A Novel Mechanism of Resistance to Multiple bNAbs Revealed by Natural Variation in Panel of 199 HCVpp

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Background: Broadly-neutralizing monoclonal antibodies (bNAbs) are informative for vaccine development for highly variable viruses including hepatitis C virus (HCV). However, HCV resistance to bNAbs is poorly understood, in part due to a lack of neutralization testing using diverse panels of HCV variants. HC33.4 and AR4A are two of the most potent bNAbs characterized to date.

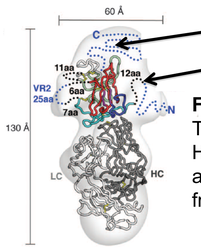


Figure 1 | Binding sites for AR4A and HC33.4. The critical residues for binding of AR4A and HC33.4 based on alanine-scanning are distinct and on opposite termini of E2. Crystal structure from *Kong et al. Science 2013*

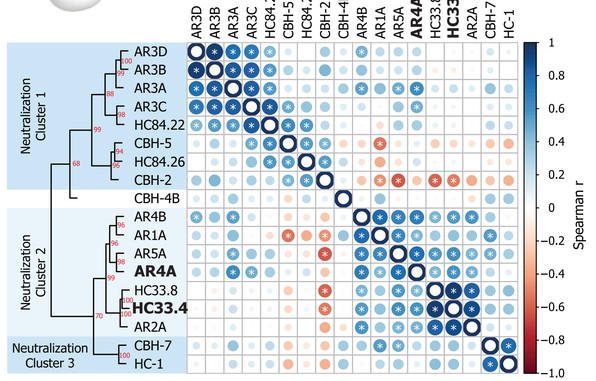


Figure 2 | AR4A and HC33.4 are part of the same neutralization cluster. Comparison of neutralization in a panel of 19 genotype 1 HCVpp indicates that some determinants of sensitivity to AR4A and HC33.4 are shared. *Bailey et al. JCI 2015*

Methods: We measured neutralization of a library of 199 genotype 1 HCV E1E2-pseudotyped particles (HCVpp). Polymorphisms at each amino acid position in E1E2 were assessed for impact on neutralization resistance using Wilcoxon rank-sum test. Findings were validated in site directed mutants in multiple E1E2 backgrounds. bNAb affinity for mutations in multiple E1E2 backgrounds was determined by ELISA.

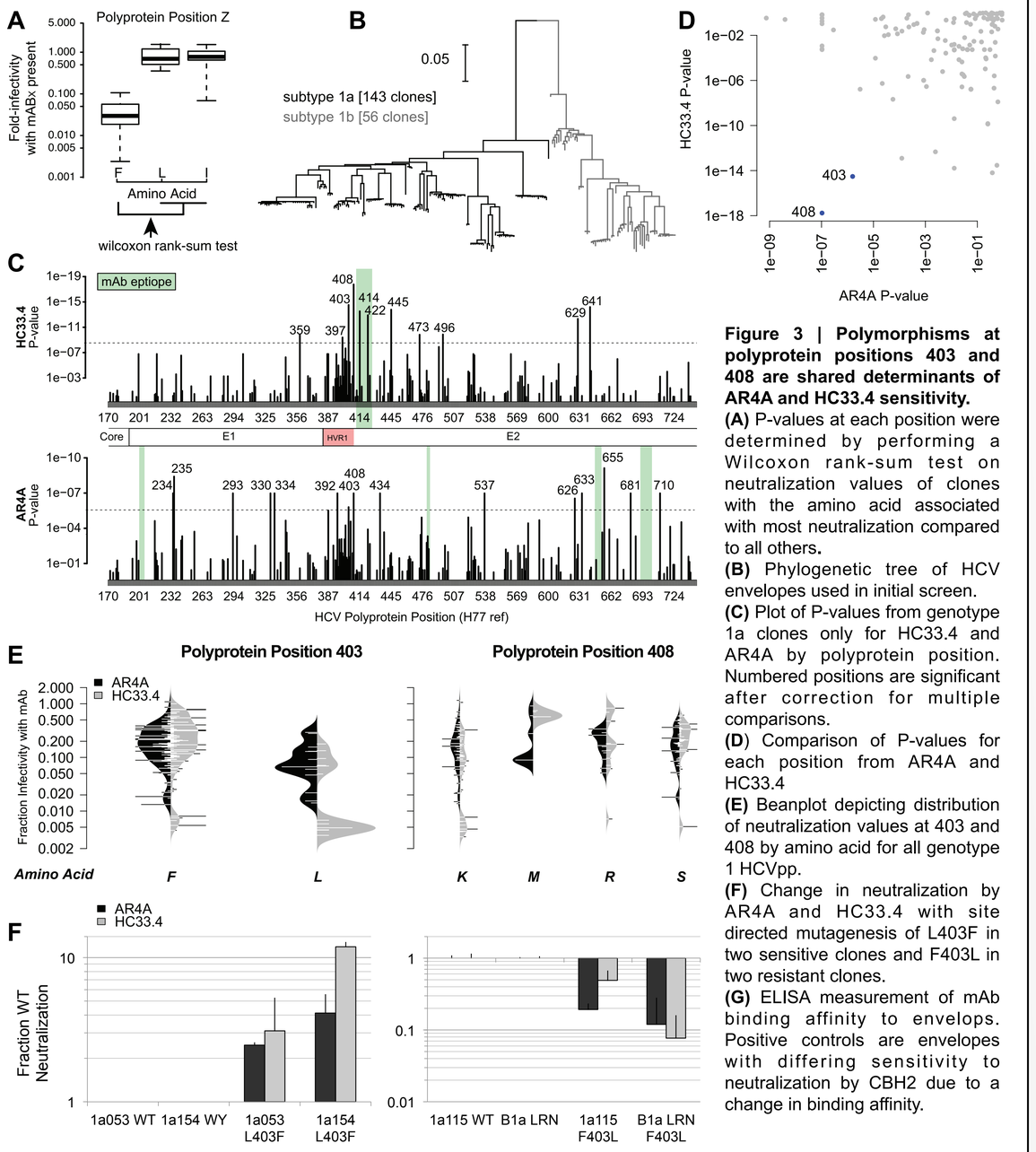
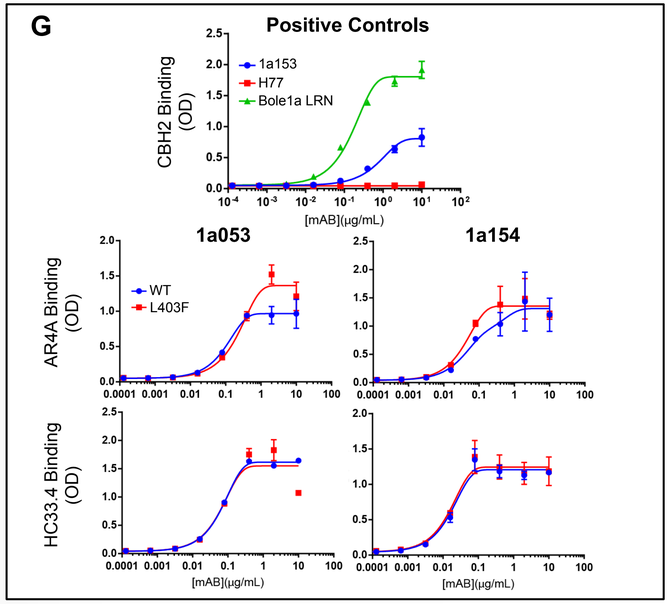


Figure 3 | Polymorphisms at polyprotein positions 403 and 408 are shared determinants of AR4A and HC33.4 sensitivity.
(A) P-values at each position were determined by performing a Wilcoxon rank-sum test on neutralization values of clones with the amino acid associated with most neutralization compared to all others.
(B) Phylogenetic tree of HCV envelopes used in initial screen.
(C) Plot of P-values from genotype 1a clones only for HC33.4 and AR4A by polyprotein position. Numbered positions are significant after correction for multiple comparisons.
(D) Comparison of P-values for each position from AR4A and HC33.4
(E) Beanplot depicting distribution of neutralization values at 403 and 408 by amino acid for all genotype 1 HCVpp.
(F) Change in neutralization by AR4A and HC33.4 with site directed mutagenesis of L403F in two sensitive clones and F403L in two resistant clones.
(G) ELISA measurement of mAb binding affinity to envelops. Positive controls are envelopes with differing sensitivity to neutralization by CBH2 due to a change in binding affinity.



Conclusions: The relationship between natural variation in HCV envelope sequence and neutralizing antibody sensitivity can be queried using a large panel of HCVpp. HCV envelope mutation L403F confers resistance to AR4A and HC33.4 without changing their binding affinity.

Acknowledgements: Steven Foung for providing mAb HC33.4, Mansun Law for providing mAb AR4A, David L. Thomas for helpful discussions, William Osburn for construction of the pseudoparticle library, and all members of the viral hepatitis group at Johns Hopkins for ongoing support. NIH grants 1K08AI102761, U19 AI088791, R37013806, and the JHU Center for AIDS Research 1P30AI094189.
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