Stability Of NS3 Q80K Polymorphism Over Time Within Hepatitis C Genotype 1a Infected Individuals

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Background

Hepatitis C (HCV) genotype 1a (GT1a) infections harbouring a baseline Q80K polymorphism in the NS3 gene display reduced virologic response to IFN-based HCV treatments containing simeprevir[1]. **In the context of individual infections, the stability of this clinically important polymorphism over time is unknown.**

Methods

Using stored first and last plasma samples collected over a 10-year period (mean 4.5 years between samples) from 200 HCV antibody positive treatment-naïve injection drug users and a public domain sequence based assay[2], we sought to investigate gain or loss of the Q80K polymorphism over time. RNA was extracted using a NucliSens easyMag. The HCV NS3 gene was amplified by nested RT-PCR and sequenced by Sanger methods. Chromatograms were interpreted automatically using in-house software (RECall) and a 1200- or 564-bp fragment was selected for analysis. Each sample was also HLA typed to confirm database annotations on source individuals. HCV sequences were multiply aligned using MAFFT v7.154b[3]. A phylogenetic tree was inferred using an approximate maximum likelihood method (FastTree2[4]) and rooted under a molecular clock model using a least-squares dating algorithm (LSD[5]).

Results

2 sequences were obtained for 121/200 included patients. We were unable to amplify sequences from multiple time points for 79 infections as a result of either clearance of the virus or PCR failure due to the age of the samples. Phylogenetic trees revealed HCV GT1a sequences grouped into 2 distinct clades (Figure 1)**. In no case did patients whose samples formed a monophyletic (unique) group (102) alter their Q80K status.** We sequenced 4 additional samples for patients initially exhibiting either a change in genotype (n=9) or a change in GT1a lineage (n=9 patients). HLA typing revealed only one sample (subsequently excluded) to be from a different individual, showing that contamination or laboratory errors do not account for our results**.** We observed a remarkably complex pattern of changes in infections over time (Table 1). Notably, of 20 total changes in infection status between time points (2 patients changed twice) we identified 11 changes in genotypes/ subtypes (7 GT3a to GT1a, 3 GT1a to GT3a, and 1 GT1b to GT1a). Furthermore, 9 GT1a infections displayed a change to a different GT1a lineage between time points (1 patient changed twice within GT1a). Importantly 6/9 changes within GT1a displayed either a switch from a Q to a K (3) or a K to a Q (3) at Q80K. The remaining 3 GT1a infection switches did not involve a change in Q80K. **These results suggest either (1) clearance followed by reinfection with a new variant or (2) a mixed genotype/subtype infection. In sum, consistent with previous work each observed case of a change in Q80K was a result of patients switching HCV lineages rather than a mutation in their original HCV lineage.**

Conclusion

Consistent with previous work[6], in the absence of therapy, the Q80K polymorphism is highly stable within HCV lineages and does not evolve in response to immune or other host effects. Future work will employ deep sequencing to evaluate the importance of mixed infections relative to clearance and reinfection by a different HCV lineage. **The observed changes in infection status amongst these patients supports genotypic and resistance testing of patients prior to starting any HCV therapy, particularly amongst those at high risk of exposure to new variants such as injection drug users.**

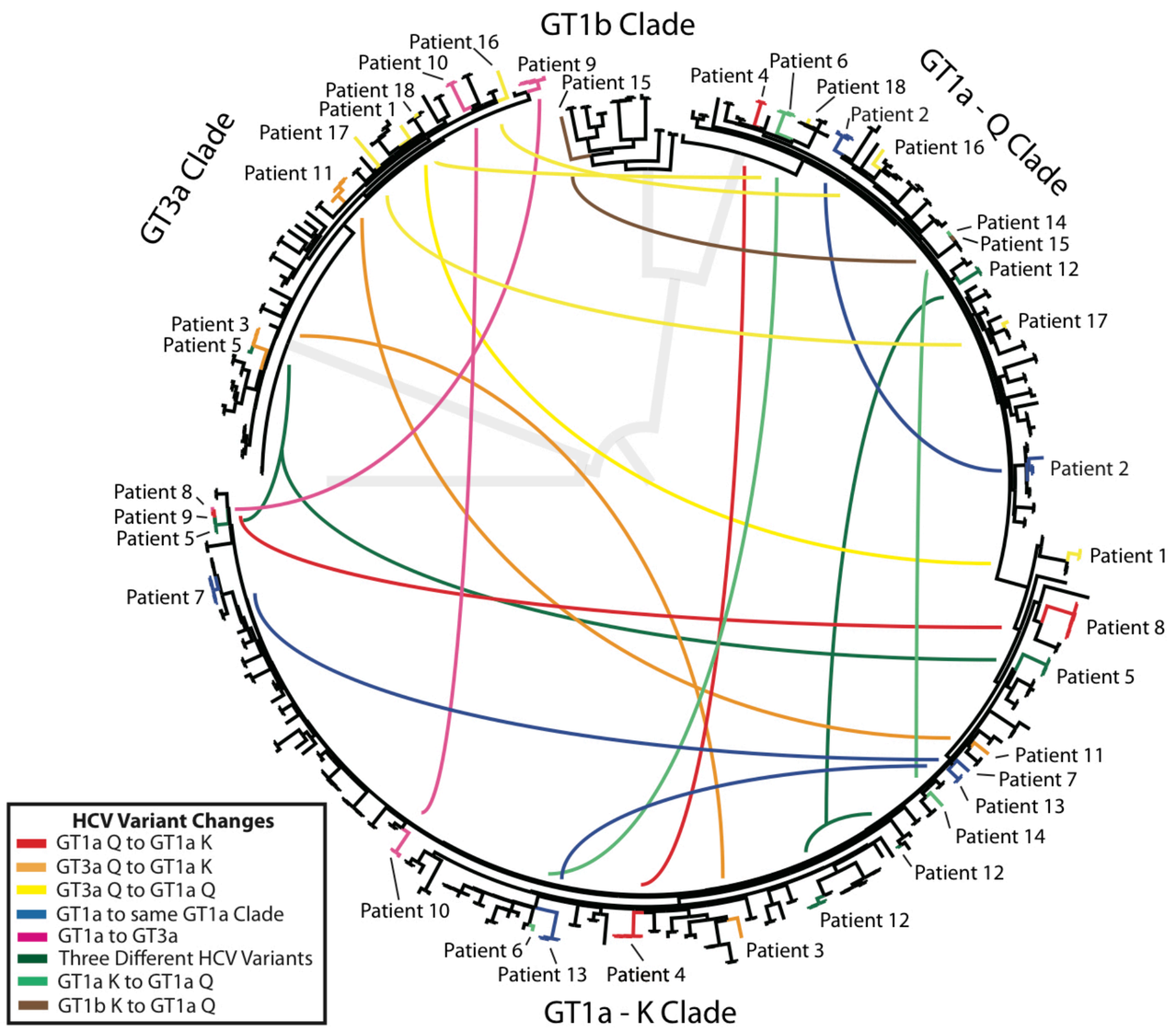

Figure 1 | Phylogenetic tree of samples from all 121 HCV patients included in this study. Patients from Table 1 illustrating changes in HCV lineages within patients between sampling points are demarcated on the tree.

Patient	Date of 1st Infection	Genotype of 1st Infection And Q80K Variant	Date of 2nd Infection	Genotype of 2nd Infection And Q80K Variant	Date of 3rd Infection	Genotype of 3rd Infection And Q80K Variant
1	1998	GT3a Q	1999-2002	GT1a Q		
2	1998	GT1a Q	2000-2001	GT1a Q		
3	1999-2001	GT3a Q	2002	GT1a K		
4	1998-1999	GT1a Q	2000-2003	GT1a K		
5	1998-1999	GT1a Q	2000	GT3a Q	2002-2003	GT1a K
6	1998	GT1a K	2001-2003	GT1a Q		
7	1998	GT1a K	2000-2003	GT1a K		
8	1998	GT1a Q	2002-2004	GT1a K		
9	1998	GT1a Q	1999-2002	GT3a Q		
10	1998	GT1a K	2002-2004	GT3a Q		
11	1999-2002	GT3a Q	2003	GT1a K		
12	1999	GT1a Q	1999	GT1a K	2002-2003	GT1a Q
13	2000-2001	GT1a K	2003-2004	GT1a K		
14	2001	GT1a K	2002-2004	GT1a Q		
15	1997	GT1b Q	2003	GT1a Q		
16	1997	GT3a Q	2004	GT1a Q		
17	2000	GT3a Q	2002	GT1a Q		
18	1997	GT3a Q	2002	GT1a Q		

Table 1 | Patients displaying more than one HCV lineage during the sampling period. Cells highlighted in red illustrate cases where the 2nd or 3rd lineage represents a clinically relevant change in Q80K as a result of either the replacement of one HCV lineage with another between sampling time points or a mixed infection.

[1]Kuntzen, T., et al. 2008. Naturally occurring dominant resistance mutations to hepatitis C virus protease and polymerase inhibitors in treatment-naïve patients. *Hepatology* 48:1769–1778.
[2]Chui, C. K. S., et al. 2015. Development and validation of two screening assays for the HCV NS3 Q80K polymorphism associated with reduced response to combination treatment regimens containing simeprevir. *Journal of Clinical Microbiology* pii: JCM.00650-15. [Epub ahead of print] doi: 10.1128/JCM.00650-15.
[3]Katoh, K., et al. 2009. Multiple alignment of DNA sequences with MAFFT. *Methods in Molecular Biology* 537:39-64.
[4]Price, M.N., et al. 2010. FastTree 2 approximately maximum-likelihood trees for large alignments. *PloS One* 5.3: e9490.
[5]To, T.H., et al. *Submitted*. Fast dating using least-squares criteria and algorithms.
[6]McCloskey, R.M. et al. 2014. Global origin and transmission of hepatitis C virus non-structural protein 3 Q80K polymorphism. *Journal of Infectious diseases* 211:1288-95.

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