

Global Spread of Norovirus GII.G17 Kawasaki 308, 2014–2016

Technical Appendix

Complete Norovirus VP1 Gene Sequencing

Five collaborating sites (Canada, Germany, Hong Kong, New Zealand, and Russia) determined complete viral protein 1 (VP1) gene sequences by first generating ≈ 2.7 -kb amplicons covering 3' end of virus genomes using high-fidelity DNA polymerase, followed by Sanger sequencing as previously described (*1*). Remaining sites used their own in-house protocols for sequencing.

Phylogenetic Analysis

Phylogenetic inference was performed using the maximum-likelihood method. Best substitution matrix, Kimura 2-parameter, was determined in MEGA6 (<http://www.megasoftware.net>). A gamma distribution of rate variation that enabled invariable sites was used. The tree with the highest log likelihood is shown. Tree confidence was assessed by bootstrapping with 1,000 iterations. All nucleotide positions with gaps and missing data were excluded. The final dataset contained 1,603 positions for analysis. The tree was rooted to an outgroup (norovirus GII.21; strain GII.21/IF1998/IQ/2003; GenBank accession no. AY675554).

Haplotype Network Analysis

Haplotype network analysis has been widely used to study virus transmission pattern, such as the Ebola virus (2,3), and it works best on closely related strains. A median-joining haplotype network was constructed using PopART version 1.7 (<http://popart.otago.ac.nz>) based on complete VP1 nucleotide sequences of norovirus GII.17 Kawasaki strains collected during 2014–2016. Kawasaki323-like strains (i.e., those collected before mid-2014) were excluded

because they were genetically distant from Kawasaki308-like strains that circulated predominantly in late 2014 onward and thus were not suitable for haplotype network analysis.

References

1. Chan MC, Lee N, Hung TN, Kwok K, Cheung K, Tin EK, et al. Rapid emergence and predominance of a broadly recognizing and fast-evolving norovirus GII.17 variant in late 2014. *Nat Commun.* 2015;6:10061. PubMed <http://dx.doi.org/10.1038/ncomms10061>
2. Ladner JT, Wiley MR, Mate S, Dudas G, Prieto K, Lovett S, et al. Evolution and spread of Ebola virus in Liberia, 2014–2015. *Cell Host Microbe.* 2015;18:659–69. PubMed <http://dx.doi.org/10.1016/j.chom.2015.11.008>
3. Mate SE, Kugelman JR, Nyenswah TG, Ladner JT, Wiley MR, Cordier-Lassalle T, et al. Molecular evidence of sexual transmission of Ebola virus. *N Engl J Med.* 2015;373:2448–54. PubMed <http://dx.doi.org/10.1056/NEJMoa1509773>

Technical Appendix Table 1. List of 254 complete viral protein 1 nucleotide sequences of norovirus genogroup II genotype 17 (GII.17) Kawasaki used in median-joining haplotype network analysis*

Counter	Strain Name	GenBank Accession no.	Year and month of collection	City/Province/Country	Remarks
1	NS-405	KP902566	2014 Sep	Hong Kong	Sublineage SL1 reference
2	NS-438	KP902567	2014 Nov	Hong Kong	
3	NS-455	KP902568	2014 Nov	Hong Kong	
4	NS-456	KP902569	2014 Nov	Hong Kong	
5	NS-463	KP902570	2014 Dec	Hong Kong	
6	NS-469	KT315668	2014 Dec	Hong Kong	
7	NS-471	KU561224	2014 Dec	Hong Kong	This study
8	NS-472	KT315669	2014 Dec	Hong Kong	
9	NS-475	KT315670	2014 Dec	Hong Kong	
10	NS-476	KT315671	2014 Dec	Hong Kong	
11	NS-477	KU561225	2014 Dec	Hong Kong	This study
12	NS-478	KT315672	2014 Dec	Hong Kong	
13	NS-480	KP902571	2014 Dec	Hong Kong	
14	NS-481	KU561226	2014 Dec	Hong Kong	This study
15	NS-482	KP902572	2014 Dec	Hong Kong	
16	NS-483	KP902573	2014 Dec	Hong Kong	
17	NS-484	KU561227	2014 Dec	Hong Kong	This study
18	NS-486	KT315673	2014 Dec	Hong Kong	
19	NS-488	KU561228	2014 Dec	Hong Kong	This study
20	NS-491	KP698928	2014 Dec	Hong Kong	
21	NS-492	KP902574	2014 Dec	Hong Kong	
22	NS-493	KP902575	2014 Dec	Hong Kong	
23	NS-494	KP698929	2014 Dec	Hong Kong	
24	NS-498	KU561229	2014 Dec	Hong Kong	This study
25	NS-500	KP902576	2014 Dec	Hong Kong	
26	NS-502	KP902577	2014 Dec	Hong Kong	
27	NS-503	KP902578	2014 Dec	Hong Kong	
28	NS-504	KU561230	2015 Jan	Hong Kong	This study
29	NS-506	KP902579	2015 Jan	Hong Kong	
30	NS-511	KP698930	2015 Jan	Hong Kong	
31	NS-512	KP902580	2015 Jan	Hong Kong	
32	NS-513	KP698931	2015 Jan	Hong Kong	
33	NS-514	KP902581	2015 Jan	Hong Kong	
34	NS-517	KP902582	2015 Jan	Hong Kong	Sublineage SL2 reference
35	NS-520	KP902583	2015 Jan	Hong Kong	
36	NS-521	KP902584	2015 Jan	Hong Kong	

Counter	Strain Name	GenBank Accession no.	Year and month of collection	City/Province/Country	Remarks
37	NS-522	KT315674	2015 Jan	Hong Kong	
38	NS-523	KT315675	2015 Jan	Hong Kong	
39	NS-528	KP902585	2015 Jan	Hong Kong	
40	NS-533	KT315676	2015 Jan	Hong Kong	
41	NS-534	KT315677	2015 Jan	Hong Kong	
42	NS-535	KU561231	2015 Jan	Hong Kong	This study
43	NS-536	KU561232	2015 Jan	Hong Kong	This study
44	NS-537	KT315678	2015 Jan	Hong Kong	
45	NS-539	KT315679	2015 Jan	Hong Kong	
46	NS-541	KU561233	2015 Jan	Hong Kong	This study
47	NS-543	KU561234	2015 Jan	Hong Kong	This study
48	NS-546	KU561235	2015 Jan	Hong Kong	This study
49	NS-548	KU561236	2015 Jan	Hong Kong	This study
50	NS-549	KP902586	2015 Jan	Hong Kong	
51	NS-556	KP902587	2015 Jan	Hong Kong	
52	NS-560	KT315680	2015 Jan	Hong Kong	
53	NS-565	KP902588	2015 Jan	Hong Kong	
54	NS-570	KT315681	2015 Jan	Hong Kong	
55	NS-574	KP902589	2015 Jan	Hong Kong	
56	NS-575	KP902590	2015 Jan	Hong Kong	
57	NS-576	KU561237	2015 Jan	Hong Kong	This study
58	NS-579	KT315682	2015 Feb	Hong Kong	
59	NS-580	KU561238	2015 Feb	Hong Kong	This study
60	NS-582	KT315683	2015 Feb	Hong Kong	
61	NS-583	KU561239	2015 Feb	Hong Kong	This study
62	NS-586	KT315684	2015 Feb	Hong Kong	
63	NS-589	KT315685	2015 Feb	Hong Kong	
64	NS-592	KT315686	2015 Feb	Hong Kong	
65	NS-593	KT315687	2015 Feb	Hong Kong	
66	NS-594	KU561240	2015 Feb	Hong Kong	This study
67	NS-595	KU561241	2015 Feb	Hong Kong	This study
68	NS-599	KT315688	2015 Feb	Hong Kong	
69	NS-600	KT315689	2015 Feb	Hong Kong	
70	NS-602	KT315690	2015 Feb	Hong Kong	
71	NS-603	KT315691	2015 Feb	Hong Kong	
72	NS-604	KT315692	2015 Feb	Hong Kong	
73	NS-606	KT315693	2015 Feb	Hong Kong	
74	NS-610	KU561242	2015 Feb	Hong Kong	This study
75	NS-611	KT315694	2015 Feb	Hong Kong	
76	NS-612	KT315695	2015 Mar	Hong Kong	Sublineage SL3 reference
77	NS-613	KU561248	2015 Mar	Hong Kong	This study; basal haplotype
78	NS-614	KU561243	2015 Mar	Hong Kong	This study
79	NS-616	KT315696	2015 Mar	Hong Kong	Basal haplotype
80	NS-619	KT315697	2015 Mar	Hong Kong	
81	NS-622	KU561244	2015 Mar	Hong Kong	This study
82	NS-627	KT315698	2015 Mar	Hong Kong	
83	NS-629	KT315699	2015 Mar	Hong Kong	
84	NS-634	KT315700	2015 Mar	Hong Kong	
85	NS-635	KU561245	2015 Mar	Hong Kong	This study
86	NS-636	KT315701	2015 Mar	Hong Kong	
87	NS-637	KT315702	2015 Mar	Hong Kong	
88	NS-639	KT315703	2015 Mar	Hong Kong	
89	NS-641	KT315704	2015 Mar	Hong Kong	
90	NS-643	KT315705	2015 Mar	Hong Kong	
91	NS-647	KT315706	2015 Mar	Hong Kong	
92	NS-648	KT315707	2015 Mar	Hong Kong	
93	NS-649	KT315708	2015 Mar	Hong Kong	
94	NS-650	KT315709	2015 Mar	Hong Kong	
95	NS-653	KT315710	2015 Apr	Hong Kong	
96	NS-655	KT315711	2015 Apr	Hong Kong	
97	NS-656	KT315712	2015 Apr	Hong Kong	
98	NS-657	KT315713	2015 Apr	Hong Kong	
99	NS-658	KT315714	2015 Apr	Hong Kong	
100	NS-659	KT315715	2015 Apr	Hong Kong	
101	NS-662	KT315716	2015 Apr	Hong Kong	
102	NS-667	KT315717	2015 Apr	Hong Kong	
103	NS-670	KT315718	2015 May	Hong Kong	
104	NS-671	KT315719	2015 May	Hong Kong	

Counter	Strain Name	GenBank Accession no.	Year and month of collection	City/Province/Country	Remarks
105	NS-679	KU561246	2015 Jun	Hong Kong	This study
106	NS-680	KU561247	2015 Jun	Hong Kong	This study
107	NS-767	KX168437	2015 Oct	Hong Kong	This study
108	NS-861	KX168438	2015 Dec	Hong Kong	This study
109	NS-863	KX168439	2016 Jan	Hong Kong	This study
110	NS-866	KX168440	2016 Jan	Hong Kong	This study
111	NS-880	KX168441	2016 Jan	Hong Kong	This study
112	NS-882	KX168442	2016 Jan	Hong Kong	This study
113	NS-892	KX168443	2016 Feb	Hong Kong	This study
114	NS-896	KX168444	2016 Feb	Hong Kong	This study
115	NS-899	KX168445	2016 Feb	Hong Kong	This study
116	NS-901	KX168446	2016 Feb	Hong Kong	This study
117	NS-907	KX168447	2016 Feb	Hong Kong	This study
118	NS-911	KX168448	2016 Feb	Hong Kong	This study
119	NS-917	KX168449	2016 Mar	Hong Kong	This study; sublineage SL1 reference
120	NS-920	KX168450	2016 Mar	Hong Kong	This study
121	NS-922	KX168451	2016 Mar	Hong Kong	This study
122	NS-928	KX168452	2016 Mar	Hong Kong	This study
123	NS-930	KX168453	2016 Mar	Hong Kong	This study
124	NS-935	KX168454	2016 Mar	Hong Kong	This study
125	NS-936	KX168455	2016 Mar	Hong Kong	This study
126	NS-942	KX168456	2016 Mar	Hong Kong	This study
127	41621	KR020503	2014 Dec	Guangzhou	
128	GZ2014-L311	KT149168	2014 Dec	Guangzhou	
129	GZ2014-L313	KT149169	2014 Dec	Guangzhou	
130	GZ2015-L324	KT149170	2015 Jan	Guangzhou	
131	GZ2015-L325	KT149171	2015 Jan	Guangzhou	
132	GZ2015-L337	KT149172	2015 Jan	Guangzhou	
133	GZ2015-L339	KT149173	2015 Jan	Guangzhou	
134	GZ2015-L340	KT149174	2015 Jan	Guangzhou	
135	GZ2015-L343	KT149175	2015 Jan	Guangzhou	
136	GZ2015-L362	KT149176	2015 Mar	Guangzhou	
137	JSSZ14313	KR270442	2015 Mar	Jiangsu	
138	JSZJ14003	KR270443	2015 Mar	Jiangsu	
139	JSCZ14010	KR270444	2015 Mar	Jiangsu	
140	JSWX14012	KR270445	2015 Mar	Jiangsu	
141	JSSZ15080	KR270446	2015 Mar	Jiangsu	
142	JSXZ15035	KR270447	2015 Mar	Jiangsu	
143	JSWX15027	KR270448	2015 Mar	Jiangsu	
144	JSNT15033	KR270449	2015 Mar	Jiangsu	
145	Kawasaki308	LC037415	2015 Feb	Japan	
146	HN01	KT992785	2015 Mar	Nanyang	
147	HN02	KT992786	2015 Mar	Nanyang	
148	HN03	KT992787	2015 Mar	Nanyang	
149	HN04	KT992788	2015 Mar	Nanyang	
150	HN05	KT992789	2015 Mar	Nanyang	
151	HNkaohao	KT992790	2015 Mar	Nanyang	
152	MIY2	LC101820	2015 Feb	Japan	
153	152642	KP864102	2015 Jan	Shanghai	
154	142700	KP864103	2014 Nov	Shanghai	
155	142661	KP864104	2014 Dec	Shanghai	
156	CGMH69	KR154230	2015 Jan	Taoyuan, Taiwan	
157	CGMH70	KR154231	2015 Feb	Taoyuan, Taiwan	
158	15-AD-2	KR052019	2015 Feb	Taichung, Taiwan	
159	15-AH-1	KR052020	2015 Feb	Changhua, Taiwan	
160	15-AP-1	KR052021	2015 Feb	Hsinchu, Taiwan	
161	15-R-4	KR052022	2015 Jan	Yunlin, Taiwan	
162	ZHITHC-12	KT253245	2015 Jan	Zhuhai	
163	Gaithersburg	KR083017	2014 Nov	USA	
164	PR668	KT346356	2015 Feb	Italy	
165	CAU-192	KU561252	2014 Nov	South Korea	
166	CAU-265	KU561253	2014 Dec	South Korea	
167	CAU-267	KU561254	2015 Jan	South Korea	
168	CAU-283	KU561255	2015 Mar	South Korea	
169	CAU-289	KU561256	2015 Apr	South Korea	
170	HUN5737	KX024652	2015 Oct	Hungary	
171	152808	KU953391	2015 May	Shanghai	This study

Counter	Strain Name	GenBank Accession no.	Year and month of collection	City/Province/Country	Remarks
172	1513140	KU953392	2015 Dec	Shanghai	This study
173	1513181	KU953393	2015 Dec	Shanghai	This study
174	1613155	KU953394	2016 Jan	Shanghai	This study
175	1613179	KU953395	2016 Jan	Shanghai	This study
176	1613225	KU953396	2016 Feb	Shanghai	This study
177	1613305	KU953397	2016 Feb	Shanghai	This study
178	1613306	KU953398	2016 Feb	Shanghai	This study
179	AlbertaEI331	KX171414	2015 Aug	Canada	This study
180	11	KX420891	2015 Dec	Canada	This study
181	12	KX420892	2015 Dec	Canada	This study
182	13	KX420893	2016 Jan	Canada	This study
183	14	KX420894	2016 Feb	Canada	This study
184	15	KX420895	2016 Feb	Canada	This study
185	22478	KX216782	2015 Mar	Russia	This study
186	22692	KX216784	2015 May	Russia	This study
187	22706	KX216787	2015 Jun	Russia	This study
188	22833	KX216783	2015 Jul	Russia	This study
189	22852	KX216786	2015 Jul	Russia	This study
190	22962	KX216794	2015 Jul	Russia	This study
191	23079	KX216785	2015 Aug	Russia	This study
192	23108	KX216795	2015 Sep	Russia	This study
193	23110	KX216796	2015 Sep	Russia	This study
194	23123	KX216789	2015 Sep	Russia	This study
195	23233	KX216793	2015 Sep	Russia	This study
196	23249	KX216798	2015 Oct	Russia	This study
197	23251	KX216799	2015 Oct	Russia	This study
198	23289	KX216806	2015 Oct	Russia	This study
199	23308	KX216797	2015 Nov	Russia	This study
200	23376	KX216800	2015 Nov	Russia	This study
201	23377	KX216801	2015 Nov	Russia	This study
202	23378	KX216792	2015 Dec	Russia	This study
203	23382	KX216802	2015 Dec	Russia	This study
204	23383	KX216803	2015 Dec	Russia	This study
205	23392	KX216788	2015 Nov	Russia	This study
206	23395	KX216791	2015 Dec	Russia	This study
207	23406	KX216790	2015 Dec	Russia	This study
208	23438	KX216804	2016 Feb	Russia	This study
209	23440	KX216805	2016 Feb	Russia	This study
210	14-273	LC148844	2015 Jan	Japan	This study
211	14-283	LC148845	2015 Jan	Japan	This study
212	14-332	LC148846	2015 Jan	Japan	This study
213	14-346	LC148847	2015 Jan	Japan	This study
214	14-394	LC148848	2015 Feb	Japan	This study
215	14-508	LC148849	2015 Mar	Japan	This study
216	15-157	LC148850	2015 Jul	Japan	This study
217	15-208	LC148851	2015 Aug	Japan	This study
218	15-377	LC148852	2016 Jan	Japan	This study
219	15-399	LC148853	2016 Jan	Japan	This study
220	15-428	LC148854	2016 Jan	Japan	This study
221	15-479	LC148855	2016 Mar	Japan	This study
222	15-493	LC148856	2016 Mar	Japan	This study
223	Ljubljana1662	KT591501	2015 Jun	Slovenia	This study
224	Ljubljana1758	KX134669	2015 Jul	Slovenia	This study
225	Ljubljana1962	KX134670	2015 Aug	Slovenia	This study
226	Ljubljana535	KX134671	2016 Mar	Slovenia	This study
227	15-G1181.01	KX244850	2015 Nov	Germany	This study
228	15-G1182.01	KX244851	2015 Nov	Germany	This study
229	15-G1200.01	KX244853	2015 Oct	Germany	This study
230	15-G1269.01	KX244852	2015 Apr	Germany	This study
231	16-G0188.01	KX244854	2016 Feb	Germany	This study
232	Veldhoven219	KX424646	2015 Feb	The Netherlands	This study
233	Almere278	KX424647	2015 May	The Netherlands	This study
234	Almere279	KX424648	2015 May	The Netherlands	This study
235	Heemskerk337	KX424649	2015 Nov	The Netherlands	This study
236	Heemskerk336	KX424650	2015 Nov	The Netherlands	This study
237	3000467356	MF172092	2015 May	USA	This study
238	3000467424	MF172093	2015 Jul	USA	This study
239	3000467425	MF172094	2015 Jul	USA	This study

Counter	Strain Name	GenBank Accession no.	Year and month of collection	City/Province/Country	Remarks
240	3000508986	MF172095	2016 Jan	USA	This study
241	3000509166	MF172096	2016 Jan	USA	This study
242	B1995	KX346699	2014 Oct	Thailand	This study
243	B2194	KX346705	2015 Sep	Thailand	This study
244	B2306	KX346700	2015 Oct	Thailand	This study
245	B2316	KX346701	2015 Nov	Thailand	This study
246	B2387	KX346702	2015 Nov	Thailand	This study
247	B2395	KX346703	2015 Nov	Thailand	This study
248	B2459	KX346704	2015 Dec	Thailand	This study
249	15NV570	KX371107	2015 Sep	New Zealand	This study
250	15NV581	KX371108	2015 Sep	New Zealand	This study
251	15NV796	KX371109	2015 Nov	New Zealand	This study
252	15NV806	KX371110	2015 Nov	New Zealand	This study
253	16NV021	KX371111	2016 Jan	New Zealand	This study
254	16NV149	KX371112	2016 Jan	New Zealand	This study

*Red text denotes the first case of norovirus GII.17 Kawasaki in this study; blue text denotes strains comprising the competent virus basal haplotype; and green text denotes reference strains of sublineages SL1, SL2 and SL3. Blank cells indicate that sequences were downloaded from GenBank.

Technical Appendix Table 2. Genomewide identification of 4-aa substitutions that can affect viral fitness of norovirus GII.17

Kawasaki in humans*

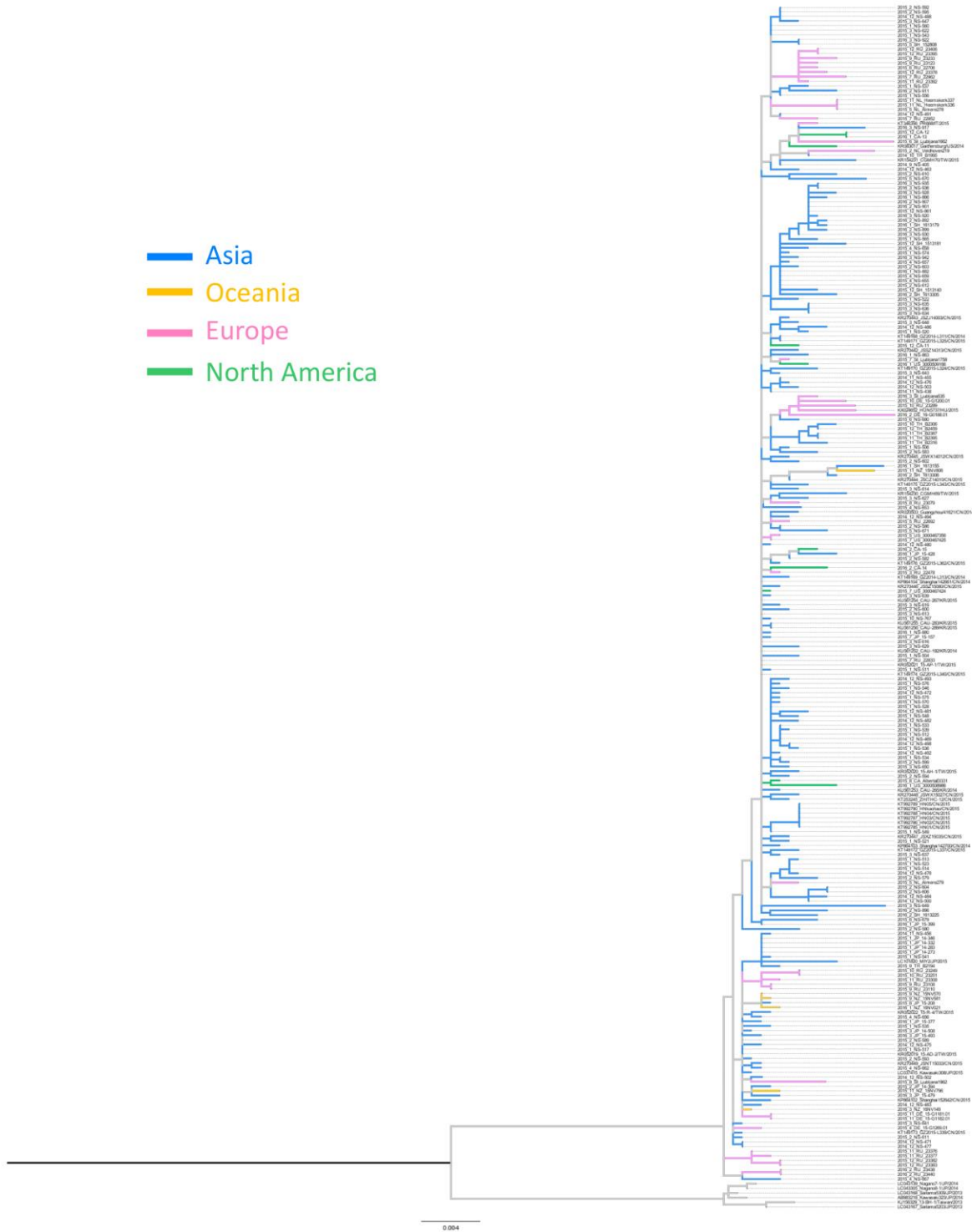
Protein/residue position	Reference†	NS-613‡	NS-616‡	CAU-192‡	CAU-267‡
Polyprotein, ORF1					
53	T	T	T	A	T
164	L	F	L	L	L
187§	A	D	D	D	D
739§	N	S	S	S	S
974	L	L	F	L	L
1194	G	G	G	G	S
1674	F	F	F	S	S
VP2, ORF3					
58§	K	R	R	R	R
89§	A	S	S	S	S
111	T	T	A	T	T
136	K	K	R	K	K

*Residues different from the reference are in bold. GII.17, norovirus genogroup II genotype 17; ORF, open reading frame; VP, viral protein.

†First case of norovirus GII.17 Kawasaki in this study (NS-405 from Hong Kong; GenBank accession no. KT326180 [complete genome]).

‡Comprised the competent basal haplotype of VP1. GenBank accession nos.: NS-613 (KU561248; Hong Kong), NS-616 (KU561249; Hong Kong), CAU-192 (KU561252; South Korea), and CAU-267 (KU561254; South Korea).

§Residue speculated to affect viral fitness of norovirus GII.17 Kawasaki in humans.



Technical Appendix Figure. Maximum-likelihood phylogenetic inference of complete viral protein 1 nucleotide sequences of norovirus genogroup II genotype 17 Kawasaki. This tree is equivalent to that in Figure 1 but with sequence details shown: Year and month of collection, followed by country and strain name. Sequences retrieved from GenBank are preceded with GenBank accession numbers.