Natural Variation in Norovirus Strains Impacts Breadth of Virus-Ligand Interactions

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INTRODUCTION

Diarrheal diseases account for 1 in 9 deaths in young children globally[1]. Norovirus is the leading cause of medically attended acute gastroenteritis in children under 5 years of age and the leading cause of viral acute gastroenteritis outbreaks in the United States[2].

Human norovirus infection is mediated by host expression of histoblood group antigen ligands, a diverse family of molecules under host genetic control, and host cofactors like bile that are present within the area of viral infection[3, 4]. Virus-ligand interactions define susceptible populations.

Natural genetic variation occurs within surface exposed amino acids on noroviruses that interact with binding ligands, shaping the pool of susceptible hosts to various norovirus strains.

Our goal is to analyze how natural variation within two GII.12 norovirus genotype isolates impacts virus-like particles (VLP) binding to different ligands.

Research Question: Can a singular amino acid change within the GII.12 norovirus genotype impact breadth in virus-like particle ligand interactions?

Impact: Understanding how natural variation within noroviruses influences ligand recognition and subsequently host susceptibility patterns can support interpretation of global virus sequence surveillance systems aimed at predicting potential epidemics and inform vaccine formulations currently in development to mitigate norovirus epidemics.

RESULTS - Carbohydrate Binding Profile of Mutants

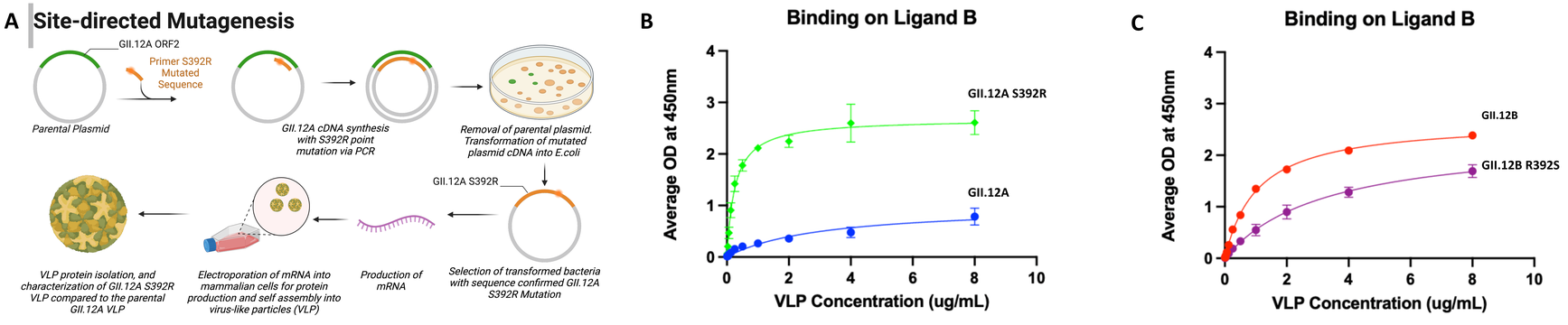
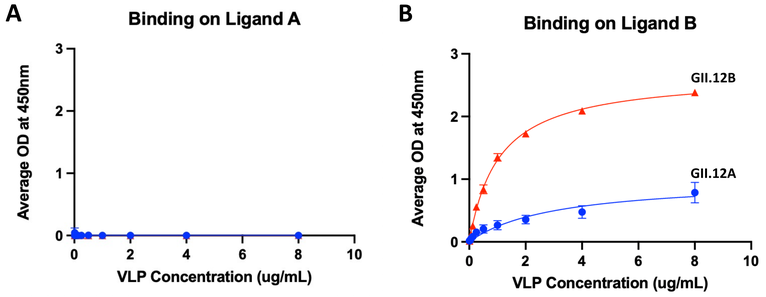
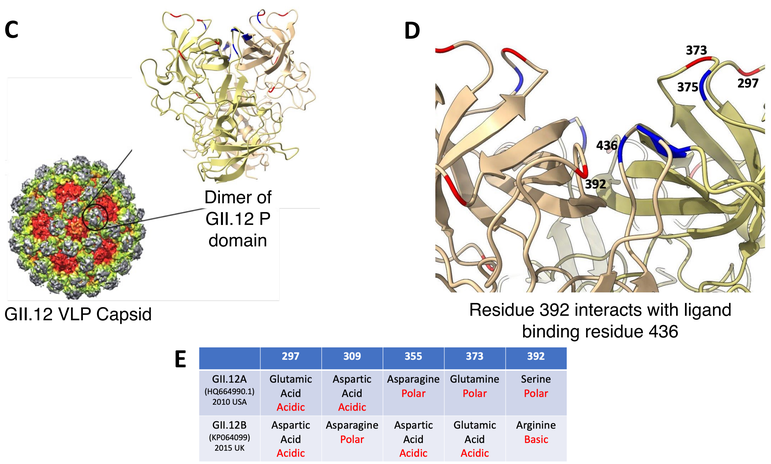


Figure 2. Residue 392 mediates affinity to Ligand B.

(A) Utilizing PCR point mutagenesis, residue 392 in the GII.12A and GII.12B strains were swapped, generating the GII.12A S392R and GII.12B R392S mutants (Panel A generated via BioRender.com). **(B and C)** Titration of VLP on ligand B reveals the S392R mutation in GII.12A shows a robust increase in B-ligand recognition, while the R392S mutation in GII.12B shows decreased B-ligand recognition. Neither the mutants nor the original strains exhibited A-ligand affinity. Together, the data suggests natural variation in the 392 residue strongly influences B-ligand recognition. Curves fitted using methods in **Figure 1**.

RESULTS - Natural Variation





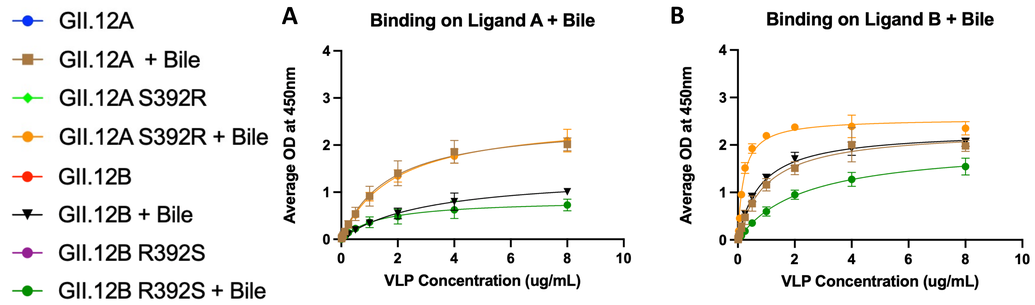
E

	297	309	355	373	392
GII.12A (HQ664990.1) 2010 USA	Glutamic Acid Acidic	Aspartic Acid Acidic	Asparagine Polar	Glutamine Polar	Serine Polar
GII.12B (KP064099) 2015 UK	Aspartic Acid Acidic	Asparagine Polar	Aspartic Acid Acidic	Glutamic Acid Acidic	Arginine Basic

Figure 1. Natural variation of amino acids within GII.12 strains of human norovirus leads to differential carbohydrate binding.

Titration of VLP against ligands A and B. **(A)** Neither strain shows affinity to ligand A. **(B)** GII.12B shows high affinity to ligand B while GII.12A shows moderate affinity to ligand B. Optical Density (OD) data were complied from three independent runs per VLP. OD were fit utilizing a one-site specific binding curve via Graphpad Prism 10 (Version 10.2.0) with error bars representing the standard deviation from the mean. **(C and D)** Natural variation between GII.12A and GII.12B occurs on surface exposed regions on the capsid P domain (Red residues). Blue residues interact with the B-trisaccharide ligand. Residue 392 in GII.12, which varies between GII.12A and GII.12B isolates **(E)**, directly interacts with the loop of GII.12 residue 436, which binds to the B-Ligand[5]. A hypothesis for the varied ligand B binding affinity is that the two positive charges at Lysine 436 and Arginine 392 in GII.12B, may repel each other altering the 436 loop conformation and improving B binding stability.

RESULTS - Carbohydrate Binding Profile Using Bovine Bile



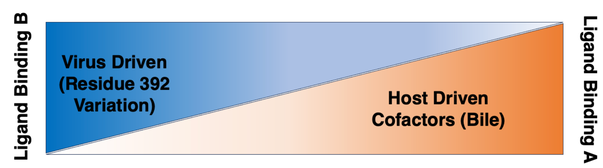


Figure 3. Bile improved A-ligand binding independently of residue 392.

(A and B) Titration of VLP against ligands A and B with 1% bovine bile solution. **(A)** Presence of bile increases A-ligand recognition for the GII.12A and GII.12A S392R strains while moderate A-ligand recognition improvement is shown for GII.12B and GII.12B R392S. **(B)** GII.12A shows significant increases in ligand B affinity while other strains binding profiles on ligand B remain relatively consistent. Assays were conducted in a similar manner in **Figures 1 and 2**, with 1% bile solution, as optimized in Mallory et al.[3] **(B)** Bile shows minimal impact on GII.12B, GII.12B R392S, and GII.12A S392R in the presence of ligand B. **(C)** B-binding recognition appears to be virus driven due to the changes in residue 392 while A-binding recognition appears to be driven by host cofactors such as bile. Optical Density (OD) data was compiled using three independent run per VLP at 450 nm. Curves were fit using methods in **Figure 1**.

CONCLUSIONS

1. Natural variation within the GII.12B isolate exemplifies increased B-ligand recognition in comparison to the GII.12A strain.
2. A singular amino acid change between the GII.12A and B isolates (GII.12A S392R and GII.12B R392S), causes a distinct switch in B-ligand recognition. GII.12A S392R shows higher B-ligand interaction in comparison to GII.12A while GII.12B R392S shows lower B-ligand interaction in comparison to GII.12B.
3. Host co-factors present in the natural environment of infection, like bile, dramatically increase the recognition of A and B ligands, particularly in the GII.12A strain. In combination with environmental factors such as bile, either natural strain variation shows affinity to ligands A and B.
4. Future studies will include evaluating the impact of 392 and other residues on viral characteristics that drive the interaction between the virus and the human population including interactions with binding ligands (host susceptibility) and antibodies (host protection).

CITATIONS

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