Published in final edited form as:
*Trends Microbiol*. 2011 August ; 19(8): 382–388. doi:10.1016/j.tim.2011.05.007.

# Norovirus-host interaction: multi-selections by human HBGAs

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## Abstract

The discovery of human histo-blood group antigens (HBGAs) as receptors or ligands of noroviruses (NoVs) raises a question about the potential role of host factors in the evolution and diversity of NoVs. Recent structural analysis of selected strains in the two major genogroups of human NoVs (GI and GII) demonstrated highly conserved HBGA binding interfaces within the two groups but not between them, indicating convergent evolution of GI and GII NoVs. GI and GII NoVs are likely introduced to humans from different non-human hosts with the HBGAs as a common niche. Each genogroup has further diverged into multiple sub-lineages (genotypes) through selections by the polymorphic HBGAs of the hosts. An elucidation of such pathogen-host interaction, including determination of the phenotypes of NoV-HBGAs interaction for each genotype, is important in understanding the epidemiology, classification and disease control and prevention of NoVs. A model of this multi-selection of NoVs by HBGAs is proposed.

## Norovirus-host cell interactions

Noroviruses (NoVs) are a major cause of epidemic acute gastroenteritis affecting millions of people worldwide. Infection by NoVs relies on recognition of human histo-blood group antigens (HBGAs) as ligands or receptors for attachment, an early infection event that most likely controls host susceptibility and resistance to NoVs [1–12]. HBGAs are complex carbohydrates on red blood cells, mucosal epithelia, saliva, milk and other body fluids, which are highly polymorphic related to the ABO, secretor, and Lewis families. The interaction between NoVs and HBGAs has been extensively studied since it was found in 2002 [7,10,12]. Early studies using variable *in vitro* binding assays to measure the binding of NoV-like particles (VLPs) with HBGAs revealed diverse binding patterns [5,6,10,12,13]. Further studies in human volunteer challenge and outbreak investigations provided direct evidence on the linkage between host blood types and susceptibility to NoV infection and thus implicating HBGAs as NoV receptors or co-receptors [7–9,11,14,15], although undefined results were also reported [16].

Recent structural and functional analysis of the HBGA-binding interfaces of NoVs has significantly advanced our understanding of the complicated interaction between NoVs and human HBGAs. For the first time, crystal structures of the HBGA binding interfaces of representative NoVs in complex with different HBGAs[17–20] precisely elucidated



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interactions between individual amino acids in the binding interfaces of NoVs with specific saccharides of HBGAs. This article summarizes these advancements, proposes a new model of NoV-HBGA interaction, and discusses the role of HBGAs in NoV evolution and resulting impacts on epidemiology and classification of human NoVs.

## Multi-interaction network between NoVs and HBGAs

The Norwalk virus (GI.1), NoV VA387 (GII.4), and NoV VA207 (GII.9) represent three major HBGA binding patterns [5,6] and three genotypes in genogroups I and II (GI and GII). The HBGA binding interfaces of these three NoVs have been resolved by co-crystallization of the P domains of the viral capsid in complex with corresponding HBGA oligosaccharides [17,19,2018]. All three NoVs revealed two symmetric HBGA binding interfaces on each arch-like P dimer, corresponding to the outermost surface of the capsid. Each interface consists of several amino acids scattered in the P domain that form a conformational pocket, which interacts with individual side chains of HBGAs [17–20]. Each interface can sterically be divided into two to three sites, each interacting with a saccharide of the HBGAs.

The two GII NoVs use a similar interface to interact with a different set of HBGA saccharides. Ten amino acids (S343, T344, R345, A346, K348, N373, D374, D391, G442, and Y443) in VA387 form up to 12 hydrogen bonds and a van der Waals interaction with the A or B antigens (Figure 1c and 1d)[19]. In VA207, a similar set of amino acids (T345, R346, D374, Y389, S439, G440, and H441) form up to 9 hydrogen bonds and a van der Waals interaction with the $Le^y$ and sialyl $Le^x$ antigens (Figure 1e and 1f)[18]. To differentiate the role of the individual saccharides in interacting with NoVs, the residue with the most interactions to the binding interface is referred as the major binding residue. For VA387 the α-1, 2 Fuc (α-1, 2 fucose, the H epitope) of the A, B and O secretor HBGAs is the major binding residue, while the α-GalNAc (α-N-Acetylgalactosamine, the A epitope), the α-Gal (α-galactose, the B epitope) and the β-Gal of the precursor of the HBGAs are the minor binding residues (Figure 1c and 1d)[19]. For VA207, however, the α-1, 3 Fuc (the Lewis residue), the Lewis x/y ($Le^{x/y}$) determinant, is the major binding residue, whereas the N-acetylglucosamine (GlcNAc) of the precursor and the α-1, 2 Fuc of $Le^y$ are the minor interaction residues (Figure 1e and 1f)[18]. In Norwalk virus, the binding interface is formed by eight residues (D327, H329, Q342, D344, W375, S377, P388, and S380) that form 6–7 hydrogen bonds and a cation-π interaction with the H or A antigens (Figure 1a and 1b)[17,20]. The binding interface of the GI Norwalk virus is composed of a completely different set of amino acids and demonstrates a different binding mode to HBGAs as compared to that of the two GII viruses. For Norwalk virus, the α-GalNAc of the A antigen is the major binding residue and α-1, 2 Fuc is the minor binding residue when Norwalk virus binds to the A antigen (Figure 1b)[17,20]. However, the Norwalk virus has an alternative binding mode with the H-type 1 antigen, in which the β-Gal from the precursor of the HBGAs is the major binding residue and α-1, 2 Fuc is the minor binding residue (Figure 1a). In summary, while the multiple interaction networks between NoVs and HBGAs appear to be a common feature, the HBGA binding modes, the involved HBGA residues and number and type of the interactions between a HBGA side chains and individual NoVs can be highly variable.

## A model of NoV-HBGA interaction

The majority of human blood types, including the secretor, ABO and Lewis blood types are determined by three unique types of saccharides (Figure 2a). The α-1, 2 Fuc (H epitope) is the determinant of the secretor blood type, this saccharide is added to the precursor of the HBGAs by the α-1, 2 fucosyl transferase encoded by FUT2. The two saccharides, α-GalNAc and α-Gal (the A/B epitopes), are the determinants for the A/B blood types and are added to

the H antigen by the A/B enzymes, respectively. The Lewis antigens are determined by the α-1, 3/4 Fuc saccharides called the Lewis epitopes that are added to the precursor by the α-1, 3/4 fucosyl transferase encoded by FUT3. Importantly, these three types of saccharides are also the major interacting sites of human NoVs [21] and thus might function as multi-selection factors of human NoVs.

We propose a new model, developed using available structural and functional analyses, in which human NoVs are sorted into three groups: the H-, A/B- and Lewis-binding groups based on the major interacting residues of the α-1, 2 Fuc, α-GalNAc/α-Gal, and α-1,3/4 Fuc saccharides, respectively (Figure 2). The two GII strains, VA387 (GII.4) and VA207 (GII. 9), belong to the H- and Lewis-binding groups, respectively. In addition, a number of GII strains revealed binding activities to the A and B secretors but not O secretors and non-secretors, such as MOH (GII.5) [5], suggesting that they target the A/B epitopes as the major residues. However, these strains have not been analyzed by co-crystallization. These NoVs should be grouped in the A/B binding group.

A similar grouping principle based on the major vs. minor binding saccharides may apply to GI NoVs. For example, the well characterized NoV Boxer (GI.8)[5] shows a similar binding pattern as that of VA207 and therefore is assigned to the Lewis-binding group. However, owing to the different genetic background and the distinct structures, amino acid composition, and binding modes of the HBGA binding interfaces between GI and GII, the assignment of binding groups for other GI strains might be different. For example, the Norwalk virus could be grouped in the A/B binding group because it targets α-GalNAc on the A antigen as its major binding residue[17,20]. However, the Norwalk virus also targets β-Gal on the H antigen as the major interacting residue (Figure 1a and 1b). Therefore, further functional and structural analyses are necessary to confirm that similar grouping principle would fit in the GI NoVs.

This proposed model emphasizes the critical roles of the major binding residues that are also the determinants of the three major human HBGA families (the ABO, secretor and Lewis families). Thus, the proposed model is likely to represent all possible repertoires of HBGAs interacting with NoVs and possibly other microbial pathogens of humans as well. The inclusion of one to two minor residues in the classification of the three binding groups, including the residues of the precursors of the HBGAs, provides further power to explain additional variations of NoV-HBGA interaction that could have biological significance.

With further information on the structure and function of the HBGA-binding interfaces of NoVs representing more binding patterns and/or binding groups, this model will be subjected to further refinement or modification. For example, as described above, the Norwalk virus is grouped in the A/B binding group, but it apparently has an alternative binding mode. Thus the final assignment of this strain could be dependent on a comparison of the binding modes of related strains in this genogroup. In addition, variation of binding affinity to the A antigen was found among different GII.4 epidemic variants [22]. Whether these variants should be classified in subgroups within the H-binding group needs to be determined. Furthermore, variable binding affinities of NoVs with similar genetic backgrounds to different antigens were reported [22–29] and need further study. Finally, GII.3 NoVs revealed an intermediate binding pattern between the A/B- and H-binding groups. They bind weakly to the H antigen in addition to strongly binding the A/B antigen[5]. Whether these strains should be grouped in the A/B- or H-binding groups needs to be determined. Thus, while the classification of the three binding groups provides a valuable tool, variations or exceptions exist. Further studies to characterize the observed variations, particularly those possibly associated with the minor binding residues, and their impacts on the clinical outcomes and epidemiology are of high importance.

## Conservation of binding interfaces

Sequence alignments of the P domain showed that the amino acid compositions of the HBGA-binding interfaces are highly conserved among strains within but not between the two major genogroups (GI and GII) of human NoVs [30]. All 8 genotypes of GI share similar residue composition of the binding interfaces with the Norwalk virus, in which D327, H329, W375, S377 and S378 (numbered according to Norwalk virus) are highly conserved, while Q342 and D344 are modestly conserved [30]. Similarly, all 17 GII NoVs except GII.13 share similar amino acid compositions of the HBGA binding interface with VA387 and VA207, in which all 7 amino acids (T344, R345, A346, H347, D374, S442 and G443, number according VA387) are highly conserved [30]. The importance of the conserved residues in binding to HBGA has been confirmed by site-directed mutagenesis and functional analysis on representative strains in both genogroups [17,20,30,31].

To summarize the available data, viruses in each of the two genogroups have unique HBGA binding interfaces formed by a different set of amino acids but interact with common HBGAs. Functionally, similar binding interfaces within each genogroup can accommodate different HBGAs, such as those of VA387 and VA207 (Figure 1c and 1f). However, viruses with distinct binding interfaces also can bind the same types of HBGAs as shown by the binding of Norwalk virus (GI) and VA387 (GII) to the H and A antigens (Figure 1b and 1c) [17,19]. These interactions follow the typical key-to-lock principle. A structural or conformational fit between the binding interface (the lock) and the HBGA (the key) is a prerequisite for binding, which is apparently independent from the genetic makeup and the primary sequences of the viral capsids. These features pave the base of the convergent evolution for NoVs which explains the diverse members of NoVs as a potential result of the selection by the polymorphic HBGAs of the human host.

## Implications for evolution of NoVs

The high conservation of the HBGA binding interfaces indicates an important role of human HBGAs in NoV evolution. The segregation of the primary sequences of the genomes, the difference of overall structures of the capsids, and the distinct structures, amino acid compositions and binding modes of the HBGA binding interfaces between GI and GII strongly suggest that these two genogroups were two species that may have developed their HBGA binding ability independently either prior to or once they become human pathogens. This hypothesis is supported by the fact that the two human genogroups, GI and GII, are phylogenetically separated by the bovine (GIII) and murine (GV) NoVs (Figure 3) [32–34]. Thus, the two genogroups most likely converged onto human HBGAs as a common attachment factor during human infection. As a result, the two genogroups developed two distinct binding interfaces to accommodate the same repertoire of the human HBGAs.

The concept of the convergent evolution towards using HBGAs as attachment factors might be extended to other members of caliciviruses and even to other viral and bacterial pathogens. Accumulating data shows that many other viral and bacterial pathogens recognize human HBGAs as receptors (reviewed in [35,36]). Thus, the human HBGAs could be a convergent factor for those pathogens as well. Recent data indicate that requiring a carbohydrate as a receptor or an attachment factor could be a common feature for caliciviruses as members in the *Lagovirus*, *Recovirus*, *Vesivirus* and *Norovirus* genera have been found to bind carbohydrates [37–43]. Recognizing that carbohydrate receptors could promote convergent evolution could bring new insights in understanding the evolution of diverse microbial pathogens that utilize carbohydrates or HBGAs as receptors or attachment factors.

The selection of human NoVs by HBGAs might be multi-functional owing to the polymorphic nature of the human HBGAs. Each of the three types of saccharides of the HBGAs denoting the A/B, H and Lewis epitopes (Figure 2) represents one such selection of convergent lineages within each of the two major human NoV genogroups. This assumption is supported by the finding of A/B-, H- and Lewis-binding NoVs in both GI and GII genogroups, although a typical GI capable of binding H-related HBGAs remains to be identified. Thus, each cluster (genotype) is expected to have a common HBGA binding profile that fits in one of the three binding groups (Figure 2). Determination of such HBGA binding profiles for all clusters of human NoVs would be important for phenotypic classification of NoVs.

## Implications for epidemiology

The structural basis of the NoV-HBGA interaction and its implication in evolution of NoVs provides new insights into the epidemiology of human NoVs. First, the separation of the two genogroups into two independent species (lineages) highlights their difference in genetic makeup, biological properties, and thus epidemiological outcomes. The GI NoVs are more frequently detected in the environmental contamination than the GII NoVs, such as in waste water [44,45]. However, the GII NoVs cause the majority (up to 92%) of reported NoV gastroenteritis incidents [46–48]. While the precise reason for these differences in infection remains unknown, their distinct HBGA binding interfaces and binding modes to HBGA could play a role (Figure 1). The GI Norwalk virus interacts with HBGAs in a more ‘upright’ mode, in which less saccharide is involved in binding through fewer interactions to the capsids (Figure 1a and 1b)[17,20]. In contrast, HBGAs bind to the GII viruses in a more ‘lying-down’ mode (Figure 1c to 1f) [1918], resulting in more interactions and possibly an overall binding mode with optimal or maximal binding affinities to GII NoVs and therefore possibly higher epidemiologic consequences. Thus, the separation of the two genogroups allows an independent definition of their epidemiologic properties.

The split of the A/B- and H-binding groups in the proposed model from the original A/B binding group[3] could also help in understanding NoV epidemiology. Since ABO secretors are the most popular blood type, representing 80% of the European and North American populations[35], strains recognizing these blood types should be more prevalent than other strains binding to less popular HBGA types. According to the proposed model, NoVs in the A/B-binding group bind the A and/or B but not O secretors and thus might have a narrower target population compared with those in the H-binding group. As a result, strains in the A/B-binding group might be less prevalent compared to strains in the H-binding groups, such as many members in the GII.4 cluster. A similar principle also applies to the Lewis-binding group. Thus, this model could provide a novel approach to understand and estimate the disease burden of NoVs.

Convergent evolution might also help to explain epochal evolution of GII.4 previously discussed in the literature [34,49–52]. In support of epochal evolution, it was proposed that an immune escape mutant might gain a new HBGA binding phenotype [24,25]. While this hypothesis is attractive and mutations in or near the HBGA binding interfaces have been observed in the laboratory that then affect binding specificity [24,30,31], the key question is whether such mutants can become dominant in epidemics. The chances for an immune escape mutant to gain a new binding pattern and become a new lineage are low because there is limited sequence space available for such mutations under the selection by human HBGAs. Even if such mutants occur they might not be able to become prevalent in epidemics because of their less competitive phenotypes. In contrast, a current NoV with a narrow host HBGA spectrum might emerge and cause a major epidemic if it could broaden its HBGA spectrum similar to that of the GII.4 NoVs. The GII.3 Mx NoV binds strongly to

A and B and weakly to O secretors [5] and GII.3 is the second most predominant cluster after GII.4 [53–55]. Thus, it would be interesting to continually monitor changes in HBGA binding specificity during epidemic surveillance of GII.3 NoVs in the future in order to determine if changes in binding specificity correlate to changes in disease burden.

Phylogenetic analysis showed that the GII.4 lineage emerged shortly after GII NoVs began infecting humans [32–34]. The H-related epitopes are the most abundant human HBGAs, which might make them a good choice to be targeted by the ancestors of the GII lineage. In fact, the amino acid compositions of the HBGA binding interfaces are nearly a 100% conserved among all known GII.4 variants found in the past decade [22,30]. This suggests that the GII.4 genotype could be the only genotype with an optimal survival phenotype since its start and no subsequent competitors afterword [32–34] (Figure 3). Thus, while future studies based on more extensive surveillance are needed, GII.4 strains with the current H-binding phenotype that is able to bind A/B/O secretors would be expected to continue to be a predominant cause of epidemics in the future.

## Concluding remarks

The crystal structure analysis of the NoV P domain-HBGA complex has led to the elucidation of the interaction between NoVs and variable HBGAs. Further sequence comparison in combination with phylogenetic analysis has resulted in the discovery of high conservation of the HBGA binding interfaces and segregation of two binding interfaces in the two genogroups of human NoVs. These new advances have led to the concept of convergent evolution of NoVs via selection by human HBGAs. This new concept partially explains several important aspects of human NoVs, including epidemiology, classification, disease control and prevention. However, the whole picture of NoV-host interaction and their clinical outcomes are far more complex than what is described here. Further understanding of this complicated picture will rely on new research on (i) structures of the HBGA binding interfaces of more representative NoVs, particularly those in the A/B-binding group in GII and those in H-binding group in GI; (ii) development of more clinically relevant assays, such as saliva- and hemoagglutination assays, on NoV-HBGA interaction; (iii) identification and characterization of other host receptors or co-receptors that could also affect the host susceptibility; and (iv) the establishment of a cell culture and/or small animal model for NoV infection. In addition, several outstanding questions on NoV epidemiology and evolution are listed in Box 1. Furthermore, it should be noted that this article focuses on a single important genetic factor, the HBGAs, and additional factors, such as the host immunity, may also play a role in NoV evolution and epidemiology.

## Acknowledgments

We thank Dr. Yutao Chen from the Institute of Biophysics, Chinese Academic of Sciences for help in preparing the figures. The research in Tan and Jiang's laboratory was supported by the National Institute of Health, the National Institute of Allergy and Infectious diseases (R01 AI37093, R01 AI055649 and R01 AI089634), and the Department of Defense (PR033018) to X.J. and by an Institutional Clinical and Translational Science Award, NIH/NCRR Grant Number 1UL1RR026314-01 to M. T.

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**Box 1**

Outstanding questions

- Is there a possibility that the observed increase of NoV activities in the past decades were not real epidemics and were rather natural fluctuations caused by non-viral factors, such as social factors?
- Although the chance is low, can a non-GII.4 cluster gain the binding ability to the H related HBGAs and cause epidemics similar to GII.4?
- Caliciviruses apparently have a broad host range, how did the GI and GII NoVs make the species jump into humans and could they go back to animals?
- With an estimated lower level of herd immunity, how can GII.4 NoVs cause major epidemics every 1–2 years which is faster than influenza?

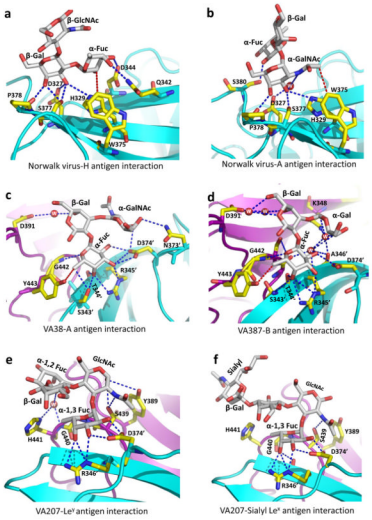


**Figure 1.**

Extensive interaction networks between norovirus P dimers and HBGA oligosaccharides. **(a)** and **(b)** Show the interaction networks between Norwalk virus (GI.1) P dimer and the H (a) and A (b) oligosaccharides. **(c)** and **(d)** Show the interaction networks between VA387 (GII. 4) P dimer and the A (c) and B (d) oligosaccharides. **(e)** and **(f)** Show the interaction networks between VA207 (GII.9) P dimer and the $Le^y$ (e) and sialyl $Le^x$ (f) oligosaccharides. Different NoVs target different residues of HBGAs as major contacts. Norwalk virus binds to H antigen through β-Gal-α-1,2 Fuc (a) and to A antigen through α-GalNAc (b). VA387 binds to both A and B antigens through the same α-1,2 Fuc (c) and (d), while VA207 binds to both $Le^y$ and sialyl-$Le^x$ through the same α-1,3 Fuc (e) and (f). The HBGA oligosaccharides are in stick representation, in which the carbon, oxygen and nitrogen atoms are in grey, red and blue, respectively. The two P protomers are in ribbon representation in cyan and purple, respectively. The amino acids involved in HBGA interactions are labeled (primes indicate the second P protomer) and shown in stick representation with carbon, oxygen and nitrogen atoms in yellow, red and blue, respectively. Hydrogen bonds are indicated by blue dotted lines, while cation-π [(a) and (b)] and van der Waals [(c) and (d)] interactions are indicated in red dotted lines. The water molecule in a water-bridged interaction is shown by a red ball and labeled by W. Abbreviations: α-1,2 Fuc, α-1,2 fucose; α-Gal, α-galactose; α-GalNAc, α-N-acetyl galactosamine; β-Gal, β-galactose; β-GlcNAc, β-N-acetyl glucosamine; α-1,3/4 Fuc, α-1,3/4 fucose. The figures were made by software PyMOL (DeLano Scientific LLC) using PDB files downloaded from Protein Data Bank (http://www.pdb.org): 2ZL6 (a), 2ZL7 (b), 2OBS (c), 2OBT (d), 3PUN (e), and 3PVD (f). The detail structural data of interaction between these NoVs and HBGAs were described in [17,18,20,56].

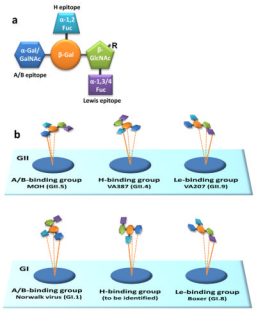


**Figure 2.**

Model of NoV-HBGA interaction. **(a)** Schematic of a HBGA product with the five saccharides indicated in different shapes and colors. The three major saccharide determinants of human blood types, H, A/B, and Lewis epitopes are indicated. R represents the position that the oligosaccharide links to the lipid or protein backbone. **(b)** Classification of GII and GI NoVs into three binding groups (A/B-, H- and Lewis [Le] binding groups) and their targets at the A/B-, H- and Lewis epitopes, respectively. HBGAs in (b) are shown in the same shape and color scheme as in (a). The thick lines indicate the major, while the thin dashed lines indicate the minor interactions between the viruses (blue round shapes) and HBGAs. Abbreviations: α-1,2 Fuc, α-1,2 fucose; α-Gal, α-galactose; α-GalNAc, α-N-acetyl galactosamine; β-Gal, β-galactose; β-GlcNAc, β-N-acetyl glucosamine; α-1,3/4 Fuc, α-1,3/4 fucose.

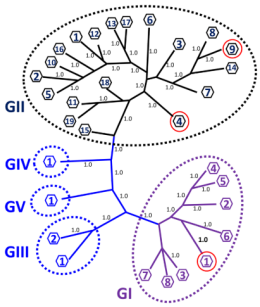


**Figure 3.**

Phylogenetic relationship of the five known genogroups (GI to GV) within the Norovirus genus based on the complete capsid amino acid sequences of NoVs strains. The tree was constructed with the structural alignment by using MrBayes program [57]. Tree topology was evaluated on the base of 1,000,000 generations (posterior possibility, 1.00. equals 100% out of 1,000,000). Numbers in pentagon boxes are the cluster numbers within the genogroups (G). The red circles indicate that crystal structure of the HBGA-binding interface of one representative NoV in this genoclusters has been determined. Figure is adapted with permission from [32]. GII.18 and GII.19 were added to the figure according to Wang et al.[58].