and reduced response to interferon alpha therapy, and has also been associated with reduced liver inflammation and disease [16]. A further common coding variant resulting from a substitution of proline at position 70 to serine (P70S) in IFNL4 was found to be associated with higher clearance rates than that of the 'wild-type' protein [17].

Sequencing of mammalian genomes and analysis of *IFNL4* sequences showed that the gene has evolved under purifying selection across a limited – but broad – selection of mammal species [18]. The *IFNL4* loss-of-function frameshift allele varies across human populations with its highest frequency in East Asia (>90%) and lowest in sub-Saharan Africa (<40%). Loss of *IFNL4* has evolved under positive selection in African and Asian populations although the cause is unknown [18].

Intrigued by the role of *IFNL4* variation in regulating HCV biology within humans and possibly between us and other species, we have shown recently that human *IFNL4* has evolved markedly reduced antiviral activity, in comparison with *IFNL4* from chimpanzees and to a lesser extent, from rhesus macaques [19]. This reduction in activity resulted from a single amino acid change arising during human evolution (glutamic acid 154 to lysine; E154K). E154 is almost 100% conserved in mammalian *IFNL4* sequences, with humans being the exception. Mechanistically, this negative-to-positive charge substitution impeded both protein secretion and potency likely through disrupting key intramolecular bonds. Furthermore, our *in vitro* observations correlated with reduced antiviral transcriptomic liver responses in humans compared with chimpanzees during HCV infection. The reduced activity is highly conserved in humans, with the E154 allele found in individuals with African descent, including African–Americans and 'pygmy' hunter-gatherers from Central Africa (but not other African hunter-gatherers). We also detected a very rare additional variant, L79F, in only one person from Sierra Leone in West Africa. Additionally, we were able to time the E154K event by identifying the variant in archaic human genomes (Neanderthal and Denisovan) and the human frameshift TT allele, suggesting a date of occurrence at some point between 6 million (post *Homo-Pan* divergence) and approximately 400,000 years ago (prior to human and Neanderthal/Denisovan divergence). The E154K mutation seems to be the initial activity-attenuating event during human evolution. This mutation likely occurred within Africa and was followed by rapid fixation in an early *Homo* ancestor before the frameshift and P70S mutations. What drove this rapid evolution of an antiviral signaling molecule with less activity is unknown.

Chimpanzees are considered a robust comparative model for HCV pathogenesis. Based on this, our work suggests that although HCV is still able to establish a chronic infection in chimpanzees, their higher *IFNL4* activity compared with modern humans may favor the host and reduce the likelihood of chronicity and disease. Of note, alongside enhanced antiviral gene expression in HCV-infected chimpanzee livers, we observed enhanced levels of gene transcripts involved in T cell immune responses, which are critical for HCV clearance [19]. We thus propose that humans evolved enhanced susceptibility to HCV relatively early during evolution. However, subsequent evolution has led to both the seemingly maladaptive (E154K) and protective variants (*IFNL4* loss-of-function, P70S) coexisting in the same population resulting in a rich assemblage of variation in infection outcome. Therefore, comparative genomics have revealed that human-specific genetic changes occurring following human–chimpanzee divergence have both enhanced and reduced our immune defences to HCV.

Significant fundamental questions remain for even this most studied association: through what mechanism(s) do *IFNL4* and its variants affect HCV chronicity and pathogenesis? As chimpanzees are no longer subjected to experimentation, and as the model would remain near impossible to genetically manipulate, this question and more can only be addressed through the development of novel experimental approaches, which captures unique aspects of HCV biology, such as chronicity and immunopathology. This could perhaps be achieved using other *Hepacivirus*–host pairs, such as cows. Recently, it has been shown that *IFNL4* drives HCV evolution within patients [20]. Thus, a broader understanding of the complex relationship between both host and pathogen genetic and phenotypic diversity should be pursued for HCV and other pathogens.

Investigating the molecular determinants of host–pathogen interactions remains critical to understanding and manipulating the outcomes of microbial infection and pathogenesis. Recent advances in comparative and functional genomics from diverse host–pathogen pairs have begun to unravel the molecular mechanisms underlying outcome of infection. Given the significant global health burden resulting from its capacity to establish a chronic infection, studies on HCV are both needed and well-suited to continue providing critical insights into the workings of the human immune system and our evolution.

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