Immune Response and Correlates of Protection from Norovirus: Lessons from Challenge Studies and Clinical Trials

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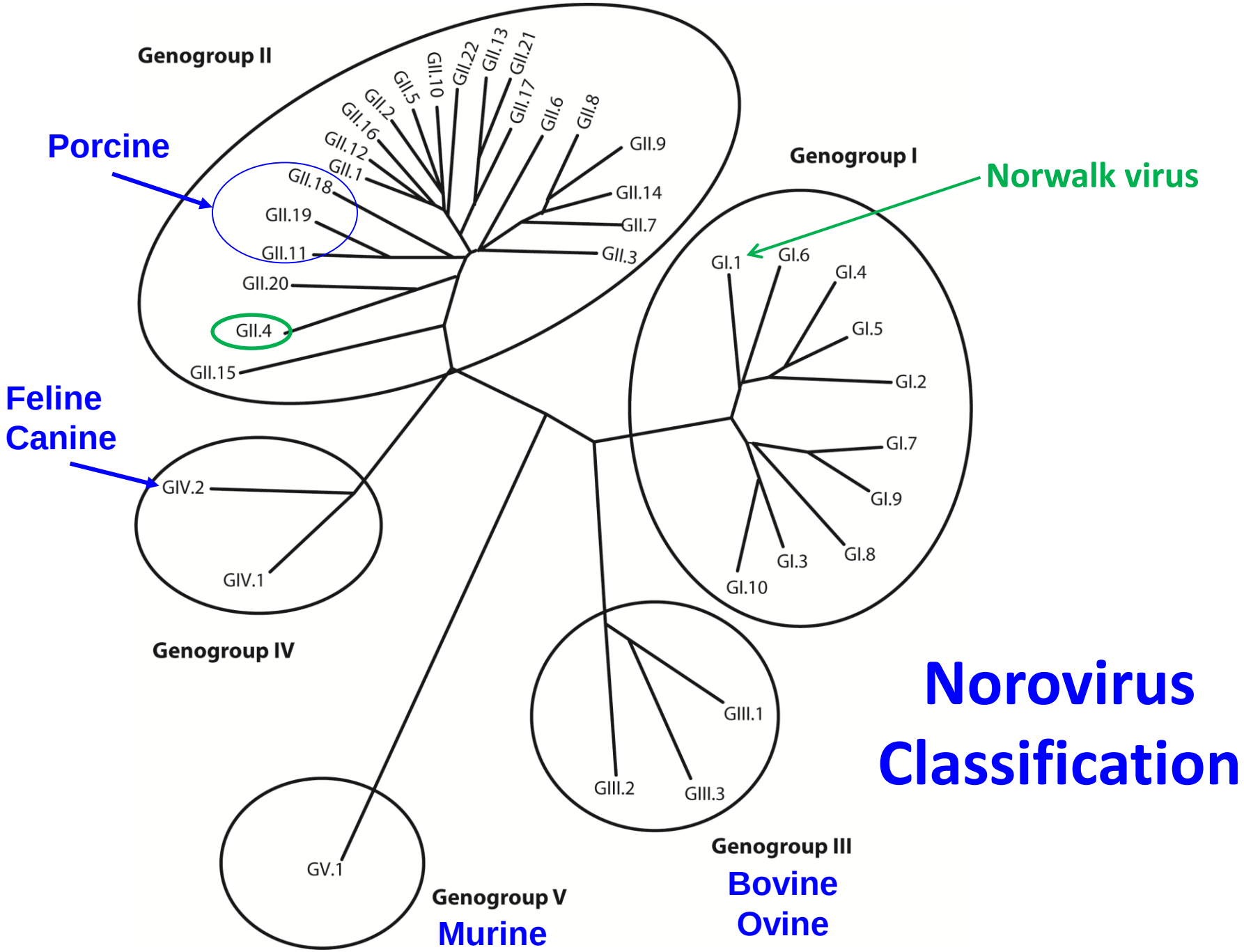
Conflict of Interest

- **Takeda Vaccines, Inc. – research support, consultant**

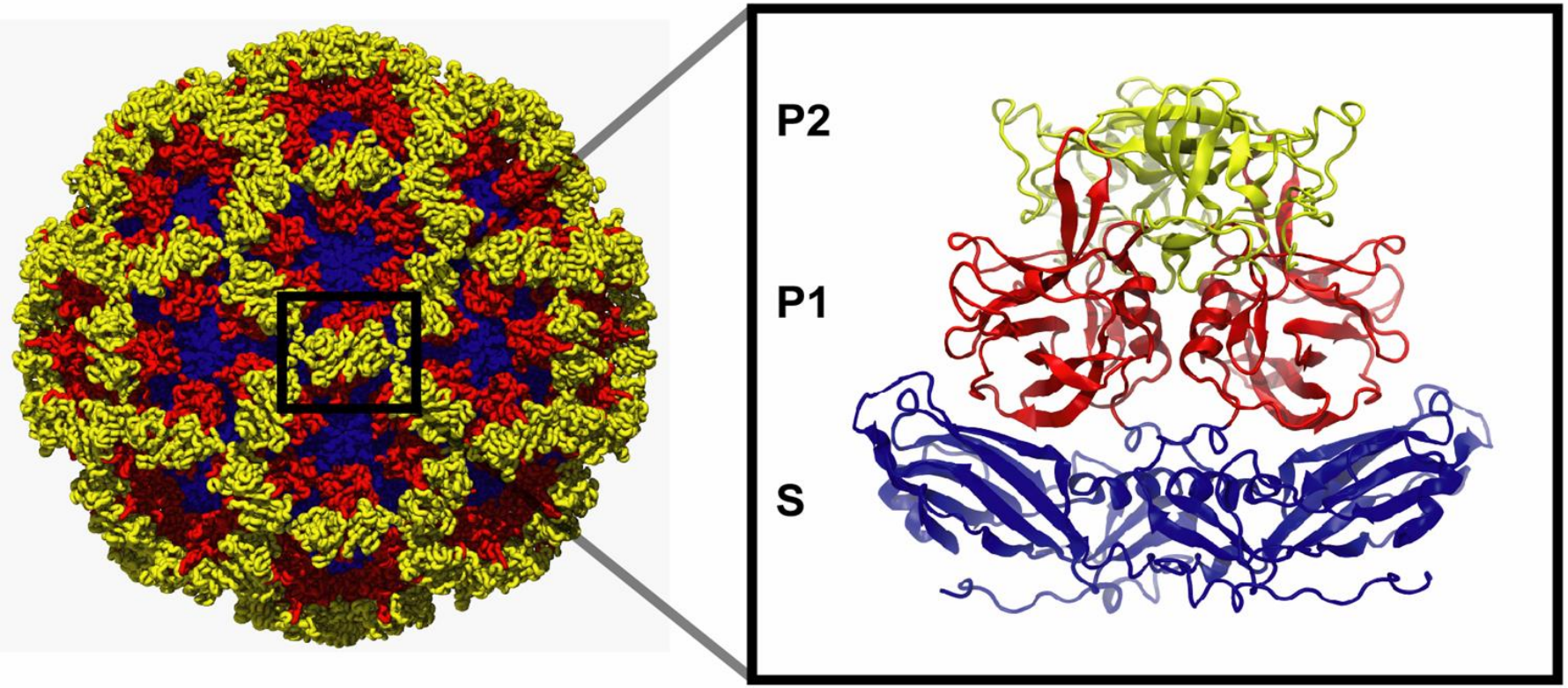
Norovirus

- **Genus in the family *Caliciviridae***
- **Prototype strain is Norwalk virus**
- **Noncultivatable (human strains)**
- **Genetically and antigenically diverse**



Ramani et al. *Curr Opin GE* 2014;30:25

One Major Capsid Protein Forms the Virus Capsid



Choi et al., PNAS 2008;105:9175

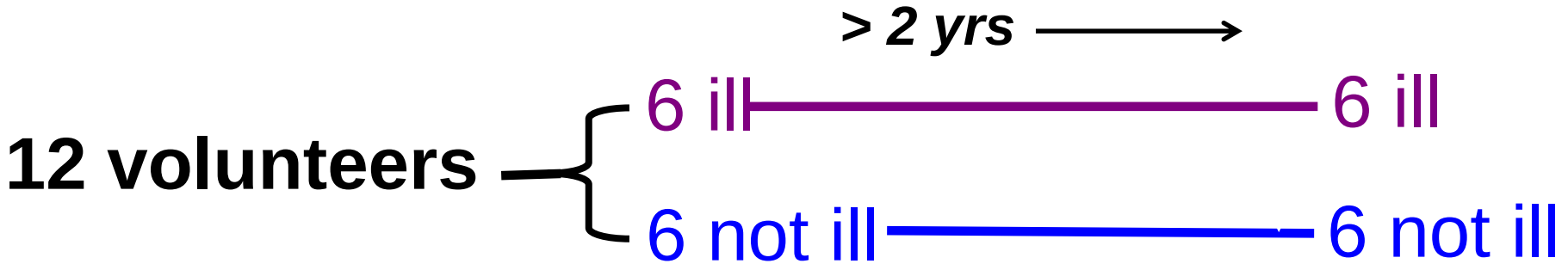
1970s – NV Challenge Studies

12 volunteers → 6 ill
12 volunteers → 6 not ill

Immunity due to previous exposure?

Parrino et al., *NEJM* 297:86 (1977)

1970s – NV Challenge Studies



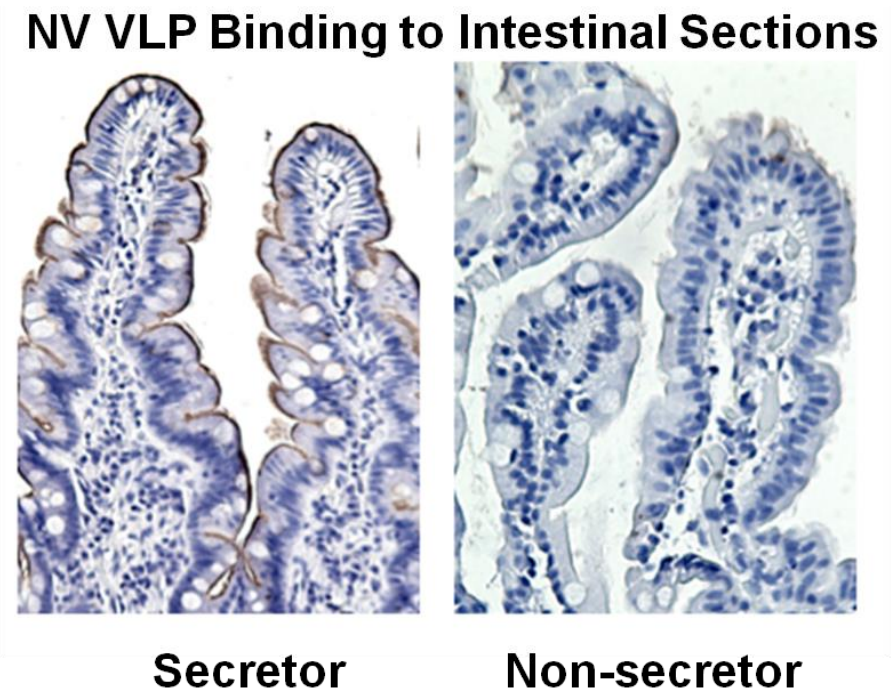
Immunity due to previous exposure?

Repeated susceptibility
Repeated resistance

Genetic factor may determine susceptibility or resistance to NV

Model for Virus-Host Cell Binding

- Norwalk VLP's bind red blood cells and intestine-derived cell lines (CaCo-2)
- HBGA's expressed on cell surface (including enterocytes) & on secreted mucins

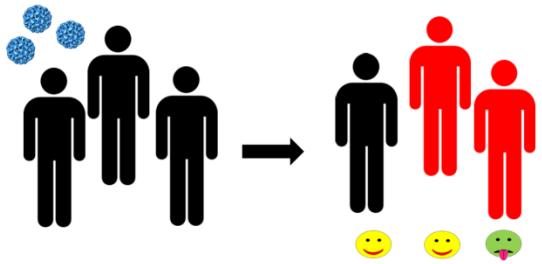


Antibody – RIA/ELISA

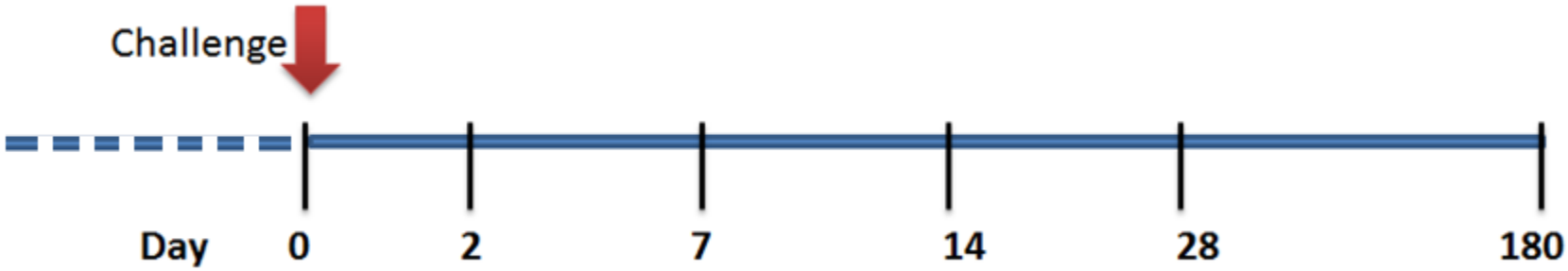
- **Higher baseline NV titer associated with lower rate of infection in Panamanian children <5 y.o** **(Ryder et al. JID 1985;151:99)**

- **No correlation between serum antibody levels and protection from experimental Norwalk virus infection** **(Greenberg et al. Persp Virol 1981;XI:163; Johnson et al. JID 1990;161:18)**

Norwalk Virus Challenge Study

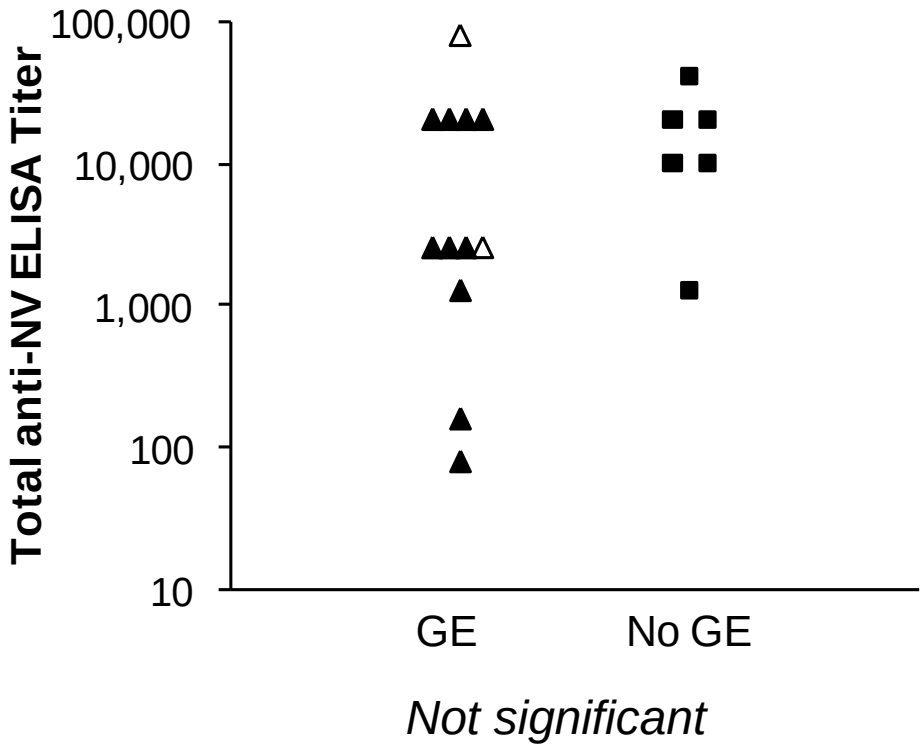


Collection of serum, stool, saliva and PBMCs



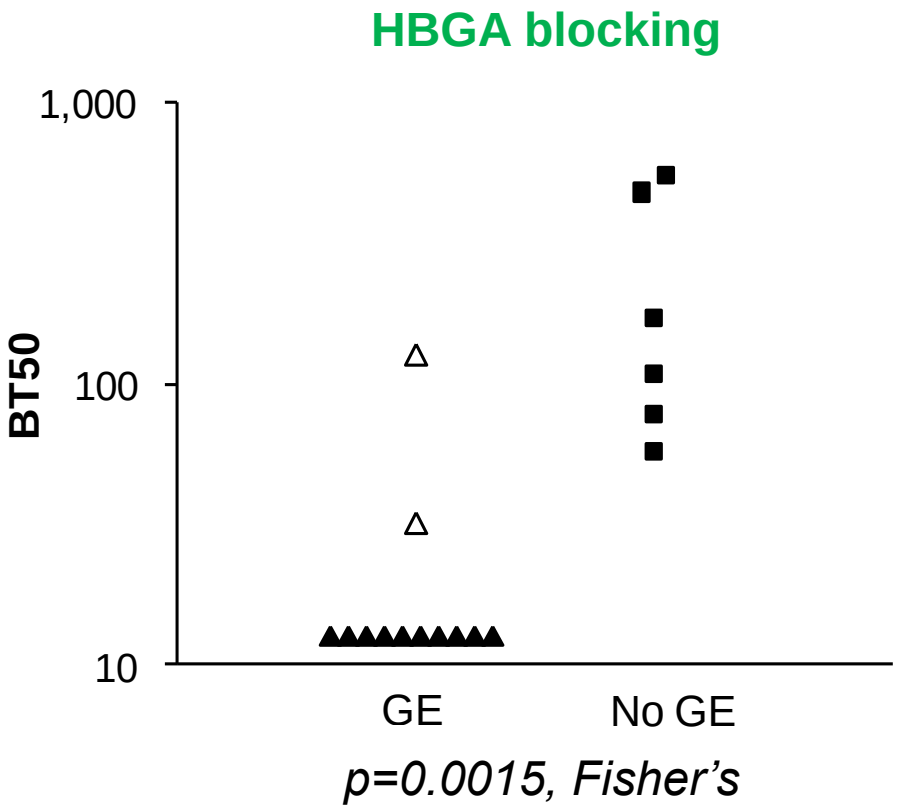
Day	Pre	2	7	14	28	180
Serology	✓	✓	✓	✓	✓	✓
Salivary IgA	✓		✓	✓	✓	
Fecal IgA	✓		✓	✓	✓	
ASC	✓		✓	✓	✓	
Memory-B cells	✓		✓	✓	✓	✓

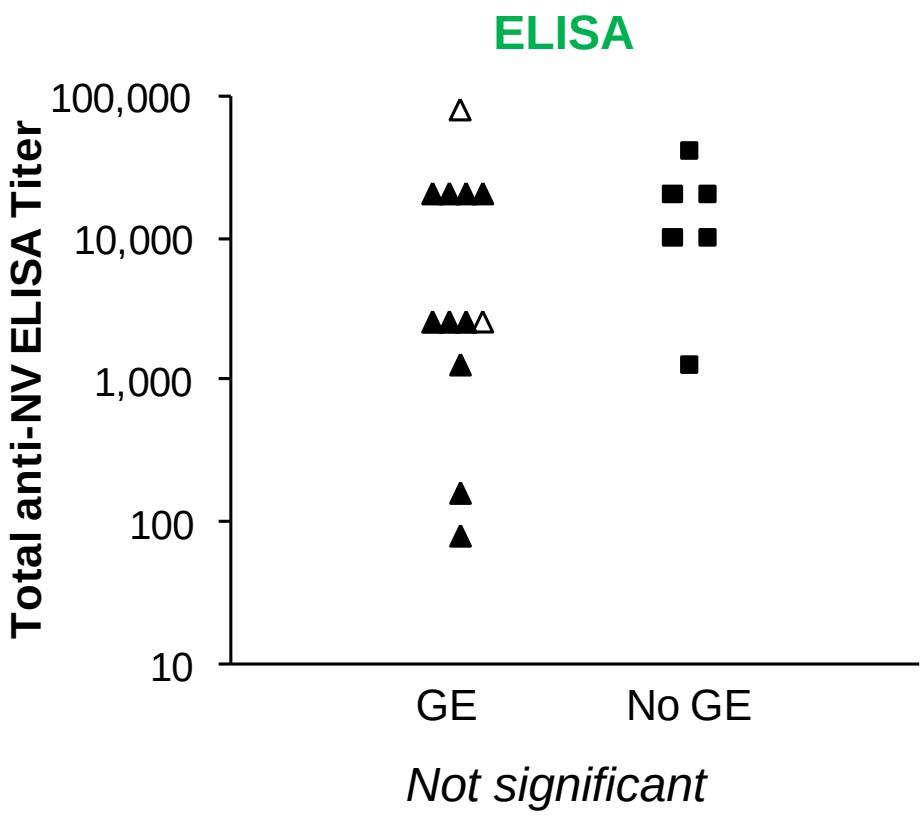
Correlation of Serum anti-NV ELISA Antibody Level with Clinical Illness after Experimental Human Infection



Reeck et al. *JID* 2010;202:1212

Correlation of Serum anti-NV HBGA Antibody Level with Clinical Illness after Experimental Human Infection



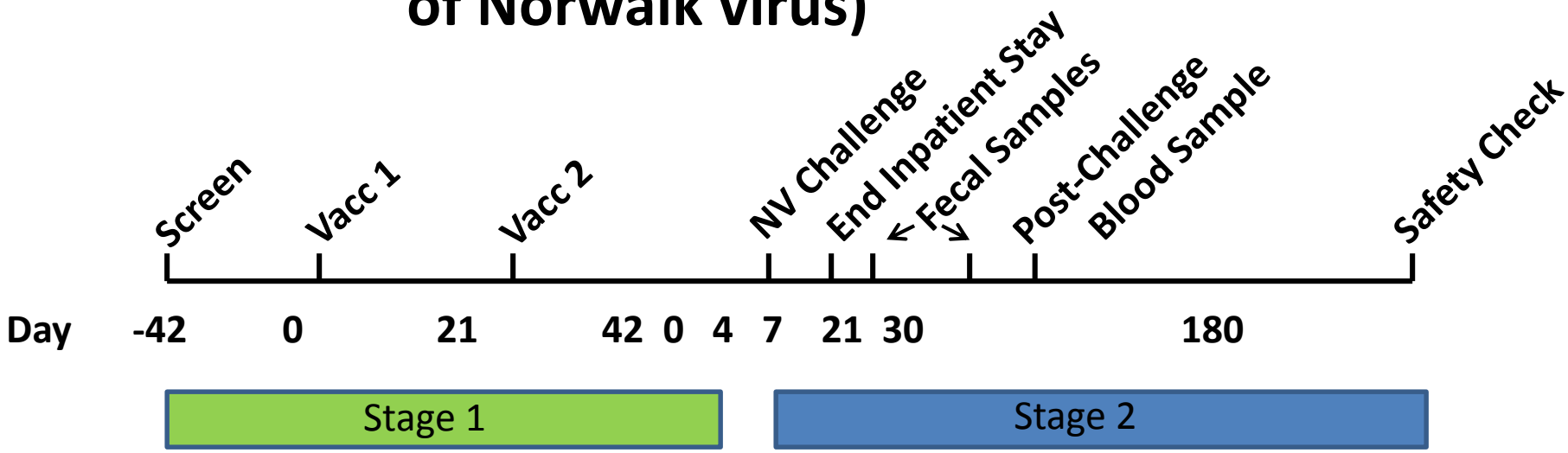


First reported correlate of protection corroborated with clinical data

Reeck et al. *JID* 2010;202:1212

IN-Delivered NoV VLP Vaccine Efficacy Trial

- **Proof of concept study (LV01-103)**
- **Two stages:**
  - **Stage 1: Vaccine or placebo (Norwalk virus VLPs)**
  - **Stage 2: Homologous virus challenge (~10 HID50 of Norwalk virus)**



Atmar et al. *NEJM* 2011;365:2178

Protection with Norwalk Virus VLP Vaccines

- **Increased pre-challenge levels of HBGA blocking antibodies correlated with protection against gastroenteritis and infection**

Outcome	Per-Protocol Analysis			
	Antibody Titer <200	Antibody Titer ≥200	Odds Ratio (95% CI)	Relative Reduction (95% CI)
	no./total no. (%)			
Viral gastroenteritis	38/60 (63)	3/17 (18)	8.1 (2.1–31.2)	72.1 (20.8–90.2)
Norwalk virus infection	49/60 (82)	6/17 (35)	8.2 (2.5–26.9)	56.8 (16.8–77.5)

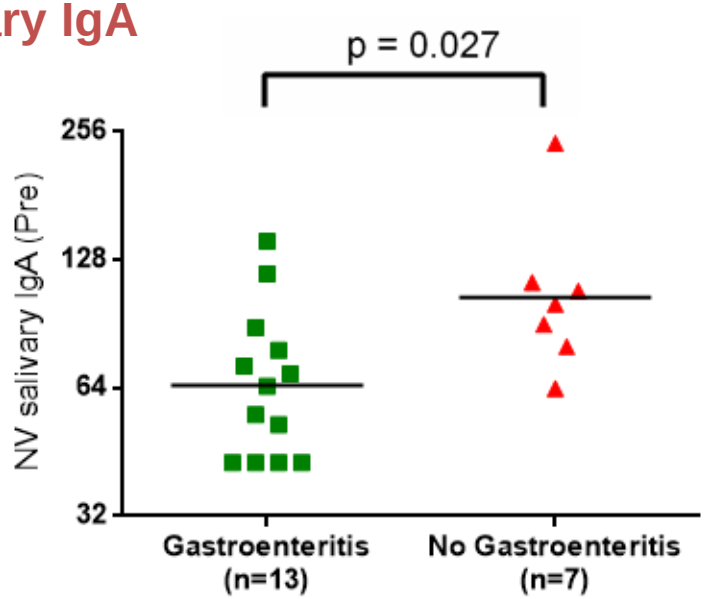
Atmar et al. *NEJM* 2011;365:2178

- **GII.4 challenge study also showed HBGA blocking antibody associated protection**

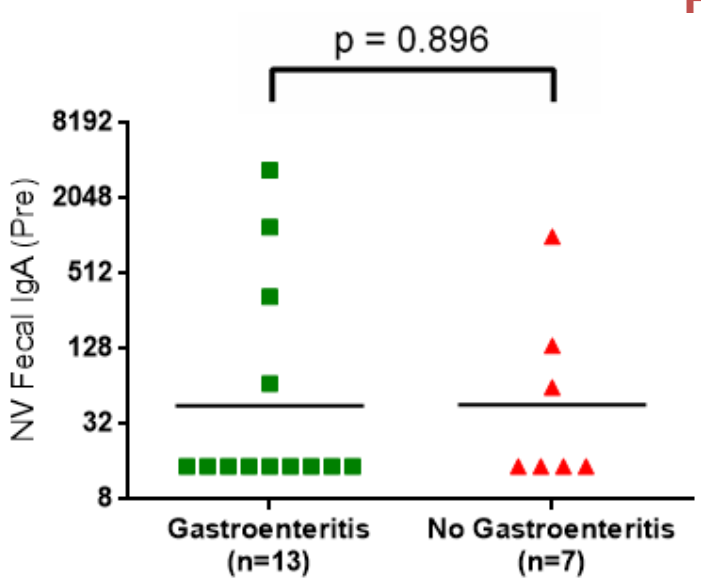
Atmar et al. *Clin Vaccine Immunol* 2015;22:923

Pre-challenge Salivary IgA is Protective against Norwalk Virus Gastroenteritis

Salivary IgA



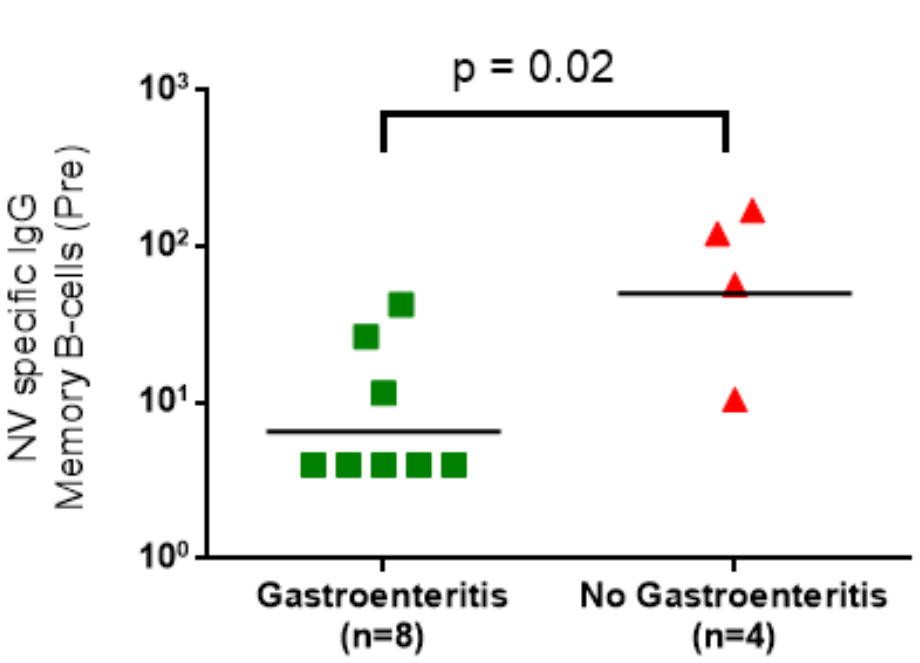
Fecal IgA



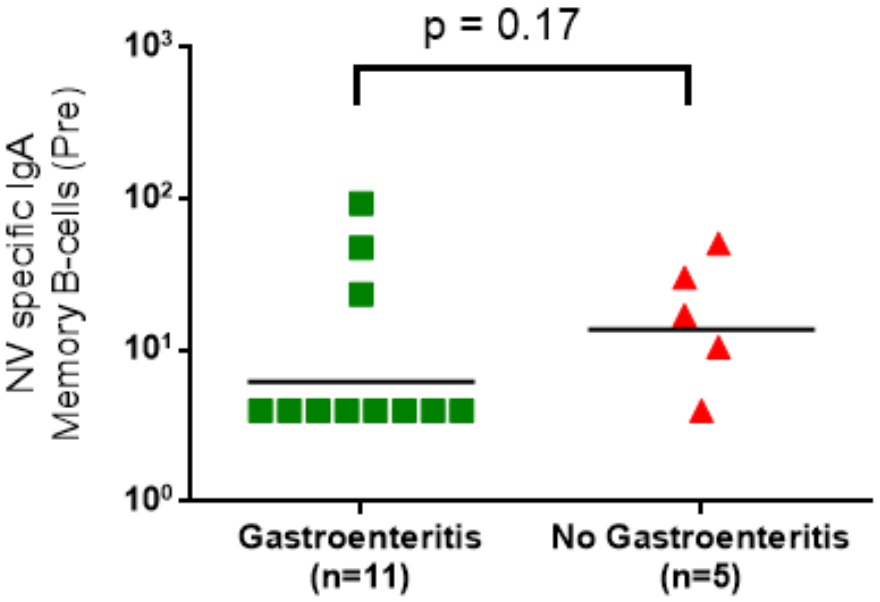
- **Pre-challenge NV-specific salivary IgA also correlates with reduced severity of gastroenteritis**
- **Pre-challenge NV-specific fecal IgA correlates with lower peak viral load**

Ramani S et al., *JID* 2015;212:397

Pre-challenge Memory IgG Cells is Protective against Norwalk Virus Gastroenteritis



IgG Memory B-cells



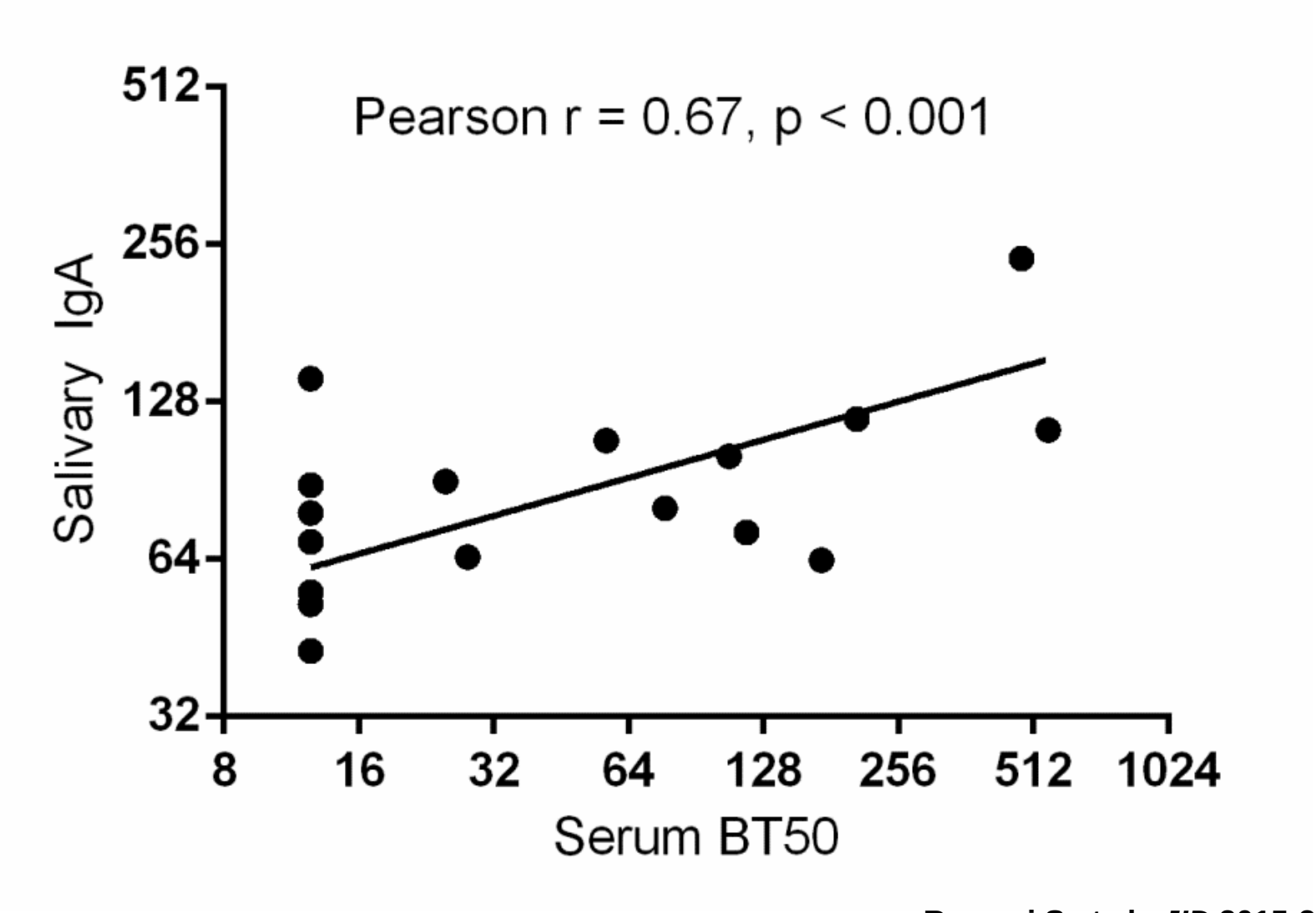
IgA Memory B-cells

Ramani S et al., *JID* 2015;212:397

Pre-challenge Immune Correlates of Protection

Assay	Persons without GE GMT (95% CI)	Persons with GE GMT (95% CI)	Correlate with protection against GE
Serum IgA	4.5 (1.2-17.1)	2.1 (1.0-4.7)	No
Serum IgG	17.9 (6.3-51.2)	6.3 (2.7-14.4)	No
Serum HBGA	127.1 (44.9-359.7)	19.0 (11.1-32.3)	Yes
Serum HAI	32.0 (15.0-68.0)	9.0 (6.0-14.0)	Yes
NV-salivary IgA	104.6 (71.2-153.8)	65.0 (51.1-82.6)	Yes
NV-fecal IgA	45.6 (10.0-206.8)	44.9 (13.91-145.0)	No
IgA ASC	6.7 (4.8-9.3)	4.7 (3.9-5.7)	No
IgG ASC	6.7 (4.8-9.3)	4.7 (3.9-5.7)	No
NV-IgA memory B cells	16.1 (4.8-54.5)	7.8 (3.5-17.5)	No
NV-IgG memory B-cells	52.3 (13.1-208.0)	6.5 (3.6-11.7)	Yes

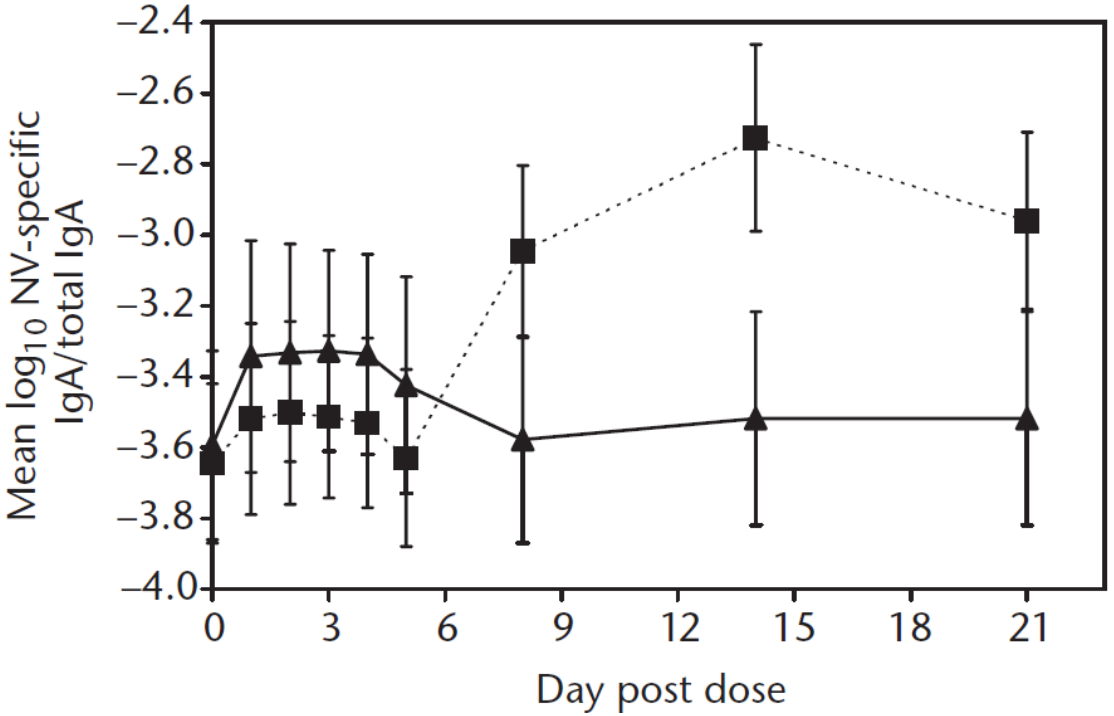
Correlation of NV Serum HBGA Blocking Antibody and Salivary IgA Titers



Ramani S et al., *JID* 2015;212:397

Post Infection Responses to Norwalk Virus

- **Early salivary IgA response seen in uninfected persons**



■ Se+ Infected

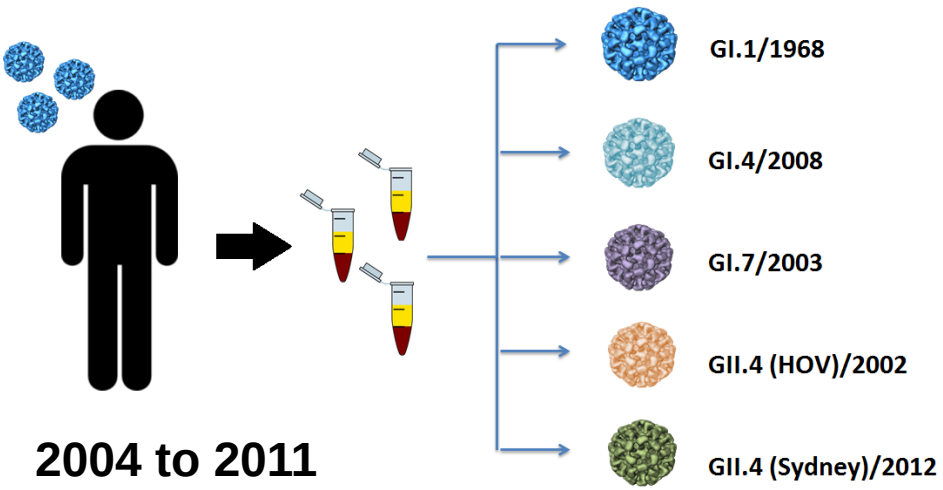
▲ Se+ Uninfected

- **Higher fecal IgA response at day 7 correlates with shorter duration of virus shedding**
- **Role of mucosal immunity in clearance?**

Lindesmith et al., *Nat Med* 2003;9:548 ; Ramani et al., *JID* 2015

Breadth of Immune Response

- Cross reactive responses within genogroup; limited evidence across genogroups
- Heterotypic HBGA-blocking activity against GI.4, GI.7, and GII.4 NoVs in individuals infected with Norwalk virus

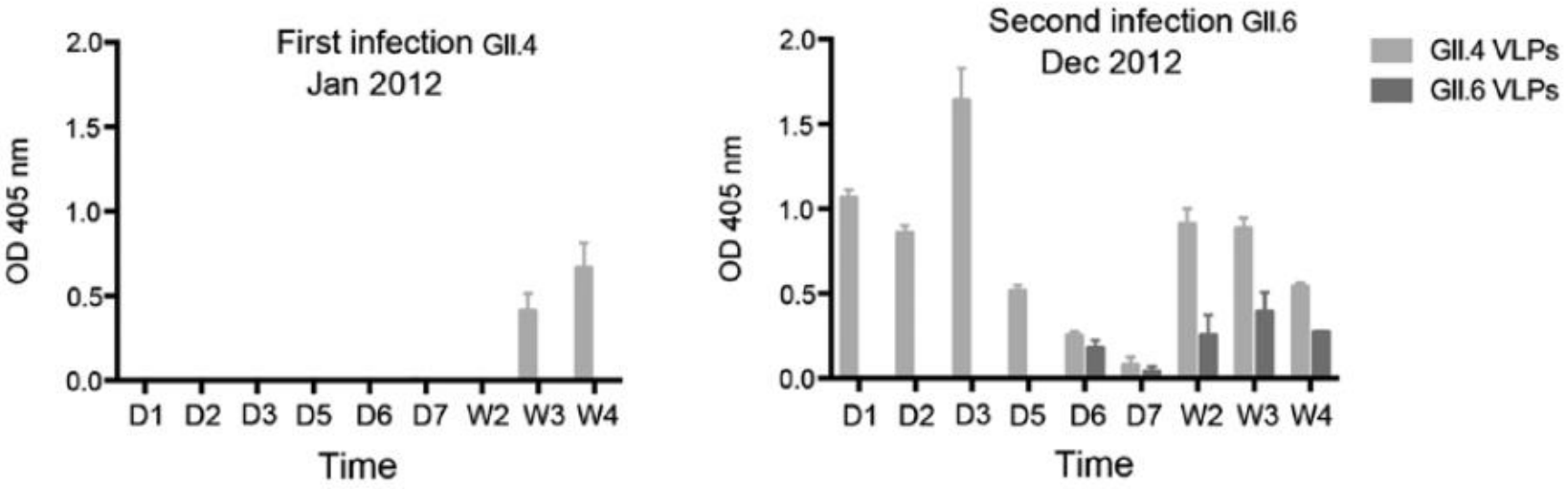


	GI.1	GI.4	GI.7	GII.4 (HOV)	GII.4 (Sydney)
701					
703					
704					
706					
707					
710					
715					
716					
717					
720					
721					
722					
723					
724					
731					
732					
733					
734					

Czako et al., *Clin Vaccine Immunol* 2015;22:221

Protective Immunity in Children

- Fewer studies on norovirus immunity in children
- Serum IgG and blocking antibodies protective in GII.4 (New Orleans) disease in children in Finland
- Genotype specific protection; also seen in birth cohort in Peru (Saito et al., 2013)
- Induction of mucosal immunity in children?



Parra & Green *EID* 2014; 20:1016

Summary and Challenges

- **Multiple immune effectors correlate with protection from gastroenteritis**
  - **Relative importance of each CoP?**
  - **Compensatory mechanisms of action?**
- **Assessment of immune effectors important to inform decisions for subsequent vaccine trials**
  - **Duration of immunity**
  - **Routes of immunization, use of specific adjuvants etc.**
- **Most data so far from adults; similar responses in children?**

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