Swann, R. E., Cowton, V. M., Robinson, M. W., Cole, S. J., Barclay, S. T., Mills, P. R., Thomson, E. c., McLauchlan, J., and Patel, A. (2016) Broad anti-hepatitis C virus (HCV) antibody responses are associated with improved clinical disease parameters in chronic HCV infection. Journal of Virology, 90(9), pp. 4530-4543.

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Broad anti-HCV antibody responses are associated with improved clinical disease parameters in chronic HCV infection

Running title: Antibody responses in chronic HCV infection

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Abstract count	244
Importance count	120
Word count	4406

Abstract

During hepatitis C virus (HCV) infection broadly neutralizing antibody (bNAb) responses targeting E1E2 envelope glycoproteins are generated in many individuals. It is unclear if these antibodies play a protective or a pathogenic role during chronic infection. In this study, we investigated whether bNAb responses in individuals with chronic infection were associated with differences in clinical

presentation. Patient-derived purified serum IgG was used to assess the breadth of HCV E1E2 binding and neutralization activity of HCV pseudoparticles. Two panels were compared, bearing viral envelope proteins representing either an inter-genotype or an intra-genotype (gt) 1 group. We found that HCV viral load was negatively associated with strong cross-genotypic E1E2 binding (*P*=0.03). Overall we observed only modest correlation between total E1E2 binding and neutralizing ability. The breadth of inter-genotype neutralization did not correlate with any clinical parameters, however, analysis of individuals with gt 1 HCV infection (n=20), using an intra-genotype pseudoparticle panel, found a strong association between neutralization breadth and reduced liver fibrosis (*P*=0.006). Broad bNAb response in our chronic cohort was associated with a single nucleotide polymorphism (SNP) in the HLA-DQB1 gene (*P*=0.038) as previously reported in an acute cohort. Furthermore bNAbs in these individuals targeted more than one region of E2 neutralizing epitopes as assessed through cross-competition of patient bNAbs with well-characterized E2 antibodies. We conclude that bNAb responses in chronic gt1 infection are associated with lower rates of fibrosis and host genetics may play a role in the ability to raise such responses.

Importance

Globally there are 130-150 million people with chronic HCV infection. Typically the disease is progressive and is a major cause of severe liver cirrhosis and hepatocellular carcinoma. While it is known that neutralizing antibodies have a role in spontaneous clearance during acute infection, little is known about their role in chronic infection. In the present work we investigate the antibody response in a cohort of chronically infected individuals and find that a broad neutralizing antibody response is protective, with reduced levels of liver fibrosis and cirrhosis. We also find an association with SNPs in class II HLA genes and the presence of a broad neutralizing response indicating that antigen presentation may be important for production of HCV neutralizing antibodies.

Introduction

Hepatitis C virus (HCV) is a significant cause of liver morbidity and mortality worldwide (1). The majority (75%) of those infected proceed to chronic infection (2). Symptomatic acute HCV infection is rare; therefore HCV has the potential to spread undetected within those at risk. In countries with high prevalence, poor healthcare infrastructure and lack of funding make eradication of HCV unlikely through curative therapies alone (1, 3). Thus, effective preventative strategies are needed to achieve global eradication of the virus (4).

Antibodies targeting the HCV envelope glycoproteins E1 and E2 can contribute significantly to viral clearance in HCV infection (5, 6). These proteins are responsible for virus attachment and entry into host cells through interaction with the receptors SR-B1, CD81, Claudin and Occludin (7-10). Previous observational studies have shown rapid onset of anti-HCV antibodies to be associated with higher likelihood of clearance (11). More recently, it has been proposed that developing an antibody profile capable of neutralizing diverse HCV strains (broadly neutralizing) predicts acute clearance in a cohort infected with gt1a HCV (12). Further studies have suggested that broadly neutralizing antibodies (bNAbs) may be able to control levels of virus and contribute to clearance even after infection has become established (13). In one case of a chronically infected patient who spontaneously cleared HCV, a bNAb response was generated, initially with subsequent restoration of T cell activity and resolution of infection (5).

Only a small number of individuals with bNAbs have been studied in detail with little information on the regions of the E1E2 glycoproteins that are preferentially targeted by these antibodies. This has largely involved epitope-mapping of patient-derived monoclonal antibodies (mAbs) (14-16). Human neutralizing and non-neutralizing mAbs have been used to identify distinct immunogenic domains of E1E2 (15, 17-21). However, *in vivo*, a polyclonal response is generated which may target multiple regions of the envelope proteins. A recent study demonstrated that some HCV-infected individuals largely target one immunogenic domain whereas others produce antibody responses to multiple

domains (21). The importance of interplay of antibodies binding different epitopes has not been fully explored, although there are conflicting reports of some antibodies conferring additive or interfering effects on virus neutralization (22, 23).

While diverse HCV strains are usually categorized by genotype, this does not correlate well with sensitivity to neutralization by mAbs (24, 25). The genetic diversification of HCV, both within and between hosts, introduces the potential to escape from monoclonal bNAbs with several studies reporting naturally occurring, single amino acid (aa) mutations conferring escape (18, 26). The recent E2 crystal structures (27, 28) provide evidence that antibody resistance is complex with mutations distant from the targeted epitope affecting antibody binding, presumably through structural changes (26). Therefore, further studies of the antibody response to a varied range of envelope proteins *in vivo* are required. As animal models of HCV infection and adaptive immunity are suboptimal, we can still gain useful information from studying the humoral responses of chronically infected individuals using *in vitro* models.

Although there is evidence that bNAbs have clinical relevance in acute infection, their role in chronic infection is not clear. Indeed, the immune response to HCV can have pathological consequences as seen in cryoglobulinaemic vasculitis (29). If large scale vaccination was to be considered, it is important to determine that stimulating such a response will not be harmful in the event of an authentic infection. Studying clinical associations in patients with bNAbs can reveal potential adverse outcomes and yield insights into factors associated with NAb production.

In this study, we investigate bNAb responses in chronically infected HCV (CHCV) patients, determine any association with clinical and host factors, and characterize the epitopes targeted by these antibodies. We identify an association between bNAb response and less severe liver disease and show that a bNAb response targets multiple neutralizing E2 epitopes within different immunodomains. We also report an association between bNab response and age, however no

association with estimated duration of infection or age at infection was found. Finally, we confirm that SNP rs2395522 in the HLA-DQ gene is strongly associated with production of a bNAb response.

Methods

Patient characteristics. Subjects with either gt1 or gt3 CHCV were prospectively recruited from 3 local liver clinics. Individuals with co-existing liver pathologies, body mass index (BMI) ≥ 31 or hepatocellular carcinoma were excluded. Healthy controls with no liver pathologies or significant co-morbidities were also recruited and their samples used as negative controls in subsequent experiments. All subjects completed a symptom questionnaire, clinical details were recorded and baseline biochemistry, virology and Interleukin-28B (rs12979860) profiles were determined. Liver stiffness was measured by transient elastography using a Fibroscan (Echosens). Serum and whole blood samples were obtained and stored at -70°C. Ethical approval was granted for this study by the West of Scotland Research Ethics Committee and all patients gave informed consent.

Cell lines. Human hepatoma Huh-7 cells, Huh7-J20 cells (30) and human epithelial kidney 293T cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 5% nonessential amino acids, Penicillin, Streptomycin and 200 mM L-glutamine. Huh7-J20 cells were supplemented with 2µg/ml puromycin.

Antibodies. The anti-E2 human monoclonal antibodies (HmAb) CBH-4B, CBH-7, HC-1, HC-11 and HC-84 (31-33) were a generous gift from Steven Foung. The anti-E2 mouse mAb AP33 has been described previously (34).

E1E2 binding assay. The enzyme-linked immunosorbent assay (ELISA) to detect antibody binding to HCV glycoproteins was performed as described (35). Briefly, HCV glycoproteins in lysates of HEK-293T cells transfected with E1E2 expression plasmids were captured onto *Galanthus nivalis* agglutinin (GNA) coated Immulon II plates (Thermolabsystems). Protein G-purified patient IgG was

isolated from serum using the Nab protein G spin kit (Thermoscientific) and the concentration was quantified by absorbance at 280nm. Purified patient IgG was added at 200 µg/ml in PBS containing 0.05% Tween-20 and 2% Skimmed Milk Powder (PBSTM) and bound antibodies were detected using HRP-conjugated anti-human IgG antibody (Sigma A0170) and TMB (3,3, 5, 5'-tetramethylbenzidine, Sigma) substrate. Two representative healthy control samples were included for quality control purposes. A dilution curve of mAb AP33 that binds to a highly conserved linear epitope was also included to enable comparison between plates. Absorbance values were measured at 450 nm and normalized according to the AP33 curve to give a value representing the concentration of AP33 (in µg/ml) which gave the same absorbance as the test sample.

Generation of HCV pseudoparticles (HCVpp), infectious virus (HCVcc) and neutralization assays. HEK-293T cells were co-transfected with plasmids expressing murine moloney leukaemia virus (MLV) Gag-pol, the MLV transfer vector carrying firefly luciferase reporter and plasmid expressing the relevant HCV E1E2. After 72 h, the medium was harvested, filtered through a 0.45 µM membrane and used as a source of HCVpp as described (36). Infectious virus was produced in Huh7 cells by electroporation of RNA encoding full-length JFH-1 or chimeric JFH-1. The intra-genotypic chimera 2B.1.1/JFH1 and the inter-genotypic chimera HQL (1a H77/JFH1) have been described previously (20, 30). For neutralization assays, HCVpp and purified patient IgG at 100 µg/ml (equivalent to serum dilution of 1:100-1:250 based on the known IgG levels in chronic HCV patient sera (37)) were mixed and incubated for 1 h at 37°C, then used to infect Huh7 cells for 4 h. The inoculum was removed and fresh media was added. Cells were lysed 3 days post-infection and infectivity assessed using the GloLysis Luciferase assay (Promega). Similarly, neutralization of HCVcc infection was tested in Huh7-J20 cells and infectivity assessed 3 days post-infection using the Phospha-Light assay system (Thermofisher) to measure secretory alkaline phosphatase (SEAP) levels (30).

Amplification of viral E1E2 sequences derived from patients. A cDNA library was created from HCV RNA isolated from patient serum using the QiaAMP Viral RNA mini kit (Qiagen). The

complete E1E2-encoding region was amplified using degenerate nested primers (Gt1 outer sense 5′ GTGAAYTAYGCRACAGGGAA, Gt1 outer antisense 5′GCAAAGCAGAARAACACGAG, Gt1 inner sense 5′CACCATGGGTTGCTCYTTYTCTATCTTC, Gt1 inner antisense 5′ AAAGTTTCTAGATTACYGCCTCYGCYTGGGAKA) and cloned into the phCMV expression vector using the Gateway recombination cloning system (Invitrogen).

DNA isolation and SNP analysis. DNA was extracted from whole blood using the QiAMP DNA Mini Kit (Qiagen). Custom primers for SNP rs2395522 and reporter dye mix (ThermoFisher) were included in a qPCR reaction using Taq mastermix on a 7500HT Fast Real-Time PCR system (Applied Biosystems). Samples were assigned as A/T heterozygotes or AA or TT homozygotes according to the fluorescence signal.

Epitope targeting. A soluble form of gt1a E2 (H77) protein (sE2) was purified following expression in High Five insect cells. Immulon II plates were coated with sE2 at 1 μg/ml and incubated with purified patient IgG at 200 μg/ml in PBSTM. Subsequently, biotinylated antibodies to known epitopes were added at a concentration close to their half maximal effective concentration (EC_{50}) (14, 17, 18, 38). Streptavidin-HRP was added and binding measured as above. The reduction in relative binding of each biotinylated antibody (calculated as percentage reduction in absorbance) on addition of patient IgG compared to PBSTM control was determined.

Analysis. Results of assays and any statistical analysis of association with clinical features were conducted using GraphPad Prism 6 Software (GraphPad Software, California) and SPSS v. 19.09 (IBM, New York). Statistical comparisons were made using non-parametric tests (Chi Squared for categorical data, Wilcoxon Rank Sum for ordinal or numeric data) unless otherwise stated.

Results

Cohort. Fifty-one HCV-infected patients (27 gt1, 24 gt3) from the CHCV cohort and 8 healthy controls were recruited. Demographics of the cohort are shown (Table 1). Apart from a higher prevalence of Asian ethnicity in the gt3 group (P=0.04), there were no significant differences in demographics between subjects infected with either gt1 or gt3 HCV.

E1E2 HCVpp panels. To determine reactivity and neutralization of patient-derived IgG across a diverse range of envelope sequences, two panels of HCV pseudoparticles bearing test envelope proteins were used. The first, termed Panel XG, enabled analysis of antibody reactivity with E1E2 proteins of different viral genotypes. This panel comprised E1E2 from 6 subgenotypes (39): gt1a H77c (Accession number: AF011751) , gt1b UKN1B5.23 (AY734976); gt2a JFH-1 (AB047639); gt2b, UKN 2B1.1 (AY734982); gt3a UKN3A13.6 (AY894683); gt4 UKN4.11.1 (AY734986). The second, termed Panel Gt1 was created to allow investigation of antibody reactivity within a single genotype as might arise during a natural infection. 103 patient-derived E1E2 sequences from eighteen gt1 HCV-infected patients collected from 3 cohorts across the UK (Trent HCV study group (40), St. Mary's Acute Hepatitis C Cohort (41), Glasgow chronic HCV cohort (42)) were tested in the HCVpp system for infectivity (data not shown). As found previously in other studies not all sequences were infectious in this system (25, 43). 64% of the E1E2 sequences tested were functional giving a robust luciferase signal >10-fold above background. All the amino acid sequences were aligned in ClustalW, the best protein model with the lowest BIC score (16423.799) was used to generate a phylogenetic tree using the Maximum Likelihood method (Fig.1). The panel selection criteria was based on the overall genetic difference (p distance) and representation of amino acid variability found in all gt1 E1E2 sequences registered with the Los Alamos HCV sequence database. Analysis of >3800 gt1 E1E2 sequences in the database identified 400aa residues that were conserved in at least 90% of sequences; of these the majority (306aa) are conserved in 99% of sequences. 154aa were found to be variable in more than 10% of gt1 E1E2 sequences. Eleven infectious sequences including the reference gt1a sequence H77 and ten-patient derived E1E2 sequences from nine HCV-infected

219 individuals were selected for inclusion in the gt1 panel Accession numbers: AF011751, AY734976,
220 AY734971.1, AY734968.1, EU155192.1 and KU645403 to KU645407. These sequences represent
221 the variability at 145/154 variable residues. In addition, 31aa residues represent minor variants found
222 in fewer than 10% of sequences in the database.
223
224 **Cross-genotypic antibody reactivity is associated with lower viral load.** Patient IgG reactivity to
225 whole E1E2 from Panel XG was tested by ELISA. Relative binding strength based on absorbance
226 readings normalized to an AP33 standard curve were ranked from 1 (highest) to 51 (lowest) for each
227 gt; all gt ranks were combined and a final rank position assigned (Table 2). This gave an overall
228 indication of the relative binding breadth for each patient. The cohort was divided in half by binding
229 rank and the top ranked half compared to the lower half to determine any clinical associations with
230 breadth of antibody binding. Those with broader ELISA reactivity profiles had a significantly lower
231 viral load (P=0.03, Fig. 2A). Individuals with a broader breadth of binding were more often infected
232 with gt1 HCV and conversely individuals with a narrower breadth of binding were more often
233 infected with gt3 HCV (P=0.04; P=0.04; Supporting Table S3). These associations appear to be
234 independent as there was no association between gt of infection and viral load (Supporting Table S3,
235 P=0.59).
236
237 **Neutralization is not associated with viral load.** Purified IgGs derived from the CHCV cohort were
238 tested for their ability to neutralize HCVpp bearing envelope proteins from Panel XG and HCVcc
239 bearing envelope proteins from Gt1a (1A-HQL), Gt2a-JFH-1 and Gt2b.1.1 (Table 3). In accordance
240 with Urbanowicz and coworkers we found that HCVpp were more readily neutralized than HCVcc
241 (25). Therefore neutralization was categorized as a reduction in infectivity of 50% in the HCVpp
242 system and 40% in the HCVcc system. In both systems, gt2B.1.1 was particularly resistant to
243 neutralization. The breadth of neutralization of HCVpp Panel XG, defined as broad (neutralization of
244 >3/6 genotypes) or narrow (<4/6 genotypes), was analyzed for association with clinical factors.
245 Twenty (40%) of the 51 individuals tested had broad cross-genotypic neutralizing IgGs. There were
246 no significant associations of breadth of cross-genotypic neutralization with viral load (Fig. 2B).

Similarly, IgG from 20 CHCV individuals with gt1 infection were tested for neutralizing activity against Panel Gt1 (Table 4) and the number of pseudoparticles neutralized to the 50% level by each individual calculated. Neutralization of >7/11 strains was defined as 'broad' while 'narrow' neutralization was defined as <8/11 strains. As with Panel XG, there was no association between breadth of neutralization of Panel Gt1 and viral load (Fig. 2B).

Correlation between ELISA binding profiles and neutralization. To determine the level of agreement between the ELISA binding profiles and neutralization activity used to determine antibody 'breadth' we calculated non-parametric correlation co-efficients between the assays. For the full cohort there was a modest correlation of cross-genotypic ELISA binding rank with number of genotypes neutralized in Panel XG and Panel Gt1 (Spearman's Rho correlation co-efficient -0.31, P=0.03 and -0.49, P=0.03, respectively) (Figs. 3A, 3B). Neutralization was determined for the full cohort against HCVpp and HCVcc (Table 3). Overall there was a significant correlation between the neutralization rank in both systems (Spearman's Rho correlation co-efficient = 0.44, P=0.001, Fig. 3C). Indeed the strongest neutralizing IgGs efficiently neutralized HCVpp and HCVcc. Interestingly, IgG isolated from some individuals neutralized HCVcc more effectively than HCVpp and vice versa, (compare C1021, C1061 with C1046, C1035, Table 3). There was also a significant correlation between the proportion of genotypes neutralized in the two HCVpp panels (Spearman's Rho correlation co-efficient =0.58 P=0.007, Fig. 3D) for the gt1-infected subgroup. Neutralization is only one possible mechanism through which HCV-binding antibodies exert potential antiviral selection. The modest correlation between ELISA binding and neutralization highlights that while an individual with strong HCV-binding antibodies often has strong neutralizing antibodies this is not always the case. This disparity is evident in the lack of association between the breadth of neutralizing antibodies and viral load, despite an association between HCV-binding and viral load.

Association of increased age and liver fibrosis with narrow intra-genotype neutralization. Neutralization breadth for Panel XG was not associated with any clinical features (Fig.4A, 4C, 5A, 5C, 5E). Interestingly, analysis of the subgroup of gt1-infected individuals tested against Panel Gt1

found an association between breadth of neutralization and age since the broadly neutralizing group was significantly younger (P=0.009; Fig. 5B). However, there was no association of neutralization breadth with the estimated duration of infection or age of acquisition for the gt1-infected subgroup (Fig.5D, 5F). Most notably, there was a striking association between breadth of neutralization activity and liver fibrosis for the gt1-infected subgroup. The broadly neutralizing group had significantly lower levels of liver fibrosis as determined by transient elastography (P=0.006; Fig. 4B) and fewer cirrhotic individuals (P=0.02; Fig. 4D). Importantly, the association between neutralization breadth and Fibroscan readings remained significant when corrected for age (P=0.025, Generalized Linear Model, SPSS V. 19.0).

Role of HLA-DQ polymorphisms in predicting antibody neutralization breadth. A recent study showed a link between breadth of neutralizing antibodies against acute gt1 HCV infection and a SNP (designated rs2395522) in an MHC Class II gene, HLA DQB1 involved in antigen presentation (12). Therefore we analyzed our cohort for any association between this and the number of HCVpp neutralized in both panels. There was no significant association between presence of these alleles and the number of HCVpp neutralized in Panel XG (P=0.41; Fig. 6A). However, the presence of the rs2395522 AA or AT allele was significantly associated with a greater number of HCVpp neutralized in Panel Gt1 (P=0.038; Fig.6C). There was no association between SNP rs2395522 and liver fibrosis for either Panel (P=0.2 and P=0.62; Figs. 6B, 6D). In addition, we tested another genetically linked HLA-DQA2 SNP rs9275224. Here the GG or AG allele was also significantly associated with increased neutralization breadth in panel Gt1 (P=0.038, data not shown as the graphs are identical due to the genetic linkage between the SNPs). SNP rs2395522 is present in the intergenic region of HLA-DQB1; the functional consequences are unknown. However, the linked SNP rs9275224 is associated with autoimmune diseases including systemic sclerosis and rheumatoid arthritis (44, 45).

E2 epitopes targeted by patient IgG. In an effort to understand the epitopes targeted by the gt1 patient IgGs used for the intra-genotypic analysis, we determined if they competed for binding to E2 with some well-characterized antibodies. The conformational antibodies selected have been

303 characterized as binding to specific hypothetical immunodomains of E2, designated A, B or C. Their
304 precise locations on E2 have not been identified to date, however, antibodies that bind
305 immunodomain A including HmAb CBH-4B are non-neutralizing. Antibodies that bind to
306 immunodomain B (HmAb HC-1, HC-11 and HC-84) and C (HmAb CBH-7) are able to neutralize
307 HCV. While immunodomain C has not been characterized, immunodomain B has been shown to
308 contain the CD81 receptor binding site (15). Lastly, the mouse NAb AP33 that binds a linear epitope
309 (aa412-423) was also selected. The majority of the broadly neutralizing samples were able to
310 efficiently compete with 3 or more of the E2 antibodies tested, in contrast most of the narrow
311 neutralizing samples could only compete with 2 or fewer E2 antibodies (Fig. 7A). Interestingly, while
312 15/20 samples tested competed with HmAb HC-11 at the >50% level, the majority of broadly
313 neutralizing samples inhibited both HmAbs CBH-7 and HC-11 binding, 6/10 that recognize different
314 neutralizing immunodomains of E2 compared to 1/10 of narrow neutralizing samples. There was also
315 a significant association with intra-genotypic 1 neutralization breadth (P=0.004, P=0.002; Fig. 7B).
316 Together, these data suggest that individuals with a broadly neutralizing phenotype compete with
317 antibodies at multiple sites on E2. Moreover, they have antibodies that efficiently bind to more than
318 one neutralizing immunodomain whereas those with a narrow neutralizing profile effectively target
319 limited numbers of neutralizing epitopes.
320
321 **Discussion**
322 Our analysis of a clinical cohort chronically infected with HCV has yielded new insights into the
323 importance of the antibody response in disease progression and factors associated with functional
324 breadth of the antibody response. A broad cross-genotype HCV-binding antibody response was
325 significantly associated with gt1 HCV infection and independently with reduced viral loads.
326 Importantly these clinical associations with broad HCV-binding were not evident when analyzing the
327 breadth of neutralization activity, highlighting distinct biological roles for non-neutralizing and
328 neutralizing anti-HCV antibodies. The association of ELISA binding and lower viral loads could
329 reflect the presence of antibodies binding to conserved non-neutralizing regions which would help

clear virus from serum through opsonisation and subsequent phagocytosis as demonstrated by Eren and coworkers or complement dependent lysis but would not directly inhibit hepatocyte cell entry (38). Indeed, there is evidence that binding of virus by non-neutralizing antibodies may prevent neutralization by antibodies targeting epitopes required for cell entry (46).

While no clinical features were significantly associated with broad or narrow neutralization as characterized using Panel XG, we did observe significant associations using a larger intra-genotype 1 panel. Most importantly, we show that individuals infected with HCV gt1 who are better able to neutralize an HCVpp panel incorporating different gt1 E1E2 sequences are less likely to have cirrhosis or significant liver fibrosis. The panel is composed of strains collected in the UK over the past decade from different geographical locations and incorporating changes at the majority of variable aa positions observed within a large sequence database. Therefore this panel represents the most common viable aa substitutions that may occur within host virus.

The association with lower levels of liver fibrosis with bNAbs is insufficient evidence alone to demonstrate a protective effect. However, this adds to case studies which have suggested that individuals with genetic or iatrogenic suppression of the antibody response show more rapid liver disease progression (47, 48). It is biologically plausible that individuals possessing antibodies capable of preventing spread of new variants of their infecting HCV strain may have a degree of protection from liver injury. Interestingly this 'broadly neutralizing' group was also significantly younger. This may suggest that the higher levels of liver fibrosis observed in the narrow neutralizing group was simply caused by longer duration of infection. However, we found no difference in duration of infection between the broad and narrow neutralizing groups and importantly the association between neutralization breadth and reduced fibrosis remained significant when corrected for age. There is already evidence that those infected in older age have more rapid disease progression (49) and that B-cell repertoire narrows with age (50). Therefore, this may also reflect an ageing effect on NAb responses.

Although the association of neutralization breadth in Panel Gt1 and host factors was clear, these were not as apparent in Panel XG. It is not clear from our data if the associations are indeed gt1-specific or simply caused by limitations due to smaller numbers of genotypes used to determine 'breadth' in the cross-genotypic panel. Although there was a significant correlation between the numbers of HCVpp isolates neutralized in both panels, some individuals who had narrow cross-genotypic neutralization profiles showed broad neutralization activity against Panel Gt1 suggesting that some bNAbs may be gt1-specific.

No association between levels of E1E2 binding and fibrosis was found. Unlike an earlier study (51), we found no relationship between antibody binding to the autologous genotype and clinical outcomes although there was a trend towards cirrhosis in gt1-infected individuals with poor binding to gt1a E1E2 (P=0.10, data not shown). Combined with the neutralization panel data, this suggests that if anti-envelope antibodies do have a protective effect, this is most marked where the antibodies target regions necessary for virus entry.

We have demonstrated that a NAb response is not closely correlated with the extent of patient IgGs binding to the whole E1E2 molecule, suggesting that some individuals preferentially target important neutralization epitopes. Our data also suggests that those gt1-infected individuals who mount a broadly neutralizing response effectively direct antibodies at more than one neutralizing domain on E2. In contrast, those with a narrower neutralizing response appear to target only one region, that recognized by the HmAb HC-11. It is possible however, that the narrow neutralizing samples do contain antibodies that target other neutralizing epitopes albeit at a lower concentration or with lower affinity than found in the broadly neutralizing group. Previous studies have shown that different regions of E2 interact to prevent neutralization, therefore it is likely that an antibody response interfering with multiple regions of E2 maybe more effective than a response targeting one epitope alone (52). Furthermore, Carlsen et al recently showed synergy in neutralization using a combination of two antibodies against differing domains (53). Our identification of epitopes targeted was constrained by the panel of monoclonal antibodies used. However, alternative methods such as

385 peptide and phage display capture have limited ability to detect antibodies directed at discontinuous
386 epitopes (21) therefore our data are a valuable complement to information from these studies.
387
388 While there are many possible explanations for why individuals might preferentially respond to
389 particular epitopes, we have confirmed that SNP rs2395522 in the HLA-DQB1 gene is associated
390 with the development of bNAbs in gt1-infected patients (12). HLA-DQB1 genotype has already been
391 identified as one of the host factors known to influence outcome of HCV infection in Caucasian
392 populations (54). This may be due to restriction in antigen presenting cell presentation of epitopes to
393 CD4 cells or may involve another mechanism. In contrast, we did not observe an association of this
394 SNP with neutralization capability across pseudoparticles of other genotypes. In particular, there was
395 no association of the SNP with the ability of IgG from gt3-infected individuals to neutralize our
396 standard gt3a HCVpp (P=0.45, data not shown). This may be due to the limitations of testing one gt3
397 isolate, alternatively it is possible that other HLA genes could be more important for adaptive
398 responses to other genotype infections. Further studies will be required to distinguish between these
399 possibilities.
400
401 Our study demonstrates that broad anti-HCV neutralizing responses are associated with lower levels
402 of liver fibrosis, raising the possibility for a protective role in chronic infection. Our data also show
403 strong indications that potent neutralizing responses target multiple key regions of E2 rather than a
404 single epitope. This has significant implications for HCV vaccine design suggesting that a successful
405 vaccine must induce NAbs to different regions of E2. If we aim to produce a universally protective
406 vaccine for HCV, a deeper understanding of the role of host genotype and presented epitope sequence
407 in determining breadth of antibody response requires further exploration across a wider range of
408 isolates from differing genotypes before vaccine candidates are tested on a wider scale.
409
410 **Funding Information:** This work was supported by the UK Medical Research Council funded grants
411 G0801822 and MC_UU_12014/2. ET is funded by the Wellcome Trust (102789/Z/13/Z).
412

Author contributions: AP and JM designed and supervised the project. RS and MR recruited the cohort with assistance from SB and PM. RS, VC, SC and MR performed the experiments. ET provided patient samples. RS, VC, MR and AP prepared the manuscript. All authors approved the manuscript.

Acknowledgments: We thank Jonathan Ball for provision of UKN E1E2 expression plasmids and Steven Foung for monoclonal E2 antibodies. We thank Carol Leitch for advice on phylogenetic analysis. The authors report no conflicts of interest.

Table 1: Demographics of Cohorts

DEMOGRAPHICS	CHCV (n=51)	
	Number	Percentage /Range
Age, Median (range)	46	(28-68)
Gender (M/F), No. male (%)	35	(68.6)
Ethnicity, Caucasian (%)	47	(92.2)
Source of infection, No. IVDU (%)	32	(62.7)
Estimated duration of infection (years), Median (range)	25	(2-58)
IL28B CC genotype, (rs12979860) **(%)**	17	(35.4)*
Anti-HBc positive, (%)	11	(24.4)**
BMI (kg/m^2), Mean (range)	26	(19-31.5)
Diabetes, Present (%)	2	(3.92)
Previously exposed to interferon based treatment$^{\$}$, (%)	21	(46.7)**
HCV RNA load pre-treatment IU/ml, Median (range)	6.8x10^5	(2272 - 1.1x10^7)
Cirrhosis, Present (%)	18	(36)
Transient elastography (kPa), Median (range)	9	(4-75)

*3 subjects not tested **no information available for 6 subjects. $^{\$}$All individuals had HCV infection at the time of testing, those previously exposed to interferon were either relapsers or null responders. No individuals were on therapy at the time of sampling.

Table 2: ELISA binding of CHCV cohort to cross-genotypic E1E2 panel.

	Gt 1A *(rank)*	Gt 1B *(rank)*	Gt 2A *(rank)*	Gt 2B *(rank)*	Gt 3A *(rank)*	Gt 4 *(rank)*	Overall rank
C1001	6.75 *(19)*	33.07 *(2)*	1.44 *(11)*	2.32 *(8)*	7.73 *(19)*	5.92 *(19)*	**6**
C1002	0.39 *(50)*	0.40 *(51)*	0.06 *(51)*	0.02 *(51)*	3.63 *(33)*	0.05 *(48)*	**50**
C1003	13.36 *(12)*	18.70 *(9)*	2.77 *(6)*	0.60 *(15)*	61.91 *(1)*	67.63 *(2)*	**1**
C1006	4.92 *(24)*	6.02 *(31)*	0.34 *(31)*	0.12 *(29)*	4.90 *(29)*	1.84 *(37)*	**34**
C1008	1.53 *(39)*	10.75 *(21)*	0.38 *(27)*	0.16 *(21)*	57.21 *(2)*	0.64 *(46)*	**28**
C1009	1.46 *(41)*	7.36 *(27)*	0.39 *(25)*	0.10 *(36)*	27.32 *(4)*	0.57 *(47)*	**33**
C1010	86.76 *(1)*	5.17 *(34)*	0.42 *(22)*	0.14 *(25)*	2.15 *(41)*	4.16 *(24)*	**27**
C1012	31.43 *(7)*	13.80 *(15)*	0.55 *(17)*	0.44 *(17)*	23.09 *(6)*	50.27 *(3)*	**4**
C1013	3.57 *(28)*	6.01 *(32)*	0.20 *(45)*	-0.15 *(49)*	3.24 *(35)*	2.70 *(34)*	**42**
C1015	0.94 *(46)*	9.19 *(25)*	0.43 *(21)*	0.15 *(23)*	34.57 *(3)*	4.02 *(27)*	**26**
C1016	7.94 *(17)*	13.10 *(19)*	0.65 *(15)*	0.23 *(18)*	19.64 *(9)*	17.69 *(7)*	**9**
C1018	8.25 *(16)*	14.50 *(13)*	0.37 *(28)*	0.19 *(19)*	7.11 *(22)*	6.57 *(17)*	**15**
C1020	2.38 *(33)*	3.01 *(43)*	0.33 *(33)*	0.10 *(35)*	22.09 *(8)*	0.72 *(45)*	**36**
C1021	26.58 *(8)*	13.18 *(18)*	1.23 *(12)*	3.05 *(5)*	3.43 *(34)*	27.45 *(4)*	**7**
C1022	39.09 *(4)*	4.54 *(37)*	0.26 *(40)*	0.08 *(39)*	2.41 *(40)*	9.83 *(11)*	**29**
C1023	14.28 *(10)*	13.46 *(16)*	0.15 *(49)*	0.12 *(28)*	16.76 *(10)*	11.21 *(9)*	**19**

C1024	2.97 *(29)*	2.87 *(45)*	0.33 *(32)*	0.11 *(32)*	3.84 *(31)*	1.15 *(41)*	**40**
C1029	6.45 *(21)*	12.76 *(20)*	0.43 *(20)*	0.10 *(37)*	22.74 *(7)*	9.31 *(13)*	**18**
C1030	6.65 *(20)*	4.86 *(35)*	0.34 *(29)*	0.11 *(31)*	0.78 *(49)*	1.60 *(38)*	**38**
C1031	34.53 *(5)*	7.04 *(29)*	0.77 *(14)*	0.13 *(27)*	2.53 *(38)*	10.70 *(10)*	**20**
C1032	8.39 *(15)*	10.08 *(23)*	0.43 *(19)*	0.09 *(38)*	6.05 *(26)*	22.85 *(5)*	**21**
C1033	0.69 *(47)*	3.67 *(40)*	0.21 *(44)*	-0.06 *(47)*	2.43 *(39)*	-0.05 *(49)*	**49**
C1034	6.38 *(22)*	13.44 *(17)*	0.38 *(26)*	0.13 *(26)*	25.72 *(5)*	18.91 *(6)*	**11**
C1035	76.17 *(2)*	8.20 *(26)*	0.30 *(35)*	0.12 *(30)*	2.06 *(42)*	13.33 *(8)*	**24**
C1036	1.72 *(36)*	20.77 *(6)*	1.53 *(9)*	2.26 *(9)*	3.77 *(32)*	5.57 *(20)*	**14**
C1037	2.71 *(31)*	7.14 *(28)*	0.13 *(50)*	0.16 *(22)*	1.50 *(44)*	3.57 *(28)*	**39**
C1038	1.87 *(35)*	9.74 *(24)*	0.26 *(39)*	0.00 *(44)*	1.34 *(47)*	2.85 *(33)*	**41**
C1040	12.29 *(14)*	27.97 *(3)*	4.51 *(3)*	4.33 *(4)*	2.96 *(36)*	9.78 *(12)*	**5**
C1041	0.98 *(45)*	5.48 *(33)*	0.21 *(43)*	-0.06 *(46)*	8.51 *(16)*	0.80 *(44)*	**43**
C1042	41.33 *(3)*	6.02 *(30)*	0.34 *(30)*	0.19 *(20)*	7.42 *(20)*	9.10 *(14)*	**17**
C1043	1.08 *(44)*	20.65 *(7)*	1.88 *(7)*	2.98 *(6)*	7.08 *(23)*	4.52 *(23)*	**12**
C1045	1.56 *(38)*	3.30 *(42)*	0.26 *(37)*	-0.04 *(45)*	0.34 *(50)*	1.50 *(39)*	**47**
C1046	14.0 *(11)*	23.83 *(5)*	1.50 *(10)*	1.69 *(12)*	5.65 *(27)*	6.88 *(16)*	**7**
C1047	32.15 *(6)*	3.53 *(41)*	0.26 *(38)*	0.10 *(34)*	1.46 *(45)*	1.95 *(36)*	**37**
C1049	2.69 *(32)*	1.54 *(49)*	0.28 *(36)*	0.14 *(24)*	10.31 *(13)*	2.93 *(32)*	**35**
C1050	18.84 *(9)*	4.29 *(38)*	0.24 *(41)*	0.10 *(33)*	10.53 *(12)*	95.25 *(1)*	**23**
C1052	0.68 *(48)*	1.65 *(48)*	0.17 *(48)*	-0.10 *(48)*	7.23 *(21)*	-0.84 *(50)*	**48**
C1054	5.78 *(23)*	2.90 *(44)*	0.32 *(34)*	0.06 *(40)*	15.58 *(11)*	4.59 *(22)*	**30**
C1055	2.72 *(30)*	2.56 *(46)*	0.23 *(42)*	0.06 *(41)*	2.85 *(37)*	1.03 *(42)*	**46**
C1056	3.89 *(27)*	2.20 *(47)*	0.20 *(46)*	0.02 *(43)*	4.84 *(30)*	1.36 *(40)*	**45**
C1057	1.11 *(43)*	1.51 *(50)*	4.00 *(4)*	16.45 *(1)*	1.36 *(35)*	2.54 *(35)*	**32**
C1060	1.49 *(40)*	4.79 *(36)*	21.84 *(1)*	-7.94 *(51)*	0.21 *(51)*	-5.13 *(51)*	**44**
C1061	6.96 *(18)*	34.01 *(1)*	3.93 *(5)*	6.43 *(2)*	9.87 *(14)*	8.07 *(15)*	**2**
C1062	4.68 *(26)*	19.17 *(8)*	1.85 *(8)*	2.46 *(7)*	9.06 *(15)*	3.05 *(31)*	**10**
C1063	0.55 *(49)*	13.81 *(14)*	0.58 *(16)*	1.06 *(13)*	6.26 *(25)*	4.06 *(26)*	**24**
C1064	0.14 *(51)*	4.19 *(39)*	0.19 *(47)*	-0.68 *(50)*	1.23 *(48)*	0.97 *(43)*	**51**
C1072	1.16 *(42)*	10.15 *(22)*	0.39 *(24)*	0.71 *(14)*	1.98 *(43)*	3.19 *(29)*	**30**
C1074	1.63 *(37)*	16.45 *(10)*	0.91 *(13)*	2.00 *(11)*	7.91 *(18)*	5.51 *(21)*	**12**
C1089	2.02 *(34)*	16.16 *(12)*	0.44 *(18)*	2.14 *(10)*	8.01 *(17)*	4.14 *(25)*	**16**
C1112	13.35 *(13)*	25.42 *(4)*	5.25 *(2)*	5.53 *(3)*	6.29 *(24)*	6.00 *(18)*	**3**
C1128	4.76 *(25)*	16.26 *(11)*	0.40 *(23)*	0.55 *(16)*	5.56 *(28)*	3.12 *(30)*	**22**

Concentration of AP33 (µg/ml) representing the A450nm absorbance in each sample. The E1E2 binding rank, strongest (1) to weakest (51) is shown in brackets.

Table 3: Neutralization activity of CHCV cohort.

Patient IgG	**HCVpp**							**HCVcc**				**Final rank**
	Gt 1A	**Gt 1B**	**Gt 2A**	**Gt 2B**	**Gt 3A**	**Gt 4**	**HCV pp rank**	**Gt 1A**	**Gt 2A**	**Gt 2B**	**HCV cc rank**	
C1001	70.1	45.1	45.8	5.1	47.8	67.3	**32**	32.5	28.7	17.6	**35**	**34**
C1002	13.7	-6.8	20.9	-4.2	34.2	15.9	**51**	9.8	6.6	14.7	**50**	**51**
C1003	83.8	61.9	84.4	30.2	66.3	89.6	**2**	54.7	51.7	13.8	**7**	**2**
C1006	61.2	16.1	54.0	18.3	36.2	77.4	**36**	26.5	16.8	14.3	**47**	**46**

C1008	54.6	42.5	65.0	23.7	77.5	73.5	**20**	29.9	21.4	1.2	**51**	**36**
C1009	50.8	39.3	67.3	20.9	48.4	71.4	**28**	24.5	28.6	6.3	**49**	**41**
C1010	72.9	51.3	73.1	37.0	45.7	86.1	**8**	36.5	20.9	20.3	**31**	**18**
C1012	77.7	51.5	84.9	45.4	76.2	86.7	**1**	44.0	40.0	20.9	**8**	**2**
C1013	85.8	43.3	39.4	30.8	48.9	70.0	**25**	51.6	24.4	31.1	**13**	**17**
C1015	68.0	37.3	66.7	26.8	48.7	82.9	**15**	37.9	47.5	15.1	**17**	**12**
C1016	79.9	61.3	88.9	7.4	74.9	89.1	**5**	42.1	46.0	32.6	**6**	**4**
C1018	76.8	50.8	40.4	-6.2	49.6	79.6	**23**	31.3	37.7	23.4	**27**	**26**
C1020	56.1	44.7	38.8	5.0	53.0	83.3	**31**	26.6	37.2	34.0	**24**	**29**
C1021	62.7	34.7	55.4	25.7	46.2	88.1	**22**	44.5	64.2	56.9	**1**	**8**
C1022	66.9	49.6	63.3	32.5	51.6	81.3	**12**	35.6	40.5	31.3	**13**	**10**
C1023	62.8	49.3	67.1	-6.8	73.5	90.1	**13**	38.6	37.3	9.3	**28**	**20**
C1024	55.4	65.6	72.5	29.0	40.2	76.8	**17**	35.2	42.1	30.3	**16**	**13**
C1029	46.4	30.9	83.2	13.4	67.1	87.8	**18**	40.9	36.1	11.9	**25**	**22**
C1030	47.2	33.3	54.9	26.4	27.9	79.6	**38**	24.5	36.6	8.0	**44**	**44**
C1031	51.7	37.4	11.0	38.2	31.6	75.3	**41**	40.1	19.3	43.2	**22**	**31**
C1032	72.6	44.8	81.0	40.3	79.1	86.0	**11**	41.8	54.2	41.4	**5**	**6**
C1033	23.5	68.3	34.4	13.7	27.8	61.9	**44**	25.7	28.6	26.5	**37**	**43**
C1034	47.3	33.7	47.3	16.6	26.4	59.1	**42**	30.8	27.1	15.2	**40**	**44**
C1035	80.7	56.0	66.7	45.1	48.3	87.5	**5**	35.1	25.5	-1.9	**45**	**26**
C1036	67.2	47.7	52.2	23.7	22.5	65.1	**35**	36.0	17.0	30.8	**29**	**32**
C1037	84.3	54.8	65.5	16.1	61.6	90.9	**9**	34.0	19.7	26.9	**33**	**21**
C1038	38.6	40.6	77.1	29.4	51.7	79.0	**21**	38.7	53.4	-12.5	**25**	**24**
C1040	75.3	53.4	34.4	29.6	29.4	63.6	**32**	30.8	39.8	3.5	**39**	**36**
C1041	66.6	-29.2	43.7	25.8	71.5	71.9	**27**	33.3	39.9	1.5	**37**	**32**
C1042	67.9	57.0	57.4	39.6	80.2	89.9	**4**	67.0	65.2	21.8	**2**	**1**
C1043	44.4	45.5	31.7	-4.1	32.9	60.7	**48**	48.6	35.0	-19.9	**31**	**42**
C1045	64.5	36.3	37.2	36.3	-22.6	65.0	**39**	51.5	47.5	-10.7	**18**	**30**
C1046	79.4	60.0	62.5	50.2	64.0	82.9	**3**	39.9	61.8	-3.5	**20**	**8**
C1047	78.2	42.2	40.3	45.6	62.9	71.4	**26**	46.3	57.0	5.9	**10**	**15**
C1049	58.1	24.0	66.8	45.0	50.3	82.0	**14**	42.0	48.8	12.8	**12**	**11**
C1050	84.4	49.5	51.5	45.5	54.3	76.2	**9**	59.6	60.3	21.8	**4**	**5**
C1052	62.0	-29.6	34.5	14.3	19.7	54.2	**49**	34.5	35.8	-5.6	**42**	**49**
C1054	13.6	-5.0	39.1	5.5	37.1	82.7	**46**	41.6	38.8	10.1	**23**	**35**
C1055	61.5	0.2	32.1	1.7	53.7	79.2	**37**	33.8	43.0	-3.6	**35**	**38**
C1056	73.8	0.3	53.1	21.4	50.1	73.3	**28**	45.4	54.2	9.6	**9**	**16**
C1057	41.3	49.1	60.4	29.4	56.8	70.0	**24**	36.4	44.4	14.5	**21**	**23**
C1060	67.0	38.2	49.7	31.2	45.9	65.8	**34**	43.1	39.6	11.7	**18**	**28**
C1061	65.2	55.5	46.0	31.9	52.8	75.8	**16**	52.1	50.5	38.5	**3**	**7**
C1062	46.3	60.5	10.2	0.3	41.2	60.2	**45**	39.4	21.4	14.6	**30**	**40**
C1063	27.8	32.4	39.1	15.2	43.5	72.2	**42**	22.1	38.1	-11.7	**48**	**48**
C1064	24.6	37.9	31.8	12.6	34.1	48.2	**50**	16.6	30.8	10.9	**46**	**50**
C1072	72.5	47.2	39.2	21.8	28.8	60.2	**40**	37.2	34.1	11.3	**33**	**39**
C1074	61.3	37.4	41.0	34.1	37.2	76.0	**28**	42.5	38.0	24.1	**11**	**18**
C1089	28.4	23.9	23.6	26.4	44.9	60.5	**47**	38.4	30.9	-6.6	**41**	**47**
C1112	71.1	48.1	63.5	38.9	50.3	69.8	**19**	50.0	36.4	17.5	**15**	**14**
C1128	81.7	70.3	48.1	39.0	65.9	81.9	**7**	32.6	39.2	-33.7	**42**	**25**

435

Relative neutralization activity (%) is shown, neutralization HCVpp >50%, HCVcc >40% is shown in blue, <20% neutralization is shown in red. The neutralization rank within the cohort, strongest (1) to weakest (51) is shown for neutralization of HCVpp, HCVcc and the final overall rank.

Table 4: Gt 1 specific neutralization activity of gt 1 panel.

Patient IgG	Gt 1 HCVpp											No. Neut >50%
	1A H77	UKN 1B 5.23	UKN 1A 14.38	UKN 1A 14.43	UKN 1B 14.818	UKN 1A 20.8	GC 12.02	GC 13.01	GC 34.11	GC 37.04	ET10	
C1001	70.1	45.1	72.0	60.6	80.5	10.6	29.3	44.6	52.4	53.4	72.9	**7**
C1002	13.7	-6.8	-13.1	-22.0	0.11	-8.8	-17.1	-24.1	-7.6	1.6	-13.1	**0**
C1003	83.8	61.9	54.4	64.1	88.6	25.4	57.2	54.5	53.1	84.5	90.2	**10**
C1010	72.9	51.3	46.1	55.8	87.0	17.7	43.7	41.4	54.8	76.6	69.5	**7**
C1012	77.7	51.5	75.7	57.9	87.1	23.7	53.6	49.5	66.6	84.4	78.7	**9**
C1013	85.8	43.3	66.6	72.2	80.9	37.2	61.2	58.3	89.0	80.6	88.0	**9**
C1016	79.9	61.3	66.2	49.3	94.0	44.0	50.2	40.5	63.5	75.3	88.7	**8**
C1022	66.9	49.6	48.9	46.4	64.7	24.1	54.7	53.1	55.1	65.7	75.6	**7**
C1023	62.8	49.3	56.1	68.5	72.1	17.2	73.5	64.8	75.9	70.9	76.8	**9**
C1030	47.2	33.3	27.3	20.1	62.9	16.5	23.1	20.4	21.3	47.9	59.6	**2**
C1031	51.7	37.4	55.7	55.5	70.1	27.6	46.2	64.3	72.3	72.2	67.8	**8**
C1032	72.6	44.8	80.6	81.7	89.7	27.6	57.2	69.5	89.2	73.7	74.4	**9**
C1034	47.3	33.7	24.0	24.5	50.3	18.1	31.9	3.9	21.6	53.4	41.7	**2**
C1035	80.7	56.0	61.0	61.9	76.0	44.7	66.6	64.6	70.9	67.2	82.5	**10**
C1036	67.2	47.7	41.6	50.0	57.5	28.3	50.7	43.8	55.4	69.1	69.5	**7**
C1037	84.3	54.8	53.7	34.3	76.8	16.2	44.6	41.5	79.2	62.1	75.0	**7**
C1045	64.5	36.3	29.1	28.2	69.6	0.2	33.1	29.6	52.8	47.9	49.5	**3**
C1047	78.2	42.2	56.0	57.8	83.3	3.6	51.5	35.4	50.7	62.8	76.8	**8**
C1072	72.5	47.2	39.5	44.3	61.3	22.8	42.0	25.6	68.2	59.1	65.7	**5**
C1112	71.1	48.1	52.4	71.3	73.2	25.7	71.3	56.7	78.7	75.0	81.6	**9**

Relative neutralization activity (%) is shown, neutralization >50% is shown in blue, <20% neutralization is shown in red.

Figure 1: Molecular Phylogenetic analysis of gt 1 E1E2 sequences. Maximum Likelihood method based on analysis of E1E2 amino-acid sequences using the JTT matrix-based model (55). A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+*G*, parameter = 0.3680)).The tree with the highest log likelihood (-7055.2655) is shown. The tree is drawn to scale with the genetic distance for each branch length indicated by the scale bar. Bootstrap analysis with 1000 replicates was performed. Branches with >70% bootstrap support are labelled. Sequences classified as functional in the HCVpp system are labelled in blue, non-functional

sequences in red and sequences included in the gt 1 panel in green. Evolutionary analyses were conducted in MEGA6 (56) .

Figure 2: Association of viral load with E1E2 binding and neutralization profiles. (A) The relative binding of CHCV cohort IgG to E1E2 from 6 subgenotypes of HCV was determined by ELISA. The IgG samples were ranked from 1 to 51 according to their binding signal for each subgenotype. The sum of these ranks was used to order the samples from highest cross-genotypic binding to lowest. The binding of the upper half of the cohort was regarded as "Broad" and that of the lower half as "Narrow". The viral load of the two groups was compared using the Mann Whitney U test. (B) Neutralization of HCVpp in both panels at the 50% level by purified IgG was determined. The full CHCV cohort was analyzed against Panel XG and 20 gt 1-infected individuals were tested against Panel Gt1. The Mann Whitney U test was used to compare viral load between broad neutralizers, those that neutralized > 3 HCVpp (n=19) in Panel XG and >7 gt 1 HCVpp (n=10) in Panel Gt1 and narrow neutralizers, that neutralized <4 genotypes (n=32) in Panel XG and <8 gt 1 HCVpp (n=10) in Panel Gt1**.**

Figure 3: Non-parametric correlation between E1E2 ELISA binding and neutralization. For each Panel the number of HCVpp neutralized was plotted against ELISA binding rank, Panel XG (A) and Panel Gt1 (B). (C) Neutralization activity of the full cohort was analyzed against Panel XG and 3 viruses and the neutralization rank in the HCVcc and HCVpp systems plotted.(D) For those gt1-infected individuals tested in both Panels, the number of HCVpp isolates neutralized in Panel XG was plotted against the number of HCVpp isolates neutralized in Panel Gt1.The Spearman's rho correlation coefficient was calculated for all graphs.

Figure 4: Association of liver fibrosis with neutralization. Neutralization of Panel XG by the full CHCV cohort IgGs (A, C) or Panel Gt1 by IgGs from the gt1-infected subgroup (B, D) was determined as described in Fig. 2. (A, B) Broad and narrow neutralizing groups were defined as in Fig. 2 and transient elastography (kPa) values measured using Fibroscan® were compared using the

Mann Whitney U test**.** (C, D) The number of HCVpp isolates neutralized by IgG from individuals with and without cirrhosis was compared using the Mann Whitney U test.

Figure 5: Association of age with breadth of neutralization. We compared the age of individuals in the broad and narrow neutralizing groups as characterized in Fig. 2 for the full CHCV cohort with Panel XG (A) and the gt1 subgroup for Panel Gt1 (B) using the Mann Whitney U test. Similarly, the broad and narrow neutralizing groups were compared for duration of infection (estimated) and age at acquisition (estimated) for the full CHCV cohort (C, E) and the gt1 subgroup (D, F) respectively. Note that, no data was available for 6 broad and 10 narrow neutralizing individuals in the full CHCV cohort and 4 broad and 4 narrow neutralizing individuals in the gt1 subgroup.

Figure 6: Association of rs2395522 SNP genotype with breadth of neutralization and liver fibrosis. All individuals in the CHCV cohort were typed for the HLA DQ-B1 SNP rs2395522. The Mann-Whitney U test was used to compare SNP type with the number of HCVpp isolates neutralized in Panel XG (A) and Panel Gt1 (C) panel. Similarly, SNP type was compared to the level of liver fibrosis as measured by transient elastography (kPa) values for the whole CHCV cohort (B) or the gt 1 subgroup (D) by the Mann Whitney U test.

Figure 7. Competition of patient IgG with monoclonal antibodies to known epitopes on E2. (A) Competition ELISA of gt1 patient IgG with E2 mAbs CBH-4B, HC-84, AP33, CBH-7, HC-1 and HC-11 was performed. The mean percentage of competition (i.e. reduction in mAb binding) from 3 independent experiments is shown. (B) Association of competition with Hmabs CBH-7 and HC-11 for broad (>7 Gt1) and narrow (<8 Gt1) neutralizing samples using the Mann Whitney U test.

References

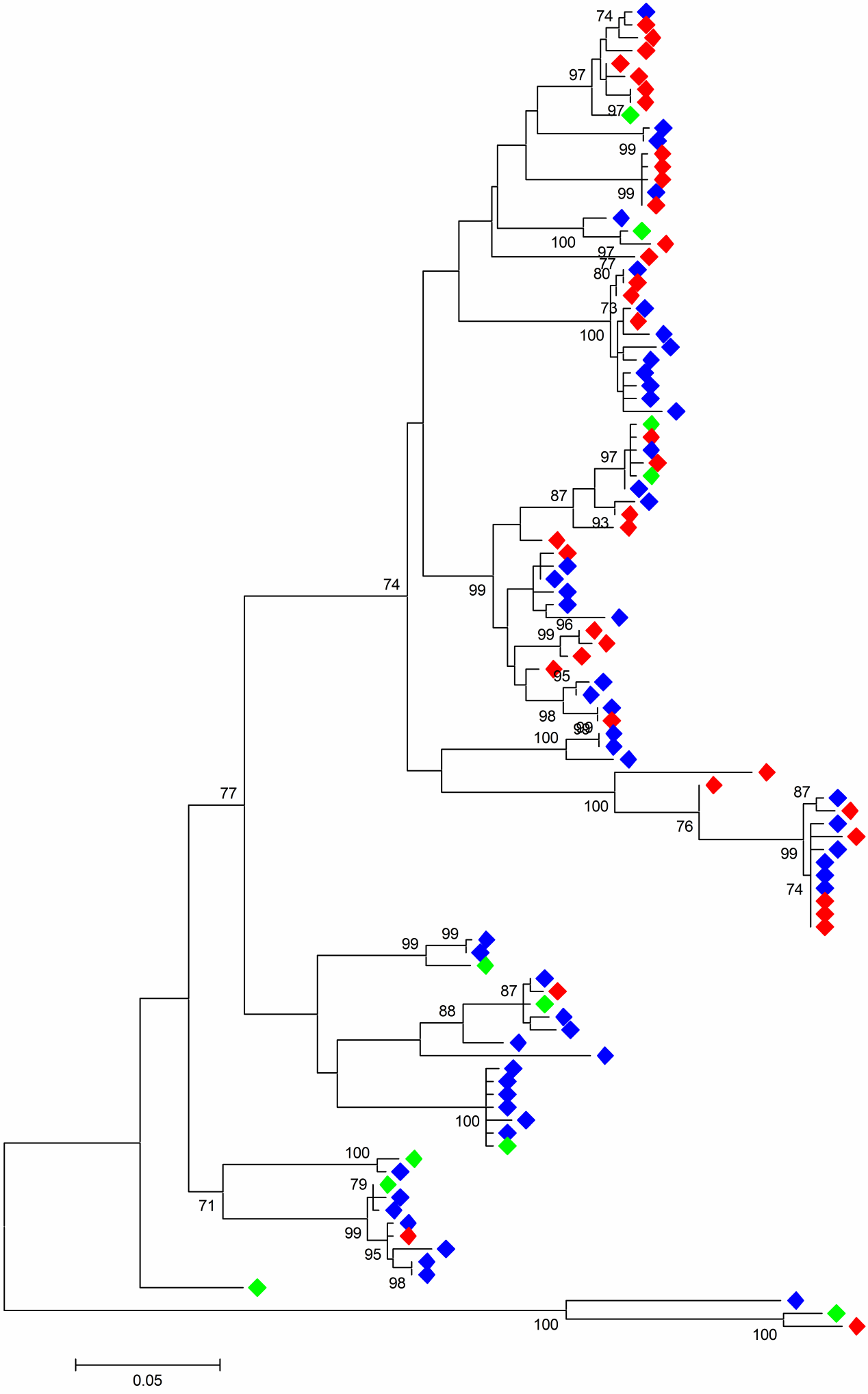
1. **WHO** 2012, posting date. WHO fact sheet: HCV vaccines number 164. [Online.]

2. **Bowen DG, Walker CM.** 2005. Adaptive immune responses in acute and chronic hepatitis C virus infection. Nature **436:**946-952.
3. **Karoney MJ, Siika AM.** 2013. Hepatitis C virus (HCV) infection in Africa: a review. The Pan African medical journal **14:**44.
4. **Hagan LM, Schinazi RF.** 2013. Best strategies for global HCV eradication. Liver Int **33 Suppl 1:**68-79.
5. **Raghuraman S, Park H, Osburn WO, Winkelstein E, Edlin BR, Rehermann B.** 2012. Spontaneous clearance of chronic hepatitis C virus infection is associated with appearance of neutralizing antibodies and reversal of T-cell exhaustion. The Journal of infectious diseases **205:**763-771.
6. **Osburn WO, Fisher BE, Dowd KA, Urban G, Liu L, Ray SC, Thomas DL, Cox AL.** 2010. Spontaneous control of primary hepatitis C virus infection and immunity against persistent reinfection. Gastroenterology **138:**315-324.
7. **Bartosch B, Cosset FL.** 2006. Cell entry of hepatitis C virus. Virology **348:**1-12.
8. **Hamilton JP, Thuluvath PJ.** 2008. Claudin-1 and its potential role in HCV entry: another piece of the puzzle. Journal of clinical gastroenterology **42:**3-4.
9. **Meredith LW, Wilson GK, Fletcher NF, McKeating JA.** 2012. Hepatitis C virus entry: beyond receptors. Reviews in medical virology **22:**182-193.
10. **Ploss A, Evans MJ.** 2012. Hepatitis C virus host cell entry. Current opinion in virology **2:**14-19.
11. **Pestka JM, Zeisel MB, Blaser E, Schurmann P, Bartosch B, Cosset FL, Patel AH, Meisel H, Baumert J, Viazov S, Rispeter K, Blum HE, Roggendorf M, Baumert TF.** 2007. Rapid induction of virus-neutralizing antibodies and viral clearance in a single-source outbreak of hepatitis C. Proc Natl Acad Sci U S A **104:**6025-6030.
12. **Osburn WO, Snider AE, Wells BL, Latanich R, Bailey JR, Thomas DL, Cox AL, Ray SC.** 2014. Clearance of hepatitis C infection is associated with the early appearance of broad neutralizing antibody responses. Hepatology **59:**2140-2151.
13. **de Jong YP, Dorner M, Mommersteeg MC, Xiao JW, Balazs AB, Robbins JB, Winer BY, Gerges S, Vega K, Labitt RN, Donovan BM, Giang E, Krishnan A, Chiriboga L, Charlton MR, Burton DR, Baltimore D, Law M, Rice CM, Ploss A.** 2014. Broadly neutralizing antibodies abrogate established hepatitis C virus infection. Science translational medicine **6:**254ra129.
14. **Keck ZY, Xia J, Cai Z, Li TK, Owsianka AM, Patel AH, Luo G, Foung SK.** 2007. Immunogenic and functional organization of hepatitis C virus (HCV) glycoprotein E2 on infectious HCV virions. Journal of virology **81:**1043-1047.
15. **Keck ZY, Op De Beeck A, Hadlock KG, Xia J, Li TK, Dubuisson J, Foung SK.** 2004. Hepatitis C virus E2 has three immunogenic domains containing conformational epitopes with distinct properties and biological functions. Journal of virology **78:**9224-9232.
16. **Wang Y, Keck ZY, Foung SK.** 2011. Neutralizing antibody response to hepatitis C virus. Viruses **3:**2127-2145.
17. **Keck ZY, Li TK, Xia J, Bartosch B, Cosset FL, Dubuisson J, Foung SK.** 2005. Analysis of a highly flexible conformational immunogenic domain a in hepatitis C virus E2. Journal of virology **79:**13199-13208.
18. **Keck ZY, Saha A, Xia J, Wang Y, Lau P, Krey T, Rey FA, Foung SK.** 2011. Mapping a region of hepatitis C virus E2 that is responsible for escape from neutralizing antibodies and a core CD81-binding region that does not tolerate neutralization escape mutations. Journal of virology **85:**10451-10463.
19. **Keck ZY, Sung VM, Perkins S, Rowe J, Paul S, Liang TJ, Lai MM, Foung SK.** 2004. Human monoclonal antibody to hepatitis C virus E1 glycoprotein that blocks virus attachment and viral infectivity. J Virol **78:**7257-7263.

20. **Owsianka AM, Tarr AW, Keck ZY, Li TK, Witteveldt J, Adair R, Foung SK, Ball JK, Patel AH.** 2008. Broadly neutralizing human monoclonal antibodies to the hepatitis C virus E2 glycoprotein. The Journal of general virology **89:**653-659.
21. **Luo K, Li S, Jiang L, Zuo T, Qing J, Shi X, Liu Y, Wu H, Chen X, Zhang L.** 2015. Combinatorial library-based profiling of the antibody response against hepatitis C virus in humans. J Gen Virol **96:**52-63.
22. **Tarr AW, Urbanowicz RA, Jayaraj D, Brown RJ, McKeating JA, Irving WL, Ball JK.** 2012. Naturally occurring antibodies that recognize linear epitopes in the amino terminus of the hepatitis C virus E2 protein confer noninterfering, additive neutralization. Journal of virology **86:**2739-2749.
23. **Sautto G, Mancini N, Diotti RA, Solforosi L, Clementi M, Burioni R.** 2012. Anti-hepatitis C virus E2 (HCV/E2) glycoprotein monoclonal antibodies and neutralization interference. Antiviral research **96:**82-89.
24. **Tarr AW, Urbanowicz RA, Hamed MR, Albecka A, McClure CP, Brown RJ, Irving WL, Dubuisson J, Ball JK.** 2011. Hepatitis C patient-derived glycoproteins exhibit marked differences in susceptibility to serum neutralizing antibodies: genetic subtype defines antigenic but not neutralization serotype. Journal of virology **85:**4246-4257.
25. **Urbanowicz RA, McClure CP, Brown RJ, Tsoleridis T, Persson MA, Krey T, Irving WL, Ball JK, Tarr AW.** 2015. A diverse panel of Hepatitis C Virus glycoproteins for use in vaccine research reveals extremes of monoclonal antibody neutralization resistance. Journal of virology.
26. **Bailey JR, Wasilewski LN, Snider AE, El-Diwany R, Osburn WO, Keck Z, Foung SK, Ray SC.** 2015. Naturally selected hepatitis C virus polymorphisms confer broad neutralizing antibody resistance. The Journal of clinical investigation **125:**437-447.
27. **Kong L, Giang E, Nieusma T, Kadam RU, Cogburn KE, Hua Y, Dai X, Stanfield RL, Burton DR, Ward AB, Wilson IA, Law M.** 2013. Hepatitis C virus E2 envelope glycoprotein core structure. Science **342:**1090-1094.
28. **Khan AG, Whidby J, Miller MT, Scarborough H, Zatorski AV, Cygan A, Price AA, Yost SA, Bohannon CD, Jacob J, Grakoui A, Marcotrigiano J.** 2014. Structure of the core ectodomain of the hepatitis C virus envelope glycoprotein 2. Nature **509:**381-384.
29. **Landau DA, Saadoun D, Calabrese LH, Cacoub P.** 2007. The pathophysiology of HCV induced B-cell clonal disorders. Autoimmun Rev **6:**581-587.
30. **Iro M, Witteveldt J, Angus AG, Woerz I, Kaul A, Bartenschlager R, Patel AH.** 2009. A reporter cell line for rapid and sensitive evaluation of hepatitis C virus infectivity and replication. Antiviral research **83:**148-155.
31. **Hadlock KG, Lanford RE, Perkins S, Rowe J, Yang Q, Levy S, Pileri P, Abrignani S, Foung SK.** 2000. Human monoclonal antibodies that inhibit binding of hepatitis C virus E2 protein to CD81 and recognize conserved conformational epitopes. Journal of virology **74:**10407-10416.
32. **Keck ZY, Li TK, Xia J, Gal-Tanamy M, Olson O, Li SH, Patel AH, Ball JK, Lemon SM, Foung SK.** 2008. Definition of a conserved immunodominant domain on hepatitis C virus E2 glycoprotein by neutralizing human monoclonal antibodies. Journal of virology **82:**6061-6066.
33. **Keck ZY, Xia J, Wang Y, Wang W, Krey T, Prentoe J, Carlsen T, Li AY, Patel AH, Lemon SM, Bukh J, Rey FA, Foung SK.** 2012. Human monoclonal antibodies to a novel cluster of conformational epitopes on HCV E2 with resistance to neutralization escape in a genotype 2a isolate. PLoS pathogens **8:**e1002653.
34. **Clayton RF, Owsianka A, Aitken J, Graham S, Bhella D, Patel AH.** 2002. Analysis of antigenicity and topology of E2 glycoprotein present on recombinant hepatitis C virus-like particles. Journal of virology **76:**7672-7682.

35. **Patel AH, Wood J, Penin F, Dubuisson J, McKeating JA.** 2000. Construction and characterization of chimeric hepatitis C virus E2 glycoproteins: analysis of regions critical for glycoprotein aggregation and CD81 binding. J Gen Virol **81:**2873-2883.
36. **Tarr AW, Owsianka AM, Szwejk A, Ball JK, Patel AH.** 2007. Cloning, expression, and functional analysis of patient-derived hepatitis C virus glycoproteins. Methods in molecular biology **379:**177-197.
37. **Musset L, Ghillani P, Lunel F, Cacoub P, Cresta P, Frangeul L, Rosenheim M, Preud'homme JL.** 1997. Variations of serum IgG subclass levels in hepatitis C virus infection during interferon-alpha therapy. Immunology letters **55:**41-45.
38. **Eren R, Landstein D, Terkieltaub D, Nussbaum O, Zauberman A, Ben-Porath J, Gopher J, Buchnick R, Kovjazin R, Rosenthal-Galili Z, Aviel S, Ilan E, Shoshany Y, Neville L, Waisman T, Ben-Moshe O, Kischitsky A, Foung SK, Keck ZY, Pappo O, Eid A, Jurim O, Zamir G, Galun E, Dagan S.** 2006. Preclinical evaluation of two neutralizing human monoclonal antibodies against hepatitis C virus (HCV): a potential treatment to prevent HCV reinfection in liver transplant patients. Journal of virology **80:**2654-2664.
39. **Owsianka A, Tarr AW, Juttla VS, Lavillette D, Bartosch B, Cosset FL, Ball JK, Patel AH.** 2005. Monoclonal antibody AP33 defines a broadly neutralizing epitope on the hepatitis C virus E2 envelope glycoprotein. Journal of virology **79:**11095-11104.
40. **Mohsen AH, Trent HCVSG.** 2001. The epidemiology of hepatitis C in a UK health regional population of 5.12 million. Gut **48:**707-713.
41. **Thomson EC, Nastouli E, Main J, Karayiannis P, Eliahoo J, Muir D, McClure MO.** 2009. Delayed anti-HCV antibody response in HIV-positive men acutely infected with HCV. Aids **23:**89-93.
42. **Robinson MW, Swann R, Sigruener A, Barclay ST, Mills PR, McLauchlan J, Patel AH.** 2015. Elevated interferon-stimulated gene transcription in peripheral blood mononuclear cells occurs in patients infected with genotype 1 but not genotype 3 hepatitis C virus. Journal of viral hepatitis **22:**384-390.
43. **Lavillette D, Tarr AW, Voisset C, Donot P, Bartosch B, Bain C, Patel AH, Dubuisson J, Ball JK, Cosset FL.** 2005. Characterization of host-range and cell entry properties of the major genotypes and subtypes of hepatitis C virus. Hepatology **41:**265-274.
44. **Jiang R, Dong J, Dai Y.** 2009. Genome-wide association study of rheumatoid arthritis by a score test based on wavelet transformation. BMC proceedings **3 Suppl 7:**S8.
45. **Allanore Y, Saad M, Dieude P, Avouac J, Distler JH, Amouyel P, Matucci-Cerinic M, Riemekasten G, Airo P, Melchers I, Hachulla E, Cusi D, Wichmann HE, Wipff J, Lambert JC, Hunzelmann N, Tiev K, Caramaschi P, Diot E, Kowal-Bielecka O, Valentini G, Mouthon L, Czirjak L, Damjanov N, Salvi E, Conti C, Muller M, Muller-Ladner U, Riccieri V, Ruiz B, Cracowski JL, Letenneur L, Dupuy AM, Meyer O, Kahan A, Munnich A, Boileau C, Martinez M.** 2011. Genome-wide scan identifies TNIP1, PSORS1C1, and RHOB as novel risk loci for systemic sclerosis. PLoS genetics **7:**e1002091.
46. **Zhang P, Zhong L, Struble EB, Watanabe H, Kachko A, Mihalik K, Virata-Theimer ML, Alter HJ, Feinstone S, Major M.** 2009. Depletion of interfering antibodies in chronic hepatitis C patients and vaccinated chimpanzees reveals broad cross-genotype neutralizing activity. Proc Natl Acad Sci U S A **106:**7537-7541.
47. **Bjoro K, Skaug K, Haaland T, Froland SS.** 1999. Long-term outcome of chronic hepatitis C virus infection in primary hypogammaglobulinaemia. QJM : monthly journal of the Association of Physicians **92:**433-441.
48. **Ennishi D, Terui Y, Yokoyama M, Mishima Y, Takahashi S, Takeuchi K, Okamoto H, Tanimoto M, Hatake K.** 2008. Monitoring serum hepatitis C virus (HCV) RNA in patients with HCV-infected CD20-positive B-cell lymphoma undergoing rituximab combination chemotherapy. Am J Hematol **83:**59-62.

49. **Davis GL, Alter MJ, El-Serag H, Poynard T, Jennings LW.** 2010. Aging of hepatitis C virus (HCV)-infected persons in the United States: a multiple cohort model of HCV prevalence and disease progression. Gastroenterology **138:**513-521, 521 e511-516.
50. **Boyd SD, Liu Y, Wang C, Martin V, Dunn-Walters DK.** 2013. Human lymphocyte repertoires in ageing. Curr Opin Immunol **25:**511-515.
51. **Hamed MR, Tarr AW, McClure CP, Ball JK, Hickling TP, Irving WL.** 2008. Association of antibodies to hepatitis C virus glycoproteins 1 and 2 (anti-E1E2) with HCV disease. Journal of viral hepatitis **15:**339-345.
52. **McCaffrey K, Gouklani H, Boo I, Poumbourios P, Drummer HE.** 2011. The variable regions of hepatitis C virus glycoprotein E2 have an essential structural role in glycoprotein assembly and virion infectivity. J Gen Virol **92:**112-121.
53. **Carlsen TH, Pedersen J, Prentoe JC, Giang E, Keck ZY, Mikkelsen LS, Law M, Foung SK, Bukh J.** 2014. Breadth of neutralization and synergy of clinically relevant human monoclonal antibodies against HCV genotypes 1a, 1b, 2a, 2b, 2c, and 3a. Hepatology **60:**1551-1562.
54. **Bengsch B, Thimme R, Blum HE.** 2009. Role of host genetic factors in the outcome of hepatitis C virus infection. Viruses **1:**104-125.
55. **Jones DT, Taylor WR, Thornton JM.** 1992. The rapid generation of mutation data matrices from protein sequences. Computer applications in the biosciences : CABIOS **8:**275-282.
56. **Tamura K, Stecher G, Peterson D, Filipski A, Kumar S.** 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular biology and evolution **30:**2725-2729.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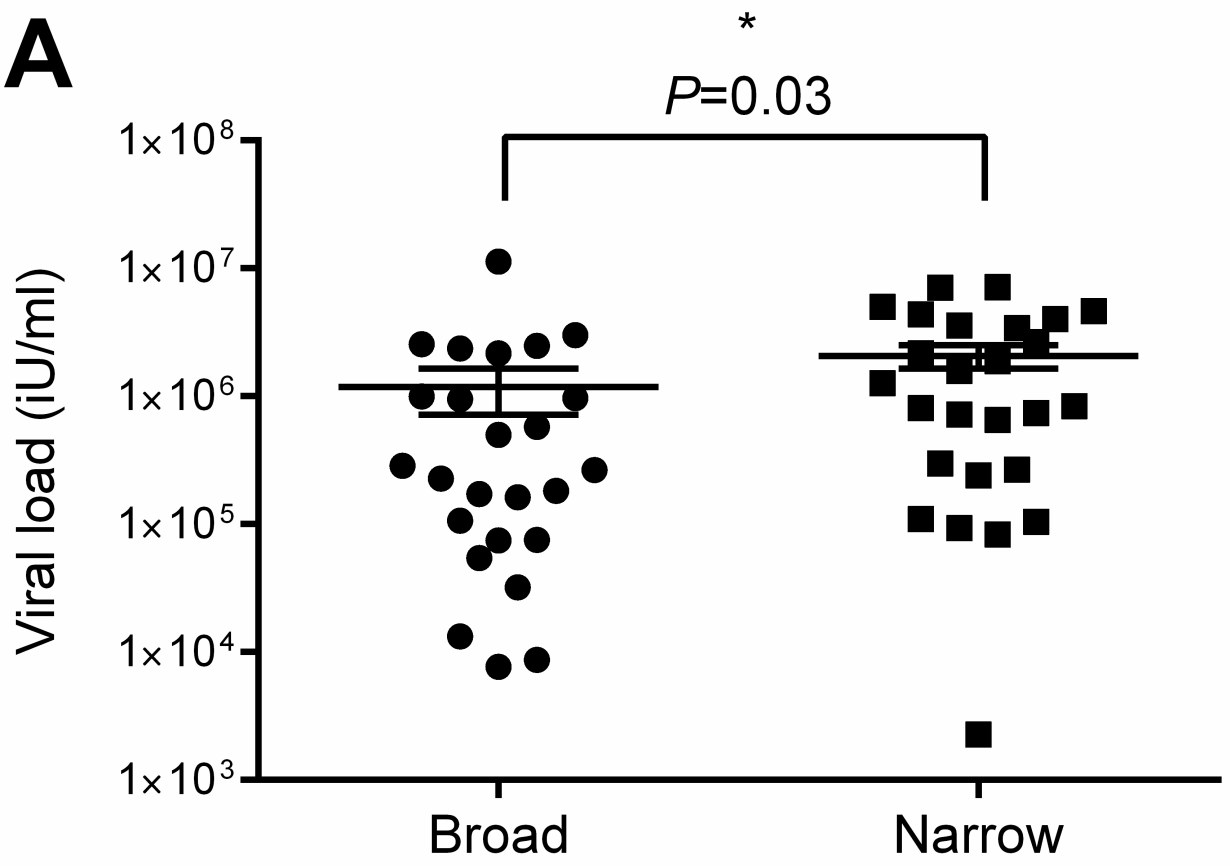
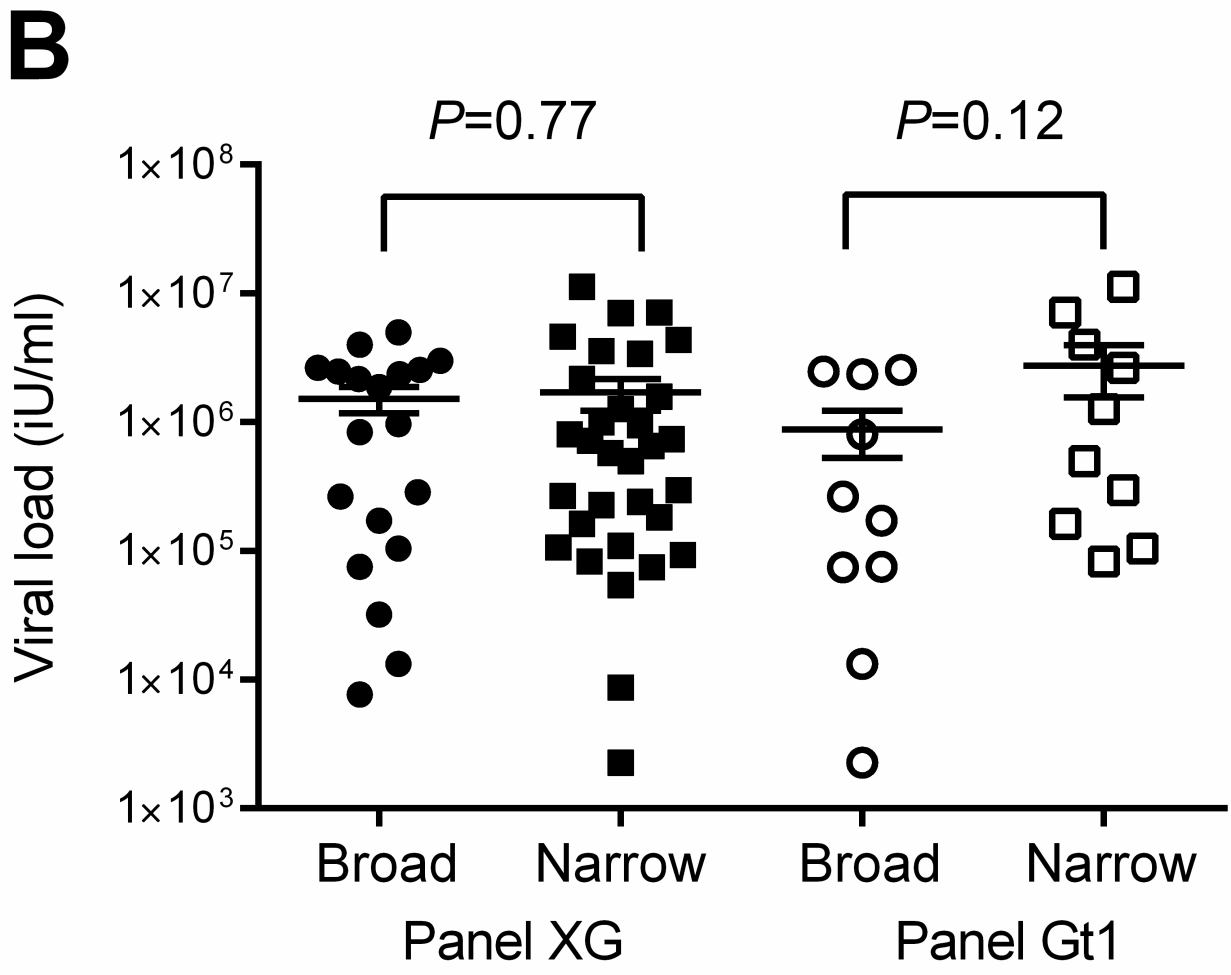
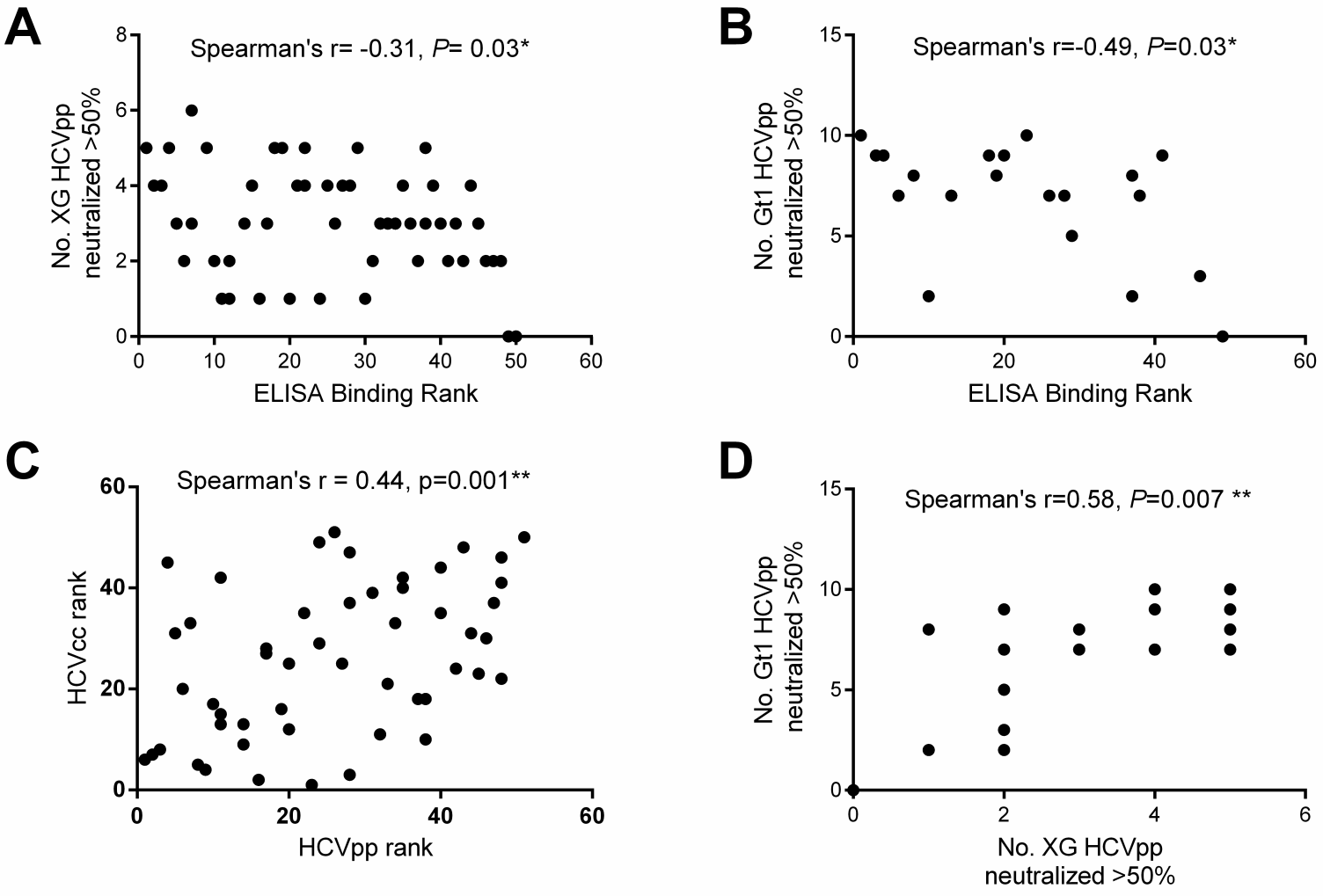
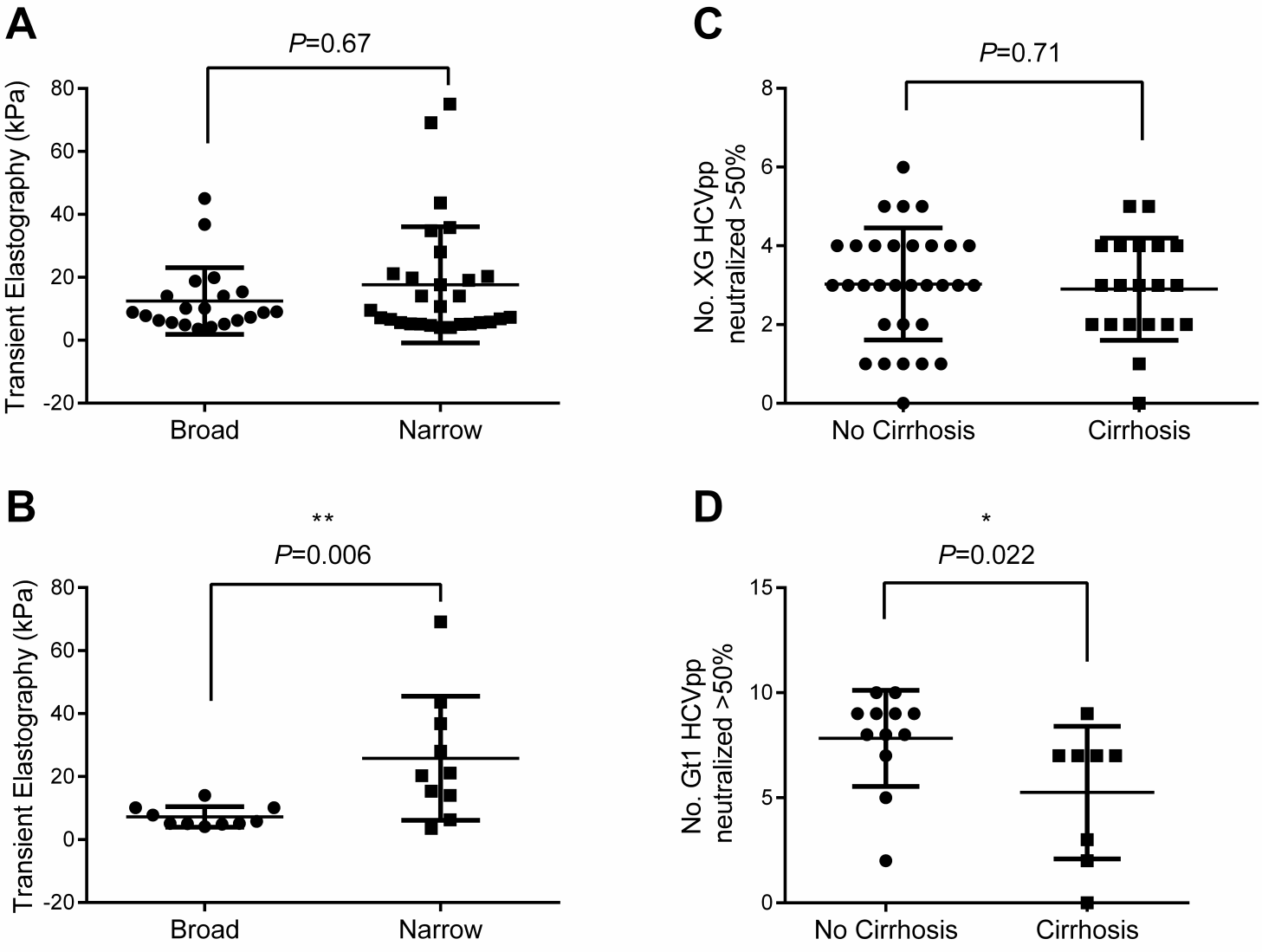
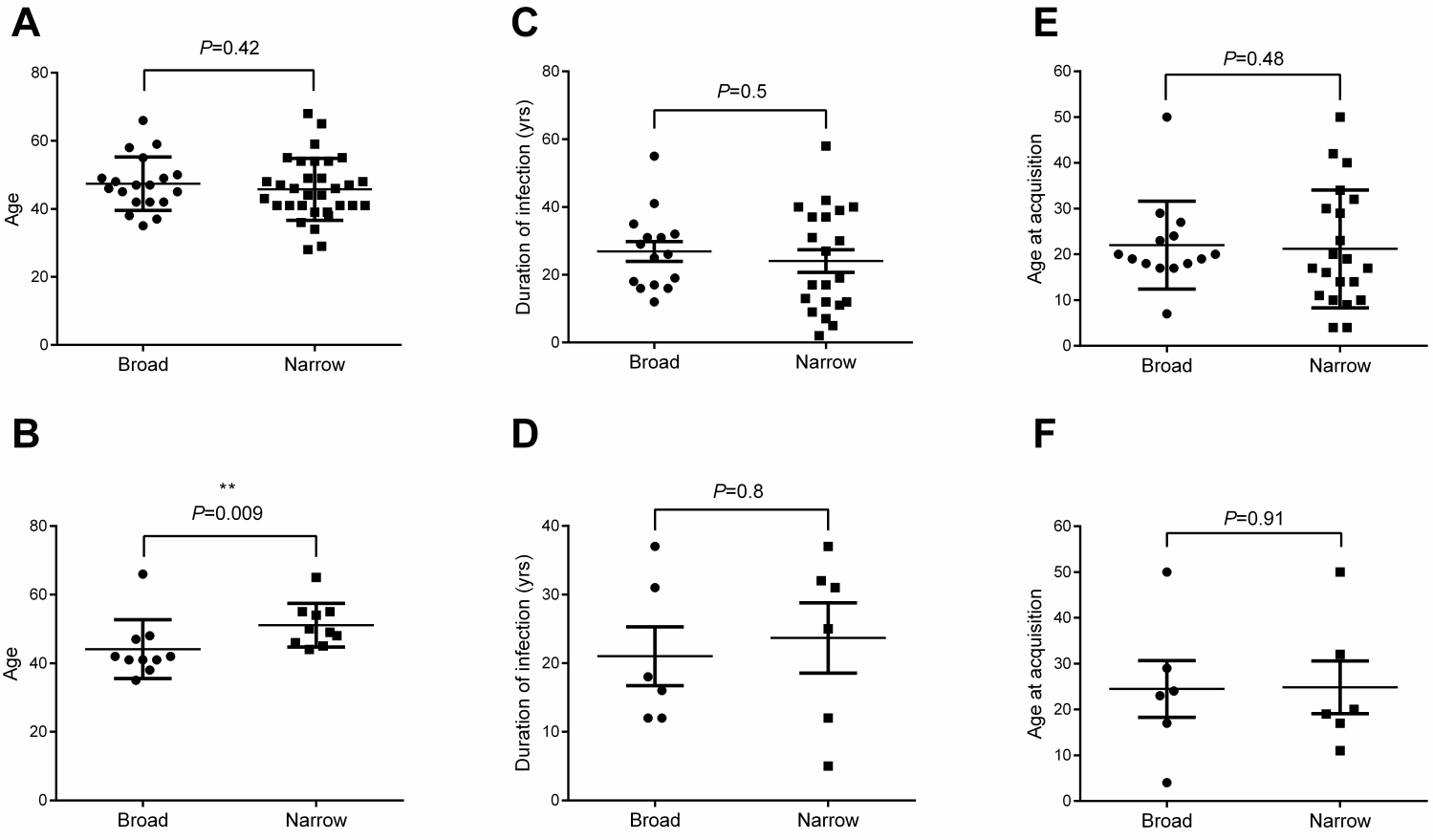
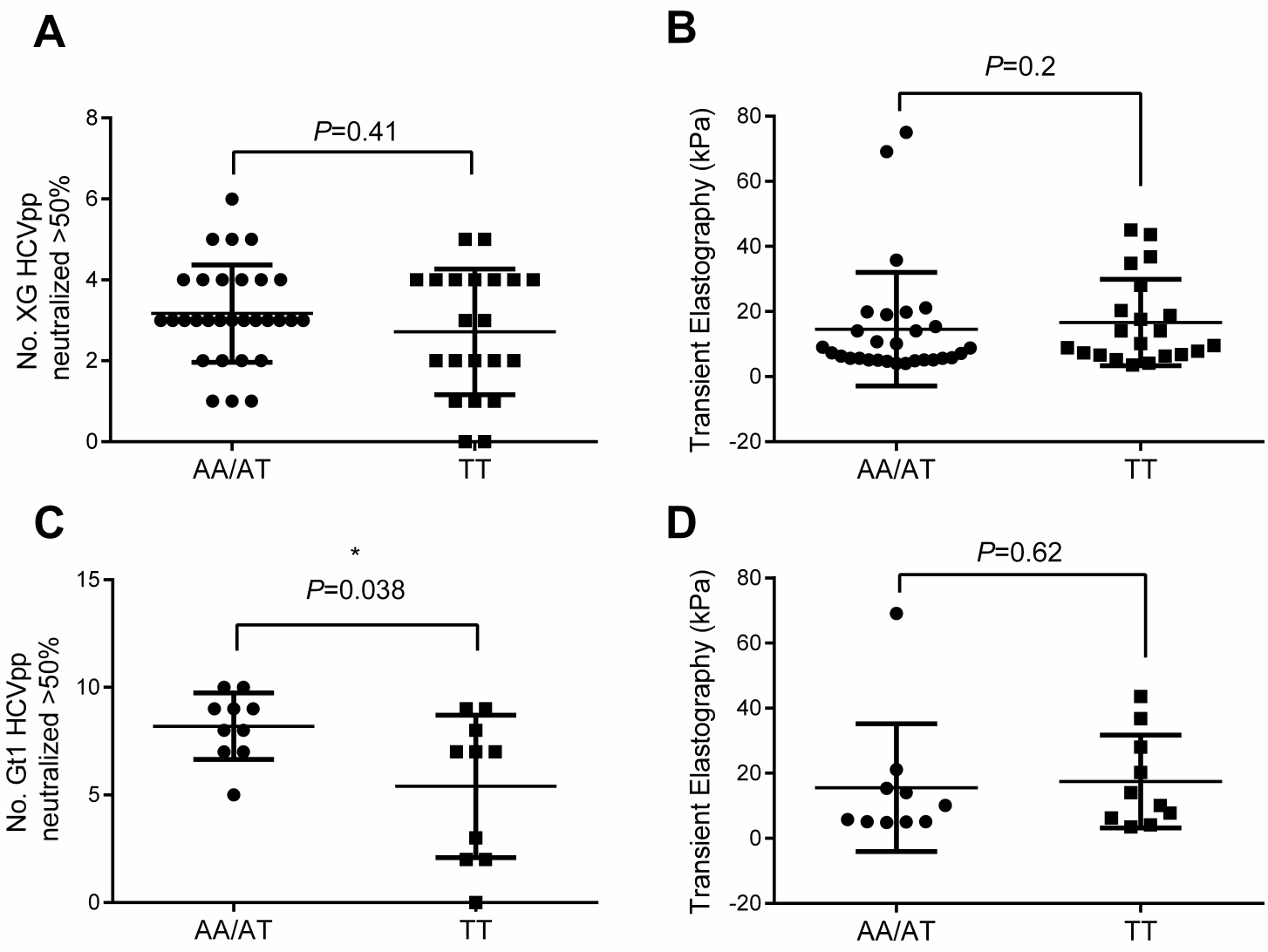

A
*
P=0.03
1×10^8
1×10^7
1×10^6
1×10^5
1×10^4
1×10^3
Viral load (iU/ml)
Broad
Narrow


B
P=0.77
P=0.12
1×10^8
1×10^7
1×10^6
1×10^5
1×10^4
1×10^3
Viral load (iU/ml)
Broad
Narrow
Broad
Narrow
Panel XG
Panel Gt1


A
Spearman's r= -0.31, P= 0.03*
No. XG HCVpp neutralized >50%
ELISA Binding Rank
B
Spearman's r=-0.49, P=0.03*
No. Gt1 HCVpp neutralized >50%
ELISA Binding Rank
C
Spearman's r = 0.44, p=0.001**
HCVcc rank
HCVpp rank
D
Spearman's r=0.58, P=0.007 **
No. Gt1 HCVpp neutralized >50%
No. XG HCVpp neutralized >50%


A
P=0.67
Transient Elastography (kPa)
80
60
40
20
0
-20
Broad
Narrow
C
P=0.71
No. XG HCVpp neutralized >50%
8
6
4
2
0
No Cirrhosis
Cirrhosis
B
**
P=0.006
Transient Elastography (kPa)
80
60
40
20
0
-20
Broad
Narrow
D
*
P=0.022
No. Gt1 HCVpp neutralized >50%
15
10
5
0
No Cirrhosis
Cirrhosis


A
P=0.42
Age
80
60
40
20
0
Broad
Narrow
C
P=0.5
Duration of infection (yrs)
80
60
40
20
0
Broad
Narrow
E
P=0.48
Age at acquisition
60
50
40
30
20
10
0
Broad
Narrow
B
**
P=0.009
Age
80
60
40
20
0
Broad
Narrow
D
P=0.8
Duration of infection (yrs)
40
30
20
10
0
Broad
Narrow
F
P=0.91
Age at acquisition
60
50
40
30
20
10
0
Broad
Narrow


A
No. XG HCVpp neutralized >50%
P=0.41
8
6
4
2
0
AA/AT
TT
B
Transient Elastography (kPa)
P=0.2
80
60
40
20
0
-20
AA/AT
TT
C
No. Gt1 HCVpp neutralized >50%
*
P=0.038
15
10
5
0
AA/AT
TT
D
Transient Elastography (kPa)
P=0.62
80
60
40
20
0
-20
AA/AT
TT

A

Patient IgG	Neutralization Breadth	Immunodomain A	aa412-423	Immunodomain C	Immunodomain B		
		CBH-4B	AP33	CBH-7	HC-1	HC-11	HC-84
C1001	Narrow	19.9	18.3	22.9	51.9	58.9	11.2
C1002	Narrow	1.0	7.2	1.1	0	4.7	-2.1
C1010	Narrow	48.5	33.2	76.4	55.2	82.7	9.5
C1022	Narrow	20.3	0.4	22.9	57.6	73.4	9.1
C1030	Narrow	16.7	13.4	15.2	0.6	36.3	6.5
C1034	Narrow	5.4	10.0	26.3	3.6	43.5	20.6
C1036	Narrow	-13.7	-0.1	1.9	35.3	65.2	14.9
C1037	Narrow	6.4	6.4	9.0	7.8	32.2	14.5
C1045	Narrow	20.7	7.9	20.9	39.6	26.4	0.7
C1072	Narrow	16.6	8.2	14.3	53.2	56.1	12.6
C1003	Broad	26.1	5.7	71.7	80.4	77.9	37.4
C1012	Broad	58.3	43.8	76.0	46.8	75.3	15.8
C1013	Broad	9.9	33.9	6.1	14.1	57.0	13.1
C1016	Broad	10.2	3.4	47.0	75.8	75.0	30.6
C1023	Broad	37.3	19.5	82.8	68.9	77.5	-1.8
C1031	Broad	49.4	10.9	50.2	51.5	83.4	13.9
C1032	Broad	44.7	28.2	85.7	70.0	92.4	26.6
C1035	Broad	18.0	8.6	53.9	63.2	77.4	20.1
C1047	Broad	16.5	12.3	29.1	62.5	71.9	12.6
C1112	Broad	24.4	14.5	48.2	69.3	74.0	18.0

Reduction in binding (%) <30 30-50 50-70 >70

B