

# GOPEN ACCESS

**Citation:** Dang Thanh H, Than VT, Nguyen TH, Lim I, Kim W (2016) Emergence of Norovirus GII.17 Variants among Children with Acute Gastroenteritis in South Korea. PLoS ONE 11(5): e0154284. doi:10.1371/journal.pone.0154284

**Editor:** Dong-Yan Jin, University of Hong Kong, HONG KONG

Received: February 13, 2016

Accepted: April 11, 2016

Published: May 5, 2016

**Copyright:** © 2016 Dang Thanh et al. This is an open access article distributed under the terms of the <u>Creative Commons Attribution License</u>, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: The nucleotide sequence data were deposited in GenBank under accession numbers KU561250–KU561256.

**Funding:** This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education (2013R1A1A2A10012148).

**Competing Interests:** The authors have declared that no competing interests exist.

**RESEARCH ARTICLE** 

# Emergence of Norovirus GII.17 Variants among Children with Acute Gastroenteritis in South Korea

#### Hien Dang Thanh<sup>1</sup>, Van Thai Than<sup>1</sup>, Tinh Huu Nguyen<sup>1</sup>, Inseok Lim<sup>2</sup>, Wonyong Kim<sup>1</sup>\*

1 Department of Microbiology, Chung-Ang University College of Medicine, Seoul 06974, South Korea,

2 Department of Pediatrics, Chung-Ang University College of Medicine, Seoul 06974, South Korea

\* kimwy@cau.ac.kr

# Abstract

Of 1,050 fecal specimens collected from January 2013 to August 2015 from children with acute gastroenteritis, 149 (14.2%) were found to be positive for norovirus. Norovirus GII was the most predominant genogroup (98.65%; 147 of 149). The genotypes detected in this study were GI (2; 1.3%), GII.Pe-GII.4 (109; 73.1%), GII.P17-GII.17 (16; 10.7%), GII.P12-GII.3 (8; 5.4%), GII.P12-GII.12 (8; 5.4%), GII.P4-GII.4 (5; 3.4%), and the recombinant GII.Pe-GII.17 (1; 0.7%). Of these, the novel GII.17 strain was the second most predominant, and the number of affected children appeared to continuously increase over time (2013 [2; 4.4%], 2014 [4; 9.3%], and 2015 [10; 16.4%]). Phylogenetic analysis of the full genome and ORF1, ORF2, and ORF3 nucleotide sequences showed that GII.17 was grouped in cluster III with other strains isolated from 2013 to 2015 and had a different evolutionary history from strains collected in 1978 to 2002 and 2005 to 2009 formed clusters I and II. However, the phylogenetic trees also showed that cluster III was divided into subclusters IIIa (CAU-55 and CAU-85) and IIIb (Kawasaki 2014) (CAU-193, CAU-265, CAU-267, CAU-283, and CAU-289). Comparative analysis of the VP1 capsid protein using 15 complete amino acid sequences from noroviruses isolated from 1978 to 2015 showed 99 amino acid changes. These results could be helpful for epidemiological studies to understand circulating norovirus genotypes in population.

# Introduction

Norovirus (NoV) is the predominant etiological viral agent of acute gastroenteritis across all age groups, but is most prevalent in young children and the elderly [1]. NoVs are recognized as the second most important cause of diarrhea in children following rotaviruses, and as an important cause of food-borne disease worldwide [2]. In most cases, NoVs cause diarrhea and vomiting, which generally last only a few days, but the symptoms can be serious for some people, especially young children and the elderly [1,3]. NoVs are responsible for an estimated 200,000 deaths among children aged less than 5 years each year in developing countries [4].

The genome of NoVs is a single-stranded positive-sense RNA approximately 7.7 kb in length that is covalently linked to VPg at the 5' end and polyadenylated at the 3' end. The

genome is organized into three open reading frames (ORFs): ORF1 encodes for a polyprotein required for replication such as NTPase, protease, and RNA-dependent RNA polymerase (RdRp); ORF2 encodes the viral protein 1 (VP1); and ORF3 encodes the viral protein 2 (VP2) [5]. Based on genetic differences in the major capsid protein, NoVs are divided into at least seven genogroups, GI–GVI, of which GI and GII are the most frequently detected in human infections, and GIV is also implicated in human gastroenteritis [6]. Within each genogroup, strains are further subdivided into genotypes based on sequence information of the RdRp and capsid genes. GII strains are responsible for 75–100% of NoV cases worldwide, whereas GII.4 genotypes have been responsible for the majority of outbreaks, as well as community cases of acute gastroenteritis, since the mid-1990s [4,7,8].

Previous studies in South Korea have reported that 13–34% of the children admitted to hospitals with acute gastroenteritis or diarrhea were diagnosed with NoV infection. GII genotypes were recognized as predominant, and most of them belonged to GII.4, GII.3, GII.6, GII.8, and GII.13 [9,10,11]. Significant diversity of NoV genotypes has emerged with several novel recombinants, owing to the accumulation of point mutations or recombination resulting in the introduction of new antigenic variants [12].

Although several NoV strains have been in circulation over the last two decades, the novel GII.17, as the predominant outbreak strain in China, has recently been reported in several countries, including Japan, Hong Kong, Taiwan, the US, Australia, France, Italy, the Netherlands, New Zealand, and Russia [13,14,15,16,17,18]. In South Korea, an environmental samples study conducted from 2004 to 2006 indicated widespread circulation of GII.17, but the novel viruses themselves were not detected and there are no matching clinical reports [13]. The aims of this study were to: (i) genetically survey NoVs in South Korea during the years 2013 to 2015, (ii) genetically characterize the novel circulating NoV strain GII.17 isolated in children with acute gastroenteritis, and (iii) study the genetic evolution of GII.17. The data obtained in this study will be useful for researchers and will lead to a better understanding of NoVs, which could contribute essential information on the epidemiology and evolution of these viruses.

# **Materials and Methods**

#### Ethics statement

The stool samples used in this study were collected and analyzed under protocol number #2010-10-02, approved by the Human Subjects Institutional Review Board (IRB) of Chung-Ang University College of Medicine, Seoul, Korea. For children enrolled in this study, written informed consent was got from parents or legal guardians. This consent included authorization to use the data for future research purposes.

#### Stool sample preparation

From January 2013 through August 2015, 1050 stool samples were collected from children with acute gastroenteritis under 5 years of age. A case of acute gastroenteritis for the stool collection in this study was defined as increased stool frequency (i.e., at least three loose and watery stools within a 24-h period) with or without vomiting and fatigue occurring within the previous 48 h. Stool samples were collected from pediatric patients presenting with acute gastroenteritis at Chung-Ang University Hospital in Seoul. For sample processing, approximately 10% suspensions of stool samples were prepared by vortexing 0.1 g of stool sample with 1 mL of phosphate-buffered saline (PBS; pH 7.4). The stool sample suspensions were centrifuged at 12,000  $\times$  g for 15 min, and the supernatants were used as the fecal suspension.

# **RNA** extraction

Viral RNA was extracted from patient fecal suspensions using a QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Extracted viral RNA was stored at -70°C until use in reverse transcription-polymerase chain reaction (RT-PCR).

# NoV detection

The presence of NoV RNA was tested by RT-PCR using GI-specific primers (Calman-29 and Calman-32) and a semi-nested GII-specific primer set (Calman-1/Calman-2 for a first-round PCR and p289/p290 for a second-round PCR). RNA from NoV GII-positive specimens was analyzed by RT-PCR using a semi-nested GII-specific primer set (GIIF1M/GIIR1M for a first-round PCR and GIIF3M/GIIR1M for a second-round PCR) to amplify the capsid gene (Table 1). RT-PCR was performed using a One-Step RT-PCR Kit (Qiagen) under previously described amplification conditions [19,20]. PCR products were purified using a QIAquick PCR Purification Kit (Qiagen). The nucleotide sequencing was conducted by Macrogen (Seoul, Korea) using a BigDye Terminator Cycle Sequencing Kit and an automated DNA sequencer (Model 3730; Applied Biosystems, Foster City, CA, USA). Each amplicon was sequenced in both the forward and reverse directions. The sequences were assembled using BioEdit software (http://www.mbio.ncsu.edu/bioedit/bioedit.html). Preliminary genotypes were assigned by using the NoV genotyping tool (http://www.rivm.nl/mpf/norovirus/typingtool).

# Full-length genome amplification and sequencing

Seven out of sixteen GII.17-positive samples were selected for complete genome sequencing based on the quality and quantity of the available RNA: CAU-55 (collected in February, 2013);

Table 1. Primers used for norovirus detection and full-length genome amplification.

			• •	•	•		
Geno-types	Reaction type	Primer	Polarity <sup>a</sup>	Region	Location	Sequence (5'-3')	Reference
I	One-step RT-PCR	Calman-29	+	ORF1	4868–4891 <sup>b</sup>	TATGGTGATGATGAAATAGTGTC	[19]
I.	One-step RT-PCR	Calman-32	-	ORF1	5338–5356 <sup>b</sup>	ATTTCGGGCAGAAGATTG	[ <u>19]</u>
П	1st PCR	Calman-1	+	ORF1	4193–4213 <sup>c</sup>	GCACACTGTGTTACACTTCC	[19]
П	1st PCR	Calman-2	-	ORF1	4997–5015 <sup>c</sup>	ACATTGGCTCTTGTCTGG	[19]
П	Nested PCR	p290	+	ORF1	4568–4590 <sup>c</sup>	GATTACTCCAAGTGGGACTCCAC	[ <u>19]</u>
П	Nested PCR	p289	-	ORF1	4865–4886 <sup>c</sup>	TGACAATGTAATCATCACCATA	[19]
П	1st PCR	GIIF1M	+	ORF2	5049–5067 <sup>c</sup>	GGGAGGGCGATCGCAATCT	[20]
П	1st PCR	GIIR1M	-	ORF2	5367–5389 <sup>c</sup>	CCRCCIGCATRICCRTTRTACAT	[20]
П	Nested PCR	GIIF3M	+	ORF2	5079–5102 <sup>c</sup>	TTGTGAATGAAGATGGCGTCGART	[20]
П	Nested PCR	GIIR1M	-	ORF2	5367–5389 <sup>c</sup>	CCRCCIGCATRICCRTTRTACAT	[20]
П	Full genome	NV-1F	+	ORF1	1–27 <sup>d</sup>	GTGAATGAAGATGGCGTCTAACGACGC	[21]
П	Full genome	NV-1R	-	ORF1	2267–2287 <sup>d</sup>	CACTATCTGRCACYTCTTGAT	[21]
П	Full genome	NV-2F	+	ORF1	2117–2139 <sup>d</sup>	ACCTTCAAYTTTGACCGCAACAA	[21]
П	Full genome	JV13	-	ORF1	4594–4614 <sup>d</sup>	TCATCATCACCATAGAAAGAG	[32]
П	Full genome	JV12	+	ORF2	4288-4308 <sup>d</sup>	ATACCACTATGATGCAGATTA	[32]
П	Full genome	NV-3R	-	ORF2	6862–6881 <sup>d</sup>	GCGCTTGGAGCATCTCTTTA	[21]
П	Full genome	COG2F	+	ORF3	5012–5037 <sup>d</sup>	CARGARBCNATGTTYAGRTGGATGAG	[ <u>33]</u>
П	Full genome	NV-4R	-	ORF3	7537–7556 <sup>d</sup>	AAAAGATACAAATTAGCCAA	[21]

<sup>a</sup> +, Forward primer; –, reverse primer.

<sup>b</sup> Norwalk virus for GI (GenBank accession number M87661).

<sup>c</sup> Lordsdale for GII (GenBank accession number X86557).

<sup>d</sup> CUHK-NS-463 for GII.17 (GenBank accession number KP998539).

doi:10.1371/journal.pone.0154284.t001

CAU-85 (collected in March, 2013); CAU-192 (collected in November, 2014); CAU-265 (collected in December, 2014); CAU-267 (collected in January, 2015); CAU-283 (collected in March, 2015); and CAU-289 (collected in April, 2015). To facilitate the sequencing of the full genome of the novel NoV strain, RT-PCR was performed using a One-Step RT-PCR Kit (Qiagen) with four pairs of primer sets (<u>Table 1</u>). RT-PCR amplification was performed at 42°C for 30 min, followed by 94°C for 1 min, and 35 cycles of PCR at 94°C for 30 s, 56°C for 30 s, 72°C for 2 min, and a final incubation at 72°C for 10 min [21]. Each PCR product was confirmed, purified, and sequenced as described above. The nucleotide sequence data were deposited in GenBank under accession numbers KU561250–KU561256.

# Phylogenetic analysis

The nucleotide sequences of NoV strains were aligned, and phylogenetic analysis was performed with other published reference strains obtained from GenBank database (<u>http://www. ncbi.nlm.nih.gov/genbank</u>) using the MEGA6 program suite [22]. The dendrograms were constructed using the neighbor-joining method with a bootstrap analysis of 1000 replicates in the MEGA 6.0 program.

# Statistical analysis

Continuous variables were analysed by the Student's t-test. P value of < 0.05 were considered statistically significant. The tests were analyzed using Microsoft Excel 2010.

# Results

#### NoV detection and molecular biology

Overall, 1050 children hospitalized for acute gastroenteritis were enrolled in this study from January 2013 to August 2015. According to RT-PCR amplification and direct sequencing using the primers in <u>Table 1</u>, there were 149 NoV-positive cases, accounting for 14.2% of acute gastroenteritis cases. The GII genogroup accounted for most of the NoV-infected cases (98.7%, 147/149), followed by the GI strain (1.3%, 2/149). Sequence analysis of the RdRp gene in ORF1 and the capsid gene in ORF2 from 147 NoV GII-positive samples and a BLAST search revealed that these 147 samples could be divided into GII.Pe-GII.4 (109; 73.1%), GII.P17-GII.17 (16; 10.7%), GII.P12-GII.3 (8; 5.4%), GII.P12-GII.12 (8; 5.4%), GII.P4-GII.4 (5; 3.4%), and the recombinant GII.Pe-GII.17 (1; 0.7%) (<u>Table 2</u>). NoV outbreaks were detected year-round, but were mainly observed in the months of October through April. NoV GII.17 detections also

#### Table 2. Distribution of genotypes in the norovirus-positive samples in this study.

	2013	2014	2015	Total
No. Samples/No.Norovirus Positive (%)	346/45 (13.0)	321/43 (13.4)	383/61 (15.9)	1050/149 (14.2)
Norovirus genotype, n (% norovirus positive)				
GI	1 (2.2)	0	1 (1.6)	2 (1.3)
GII.Pe-GII.4	34 (75.6)	31 (72.0)	44 (72.2)	109 (73.1)
GII.P4-GII.4	0	2 (4.7)	3 (4.9)	5 (3.4)
GII.P17-GII.17	2 (4.4)	4 (9.3)	10 (16.4)	16 (10.7)
GII.P12-GII.3	4 (8.9)	2 (4.7)	2 (3.3)	8 (5.4)
GII.P12-GII.12	3 (6.7)	4 (9.3)	1 (1.6)	8 (5.4)
GII.Pe-GII.17	1 (2.2)	0	0	1 (0.7)

doi:10.1371/journal.pone.0154284.t002



Fig 1. Monthly distribution of NoVs and genotype GII.17 in children with acute gastroenteritis from 2013 to 2015 in Seoul, Korea.

occurred from November to March throughout the year, with peaks in January 2015 (Fig 1). Among the 149 NoV-positive cases, GII.17 was the second most predominant (n = 16) and accounted for 10.7% of these cases. The proportion of NoV GII.17 increased constantly from 4.4% in 2013 to 9.3% in 2014 and to 16.4% in 2015. In the NoV-positive patients hospitalized with acute gastroenteritis, there were no significant differences in clinical characteristics of patients infected with either the GII.4 or GII.17 genotype, such as daily diarrheal frequency with a mean of four liquid stools a day, diarrhea, abdominal pain, nausea, and fever (Table 3).

#### **ORF** analysis

From the NoV GII.17 specimens collected from January 2013 to August 2015, seven available specimens (CAU-55, CAU-85, CAU-192, CAU-265, CAU-267, CAU-283, and CAU-289) were selected for full-genome analyses. The NoV GII.17 genome is composed of three open reading frames: ORF1, ORF2, and ORF3.

Phylogenetic analysis was performed with the target 5108-bp sequence of the ORF1 gene of the study strains and reference strains frequently isolated worldwide (S1 Table). As shown in the analysis of ORF1 (Fig 2A), cluster III contains the emerging GII.17 strain isolated in Japan, China, and the US from 2013 to 2015 as well as strains in this study, and the nucleotide

Table 3.	Clinical information of	f children with NoV	GII.17 and GII.4	genogroups infection.
----------	-------------------------	---------------------	------------------	-----------------------

Characteristic	Gll.17 group (n = 16)	GII.4 group (n = 22)	P value
Age (months)	34 (18–58)	28 (14–58)	0.09
Male to female ratio	6:9	12:10	0.12
Frequency of vomiting (times/day)	2.1 (0-4)	2.6 (0-4)	0.12
Duration of vomiting (days)	2 (0–4)	1.6 (0–3)	0.15
Frequency of diarrhoea (times/day)	5.3(4–7)	4.8 (4–7)	0.07
Duration of diarrhoea (days)	3.7(2–5)	4.0 (2–6)	0.22
Fever of $\geq$ 37.5°C	11 (68.8)	16 (72.7)	0.22
Abdominal pain	75	66.7	0.29

doi:10.1371/journal.pone.0154284.t003

doi:10.1371/journal.pone.0154284.g001



Fig 2. Phylogenetic tree based on the complete sequence of ORF1 (about 5.2 kb) (a), ORF2 (about 1.6 kb) (b), and ORF3 (about 0.8 kb) (c).

doi:10.1371/journal.pone.0154284.g002

sequences of the ORF1 genes show 98.1–99.8% identity with the reference strains. Although sharing a common ancestor in cluster III, the strains isolated in 2014 and 2015 are localized within Kawasaki 2014, whereas strains in 2013 formed a distinct branch inside subcluster

Kawasaki 2014 and group with known GII.17 NoV strains from Japan and Taiwan isolated in the 2013/14 winter season.

Phylogenetic analysis of the ORF2 (VP1 protein) region was performed with the target 1623-bp sequence of the strains in this study and reference strains. As shown in Fig 2B, all strains isolated in South Korea were classified as members of cluster III, which was distinct from other strains isolated before 2011 (cluster II) and 2003 (cluster I). However, further diversification of the cluster strains led to two subclusters: subcluster IIIa comprised the CAU-55, CAU-85, and GII.17 strains from Japan and Taiwan in 2013–2014 (98.9–99.2% nucleotide sequence identity), whereas subcluster Kawasaki 2014 included the other five recent GII.17 strains identified in this study and strains isolated in Japan, China, the US, Italy, and Hong Kong from 2014–2015 (99–99.8% nucleotide sequence identity).

Phylogenetic analysis of ORF3 (VP2 protein) is described in Fig 2C. With respect to the ORF3 780-bp region, the CAU-55 and CAU-85 strains showed the highest similarity with the Kawasaki323 and 13-BH-1 strains, exhibiting 98.2–99.3% and 95.7–98.9% nucleotide and amino acid sequence identities, respectively. The other five recent GII.17 strains shared high sequence identity with ZHITHC-12, Kawasaki308, and Gaithersburg (98.8–99.7% and 97.7–100% nucleotide and amino acid sequence identities, respectively). Interestingly, high sequence identity was observed among strains isolated in 2014 and 2015 (99–99.7%), but GII.17 strains isolated in 2013 displayed only 96.7% and 96% nucleotide and amino acid sequence identities, respectively.

#### Phylogenetic analysis of the NoV genome

For phylogenetic analysis of the NoV genome, NoV GII.17 full-genome sequences obtained in this study from South Korea between 2013 and 2015 (n = 7), reported GII.17 reference sequences of past global epidemics, and representative sequences of the non-GII.17 genotypes reported to date in the GenBank database were used (S1 Table). As observed in the maximum-likelihood ORF1 and ORF2 tree (Fig 3), most of the non-GII.17 sequences were located far from the GII.17 cluster, and NoV GII.17 strains genetically formed the same cluster. Within the GII.17 cluster, the 2013/14 season strains were grouped separately from the GII.17 strains detected in 2014/15. The 2014/15-season strains were genetically related (99.8%) and clustered with the GII.P17-GII.17 strain detected in the US, Italy, and Asia in the 2014/15 seasons; these belonged to the novel NoV GII.17. It is interesting to note that the GII.17 strains are genetically most similar to GII. 3 reference strains.

#### Amino acid variation in the viral structural protein VP1

Seven GII.17 capsid protein VP1 sequences in this study were aligned with representative viruses of the other GII.17 clusters isolated from 1978 to 2015, including one or two representative strains from each of three clusters previously released and deposited in GenBank. Alignment of the derived amino acid sequences revealed 99 variable residues across the VP1 domain of the study strains as compared to the strains in the other two clusters, representing up to 18% of the total VP1 amino acids; their locations in the structure are shown in Fig 4. Among the 99 variable amino acids, 13 (13.13%) were observed in the shell domain and 25 (25.25%) in the P1 region; most of the substitutions and insertions were located in the P2 region, which contains the antigenic epitopes and host receptor-binding domain. In the capsid-protruding hypervariable P2 region, 60 amino acids accumulated changes at several positions, including epitope A amino acids 297–298, 374, and 378; epitope B amino acids 307 and 395; epitope C amino acid 383; epitope D amino acids 400 and 402; epitope E amino acids 407 and 414; and 1 substitution at epitope C amino acid 343. Changes in these residues likely alter the ability of preexisting immunity to neutralize the virus, thereby facilitating the emergence of new epidemic strains.





doi:10.1371/journal.pone.0154284.g003

Compared with the GII.17 strains identified before 2013/14, mutations were observed in the RGD/K-like motif (K289R), located at positions 287–289 of five recent strains. Three deletions (residues 295, 296, and 384) and one insertion (344) of all the strains in this study differed markedly from the oldest GII.17. Of note, the 2014/15 winter season strains contained 2 amino acid insertions (-380D and -396D; C142 numbering) in the P2 region as compared to the previous strains, resulting in different lengths of VP1. Among cluster III, five strains in the 2014/15 winter season presented 25 amino acid site substitutions (sites N169S, E293Q, T294I, D297N,

GAU276       K       A       E       N       T       P       Q       I       S       Q       N       S       D       L       V       F       S       V       N       Q       I       S       A       H       V       R       A       V       R       A       L       N       V       A       H       V       R       A       H       V       R       A       H       V       R       A       H       V       R       A       H       V       R       A       H       V       R       A       H       V       R       A       H       V       R       A       H       V       R       A       H       V       R       A       H       V       R       A       H       V       R       A       H       V       R       A       A       H       V       R       A       H       V       R       A       A       H       V       R       A       A       V       R       A       A       V       R       A       A       V       R       A       A       V       R       A       A       A <th>Strains</th> <th>n</th> <th>2</th> <th>16</th> <th>22</th> <th>24</th> <th>37</th> <th>7 4</th> <th>75</th> <th>169</th> <th>171</th> <th>173</th> <th>174</th> <th>193</th> <th>23</th> <th>244</th> <th>253</th> <th>256</th> <th>258</th> <th>425</th> <th>440</th> <th>444</th> <th>447</th> <th>449</th> <th>451</th> <th>452</th> <th>456</th> <th>463</th> <th>480</th> <th>497</th> <th>504</th> <th>511</th> <th>514</th> <th>517</th> <th>524</th> <th>541</th> <th>542</th> <th></th>	Strains	n	2	16	22	24	37	7 4	75	169	171	173	174	193	23	244	253	256	258	425	440	444	447	449	451	452	456	463	480	497	504	511	514	517	524	541	542	
CAU-283       K       A       E       N       T       P       Q       I       S       D       L       V       F       Q       V       F       S       V       N       Q       I       I       I       H       V       A       H       V       A       H       V       A       H       V       A       H       V       A       H       V       A       H       V       A       H       V       A       H       V       A       H       V       A       H       V       A       H       V       A       H       V       A       H       V       A       H       V       A       H       V       A       H       V       A       H       V       A       H       V       A       H       V       A       H       V       A       H       V       A       H       V       A       H       V       A       H       V       A       H       V       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A </td <td>CAU-276</td> <td></td> <td>ĸ</td> <td>Α</td> <td>Е</td> <td>N</td> <td>т</td> <td><b>,</b></td> <td>2 1</td> <td>S</td> <td>Q</td> <td>Ν</td> <td>S</td> <td>D</td> <td>L</td> <td>V I</td> <td>FQ</td> <td>V</td> <td>Q</td> <td>v</td> <td>F</td> <td>S</td> <td>YN</td> <td>Q</td> <td>1</td> <td>Т</td> <td>1</td> <td>н</td> <td>Y</td> <td>S</td> <td>н</td> <td>۷</td> <td>Α</td> <td>н</td> <td>V</td> <td>R</td> <td>Α</td> <td></td>	CAU-276		ĸ	Α	Е	N	т	<b>,</b>	2 1	S	Q	Ν	S	D	L	V I	FQ	V	Q	v	F	S	YN	Q	1	Т	1	н	Y	S	н	۷	Α	н	V	R	Α	
CAU-289       K       A       E       N       T       P       Q       I       S       Q       N       Q       I       V       R       A       H       V       R       A       H       V       R       A       H       V       R       A       H       V       R       A       H       V       R       A       H       V       R       A       H       V       R       A       H       V       R       A       H       V       R       A       H       V       R       A       H       V       R       A       H       V       R       A       H       V       R       A       H       V       R       A       H       V       R       A       H       V       R       A       H       V       R       A       H       V       R       A       H       V       R       A       H       V       R       A       H       V       R       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A </td <td>CAU-283</td> <td></td> <td>к</td> <td>Α</td> <td>Е</td> <td>Ν</td> <td>тι</td> <td>• •</td> <td>2 1</td> <td>S</td> <td>Q</td> <td>Ν</td> <td>S</td> <td>D</td> <td>L</td> <td>V I</td> <td>FQ</td> <td>v</td> <td>Q</td> <td>v</td> <td>F</td> <td>S</td> <td>YN</td> <td>Q</td> <td>1</td> <td>Т</td> <td>1</td> <td>н</td> <td>Y</td> <td>S</td> <td>н</td> <td>v</td> <td>А</td> <td>н</td> <td>v</td> <td>R</td> <td>Α</td> <td></td>	CAU-283		к	Α	Е	Ν	тι	• •	2 1	S	Q	Ν	S	D	L	V I	FQ	v	Q	v	F	S	YN	Q	1	Т	1	н	Y	S	н	v	А	н	v	R	Α	
Kawasaki0303       K       A       E       N       T       P       Q       I       S       D       L       V       F       Q       V       F       S       N       Q       I       I       H       Y       S       H       V       R       A         Gallbersburg       K       A       E       N       T       P       Q       I       S       D       V       F       S       V       N       Q       I       I       H       Y       S       H       V       A       H       V       R       A         Gallersburg       K       A       E       N       T       P       Q       I       S       D       U       V       F       S       N       Q       I       I       H       Y       S       H       V       A       H       V       R       A       I       N       A       I       N       N       N       N       N       N       N       N       N       N       N       N       N       N       N       N       N       N       N       N       N       N       N       N	CAU-289		к	Α	Е	Ν	т	° (	2 1	S	Q	Ν	S	D	L	V I	FQ	v	Q	v	F	S	YN	Q	1	1	1	н	Y	S	н	v	Α	н	v	R	Α	
Gallesburg       K       A       E       N       T       P       Q       I       S       Q       N       S       D       L       V       F       Q       V       Q       I       I       H       Y       S       H       V       R       A         CAU-285       K       A       E       N       T       P       Q       I       S       Q       N       S       D       L       V       F       Q       V       F       S       Y       N       Q       I       I       H       Y       S       H       V       R       A         CAU-55       K       A       E       N       T       P       Q       I       N       S       D       L       V       F       Q       V       F       S       N       N       V       I       H       Y       R       A       V       R       A       V       R       A       V       R       A       V       R       A       V       R       A       V       R       A       V       R       A       V       R       A       V       R       A<	Kawasaki308		к	А	Е	Ν	TF	° (	ו ג	S	Q	Ν	S	D	L	V I	FQ	V	Q	V	F	S	YN	I Q	1	1	1	н	Y	S	н	V	А	н	v	R	A	
CAU-285       K       A       E       N       T       P       Q       I       S       Q       N       S       D       L       V       F       S       Y       N       Q       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I </td <td>Gaithersburg</td> <td></td> <td>к</td> <td>А</td> <td>E</td> <td>Ν</td> <td>TF</td> <td>° (</td> <td>2 1</td> <td>S</td> <td>Q</td> <td>Ν</td> <td>S</td> <td>D</td> <td>L</td> <td>V I</td> <td>FQ</td> <td>V</td> <td>Q</td> <td>V</td> <td>F</td> <td>S</td> <td>YN</td> <td>Q</td> <td>1</td> <td>1</td> <td>1</td> <td>н</td> <td>Y</td> <td>S</td> <td>н</td> <td>V</td> <td>A</td> <td>н</td> <td>V</td> <td>R</td> <td>A</td> <td></td>	Gaithersburg		к	А	E	Ν	TF	° (	2 1	S	Q	Ν	S	D	L	V I	FQ	V	Q	V	F	S	YN	Q	1	1	1	н	Y	S	н	V	A	н	V	R	A	
CAU-192       K       A       E       N       T       P       Q       I       S       Q       N       S       D       L       V       F       Q       Q       V       F       S       N       Q       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       V       I       I       V       I       I       V       I       I       V       I       I       V       I       I       V       I       I       V       I       I       V       I       I       V       I       I       V       I       I       V       I       I       V       I       I       V       I       I       V       I       I       V       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I </td <td>CAU-265</td> <td></td> <td>ĸ</td> <td>Α</td> <td>E</td> <td>N</td> <td>т</td> <td>° (</td> <td>2 1</td> <td>S</td> <td>Q</td> <td>N</td> <td>S</td> <td>D</td> <td>L</td> <td>V I</td> <td>FQ</td> <td>V</td> <td>Q</td> <td>v</td> <td>F</td> <td>S</td> <td>YN</td> <td>Q</td> <td>1</td> <td>1</td> <td>1</td> <td>н</td> <td>Y</td> <td>S</td> <td>н</td> <td>v</td> <td>Α</td> <td>н</td> <td>v</td> <td>R</td> <td>Α</td> <td></td>	CAU-265		ĸ	Α	E	N	т	° (	2 1	S	Q	N	S	D	L	V I	FQ	V	Q	v	F	S	YN	Q	1	1	1	н	Y	S	н	v	Α	н	v	R	Α	
Nagano71702       K       A       A       A       E       N       T       P       Q       I       N       S       D       L       V       F       Q       Q       V       F       S       N       Q       I       N       A       Y       R       A       Y       V       R       A       Y       V       R       A       Y       V       R       A       Y       V       R       A       Y       V       R       A       Y       V       R       A       Y       V       R       A       Y       V       R       A       Y       V       R       A       Y       V       R       X       V       V       F       Y       V       V       F       Y       N       Q       V       R       X       L       V       R       X       Y       R       A       Y       R       A       Y       R       A       Y       N       A       Y       N       A       Y       N       A       Y       N       A       Y       N       A       Y       N       A       Y       N       A       Y <th< td=""><td>CAU-192</td><td></td><td>ĸ</td><td>Α</td><td>Е</td><td>N</td><td>т</td><td>° (</td><td>2 1</td><td>S</td><td>Q</td><td>N</td><td>S</td><td>D</td><td>L</td><td>V I</td><td>FQ</td><td>v</td><td>Q</td><td>v</td><td>F</td><td>S</td><td>YN</td><td>Q</td><td>1</td><td>1</td><td>1</td><td>н</td><td>Y</td><td>S</td><td>н</td><td>v</td><td>Α</td><td>н</td><td>v</td><td>R</td><td>A</td><td></td></th<>	CAU-192		ĸ	Α	Е	N	т	° (	2 1	S	Q	N	S	D	L	V I	FQ	v	Q	v	F	S	YN	Q	1	1	1	н	Y	S	н	v	Α	н	v	R	A	
13:8H-11       K       A       E       N       T       P       Q       I       N       S       D       L       V       F       S       V       Q       V       V       F       S       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V<	Nagano7-1 POL		к	А	Е	Ν	TR	° (	ו ג	Ν	Q	Ν	S	D	L	V I	FQ	V	Q	V	F	S	YN	I Q	1	V	1	н	Y	S	н	V	А	Y	V	R	A	
CAU-55       K       A       E       N       T       P       Q       I       N       Q       N       S       D       L       V       F       S       V       N       Q       I       N       Q       N       V       T       N       Q       V       V       F       S       V       N       Q       I       V       T       A       Y       V       T       A       Y       V       T       A       Y       V       T       A       Y       V       T       A       Y       V       T       A       Y       V       T       A       Y       V       T       A       Y       V       T       A       Y       V       T       A       Y       V       T       A       Y       V       T       A       Y       V       T       A       Y       V       T       A       Y       V       T       A       Y       V       T       A       Y       V       T       A       Y       V       T       A       Y       V       T       A       Y       V       T       A       Y       V       T <td>13-BH-1</td> <td></td> <td>к</td> <td>А</td> <td>Е</td> <td>Ν</td> <td>TF</td> <td>° (</td> <td>ו ג</td> <td>Ν</td> <td>Q</td> <td>Ν</td> <td>S</td> <td>D</td> <td>L</td> <td>V I</td> <td>FQ</td> <td>V</td> <td>Q</td> <td>V</td> <td>F</td> <td>S</td> <td>YN</td> <td>I Q</td> <td>1</td> <td>V</td> <td>1</td> <td>н</td> <td>Y</td> <td>S</td> <td>н</td> <td>V</td> <td>Α</td> <td>Υ</td> <td>V</td> <td>R</td> <td>A</td> <td></td>	13-BH-1		к	А	Е	Ν	TF	° (	ו ג	Ν	Q	Ν	S	D	L	V I	FQ	V	Q	V	F	S	YN	I Q	1	V	1	н	Y	S	н	V	Α	Υ	V	R	A	
CAU-35       K       A       E       N       T       P       Q       I       N       R       S       E       D       L       V       F       S       Y       N       Q       I       H       S       H       V       I       H       H       S       H       V       I       H       H       S       I       V       I       H       H       V       I       H       H       V       I       H       H       V       I       H       H       V       I       I       H       V       I       I       H       V       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I       I <td>CAU-55</td> <td></td> <td>к</td> <td>Α</td> <td>Е</td> <td>Ν</td> <td>т</td> <td>° (</td> <td>2 1</td> <td>N</td> <td>Q</td> <td>Ν</td> <td>s</td> <td>D</td> <td>L</td> <td>V I</td> <td>FQ</td> <td>V</td> <td>Q</td> <td>2 V</td> <td>F</td> <td>S</td> <td>YN</td> <td>Q</td> <td>1</td> <td>v</td> <td>1</td> <td>н</td> <td>Y</td> <td>S</td> <td>н</td> <td>v</td> <td>Α</td> <td>Y</td> <td>v</td> <td>R</td> <td>Α</td> <td></td>	CAU-55		к	Α	Е	Ν	т	° (	2 1	N	Q	Ν	s	D	L	V I	FQ	V	Q	2 V	F	S	YN	Q	1	v	1	н	Y	S	н	v	Α	Y	v	R	Α	
Zunchy/Jo384       K       A       I       I       L       A       G       I       A       F       Q       N       N       V       N       A       V       N       A       V       N       A       V       N       A       V       N       A       V       N       A       V       N       A       V       N       A       V       N       A       V       N       A       V       N       A       V       N       A       V       N       A       V       N       A       V       N       A       V       N       A       V       N       A       V       N       A       V       N       A       V       N       A       V       N       A       V       N       A       V       N       A       V       N       A       V       N       A       V       N       A       V       N       A       V       N       A       A       C       B       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D <t< td=""><td>CAU-85</td><td></td><td>K</td><td>A</td><td>E</td><td>N</td><td>т</td><td>° (</td><td>2 1</td><td>N</td><td>Q</td><td>N</td><td>S</td><td>D</td><td>L</td><td>V</td><td>F H</td><td>V</td><td>Q</td><td>V</td><td>F</td><td>S</td><td>YN</td><td>Q</td><td>. !</td><td>V</td><td></td><td>н</td><td>H</td><td>S</td><td>н</td><td>v</td><td>A</td><td>Y</td><td>V</td><td>т</td><td>Α</td><td></td></t<>	CAU-85		K	A	E	N	т	° (	2 1	N	Q	N	S	D	L	V	F H	V	Q	V	F	S	YN	Q	. !	V		н	H	S	н	v	A	Y	V	т	Α	
Ratma-17       K       A       I       I       L       A       S       V       N       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       N       V       N       V       N       V       N       N       V       N       V       N       V       N       V       N       N       N       N       N       N       N<	Zurich/P7d384		ĸ	A	1	1	L /		2 1	N	R	S	E	E	1	A		N	N	V	N	A	VS				M		Y	-	S		S	Y	v	-		
Saltamartor       M       T       I       N       M       A       N       D       I       A       Y       N       A       V       N       A       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       V       N       N       V       N       N       V       N       N       N       N       N       N       N       N       N       N       N       N       N       N       N       N       N       N       N       N       N       N       N       N       N       N       N       N       N       N       N       N       N       N       N       N <td< td=""><td>Katrina-17</td><td></td><td>K</td><td>A T</td><td></td><td></td><td>L /</td><td></td><td></td><td>N</td><td>R</td><td>5</td><td>E</td><td>D</td><td>1</td><td>A  </td><td></td><td>N</td><td>N</td><td>v</td><td>N</td><td>A</td><td>v a</td><td>E</td><td></td><td></td><td>IVI</td><td></td><td>Ţ</td><td>+</td><td>н</td><td>v</td><td>5</td><td>T V</td><td></td><td>R</td><td>v.</td><td></td></td<>	Katrina-17		K	A T			L /			N	R	5	E	D	1	A		N	N	v	N	A	v a	E			IVI		Ţ	+	н	v	5	T V		R	v.	
aa       A       A       A       A       A       A       A       C       B       D       D       C       E       E         Strains       A       A       A       A       A       C       B       D       D       D       D       D       E       E         Strains       A       A       A       A       A       C       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B       B	Saltama/18/		K	4	1	N				IN N	Q	~	N	0	1.1			N	N	V V	N	A	ve		Ň	1	1	H	T V	÷.	н	v	5	T V	v	R	Ň	
A       A       A       A       A       A       A       C       B       D       E       E       E         Strains       5       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6	0142					п.			2 1	IN	Q	A	IN	U		~		IN .	IN	v	N	~	V 3		•		-					v	3		v	R	v	
A       A       A       A       A       A       C       B       D       D       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E       E			M						9								D1																					
A       A       A       A       A       A       A       A       A       A       C       B       D       D       E       E       E         Strains       A       A       A       A       A       A       C       B       B       C       B       B       C       B       B       C       B       B       C       B       B       C       B       B       C       B       B       C       B       B       C       B       B       C       B       B       C       B       B       C       B       B       C       B       B       C       B       B       C       B       B       C       B       B       C       B       B       C       B       B       C       B       B       C       B       B       C       B       B       C       B       B       C       B       B       B       C       B       B       C       B       B       C       B       B       C       B       B       C       B       B       C       B       B       C       B       B       C       C       B			M						S								P1			_	_	_	_				PT											
A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A       A			M						S						_	_	P1	-	_	_	-	-	_	_	_	_	P1											
ADV-26       Strains       5       6       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7       7 <t< td=""><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td>S</td><td>_</td><td>_</td><td>_</td><td>_</td><td>_</td><td>-</td><td>-</td><td>P1</td><td></td><td></td><td></td><td>-</td><td>-</td><td>_</td><td>_</td><td>_</td><td>_</td><td>PI</td><td>_</td><td>_</td><td>_</td><td>_</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></t<>									S	_	_	_	_	_	-	-	P1				-	-	_	_	_	_	PI	_	_	_	_							
CALI-276       S       R       V       T       Q       I       Y       D       K       V       M       V       G       N       D       A       P       G       S       Q       A       W       S       Y       P       L       R       I       S       D       D       D       D       D       G       P       F       R       L       N       R       R       C       Y       D       K       V       M       V       N       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D<	- <sup>a</sup> a n	+			A	A A	A	_	S		_		E	вс	-	_	P1	_		-	_	_	-		_	A	0	;		в	_	_		DC	)	Ē		E
Adv230       S       R       V       T       Q       T       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V <td>aa position</td> <td>289</td> <td>291</td> <td>293</td> <td>295 Y</td> <td>A 062</td> <td>298 V</td> <td>301</td> <td>307 313 S</td> <td>314 318</td> <td>330</td> <td>332</td> <td>336 336</td> <td>343 C</td> <td>344</td> <td>346</td> <td>347 348</td> <td>349</td> <td>354 356</td> <td>356</td> <td>358</td> <td>360</td> <td>371</td> <td>374 &gt; 375</td> <td>376</td> <td>378 Þ</td> <td>380</td> <td>384</td> <td>385</td> <td>395 CD</td> <td>396</td> <td>397</td> <td>399</td> <td>400 D</td> <td>403</td> <td>404 407 m</td> <td>408</td> <td>414 H</td>	aa position	289	291	293	295 Y	A 062	298 V	301	307 313 S	314 318	330	332	336 336	343 C	344	346	347 348	349	354 356	356	358	360	371	374 > 375	376	378 Þ	380	384	385	395 CD	396	397	399	400 D	403	404 407 m	408	414 H
Au-280       S       R       V       T       Q       R       Q       T       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V <td>aa position s</td> <td>289</td> <td>- 291</td> <td>293</td> <td>295 Y</td> <td>297 b</td> <td>298 V 299</td> <td>301</td> <td>a 313 S</td> <td>314 318</td> <td>330</td> <td>c 332</td> <td>336</td> <td>343.0</td> <td>344</td> <td>345 346</td> <td>a 347 348 14</td> <td>349</td> <td>354 355</td> <td>356</td> <td>358</td> <td>360</td> <td>371</td> <td>a 374 P</td> <td>376</td> <td>378 P</td> <td>380</td> <td>384</td> <td>385</td> <td>395 CD</td> <td>396</td> <td>397</td> <td>399</td> <td>400 C</td> <td>403</td> <td>404 407 m</td> <td>408</td> <td>414 H</td>	aa position s	289	- 291	293	295 Y	297 b	298 V 299	301	a 313 S	314 318	330	c 332	336	343.0	344	345 346	a 347 348 14	349	354 355	356	358	360	371	a 374 P	376	378 P	380	384	385	395 CD	396	397	399	400 C	403	404 407 m	408	414 H
XAWasaki308       T       V       T       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V <td< td=""><td>aa position Strains CAU-276</td><td>107 S R</td><td>H H 291</td><td><b>2 2</b> 293</td><td>1 295 V</td><td>Z 297 P</td><td>A 298 A 298</td><td>301 R R</td><td>1 1 313 S</td><td><b>X X</b> 314 <b>D</b> 318</td><td>× × 330</td><td></td><td>336 237 H</td><td>5 537 W 3 6 343 C</td><td><b>N</b> 344</td><td><b>A A</b> 346</td><td>a a 347</td><td><b>6 10</b> 349</td><td>0 0 354 0 0 354 7 7 366</td><td><b>8</b> 356 <b>8</b> 357</td><td><b>5 5 5 5 5 5 5 5 5 5</b></td><td><b>d A</b> 360 <b>d</b> 362</td><td><ul> <li>A 371</li> <li>A 373</li> </ul></td><td>- 374 P</td><td>376 377</td><td><b>N</b> 378 <b>N</b></td><td></td><td>1 384</td><td>0 0 385</td><td><b>Z</b> 395 B</td><td>a a 396</td><td>0 0 397 0 0 398</td><td>399 0 399</td><td>400 C</td><td>0 201 F F</td><td>404 407 107</td><td>- <b>7</b></td><td>► 410 ►</td></td<>	aa position Strains CAU-276	107 S R	H H 291	<b>2 2</b> 293	1 295 V	Z 297 P	A 298 A 298	301 R R	1 1 313 S	<b>X X</b> 314 <b>D</b> 318	× × 330		336 237 H	5 537 W 3 6 343 C	<b>N</b> 344	<b>A A</b> 346	a a 347	<b>6 10</b> 349	0 0 354 0 0 354 7 7 366	<b>8</b> 356 <b>8</b> 357	<b>5 5 5 5 5 5 5 5 5 5</b>	<b>d A</b> 360 <b>d</b> 362	<ul> <li>A 371</li> <li>A 373</li> </ul>	- 374 P	376 377	<b>N</b> 378 <b>N</b>		1 384	0 0 385	<b>Z</b> 395 B	a a 396	0 0 397 0 0 398	399 0 399	400 C	0 201 F F	404 407 107	- <b>7</b>	► 410 ►
Saithersburg       S       R       V       T       Q       R       R       Q       T       Y       D       K       V       M       V       M       V       S       Y       P       I       L       R       I       N       D       C       O       V       N       D       D       D       G       P       F       R       L       N       R       R       C       T       Y       D       K       V       M       V       S       Y       P       I       L       R       I       S       D       D       F       Q       V       N       D       D       D       G       T       F       R       L       N       E       L       N       E       L       N       E       L       N       D       D       D       G       T       F       R       L       N       D       D       D       D       G       T       T       T       T       T       T       T       T       T       T       T       T       T       T       T       T       T       T       T       T       T       T <t< td=""><td>Alposition Strains Strains CAU-276 CAU-283 CAU-289</td><td>107 S 701</td><td></td><td>293 294 294</td><td>1 1 295 V</td><td>Z Z Z 297 &gt;</td><td>298 V 299 V 299</td><td>301 R R R</td><td>0 0 307 0 1 1 313 0 2 0 307</td><td><b>X X X</b> 314 <b>D D D</b> 318</td><td><b>X X X</b> 330</td><td><pre>&lt; &lt; 332</pre></td><td>M M 336</td><td>0 0 0 343 C</td><td><b>Z Z 3</b>44</td><td><b>A A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A A A A A A A A A A</b></td><td><b>a a b b b b b b b b c b c c c c c c c c c c</b></td><td><b>6 6 6</b> 349</td><td>0 0 0 0 354 0 7 7 3 356</td><td>356 ∧ ∧ 356 ∧ ∧ 357</td><td><b>5 5 5 5 5 5 5 5 5 5</b></td><td>4 Y X 360 4 A X 362</td><td>× × × 371 - T T 373</td><td><b>A 74 P</b>  375</td><td>376 376 377</td><td><b>Z Z Z</b> 378 <b>D</b></td><td></td><td>1 1 384</td><td>0 0 0 0 385</td><td></td><td>1 0 396</td><td>0 0 0 397 0 0 0 398</td><td><b>0 0 0 0 0 0 0 0 0 0</b></td><td>0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0</td><td></td><td><b>A 2 2</b> 404</td><td>- T T 408</td><td>х х х 410 ▼ п т т 414 П</td></t<>	Alposition Strains Strains CAU-276 CAU-283 CAU-289	107 S 701		293 294 294	1 1 295 V	Z Z Z 297 >	298 V 299 V 299	301 R R R	0 0 307 0 1 1 313 0 2 0 307	<b>X X X</b> 314 <b>D D D</b> 318	<b>X X X</b> 330	<pre>&lt; &lt; 332</pre>	M M 336	0 0 0 343 C	<b>Z Z 3</b> 44	<b>A A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A B A A A A A A A A A A</b>	<b>a a b b b b b b b b c b c c c c c c c c c c</b>	<b>6 6 6</b> 349	0 0 0 0 354 0 7 7 3 356	356 ∧ ∧ 356 ∧ ∧ 357	<b>5 5 5 5 5 5 5 5 5 5</b>	4 Y X 360 4 A X 362	× × × 371 - T T 373	<b>A 74 P</b> 375	376 376 377	<b>Z Z Z</b> 378 <b>D</b>		1 1 384	0 0 0 0 385		1 0 396	0 0 0 397 0 0 0 398	<b>0 0 0 0 0 0 0 0 0 0</b>	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0		<b>A 2 2</b> 404	- T T 408	х х х 410 ▼ п т т 414 П
CAU-265       S       R       V       T       Q       I       Q       R       Q       T       V       V       V       V       Q       R       Q       T       V       V       V       V       Q       Q       Q       V       L       R       I       S       D       D       F       Q       V       N       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D       D </td <td>Strains Strains CAU-276 CAU-283 CAU-289 Stawasaki308</td> <td>4687 R V 5 R R V 5 R R V</td> <td></td> <td>0 0 0 0 293</td> <td></td> <td></td> <td><b>2000</b> 298 V</td> <td>301 R R R R</td> <td>0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0</td> <td>A A A 314 D D D 318</td> <td>х <b>х х х</b> 330</td> <td>&lt; &lt; &lt; &lt; 332 &lt; &lt; &lt; 333</td> <td>M M 336 M M 336</td> <td>0 0 0 0 343 C</td> <td><b>Z Z Z Z</b> 344</td> <td><b>V V V V V V V V V V</b></td> <td><b>1</b> <b>1</b> <b>1</b> <b>1</b> <b>1</b> <b>1</b> <b>1</b> <b>1</b> <b>1</b> <b>1</b></td> <td>0 0 0 353</td> <td>0 0 0 0 0 354 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5</td> <td><ul> <li>≤</li> <li>≤</li></ul></td> <td>0 <b>5 5 3 5 8</b></td> <td><b>A A A</b> <b>A A A</b> 362</td> <td>×××× 7773 373</td> <td><b>X X X X</b> 374 Y</td> <td>376 2 2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2</td> <td></td> <td></td> <td>1 1 384</td> <td>0 0 0 0 385</td> <td></td> <td><b>1 1</b> 396</td> <td>0 0 0 0 397 0 0 0 398</td> <td><b>D D D D D D D D D D</b></td> <td>0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0</td> <td>4 4 4 03 C</td> <td>404 R R R R R R R R R R R R R R R R R R</td> <td>1 L L L L</td> <td>х х х 410 ▼ <b>п п п 4</b>14 П</td>	Strains Strains CAU-276 CAU-283 CAU-289 Stawasaki308	4687 R V 5 R R V 5 R R V		0 0 0 0 293			<b>2000</b> 298 V	301 R R R R	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	A A A 314 D D D 318	х <b>х х х</b> 330	< < < < 332 < < < 333	M M 336 M M 336	0 0 0 0 343 C	<b>Z Z Z Z</b> 344	<b>V V V V V V V V V V</b>	<b>1</b> <b>1</b> <b>1</b> <b>1</b> <b>1</b> <b>1</b> <b>1</b> <b>1</b> <b>1</b> <b>1</b>	0 0 0 353	0 0 0 0 0 354 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	<ul> <li>≤</li> <li>≤</li></ul>	0 <b>5 5 3 5 8</b>	<b>A A A</b> <b>A A A</b> 362	×××× 7773 373	<b>X X X X</b> 374 Y	376 2 2 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2			1 1 384	0 0 0 0 385		<b>1 1</b> 396	0 0 0 0 397 0 0 0 398	<b>D D D D D D D D D D</b>	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	4 4 4 03 C	404 R R R R R R R R R R R R R R R R R R	1 L L L L	х х х 410 ▼ <b>п п п 4</b> 14 П
CAU-192       S       R       V       V       Q       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V       V </td <td>Ala position of Strains CAU-276 SCAU-283 SCAU-289 SCAU-280 SCAU-28</td> <td>200 200 200 200 200 200 200</td> <td></td> <td>0 0 0 0 0 293</td> <td></td> <td></td> <td>0 0 0 0 0 298 V 2 2 2 2 299 V</td> <td>2 2 2 2 301</td> <td>0 0 0 0 307 1 1 1 1 313 313 8</td> <td>A A A A 314 D D D D 318 318</td> <td>х х х х 330</td> <td>&lt; &lt; &lt; &lt; &lt; 332 &lt; &lt; &lt; &lt; 333</td> <td>M M M 336 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0</td> <td>0000003430</td> <td>Z Z Z Z Z 344</td> <td><b>V V V V V V V V V V</b></td> <td>H         H         347           D         D         D         348           D         D         D         348</td> <td>0 0 0 0 349</td> <td>0 0 0 0 0 354 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5</td> <td></td> <td>0 0 <b>5 8 3</b>58</td> <td>4 4 4 4 360 4 4 4 362 3 62</td> <td>- × × × 371 </td> <td>A 74 A 74</td> <td>376 277 277</td> <td>Z Z Z Z Z 378 P</td> <td></td> <td></td> <td>0 0 0 0 385</td> <td></td> <td>0 0 0 396</td> <td>0 0 0 0 397 0 0 0 0 398</td> <td></td> <td>0 0 0 0 0 400 C</td> <td>403</td> <td>404 RRRR</td> <td>1 L L L</td> <td>ZZZZZ410 ▼ ШШШ 414Ш</td>	Ala position of Strains CAU-276 SCAU-283 SCAU-289 SCAU-280 SCAU-28	200 200 200 200 200 200 200		0 0 0 0 0 293			0 0 0 0 0 298 V 2 2 2 2 299 V	2 2 2 2 301	0 0 0 0 307 1 1 1 1 313 313 8	A A A A 314 D D D D 318 318	х х х х 330	< < < < < 332 < < < < 333	M M M 336 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	0000003430	Z Z Z Z Z 344	<b>V V V V V V V V V V</b>	H         H         347           D         D         D         348           D         D         D         348	0 0 0 0 349	0 0 0 0 0 354 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5		0 0 <b>5 8 3</b> 58	4 4 4 4 360 4 4 4 362 3 62	- × × × 371 	A 74	376 277 277	Z Z Z Z Z 378 P			0 0 0 0 385		0 0 0 396	0 0 0 0 397 0 0 0 0 398		0 0 0 0 0 400 C	403	404 RRRR	1 L L L	ZZZZZ410 ▼ ШШШ 414Ш
Vagano7-1 POL       S       R       V       T       E       T       C       I       D       G       D       P       G       S       H       E       V       F       R       S       N       D       G       D       P       R       K       V       V       V       A       G       N       D       S       V       F       R       S       N       D       L       Q       I       N       D       G       D       P       R       L       Q       I       N       D       G       D       P       R       K       V       V       V       V       A       G       N       D       V       P       V       R       S       N       D       L       Q       I       N       D       G       D       P       R       K       D       N       D       N       D       N       D       N       D       N       D       N       D       N       D       N       D       N       D       N       D       N       D       N       D       D       D       D       D       D       D       D       <	Paposition       Strains       CAU-276       SCAU-283       CAU-289       Stavasaki308       Gaithersburg       CAU-265	4682 R N N N N N N N N N N N N N N N N N N		<b>D</b> D D D D D D 293 294		ZZZZZ 297 >	<b>00000</b> 298 V 2 2 2 2 2 2 299	301 <b>8 8 8 8</b>	<b>D</b> D D D D D D D D D D D D D D D D D D	A A A A A 314 D D D D 0 318 318	<mark>х х х х х</mark> 330	< < < < < < 332 < < < < < 333	M M 336 M M M 336 M M M M 336	<b>000000</b> 3430	<b>N</b> Z Z <b>N</b> 344	<b>A</b> A A A A 345 <b>A</b> A A A A 346	<b>H</b> <b>H</b> <b>H</b> <b>H</b> <b>H</b> <b>H</b> <b>H</b> <b>H</b> <b>H</b> <b>H</b>	<b>8 0 0 8 8 8</b> 349	D D D D D D 354	★ ★ ★ ★ ★ 356	<b>5</b> 58 <b>5 5 5 5 5 5 5 5 5 5</b>	<b>A A A A A A A A A A</b>	<pre><pre><pre><pre><pre><pre><pre><pre></pre></pre></pre></pre></pre></pre></pre></pre>	<b>X</b> X X X X 374 Y	376 377 377			1 1 1 1 384	0 0 0 0 0 385		<b>1</b> <b>1</b> <b>1</b> <b>1</b> <b>1</b> <b>1</b> <b>1</b> <b>1</b>	<b>0</b> 00 <b>00</b> 397		0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	<b>1 1 1 1 1 1 1 1 1 1</b>	404 R R R R R R R R R R R R R R R R R R		х Z Z Z Z Z 410 ▼ ппппп 414П
ADJ-51       S       K       V       T       K       V       V       V       A       N       D       S       F       N       D       L       Q       I       N       D       G       D       F       R       L       L       Q       I       N       D       G       D       F       R       L       L       Q       I       N       D       G       D       F       R       L       L       Q       I       N       D       G       D       F       R       L       L       Q       I       N       D       G       D       F       R       L       L       Q       I       N       D       G       D       F       R       L       L       L       L       L       D       N       D       L       Q       I       N       D       G       D       F       R       L       D       L       L       D       Q       N       N       D       N       N       D       N       N       D       N       N       D       N       N       D       N       N       D       N       D       N <td>Aa position S Strains S CAU-276 S CAU-289 S CAU-289 S Gauthersburg S CAU-265 S CAU-192 S</td> <td></td> <td></td> <td><b>200000</b> 293</td> <td></td> <td></td> <td><mark>ООООО</mark> 298 У У У У У У У У У У У У У У У У У У У</td> <td>100 RRRRRR R</td> <td>S 301 302 303 303 303 304 305 305 305 305 305 305 305 305</td> <td><b>XXXXX</b> 314 <b>D</b> D D D D D 318 318</td> <td><mark>х х х х х х</mark> 330</td> <td><pre>&lt; &lt; &lt; &lt; &lt; &lt; 332</pre></td> <td>M M M 336 M M M 336 M M M M 136 M M M M M 136 M M M M M M 136 M M M M M M M M M M M M M M M M M M M</td> <td><b>3 3 3 3 3 3 3 3 3 3</b></td> <td><b>Z Z Z Z Z Z Z 344</b></td> <td></td> <td>Р1 9 9 9 9 9 9 347 347 9 9 9 9 9 9 3 348</td> <td><b>8 8 8 8 8 8 3</b>49</td> <td></td> <td><b>3</b>56 <b>A A A A A A A A A A</b></td> <td><b>6 6 6 6 8</b> 358</td> <td><b>A A A A A A A A A A</b></td> <td><b>× × - × × ×</b> 371 <b>7 1 1 1 1 3</b>73</td> <td><b>X X X X X X</b> 374 Y</td> <td>376 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2</td> <td></td> <td></td> <td></td> <td>0 0 0 0 0 0 385 0 0 0 0 0 385</td> <td></td> <td><b>D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D D I D D I D D D D D D D D D D</b></td> <td><b>0</b> 0 0 0 0 397 0 0 0 0 0 398</td> <td></td> <td><b>000000</b> 4000</td> <td><b>4</b>02 <b>4 4</b>02 <b>4</b></td> <td>HOT RRRRR R HOT</td> <td></td> <td>х х Х Х Х Х Х 410 ▼ п п п п п 414 П</td>	Aa position S Strains S CAU-276 S CAU-289 S CAU-289 S Gauthersburg S CAU-265 S CAU-192 S			<b>200000</b> 293			<mark>ООООО</mark> 298 У У У У У У У У У У У У У У У У У У У	100 RRRRRR R	S 301 302 303 303 303 304 305 305 305 305 305 305 305 305	<b>XXXXX</b> 314 <b>D</b> D D D D D 318 318	<mark>х х х х х х</mark> 330	<pre>&lt; &lt; &lt; &lt; &lt; &lt; 332</pre>	M M M 336 M M M 336 M M M M 136 M M M M M 136 M M M M M M 136 M M M M M M M M M M M M M M M M M M M	<b>3 3 3 3 3 3 3 3 3 3</b>	<b>Z Z Z Z Z Z Z 344</b>		Р1 9 9 9 9 9 9 347 347 9 9 9 9 9 9 3 348	<b>8 8 8 8 8 8 3</b> 49		<b>3</b> 56 <b>A A A A A A A A A A</b>	<b>6 6 6 6 8</b> 358	<b>A A A A A A A A A A</b>	<b>× × - × × ×</b> 371 <b>7 1 1 1 1 3</b> 73	<b>X X X X X X</b> 374 Y	376 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2				0 0 0 0 0 0 385 0 0 0 0 0 385		<b>D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D I D D I D D I D D D D D D D D D D</b>	<b>0</b> 0 0 0 0 397 0 0 0 0 0 398		<b>000000</b> 4000	<b>4</b> 02 <b>4 4</b> 02 <b>4</b>	HOT RRRRR R HOT		х х Х Х Х Х Х 410 ▼ п п п п п 414 П
AU-85 SRVTET_DHRKQTYDKVVVAGNDAPGSHEAVISYPVFRSNDN_L_QIN_DGGDPFRELDL Curich/P7d384 TKISDVHSSHQRTPFDTLLVAGEPNXNTHEVVITTTIFGSESD_ISPIK_IETGPCNDLNA Katrina-17 TKISDVQNSHQRTPFDTLLVAGGTGNNTHEVVIATTIFGSESE_VGPIK_IETGSFRDPNA Saitama/T87 SRLTDVDGSHDRTPFETLLVAG_SNPNTHEAVISTSVFGSTSD_LQQIK_VESGDFDALRH 2142	Aa position S Strains CAU-276 S CAU-283 S CAU-289 S Kawasaki308 S Gaithersburg S CAU-265 S CAU-265 S CAU-192 S CAU-192 S			1 1 0 0 0 0 0 293			сносости 298 У 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	101 RRRRRR R R R R R R R R R R R R R R R	S S S S S S S S S S S S S S S S S S S	2 4 4 4 4 4 4 314 2 0 0 0 0 0 0 318 3 18	х <b>х х х х х х 3</b> 30		E 100 M 336 M M 336 M M M 336 M M M M M M M M M M M M M M M M M M M	<b>5 5 5 5 5 5 5 5 5 5</b>	ZZZZZZZ344	A         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C         C	0 0 0 0 0 0 0 0 347	5 0 0 0 0 0 0 0 349			0 0 0 0 0 0 0 0 358	<b>X X X X X X 360</b> <b>A A A A A A A</b> 360 362	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	374 Y	376 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2		082 0 F D F D F D F D F D F D F D F D F D F		0 0 0 0 0 0 0 0 385 		960 <b>D</b> 1 D	0 0 0 0 0 0 397 0 0 0 0 0 0 338 3 3 0 0 0 0 0 0 338		D D D D D D D D D D D D D D D D D D D	•         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •         •	404 RRRRRRRRR		0 <b>0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 </b>
Curich/P7d384       TKISDVHSSHQRTPFDTLLVAGEPNXNTHEVVITTTIFGSESD_ISPIK_IETGPCNDLNA         Katrina-17       TKISDVQNSHQRTPFDTLLVAGGTGNNTHEVVIATTIFGSESE_VGPIK_IETGSFRDPNA         Saitama/T87       SRLTDVDGSHDRTPFETLLVAG_SNPNTHEAVISTSVFGSTST_LQQIK_IESGEFDALRH         2142       SKLTDVHQSHDRTPFETLLVAV_SNPNTHEAVISTSIFGSTSD_LQQIK_VESGDFDALRH	Aa position S Strains CAU-276 S CAU-289 S CAU-289 S CAU-289 S CAU-285 S CAU-192 S Nagano7-1 POL S CAU-192 S Nagano7-1 POL S CAU-55 S			<b>1 1 1 1 1 1 1 1 1 1</b>			н н н <mark>о о о о о о</mark> 298 У и л и и и и и и и и 299	301 RRRRRR 201	S C C C C C C C C C C C C C C C C C C C	A A A A A A A A 314 D D D D D D D D 318 318	<b>х х х х х х х х х</b> 330	<pre>&lt; &lt; &lt; &lt; &lt; &lt; &lt; &lt; 332</pre>	E 1 336 M M M 1 336 M M M 1 1 336 M M M 1 1 336 M M 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1			<b>V V V V V V V V V V</b>	B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B         B	<b>8 0 0 8 0 0 0 0 0 3</b> 19 <b>7</b> 7 7 <b>0 0 0 0 0 0 3</b> 3 3 3		= -	<b>5 5 5 5 5 5 5 5 5 5</b>	4 Y Y Y Y 360 4 A Y Y Y 360 4 A Y Y Y 360 362 362	<b>× &lt; &lt; × &lt; </b> 371 <b>1</b> 1 1 1 1 1 373 <b>1</b> 2 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	A 727 R R R R R R R R R R R R R R R R R R	<b>X X X X X X X X X X</b>		0 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5				<b>1 1 1 1 1 1 1 1 1 1</b>	0 0 0 0 0 0 0 397 0 0 0 0 0 0 0 398 0 3 3 8		D D D D D D D D D D D D D D D D D D D	<b>4</b> 02 <b>4</b> 03 <b>4</b> 03 <b>4</b> 03	404 RRRRRRR 404		0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
Katrina-17 TKISDVQNSHQRTPFDTLLVAGGTGNNTHEVVIATTIFGSESE_VGPIK_IETGSFRDPNA Saitama/1787 SRLTDVDGSHDRTPFETLLVAG_SNPNTHEAVISTSVFGSTST_LQQIK_IESGEFDALRH 2142 SKLTDVHQSHDRTPFETLLVAV_SNPNTHEAVISTSIFGSTSD_LQQIK_VESGDFDALRH	aposition       Strains       CAU-276       Strains       CAU-283       SCAU-289       SCAU-289       SCAU-289       CAU-289       SCAU-289       SCAU-289       SCAU-289       SCAU-289       SCAU-289       SCAU-289       SCAU-289       SCAU-289       SCAU-289       SCAU-192       SCAU-192       SCAU-55       CAU-55       SCAU-385			<b>H H H H H H H H H H</b>			<u>ннноооо</u> 298 У <b>жилии и и и 1</b> 299 У	301 RRRRRR R 301	8 8 8 8 8 8 8 8 8 8 8 8 8 8	<b>A A A A A A A A A A</b> 314 <b>D D D D D D D D D 0</b> 318 318	<b>X X X X X X X X X</b> 330	<pre>&lt; &lt; &lt; &lt; &lt; &lt; &lt; &lt; &lt; 332</pre>	M M M M M M M M M M M M M M M M M M M	<b>9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 </b>		<b>A C C C C C C C C C C</b>	<b>d d d d d d d d d d</b>	<b>5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 </b>			<b>5 5 5 5 5 5 5 5 5 5</b>	<b>A A A A A A A A A A</b>	<b>×××××××</b> - <b>×××××</b> 371 	A 422 R R R R R R R R R R R R R R R R R R	376 2 2 2 5 5 5 2 3 76 2 2 2 2 2 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	<b>X X X X X X X X X X X X X X X X X X X </b>	082 D F D 0 F D D F F D D F F D D F F D I L L L L L L L L L L L L L L L L L L					<b>0 0 0 0 0 0 0 0 0 0</b>		D D D D D D D D D D D D D D D D D D D	<b>4 4 4 4 4 4 4 4 4 4</b>	404 R R R R R R R R R R R R R R R R R R		<pre>0 0 0 2 2 4 10 4 1 1 1 1 2 3 4 4 4 4 4 1 4 1 4 4 4 4 1 4 1 4 4 1 4 1 0 4 1 4 1 0 4 1 4 1 0 4 1 0 1 0 4 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1</pre>
Saitama/187 SIRLTDVDGSHDRTPFETLLVAG_SNPNTHEAVISTSVFGSTST_LQQIK_IESGEFDALRH 2142 SKLTDVHQSHDRTPFETLLVAV_SNPNTHEAVISTSIFGSTSD_LQQIK_VESGDFDALRH	Ba position     Strains       Strains     Strains       CAU-276     Strains       CAU-283     Strains       Gatthersburg     Strains-strains       Gatthersburg     Strains-strains       CAU-289     Strains-strains       Kawasaki308     I       Gatthersburg     Strains-strains       CAU-265     Strains-strains       CAU-192     Strains-strains       Nagano7-1 POL     Strains-strains       CAU-55     Strains-strains       CAU-55     Strains-strains       Zürich/P7d384     1	500 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	<b>2 1 1 1 1 1 1 1 1 1 1</b>	а <b>в в е в б б б б б б б</b> 293 А <b>1 1 1 1 1 1 1 1 1 1 2</b> 94	HIIIIII 295 Y		Н <b>НННООООО</b> 298 V Салалалала 23 299	301 <b>8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8</b>	S 301 302 304 304 305 307 307 307 307 307 307 307 307	4 4 4 4 4 4 4 4 4 4 4 314 0 0 0 0 0 0 0 0 0 0 318 318	Т <mark>Х Х Х Х Х Х Х Х Х Х</mark> 330		→ → → → → → → → → → → → → → → → → → →	9         9         9         5         7         5         8           9         9         9         9         5         6         9         8         8           9         9         9         9         9         9         3         3         8		<b>V C C C C C C C C C C</b>	X 4 4 4 4 4 4 4 4 4 4 4 4 347 X 9 9 9 9 9 9 9 9 9 9 3 348 X 9 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	1         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5         5			828 <b>S S S S S S S S S S</b>	L L L L L L L L L L L L L L L L L L L	- <mark>&lt; &lt; &lt;</mark>	974 S S S S S S S S S S S S S S S S S S S	E Z Z Z Z S S S S S 376			01         1         1         1         1         1         1         1         384				<b>1 0 0 0 0 0 0 0 0 3</b> 97 <b>2 0 0 0 0 0 0 0 0 3</b> 98 <b>3 0 0 0 0 0 0 0 0 0 0 3</b> 98	D D D D G G G G G G G G G G G G G G G G	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	<b>244</b>	HOF RRRRRRRRRRRRR		V D D D Z Z Z Z Z Z Z Z Z Z Z Z Z Z Z Z
J142 SKLIDVHQSHDRTPFETLLVAV_SNPNTHEAVISTSIFGSTSD_LQQTK_VESGDFDALRH	ap position     strains       Strains     strains       CAU-276     strains       CAU-283     strains       CAU-289     strains       Gaithersburg     strains       CAU-192     strains       Nagano7-1 POL     strains       CAU-55     strains       CAU-55     strains       CAU-55     strains       CAU-55     strains       CAU-57     strains		<b>1 1 1 1 1 1 1 1 1 1</b>	0 0 <b>3 3 3 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 </b>			НН <b>НННООООО</b> 298 2000 2000 2000 2000 2000 2000 2000	301 <b>5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5</b>	S T T D D D D D D D D D D D D D D D D D D	4 4 4 4 4 4 4 4 4 4 4 3 3 4 3 4 0 0 0 0	330 <b>5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5</b>		→ → → → → → → → → → → → → → → → → → →	9       9       9       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5       5		<b>1 0 0 0 0 0 0 0 0 0 0</b>	X X 4 4 4 4 4 4 4 4 4 4 4 347         X 1 347           X X 0 0 0 0 0 0 0 0 0 0 0 348         348	1 1 <b>6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6</b>			828 S S S S S S S S S S S S S S S S S S	11144444444444444444444444444444444444	12 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	A 72 R R R R R R R R R R R R R R R R R R	<b>H H K K K S S S S S S S S S S S S S S S </b>		D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F F D F D F F D F D F F D F D F F D F D F F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F D F	O SI I I I I I I I I 384	<b>A A D D D D D D D D D D</b>	х х <mark>х х х х х х х х х х х</mark> 395 в		<b>1 1 0 0 0 0 0 0 0 0 0 0</b>			2 2 4 4 2 4 2 4 4 4 4 4 7 4 7 4 7 4 7 4	404 RRRRRRRRRR 404		V U O O O O Z Z Z Z Z Z Z Z Z Z Z Z Z Z Z
	aposition         Strains         CAU-276         CAU-276         CAU-289         Staussaki308         Gaithersburg         Gaithersburg         CAU-265         SCAU-192         Saithersburg         CAU-265         Saithersburg         CAU-365         Saithersburg         Sai	187 S S S T S S S S S S T T S S		C D D D <b>D D D D D D D D 2</b> 93		500 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	: Н Н Н <b>Н Н Н Р Р Р Р Р Р Р 2</b> 98 Р	301 <b>8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8</b>	S S S S S S S S S S S S S S S S S S S	- F - E - E - E - E - E - E - E - E - E	330 <b>K K K K K K K K K K T</b> T T T	. T T T <b>&lt; &lt; 332</b> . T T T <b>&lt; &lt; 333</b>	■ 1 × 1 × 1 × 1 × 1 × 1 × 1 × 1 × 1 × 1	0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0     0 <td></td> <td></td> <td>L         Z         Z         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d          d         d         d</td> <td>1 1 1 1 <b>6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6</b></td> <td></td> <td></td> <td>825 <b>S S S S S S S S S S S S S S S S S S S</b></td> <td>2 T T T T Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y</td> <td></td> <td>0000<b>023483348345545</b>000000000000000000000000000000000</td> <td></td> <td></td> <td>D F F D 3330 D F F D D F F D D F F D D F F D D F F D D F F D D F F D D F F D D F F D D F F D D F F D D F D D F D D F D D F D D F D D F D D F D D F D D F D D F D D F D D F D D F D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D D F D D D D F D D D D F D D D D F D D D D F D D D D F D D D D F D D D D F D D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D D F D D D D F D D D F D D D F D D D D F D D D F D D D D F D D D D F D D D D F D D D D F D D D D F D D D D D F D D D D F D D D D D F D D D D D D F D D D D D D D D D D D D D D D D D D D D</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>66C D D D D D G G G G T T S</td> <td></td> <td>1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1</td> <td>404 RRRRRRRRRRR 404</td> <td></td> <td>N N N N N N N N N N N N N N N N N N N</td>			L         Z         Z         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d         d          d         d         d	1 1 1 1 <b>6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6</b>			825 <b>S S S S S S S S S S S S S S S S S S S</b>	2 T T T T Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y		0000 <b>023483348345545</b> 000000000000000000000000000000000			D F F D 3330 D F F D D F F D D F F D D F F D D F F D D F F D D F F D D F F D D F F D D F F D D F F D D F D D F D D F D D F D D F D D F D D F D D F D D F D D F D D F D D F D D F D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D D F D D D D F D D D D F D D D D F D D D D F D D D D F D D D D F D D D D F D D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D F D D D D F D D D D F D D D F D D D F D D D D F D D D F D D D D F D D D D F D D D D F D D D D F D D D D F D D D D D F D D D D F D D D D D F D D D D D D F D D D D D D D D D D D D D D D D D D D D						66C D D D D D G G G G T T S		1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1	404 RRRRRRRRRRR 404		N N N N N N N N N N N N N N N N N N N

Fig 4. Amino acid substitutions in the VP1 sequence of norovirus GII.17 strains over time. The putative blockade epitopes A–E are indicated. Dashes indicate deletions/insertions of the amino acid residues. Amino acid numbering is based on the sequence of the C142 strain (JN699043).

doi:10.1371/journal.pone.0154284.g004

PLOS

aa ...

H298Q, K301R, V336M, A337V, H353Q, E354Q, I357V, F373L, S375I, N376S, -380D, L383F, I394V, -396D, G398D, G399D, D400G, D410N, L414E, V452I, and Y517H) and accumulated changes at five positions (H253Q, A356W, P402T, H480Y, and T541R) as compared with viruses isolated in 2013 (CAU-55 and CAU-85).

#### Discussion

Enteric viruses such as rotaviruses, NoVs, astroviruses, and adenoviruses are the most significant etiological agents of childhood viral gastroenteritis in both developing and developed countries [23]. NoV is considered to be the second most common cause of acute gastroenteritis requiring hospitalization among children below five years of age [2]. Laboratory-confirmed NoV infections involving 149 strains (14.2%) were isolated from children with acute gastroenteritis in this study. Similar findings have been reported with a figure of 13% in patients with acute gastroenteritis symptoms from five participating hospitals in three regions (Seoul, Gyeonggi Province, and Gangwon Province) from 2007 to 2010 [10]. However, this was a lower percentage of NoV-positive samples than those reported in previous studies [9,11]. This discrepancy might be due to differences in the duration of the studies, the study design, and the target population, for example.

Six different NoV genotypes were identified by BLAST using 300-bp sequences of the polymerase and capsid genes. The predominant genotype in this study was NoV GII.4, which accounted for 114 of 149 (76.5%) of the detected NoV strains. Several other previous studies in Korea have also shown that most of the NoV GII genotypes belonged to GII.4, followed by GII.3, GII.6, and GII.8 [9,24,25]. However, the relatively high detection rate (10.75%) of the previously rare GII.17 genotype reported in South Korea indicated changing patterns of the circulating NoV strains. Although it is still too early to predict whether NoV GII.17 will replace NoV GII.4, GII.17 is the predominant genotype in Asian countries such as Hong Kong and Taiwan, and in Japan the number of cases caused by this novel virus sharply increased from 2013 to 2015 [13].

Phylogenetic analysis of the full-length sequences established a separate cluster for the NoV GII.17 strains in a sister relationship with other known NoV GII.17 strains identified earlier. NoV GII.17 strains in Korea had evolutionary relationships with those obtained from 2013–2015 in the US, Italy, China, Japan, and Hong Kong. However, it is surprising to note that the isolated Korean NoVs diverged into two distinct subclusters within a relatively short period of time. The rapid evolution of the novel GII.17 strain may lead to it evading the host immune response, driving changes in histo-blood group antigen affinities, and altering population susceptibility patterns [26,27,28]. Interestingly, the GII.3 strains detected in the 2000s have an evolutionary relationship with the recent GII.17 cluster III viruses.

Analysis of VP1 protein sequences revealed 99 amino acid changes. VP1 is further divided into the shell (S) comprised of amino acids 1–220 and a protruding (P) domain comprised of amino acids 221–541. The S domain contains elements essential for formation of the contiguous shell of the virus [29]. The P domain is divided into two subdomains, P1 and P2 [5]. P1, a conserved region among NoV strains across genogroups, consists of amino acid residues 221 to 276 and 415 to 541. P2, which consists of amino acids 277 to 414 as an insertion into P1, is hypervariable in sequence. Meanwhile, the P2 region is thought to play an important role in receptor binding and immune reactivity, and is likely to be primarily responsible for ABO histo-blood group antigen interactions associated with susceptibility to NoV infections [14,30]. Mutations in this region may have a significant effect on binding interactions [26,29,31]. Comparison of amino acid sequences between Korean NoV strains of 2013 and 2014/15 revealed the accumulation of mutations at several sites in VP1, and particularly in the main blockade epitopes located in the P2 region.

# Conclusions

The present findings demonstrate that the emerging new variants of NoV GII.17 were the second most predominant in all NoV-positive children with acute gastroenteritis. Amino acid analysis revealed several mutations in the P2 region of the recent strains. Thus, it is necessary to continue epidemiological surveillance of the emergence of GII.17 and monitor trends in its geographical spread and evolution.

#### **Supporting Information**

S1 Table. The norovirus in this study and reference strains used to construct phylogenetic tree.

(DOC)

#### Acknowledgments

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education (2013R1A1A2A10012148).

#### **Author Contributions**

Conceived and designed the experiments: WK. Performed the experiments: HDT THN. Analyzed the data: HDT VTT WK. Contributed reagents/materials/analysis tools: IL WK. Wrote the paper: HDT WK.

#### References

- 1. Hall AJ, Lopman BA, Payne DC, Patel MM, Gastanaduy PA, Vinje J, et al. (2013) Norovirus disease in the United States. Emerg Infect Dis 19: 1198–1205. doi: 10.3201/eid1908.130465 PMID: 23876403
- Kowalzik F, Riera-Montes M, Verstraeten T, Zepp F (2015) The burden of norovirus disease in children in the European Union. Pediatr Infect Dis J 34: 229–234. doi: <u>10.1097/INF.0000000000546</u> PMID: <u>25742072</u>
- 3. Mayet A, Andreo V, Bedubourg G, Victorion S, Plantec J, Soullie B, et al. (2011) Food-borne outbreak of norovirus infection in a French military parachuting unit, April 2011. Euro Surveill 16.
- Patel MM, Widdowson MA, Glass RI, Akazawa K, Vinje J, Parashar UD (2008) Systematic literature review of role of noroviruses in sporadic gastroenteritis. Emerg Infect Dis 14: 1224–1231. doi: <u>10.3201/</u> eid1408.071114 PMID: <u>18680645</u>
- Hardy ME (2005) Norovirus protein structure and function. FEMS Microbiol Lett 253: 1–8. PMID: <u>16168575</u>
- Vinje J (2015) Advances in laboratory methods for detection and typing of norovirus. J Clin Microbiol 53: 373–381. doi: <u>10.1128/JCM.01535-14</u> PMID: <u>24989606</u>
- Zheng DP, Widdowson MA, Glass RI, Vinje J (2010) Molecular epidemiology of genogroup II-genotype 4 noroviruses in the United States between 1994 and 2006. J Clin Microbiol 48: 168–177. doi: <u>10.1128/</u> JCM.01622-09 PMID: <u>19864482</u>
- Kumazaki M, Usuku S (2015) Genetic Analysis of Norovirus GII.4 Variant Strains Detected in Outbreaks of Gastroenteritis in Yokohama, Japan, from the 2006–2007 to the 2013–2014 Seasons. PLoS One 10: e0142568. doi: 10.1371/journal.pone.0142568 PMID: 26544040
- Truong TC, Than VT, Kim W (2014) Evolutionary phylodynamics of Korean noroviruses reveals a novel GII.2/GII.10 recombination event. PLoS One 9: e113966. doi: <u>10.1371/journal.pone.0113966</u> PMID: <u>25500567</u>
- Park DJ, Kim JS, Park JY, Kim HS, Song W, Kim HS, et al. (2010) Epidemiological Analysis of Norovirus Infection between March 2007 and February 2010. Korean J Lab Med 30: 647–653. doi: <u>10.3343/kjlm.2010.30.6.647</u> PMID: <u>21157152</u>
- Cho HG, Park PH, Lee SG, Kim JE, Kim KA, Lee HK, et al. (2015) Emergence of Norovirus GII.4 variants in acute gastroenteritis outbreaks in South Korea between 2006 and 2013. J Clin Virol 72: 11–15. doi: <u>10.1016/j.jcv.2015.08.012</u> PMID: <u>26356986</u>
- Rohayem J, Munch J, Rethwilm A (2005) Evidence of recombination in the norovirus capsid gene. J Virol 79: 4977–4990. PMID: <u>15795283</u>
- de Graaf M, van Beek J, Vennema H, Podkolzin AT, Hewitt J, Bucardo F, et al. (2015) Emergence of a novel GII.17 norovirus—End of the GII.4 era? Euro Surveill 20.
- Chan MC, Lee N, Hung TN, Kwok K, Cheung K, Tin EK, et al. (2015) Rapid emergence and predominance of a broadly recognizing and fast-evolving norovirus GII.17 variant in late 2014. Nat Commun 6: 10061. doi: 10.1038/ncomms10061 PMID: 26625712
- Parra GI, Green KY (2015) Genome of Emerging Norovirus GII.17, United States, 2014. Emerg Infect Dis 21: 1477–1479. doi: <u>10.3201/eid2108.150652</u> PMID: <u>26196235</u>
- 16. Lee CC, Feng Y, Chen SY, Tsai CN, Lai MW, Chiu CH (2015) Emerging norovirus GII.17 in Taiwan. Clin Infect Dis 61: 1762–1764. doi: <u>10.1093/cid/civ647</u> PMID: <u>26306886</u>
- Lu J, Sun L, Fang L, Yang F, Mo Y, Lao J, et al. (2015) Gastroenteritis Outbreaks Caused by Norovirus GII.17, Guangdong Province, China, 2014–2015. Emerg Infect Dis 21: 1240–1242. doi: <u>10.3201/</u> <u>eid2107.150226</u> PMID: <u>26080037</u>
- Matsushima Y, Ishikawa M, Shimizu T, Komane A, Kasuo S, Shinohara M, et al. (2015) Genetic analyses of GII.17 norovirus strains in diarrheal disease outbreaks from December 2014 to March 2015 in Japan reveal a novel polymerase sequence and amino acid substitutions in the capsid region. Euro Surveill 20.
- Rohayem J, Berger S, Juretzek T, Herchenroder O, Mogel M, Poppe M, et al. (2004) A simple and rapid single-step multiplex RT-PCR to detect Norovirus, Astrovirus and Adenovirus in clinical stool samples. J Virol Methods 118: 49–59. PMID: <u>15158068</u>

- Kim EJ, Cheon DS, Jeong HS, Park SH, CK. K, Choi SS, et al. (2010) Genetic Diversity of Norovirus in Outbreaks of Gastroenteritis in Seoul. Food Sci Biotechnol 19: 1089–1092.
- Wang HB, Wang Q, Zhao JH, Tu CN, Mo QH, Lin JC, et al. (2015) Complete nucleotide sequence analysis of the norovirus GII.17: A newly emerging and dominant variant in China, 2015. Infect Genet Evol 38: 47–53. doi: 10.1016/j.meegid.2015.12.007 PMID: 26687061
- Tamura K, Stecher G, Peterson D, Filipski A, K S. (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30: 2725–2729. doi: <u>10.1093/molbev/mst197</u> PMID: <u>24132122</u>
- Lu L, Jia R, Zhong H, Xu M, Su L, Cao L, et al. (2015) Molecular characterization and multiple infections of rotavirus, norovirus, sapovirus, astrovirus and adenovirus in outpatients with sporadic gastroenteritis in Shanghai, China, 2010–2011. Arch Virol 160: 1229–1238. doi: <u>10.1007/s00705-015-2387-1</u> PMID: <u>25772574</u>
- Ham H, Oh S, Seung H, Jo S (2015) Molecular characteristics of noroviruses genogroup I and genogroup II detected in patients with acute gastroenteritis. Ann Lab Med 35: 242–245. doi: <u>10.3343/alm.</u> <u>2015.35.2.242</u> PMID: <u>25729728</u>
- Park S, Jung J, Oh S, Jung H, Oh Y, Cho S, et al. (2012) Characterization of norovirus infections in Seoul, Korea. Microbiol Immunol 56: 700–707. doi: <u>10.1111/j.1348-0421.2012.00494.x</u> PMID: 22823184
- Lochridge VP, Jutila KL, Graff JW, Hardy ME (2005) Epitopes in the P2 domain of norovirus VP1 recognized by monoclonal antibodies that block cell interactions. J Gen Virol 86: 2799–2806. PMID: <u>16186235</u>
- 27. Debbink K, Lindesmith LC, Donaldson EF, Baric RS (2012) Norovirus immunity and the great escape. PLoS Pathog 8: e1002921. doi: 10.1371/journal.ppat.1002921 PMID: 23093932
- Chen H, Qian F, Xu J, Chan M, Shen Z, Zai S, et al. (2015) A novel norovirus GII.17 lineage contributed to adult gastroenteritis in Shanghai, China, during the winter of 2014–2015. Emerg Microbes Infect 4: e67. PMID: 26975060
- Allen DJ, Gray JJ, Gallimore CI, Xerry J, Iturriza-Gomara M (2008) Analysis of amino acid variation in the P2 domain of the GII-4 norovirus VP1 protein reveals putative variant-specific epitopes. PLoS One 3: e1485. doi: <u>10.1371/journal.pone.0001485</u> PMID: <u>18213393</u>
- 30. Zhang XF, Huang Q, Long Y, Jiang X, Zhang T, Tan M, et al. (2015) An outbreak caused by GII.17 norovirus with a wide spectrum of HBGA-associated susceptibility. Sci Rep 5: 17687. doi: <u>10.1038/</u> srep17687 PMID: 26639056
- Lochridge VP, Hardy ME (2007) A single-amino-acid substitution in the P2 domain of VP1 of murine norovirus is sufficient for escape from antibody neutralization. J Virol 81: 12316–12322. PMID: 17804495
- Vinje J, Vennema H, Maunula L, von Bonsdorff CH, Hoehne M, Schreier E, et al. (2003) International collaborative study to compare reverse transcriptase PCR assays for detection and genotyping of noroviruses. J Clin Microbiol 41: 1423–1433. PMID: 12682125
- Kojima S, Kageyama T, Fukushi S, Hoshino FB, Shinohara M, Uchida K, et al. (2002) Genogroup-specific PCR primers for detection of Norwalk-like viruses. J Virol Methods 100: 107–114. PMID: <u>11742657</u>