Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Glass RI, Parashar UD, Estes MK. Norovirus gastroenteritis. N Engl J Med 2009;361:1776-85.

Supplement or Appendix

Table 1. Noroviruses used to construct the phylogenetic tree

Table includes reference NoV strains, their GenBank Accession numbers and the location name of other well-known viruses in the genogroup.

Supplemental Figure 1. Histoblood Group Antigens and Noroviruses.

Top left panel: Flow chart of histoblood group antigen synthesis. Only type-1 histoblood group antigens are shown. Histoblood group antigens are oligosaccharide epitopes with varying carbohydrate compositions and linkages between them. They are synthesized by sequential addition of a monosaccharide starting from a precursor oligosaccharide with a terminal [β-galactose (1-3)-N-acetyl-glucosamine] disaccharide (type 1). This modification is made by glycosyl transferases such as fucosyl transferases (FUT) 2 and 3 that catalyze linkage-specific addition of an α-fucose (α-fuc) residue, and enzymes A and B, which catalyze the linkage specific addition of N-acetyl-galactosamine (GalNAc) and β-galactose (β-gal), respectively. Carbohydrate addtions by enzymes A, B and FUT2 to the β-galactose residue of the precursor, result in (type 1) ABH histoblood group antigens or the precursor disaccharide results in Lewis antigens (Lewis antigens (Le^a, Le^b, ALe^b, BLe^b). A similar pathway with the precursor having 1-4 linkage results in type 2 HBGA and Lewis antigens (Le^x and Le^y) (not shown) (Modified from Tan M, Jiang X: Norovirus and its histo-blood group antigen receptors: an answer to a historical puzzle; copyright *Trends in Microbiology* June 2005, pgs 285-93, with permission from Elsevier LTD) (1).

Middle Top Panel: Histo-blood group antigen binding in the P2 domain of GI.1 Norwalk virus (green ribbon backbone) and GII.4 VA387 virus (yellow ribbon backbone). The carbohydrate binding sites in these two viruses is distinctly different both in its location and in its structural characteristics. The NV binding site located on the other site of the P domain is formed by residues that project from a well structured anti-parallel β-sheet in contract to the reported GII.4 binding site that is predominantly formed

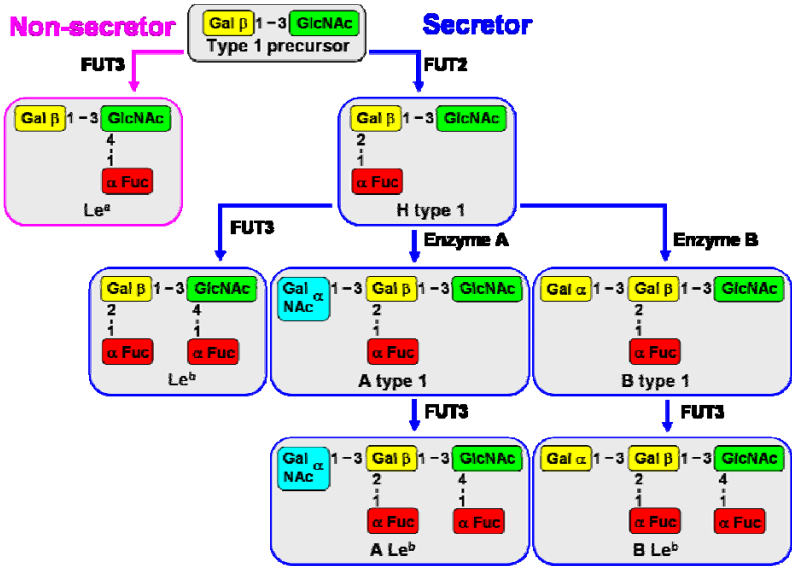
by residues from two surface-exposed loops that are close to the P domain dimeric interface. **Right Top Panel**: In spite of the distinct binding features of these two NoVs with HGBAs, the location of the binding sites in both GI.1 (red circles) and the GII.4 (blue circles) viruses are close to one another in the context of the P2 domain dimeric interface (black dotted line). **Bottom Panels**: Structural basis of HGBA recognition for Norwalk virus. Molecular interactions at the ligand binding site between the Norwalk virus P domain residues and H-type 1 pentasaccharide (left panel) and A-type trisaccharide (right panel). Hydrogen bonds and hydrophobic interactions are shown in black and red dotted lines, respectively, along with water molecules as red spheres. Despite differences in their carbohydrate sequence and linkage, both H-type 1 and A-type HBGAs bind to the same surface-exposed site in the P2 subdomain and project outward from the capsid surface consistent with their possible role in initiating cell attachment. In the binding site, precisely juxtaposed polar side chains that engage the sugar hydroxyls of the HBGA in a cooperative hydrogen bonding and a His/Trp pair that is involved in a cation-π interaction contribute to selective and specific recognition of A- and H-type HBGAs. H-type HBGA pentasaccharide binds to the P domain with its two terminal residues, α-Fuc and β-Gal. Interactions with β-Gal are predominantly through hydrogen bonds, whereas α-Fuc is involved in both hydrogen bond and hydrophobic interactions (**bottom, left panel**). Involvement of only the terminal fucose and galactose of the H-type 1 in the binding is consistent with the observation that NV also interacts with other HBGAs that have the same two carbohydrates as terminal residues, such as H-types 2 and 3, Le^y, and Le^b (2-4)

The A-type differs from H-type by having an N-acetyl-galactosamine (GalNAc) residue attached to the β-Gal through an α1-3 linkage (**Top Panel, left diagram**). In the P dimer-A-type (trisaccharide) complex structure, the terminal GalNAc and α-Fuc moieties that are attached to the central β-Gal make all the contacts with the P domain (**bottom, right panel)**. The hexose ring of the GalNAc occupies the same position as the penultimate β-Gal residue of the H-type with essentially the same cooperative hydrogen

bond interactions. As a result, the acetamido group of the GalNAc is positioned in the same direction as the α-Fuc attached to β-Gal in the H-type 1 HBGA such that the acetamido group makes the same hydrogen bond and hydrophobic interactions as the α-Fuc in the H-type 1. Thus, the binding site in NV is optimally configured to primarily recognize a terminal Gal-Fuc or Gal-acetamido (as in GalNac) combination through well coordinated hydrogen bond and hydrophobic interactions, thus limiting the number of HBGAs with which these NoVs can interact. Hydrogen bonding interactions with Gal alone are not sufficient and hydrophobic interactions involving (α-Fuc or acetamido) are required for efficient binding. For instance, NV binds to H-types 2 and 3, and Lewis blood group antigen Le^b but not to Le^a (2-4). The main difference between the Le^a and other H-type HBGAs is that Le^a does not have the terminal fucose residue attached to β-Gal. Also, as mentioned earlier NV does not bind the B-type HBGA, which differs from the A-type by having a terminal α-Gal instead of GalNAc. (1).

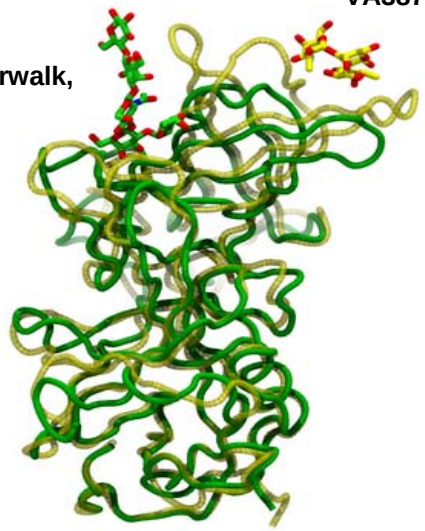
Supplementary Table 1. Noroviruses used to construct phylogenetic tree		
Reference Norovirus	**GenBank Accession**	**Other well-known viruses in genotype**
Hu/NoV/GI.1/**Norwalk**/1968/US	AAB50466	
Hu/NoV/GI.2/Southampton/1991/UK	AAA92984	
HuNoV/GI.3/Desert Shield 395/1990/SA	AAA16285	
HuNoV/GI.4/Chiba407/1987/JP	BAB18267	
HuNoV/GI.5/Musgrove/1989/UK	CAB89095	
HuNoV/GI.6/Hesse 3 (BS5)/1997/DE	AAC64603	
HuNoV/GI.7/Winchester/1994/UK	CAB89090	
HuNoV/GI.8/Boxer/2001/US	AAN15140	
HuNoV/GII.1/Hawaii/71/US	AAB97768	
HuNoV/GII.2/Snow Mountain/1976/US	AAB16915	Melksham/94
HuNoV/GII.3/Toronto24/1991/CA	AAA18930	
HuNoV/GII.4 Bristol/1993/UK	CAA54134	Lordsdale/94; Grimsby/96; VA387/98; Farmington Hills/02; Hunter/04; 2006a; 2006b
HuNoV/GII.5/Hillingdon/1990/UK	CAB89088	
HuNoV/GII.6/Seacroft/1990/UK	CAB89101	
HuNoV/GII.7/Leeds/1990/UK	CAB89089	
HuNoV/GII.8/Amsterdam/1998/NL	AAF05820	
HuNoV/GII.9/VA97207/1997/US	AAK84676	
HuNoV/GII.10/Erfurt546/2000/DE	AAL18874	
PoNoV/GII.11/SW918/1997/JP	BAB83516	
HuNoV/GII.12/Wortley/1990/UK	CAB89099	
HuNoV/GII.13/Fayetteville/1998/US	AAM56034	
HuNoV/GII.14/M7/1999/US	AAN05735	
HuNoV/GII.15/J23/1999/US	AAN05736	
HuNoV/GII.16/Tiffin/1999/US	AAS86789	
HuNoV/GII.17/CS-E1/2002/US	AAS86786	
PoNoV/GII.18/OH-QW101/2003/US	AAX32877	
PoNoV/GII.19OH-QW170/2003/US	AAX32883	
Bo/NoV/GIII.1/Jena/1989/DE	CAA09481	
Bo/NoV/GIII.2/CH126/1998/NI	AAL40190	Bovine Newbury2/76/UK
Hu/NoV/GIV.1/Alphatron 98-2/1998/NL	AAF05819	
Lion/NoV/GIV.2/387/06/IT	ABR15783	Canine NoV/170/07/IT
Mu/NoV/GV.1/MNV-1/2003/US	AA063099	

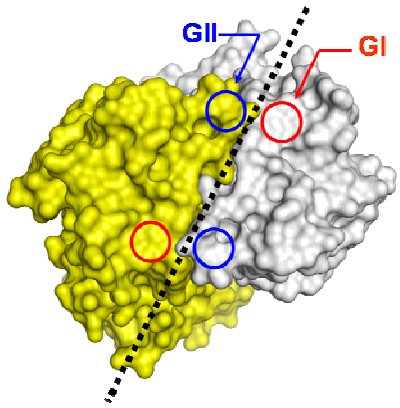
Country abbreviations: CA, Canada; DE, Germany; IT, Italy; JP, Japan; NL, Netherlands; SA, Saudi Arabia; US, United Kingdom; US, United States.
Species abbreviations: Bo, bovine; Ca, canine; Hu, human, Mu, murine; Po, porcine
Compiled from (5-8)

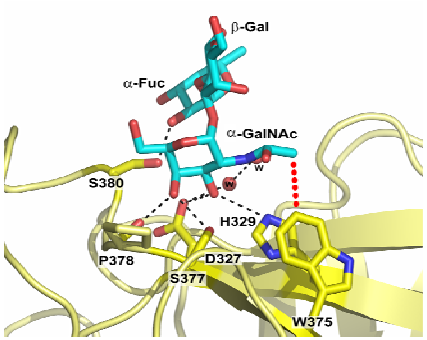


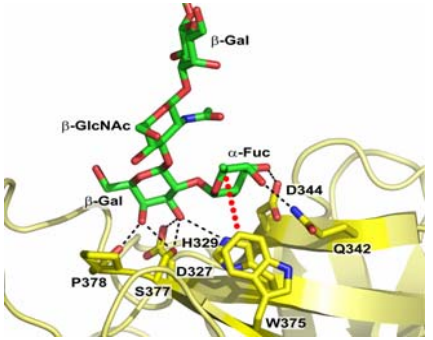
H type 1 (GI.1 Norwalk, Green)

A type (GII.4 VA387, Yellow)









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