CHAPTER 5

Results

5.1 Molecular epidemiology of noroviruses and sapoviruses

5.1.1 Prevalence and genotypic distribution of norovirus and sapovirus infection

A total of 812 stool samples collected from pediatric patients with acute gastroenteritis during January, 2015 to December, 2016 were screened for NoV GI, GII, and SaV by reverse transcription (RT) reaction, multiplex PCR, and semi-nested PCR. Of these, 335 and 477 samples were obtained in 2015 and 2016, respectively. An example of expected PCR product sizes of each virus demonstrated by agarose gel electrophoresis is shown in Figure 5.1 and 5.2. In this study, 164 samples (20.2%) were positive for NoV. Among these, 161 (98.2%) were positive for NoV GII and 2 (1.2%) were positive for NoV GI (Table 5.1). Moreover, a detected sample in 2015 was positive for both GI and GII. Focusing on the detection rate by year, the prevalence rate of NoV GII in 2015 was 12.8% (43 out of 335), while in 2016 the prevalence increased to 24.7% (118 out of 477). For NoV GI, the detected rates NoV GI in 2015 and 2016 were 0.3% (1 out of 335) and 0.2% (1 out of 477), respectively. As shown in Table 5.1, the NoV GI was identified as GI.5 (0.6%) and GI.6 (0.6%) genotypes. For NoV GII, the most predominant genotype was GII.4 (58.9%, 96 out of 163), followed by GII.2 (10.4%, 17 out of 163), GII.3 (7.4%, 12 out of 163), and GII.17 (6.1%, 10 out of 163). Other minor genotypes were GII.6 (2.5%), GII.7 (3.1%), GII.13 (4.3%), GII.14 (1.8%), GII.15 (3.1%), and GII.21 (1.2%). Within the NoV GII.4, Sydney 2012 variant was the most predominant variant found (96.0%, 94 out of 98) in this study followed by New Orleans 2009 and Asia 2003 (2.0% each). It was observed that NoV GII.2, GII.6, GII.13, and

GII.14 genotypes were detected only in 2016, while GI.6 and GII.21 genotypes were found only in 2015. However, one sample (CMH-S082-15) showed positive for both NoV GI.6 and NoV GII.4.

Focusing on the monthly distribution of NoV, as shown in Figure 5.3, NoVs were detected all year round with the high detection rate in dry and cold seasons from December, 2015 to March, 2016. In addition, minor peak of NoV distribution was observed in July 2015 and 2016. NoV GI was sporadically found in April and December, 2015 and January, 2016.

For SaV, the detection rate of SaV was 1.8% (15 out of 812). Of these, 1.5% (5 out of 335) and 2.1% (10 out of 477) were detected in 2015 and 2016, respectively. Based on the phylogenetic analysis, 3 SaV genotypes GI.1, GI.2, and GII.5 were identified in this study. As shown in Table 5.1, 9 SaV strains were identified as SaV GI.1 (52.9%), 6 were GI.2 (35.3%), and the other 2 were GII.5 (11.8%). The GII.5 genotype was found only in 2015 whereas the other genotypes, GI.1 and GI.2, were detected in both 2015 and 2016 of the study period. Interestingly, two samples were found to be positive for both NoV and SaV. One sample (CMH-S254-15) was co-infected between NoV GII.4 and SaV GI.2, and another sample (CMH-ST090-16) was co-infected between NoV GII.2 and SaV GI.1. Focusing on the monthly distribution of SaV, as shown in Figure 5.4, most of SaV in this study were detected in cool season from December 2015 to March 2016.

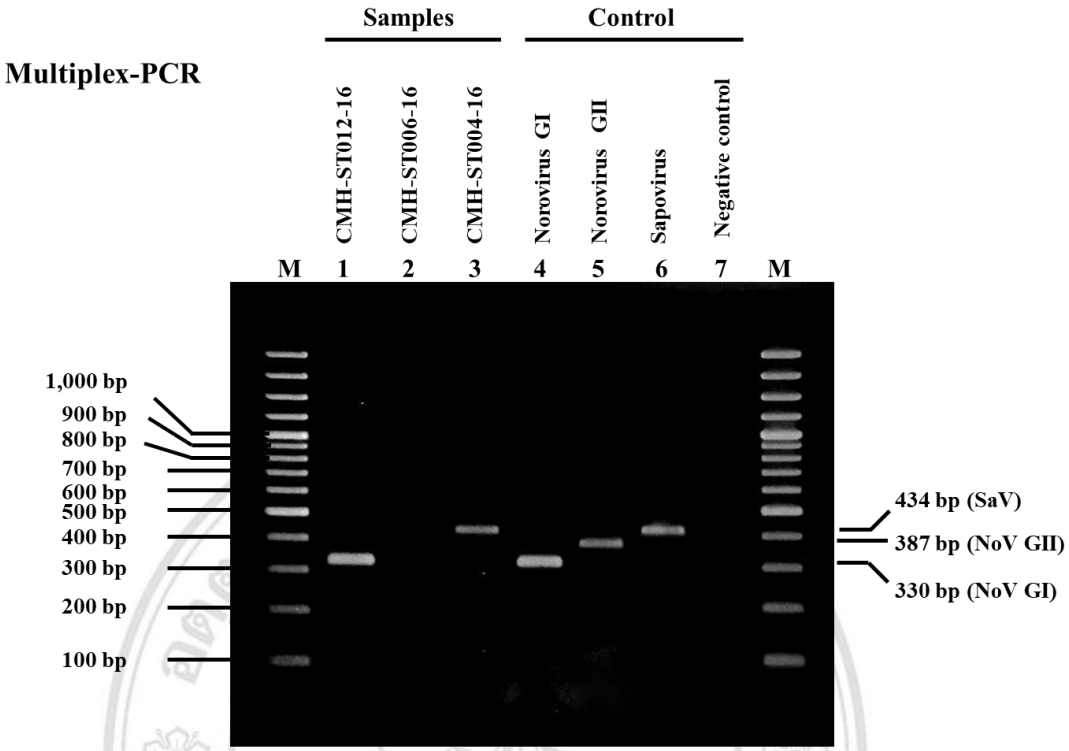


Figure 5.1 Agarose gel electrophoresis illustrating the predicted PCR product sizes of norovirus GI, GII, and sapovirus generating by multiplex-PCR in comparison with the reference strains. Lane M represents a 100 bp DNA marker. Lane 1-3 represent tested samples which were positive for NoV GI, negative for NoV GII, and positive for SaV, respectively. Lane 4-6 represent reference strains of NoV GI, GII, and SaV with the PCR product sizes of 330 bp, 387 bp, and 434 bp, respectively. Lane 7 is a negative control of the reaction in which the DNA template was omitted. The molecular sizes of marker used in this study are shown on the left and the expected PCR product sizes of NoV GI, GII, and SaV are indicated on the right.

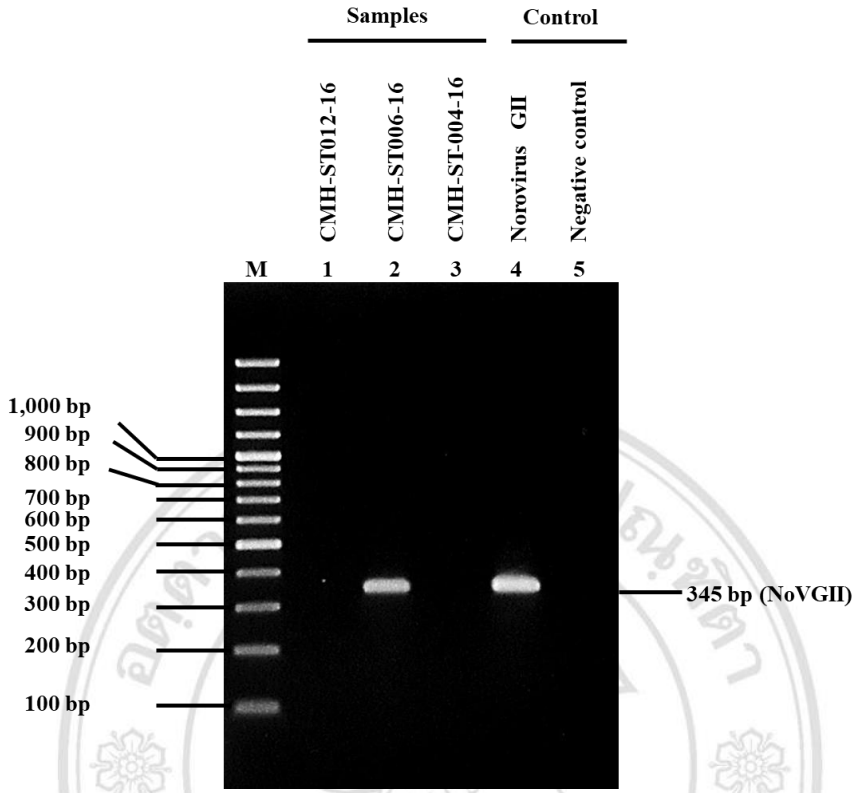


Figure 5.2 Agarose gel electrophoresis illustrating the predicted PCR product sizes of norovirus GII generating by semi-nested PCR from negative samples for norovirus GII from multiplex-PCR in comparison with the reference strains. Lane M represents a 100 bp DNA marker. Lane 1 and 3 represent tested samples which were negative for NoV GII. Lane 2 represents tested sample which was positive for NoV GII. Lane 4 is reference strain of NoV GII with PCR product size of 345 bp. Lane 5 is a negative control of the reaction in which the DNA template was omitted. The molecular sizes of marker used in this study are shown on the left and the expected PCR product size of NoV GII is indicated on the right.

Table 5.1 Prevalence and genotypic distribution of norovirus and sapovirus detected in Chiang Mai during 2015-2016

Year of specimen collection	2015 (%)	2016 (%)	Total (%)
No. of sample tested	335	477	812
NoV positive	45 (13.4)	119 (24.9)	164 (20.2)
NoV GI positive	1 (2.2)	1 (0.8)	2 (1.2)
NoV GII positive	43 (95.6)	118 (99.2)	161 (98.2)
NoV GI + GII positive	1 (2.2)	0 (0.0)	1 (0.6)
SaV positive	5 (1.5)	10 (2.1)	15 (1.8)
NoV + SaV positive	1 (0.3)	1 (0.2)	2 (0.2)
NoV genotype distribution	44	119	163
GI.5	0 (0.0)	1 (0.8)	1 (0.6)
GI.6	1 (2.3)	0 (0.0)	1 (0.6)
GII.2	0 (0.0)	17 (14.3)	17 (10.4)
GII.3	1 (2.3)	11 (9.2)	12 (7.4)
GII.4	33 (75.0)	63 (53.0)	96 (58.9)
GII.6	0 (0.0)	4 (3.4)	4 (2.5)
GII.7	2 (4.5)	3 (2.5)	5 (3.1)
GII.13	0 (0.0)	7 (5.9)	7 (4.3)
GII.14	0 (0.0)	3 (2.5)	3 (1.8)
GII.15	2 (4.5)	3 (2.5)	5 (3.1)
GII.17	3 (6.9)	7 (5.9)	10 (6.1)
GII.21	2 (4.5)	0 (0.0)	2 (1.2)
NoV GII.4 variants	35	63	98
GII.4 Asia 2003	0 (0.0)	2 (3.2)	2 (2.0)
GII.4 New Orleans 2009	0 (0.0)	2 (3.2)	2 (2.0)
GII.4 Sydney 2012	35 (100.0)	59 (93.6)	94 (96.0)
SaV genotype distribution	6	11	17
GI.1	3 (50.0)	6 (54.5)	9 (52.9)
GI.2	3 (50.0)	3 (27.3)	6 (35.3)
GII.5	0 (0.0)	2 (18.2)	2 (11.8)

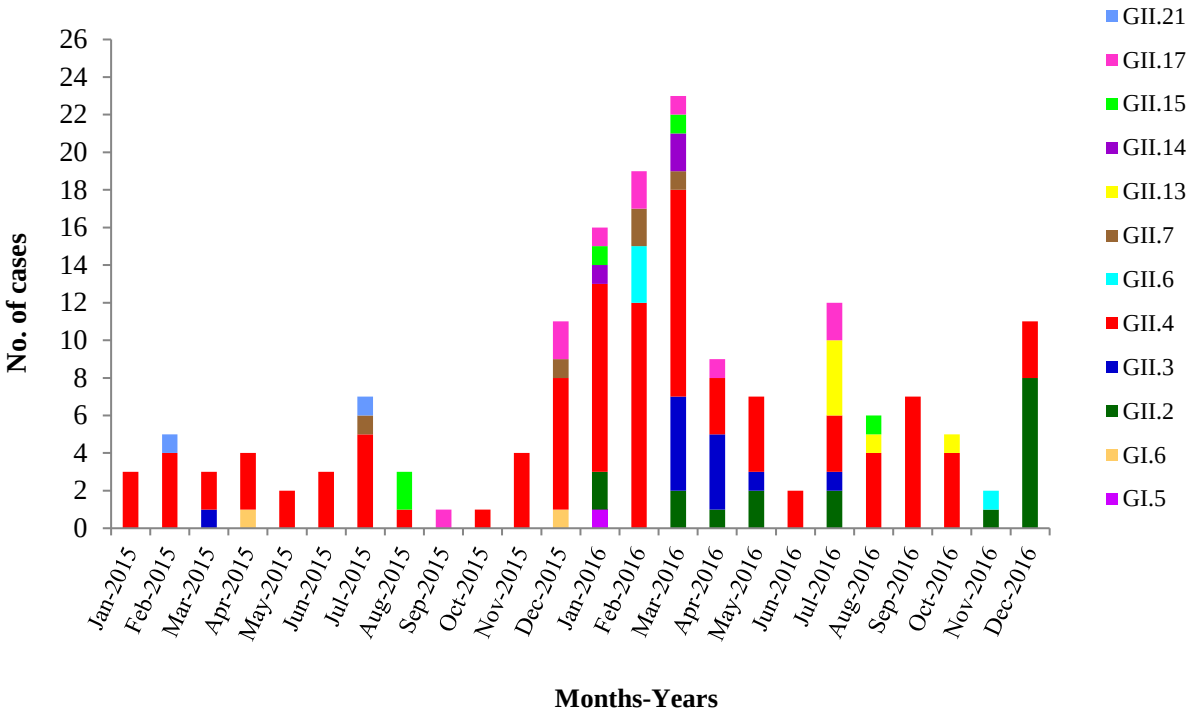


Figure 5.3 Monthly genotypic distribution of norovirus in children hospitalized with diarrhea in Chiang Mai, Thailand during 2015-2016

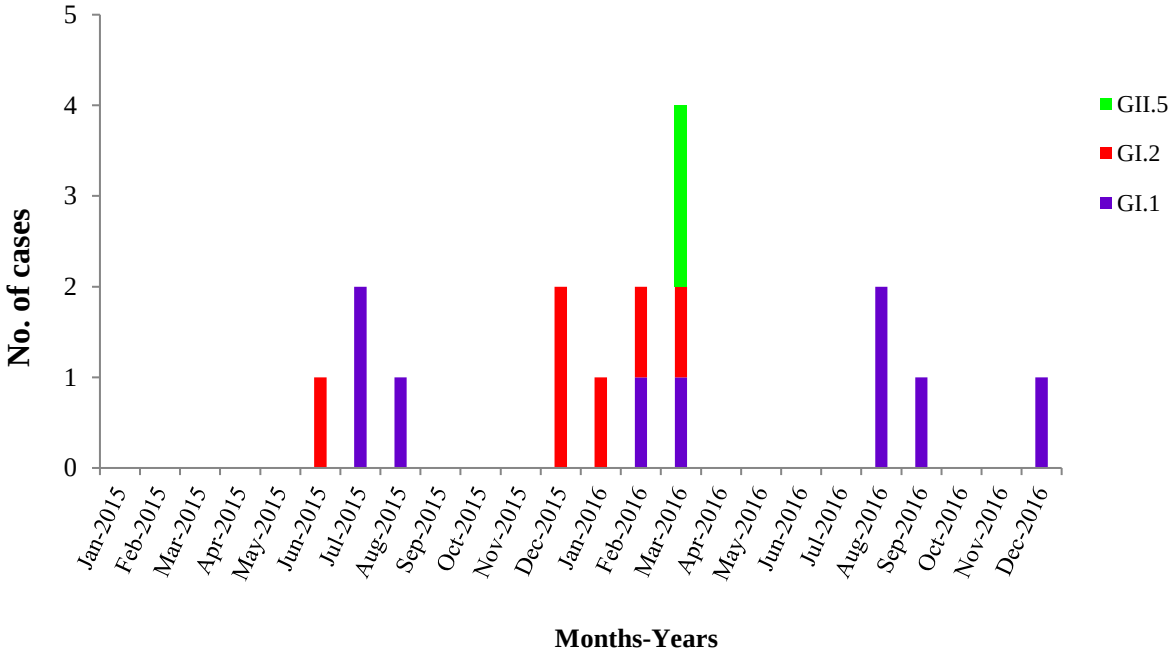


Figure 5.4 Monthly genotypic distribution of sapovirus in children hospitalized with diarrhea in Chiang Mai, Thailand during 2015-2016

5.1.2 Phylogenetic analysis of noroviruses

To characterize the NoV genotypes, nucleotide sequencing and phylogenetic tree analysis based on partial VP1 region of NoV GI and GII were performed (Figure 5.5 and 5.6). Based on the phylogenetic analysis of NoV GI, one NoV strain (CMH-ST012-16) detected in 2015 was identified as NoV GI.5 and shared nucleotide sequence identity with a strain from Taiwan in 2014 (KP027329) at 98.7%. Two strains (CMH-S082-15 and CMH-S253-15) detected in 2016 were identified as NoV GI.6 and showed high nucleotide sequence identity with a strain from Brazil in 2014 (KP963774) at 98.7%.

For the phylogenetic analysis of NoV GII, the NoV detected in this study (164 strains) were clustered separately into 10 different genotypes (Figure 5.6A and 5.6B). Majority of the NoV strains (98 strains) were identified as NoV GII.4. Twelve samples (CMH-S051-15, CMH-S122-16, CMH-S142-16, CMH-S175-16, CMH-ST064-16, CMH-ST085-16, CMH-ST086-16, CMH-ST087-16, CMH-ST096-16, CMH-ST101-16, CMH-ST102-16, and CMH-ST118-16) were assigned as NoV GII.3 and clustered together with the NoV GII.3 strains from China (KR007958) and Thailand (KR093998) in 2014. In addition, 18 strains were found to be NoV GII.2 and it was interesting to observe that the NoV GII.2 in this study were located separately into two distinct clusters. Nine of them (CMH-N010-16, CMH-S070-16, CMH-S117-16, CMH-S121-16, CMH-ST071-16, CMH-ST090-16, CMH-ST123-16, CMH-ST149-16, and CMH-ST154-16) were clustered together and closely related to the reference strains from China in 2003 (KR107724), Japan in 2004 (DQ456824), 2008 (AB662869), 2010 (AB662898), and Belgium in 2008 (JF697283) ranging from 98.7-100% nucleotide sequence identity. However, the other nine strains (CMH-S237-16, CMH-S238-16, CMH-S241-16, CMH-S244-16, CMH-S246-16, CMH-S249-16, CMH-S251-16, CMH-ST197-16, and CMH-ST200-16) were clustered closely with the reference strain from Viet Nam in 2010 (HE716731) and recombinant GII.P16/GII.2 strains from Japan in 2011 (LC147193), Hong Kong in 2016 (KY771081), and Germany in 2016

(KY357462) with the nucleotide sequence identity ranging from 99.5-100%. Seven strains of GII.13 (CMH-S161-16, CMH-S170-16, CMH-S185-16, CMH-S204-16, CMH-ST116-16, CMH-ST155-16, and CMH-ST184-16) were closely related to the reference strains from Nepal in 2010 (AB810007), Germany in 2012 (KC832472), Taiwan in 2013 (KM036380), Australia in 2013 (KT239632) and 2014 (KT239639), and a GII.13 strain detected in sewage from China in 2013 (KR107722) with the nucleotide sequence identity ranging from 99.1-100%. Two GII.21 strains (CMH-N031-15 and CMH-S015-15) showed high nucleotide sequence identity (97.8-98.7%) with the reference strains from Japan (KJ196284) and China (HM104665) in 2007, and USA in 2011 (JN899245). Ten GII.17 strains (CMH-N007-16, CMH-S200-15, CMH-S251-15, CMH-S101-16, CMH-S145-16, CMH-S158-16, CMH-S160-16, CMH-ST014-15, CMH-ST034-16, and CMH-ST057-16) were closely related to the reference strains from China in 2014 (KU557785), Taiwan in 2015 (KT732276), Australia in 2015 (KT190704), and Brazil in 2016 (KX353835) with the nucleotide sequence identity ranging from 97.4-100%. As shown in Figure 5.6B, three NoV GII.14 (CMH-ST005-16, CMH-ST074-16, and CMH-ST093-16) showed nucleotide sequence identity at 98.7-99.1% with a reference strain from South Africa in 2013 (KR904230). In addition, among 4 strains of GII.6, one strain (CMH-ST191-16) showed highly nucleotide sequence identity at 99.5% with a reference strain from Taiwan in 2013 (KM267742), whereas the others (CMH-ST021-16, CMH-ST032-16, and CMH-ST039-16) were closely related to reference strains from Japan in 1982 (AB684718) and USA in 1994 (AF414410) with the nucleotide sequence identity ranging from 94.3-94.8%. Five GII.7 strains (CMH-N025-15, CMH-S040-16, CMH-S100-16, CMH-ST027-15, and CMH-ST018-16) showed high nucleotide sequence identity (98.2-99.5%) to the strains from Japan in 2010 (KJ196295), Italy in 2011 (KF846529), and Russia in 2012 (KJ634709). Moreover, 5 strains of GII.15 (CMH-S175-15, CMH-S177-15, CMH-S011-16, CMH-S088-16, and CMH-S201-16) were closely related to the reference strains from China in 2008 (GQ856474) and 2011 (KM044168),

and Brazil in 2011 (KR074189) with the nucleotide sequence identity ranging from 98.2-99.1%.

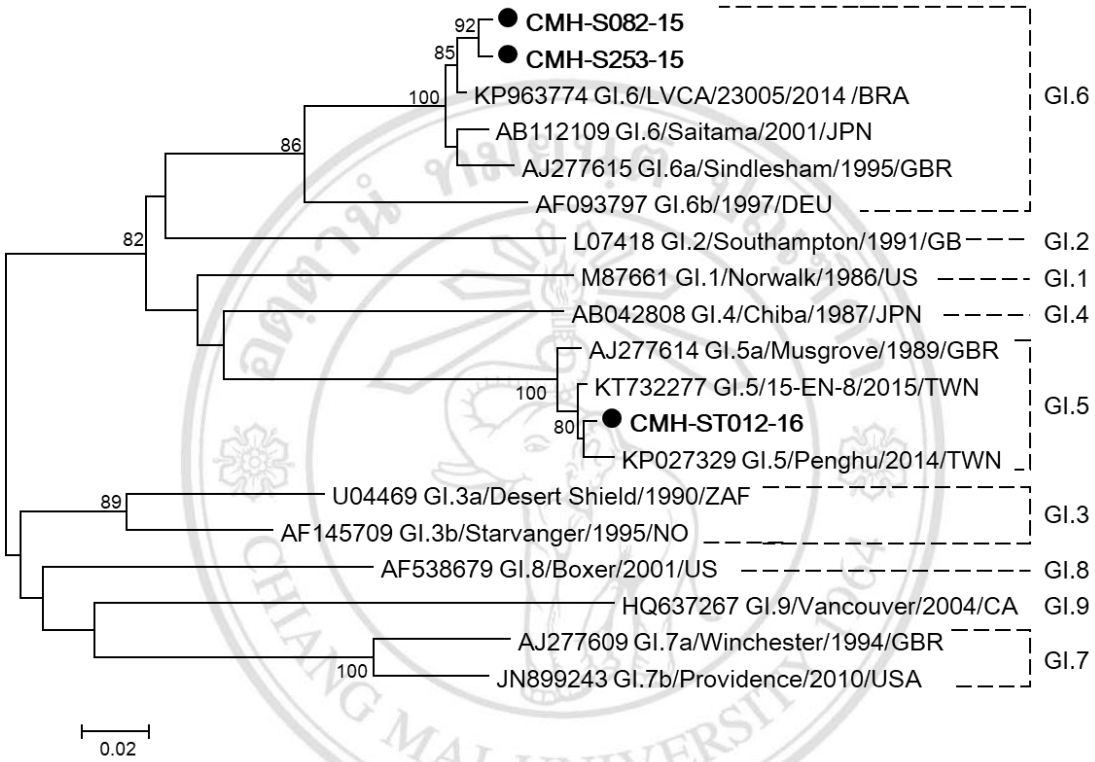


Figure 5.5 Phylogenetic tree of partial VP1 nucleotide sequences of norovirus GI detected in Chiang Mai during 2015-2016. The phylogenetic tree was constructed using MEGA7 program based on the neighbor-joining method. The detected strains are indicated by boldface with black dot symbol.

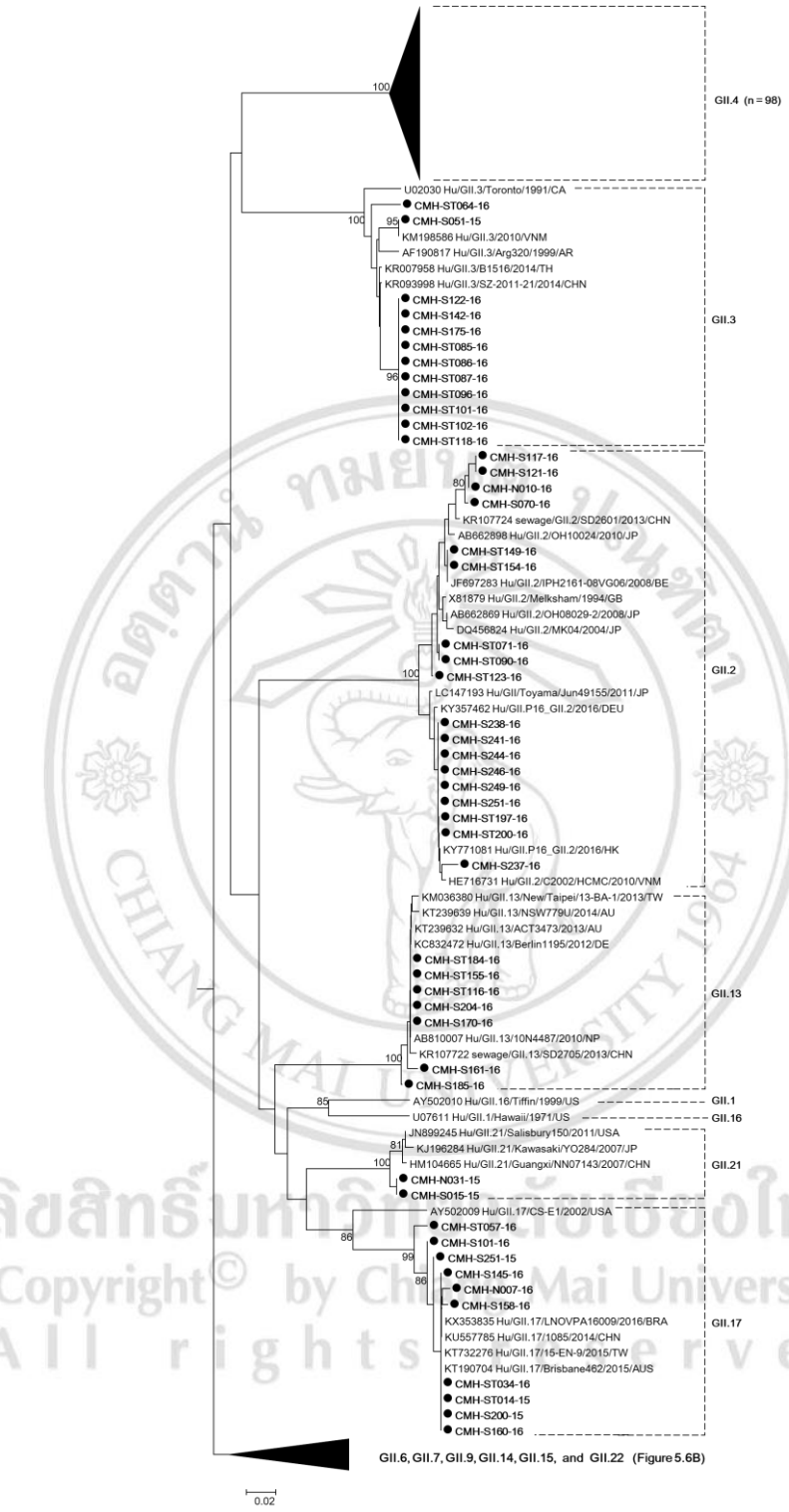


Figure 5.6A Phylogenetic tree of partial nucleotide VP1 gene of norovirus GII. The phylogenetic tree was constructed by using the neighbor-joining method in MEGA7 program. The detected strains are presented in boldface with black dot symbol.

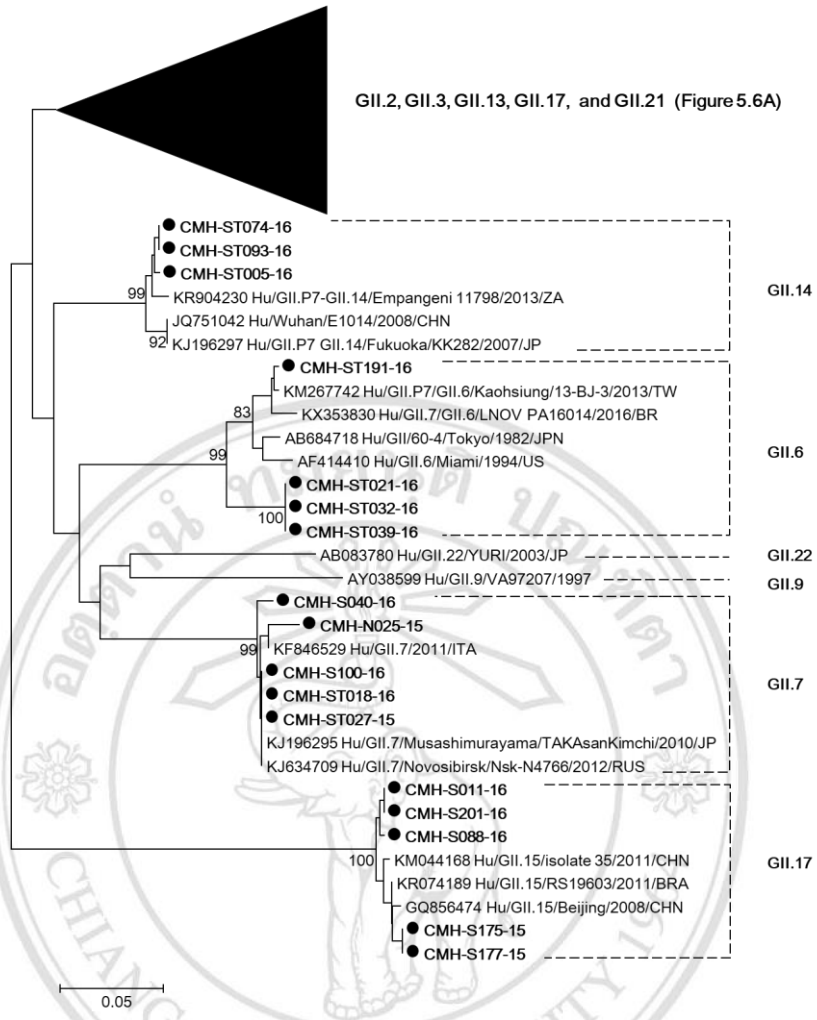


Figure 5.6B Phylogenetic tree of partial nucleotide VP1 gene of norovirus GII. The phylogenetic tree was constructed by using the neighbor-joining method in MEGA7 program. The detected strains are presented in boldface with black dot symbol.

Focusing on NoV GII.4, the strains circulated in pediatric patients with acute gastroenteritis included in this study were classified into three distinct variants including NoV GII.4 Asia 2003, New Orleans 2009, and Sydney 2012 as shown in Figure 5.7. Most of the isolated GII.4 strains showed highly nucleotide sequence identity (97.4-100%) with the Sydney 2012 variant strain from Australia (JX459908). All the NoV GII.4 strains (n=35) detected in 2015 and most of the strains in 2016 (n=59) were GII.4 Sydney 2012 variant. Thus, Sydney 2012 variant was the major variant that circulated in Chiang Mai area in 2015-2016. In addition, 2 strains (CMH-N029-16 and CMH-S233-16) were clustered with a reference GII.4 Asia 2003 variant (HM802551) with the nucleotide sequence identity of 97.8%, while the other 2 strains (CMH-S015-16 and CMH-ST033-16) showed 100% nucleotide sequence identity closely related to a reference strain GII.4 New Orleans 2009 (GU445325).

5.1.3 Phylogenetic analysis of sapoviruses

The phylogenetic tree of SaV partial nucleotide VP1 gene has been constructed and demonstrated in Figure 5.8. Among 17 SaV strains, 9 strains (CMH-N006-15, CMH-N007-15, CMH-S166-15, CMH-S198-16, CMH-S229-16, CMH-ST016-16, CMH-ST090-16, CMH-ST163-16, and CMH-ST199-16) were clustered together with SaV GI.1 reference strains and showed most closely related to SaV GI.1 prototype strain Manchester isolated in England in 1997 (X86560), Thailand in 2002 (AY646853 and AY646854), Venezuela in 2003 (GU296663), Japan (AY237422) and Germany (AY694184) in 2004, Canada (KU973909) and Brazil (KJ826503) in 2009, Korea in 2013 (KP28674) and China in 2010 (HM195198) and 2014 (KT327081) with the nucleotide sequence identity ranging from 98.8-100%. In addition, 6 strains of GI.2 (CMH-S152-15, CMH-S252-15, CMH-S254-15, CMH-S108-16, CMH-ST004-16, and CMH-ST029-16) shared nucleotide sequence identity with the reference strains from Germany in 2000 (AF294739), Japan in 2010 (AB607855) and 2013 (LC071893), and China in 2012 (KF649133) with the nucleotide sequence identity ranging

from 97.4-100%. Moreover, 2 SaV strains (CMH-ST091-16 and CMH-ST095-16) were closely related to the reference strain from Thailand in 2005 (EU872292) with 97.0% nucleotide sequence identity.

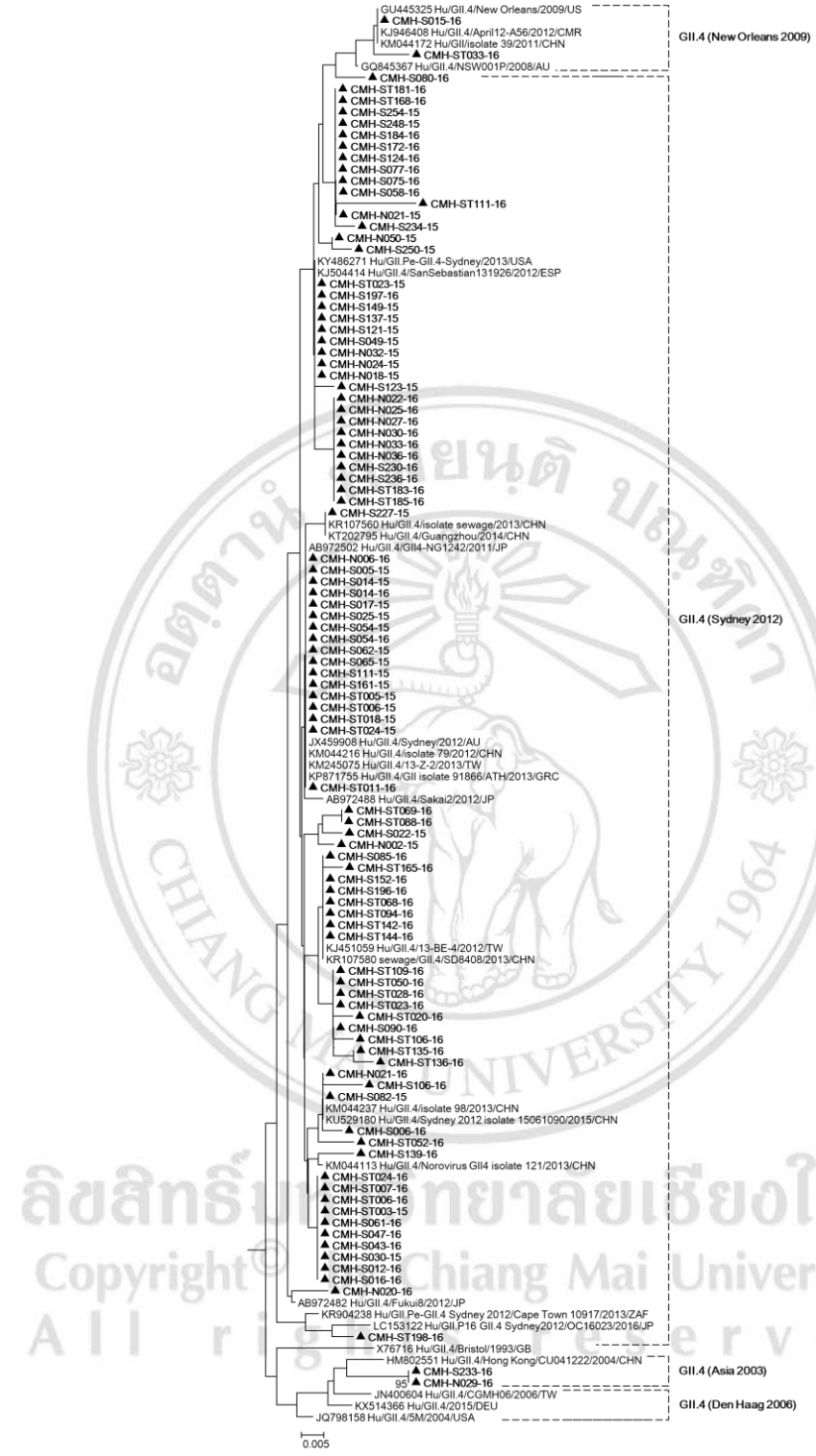


Figure 5.7 Phylogenetic tree of partial nucleotide VP1 gene of norovirus GII.4 variants. The phylogenetic tree was constructed by using the neighbor-joining method in MEGA7 program. The detected strains are presented in bold-face with triangular symbol.

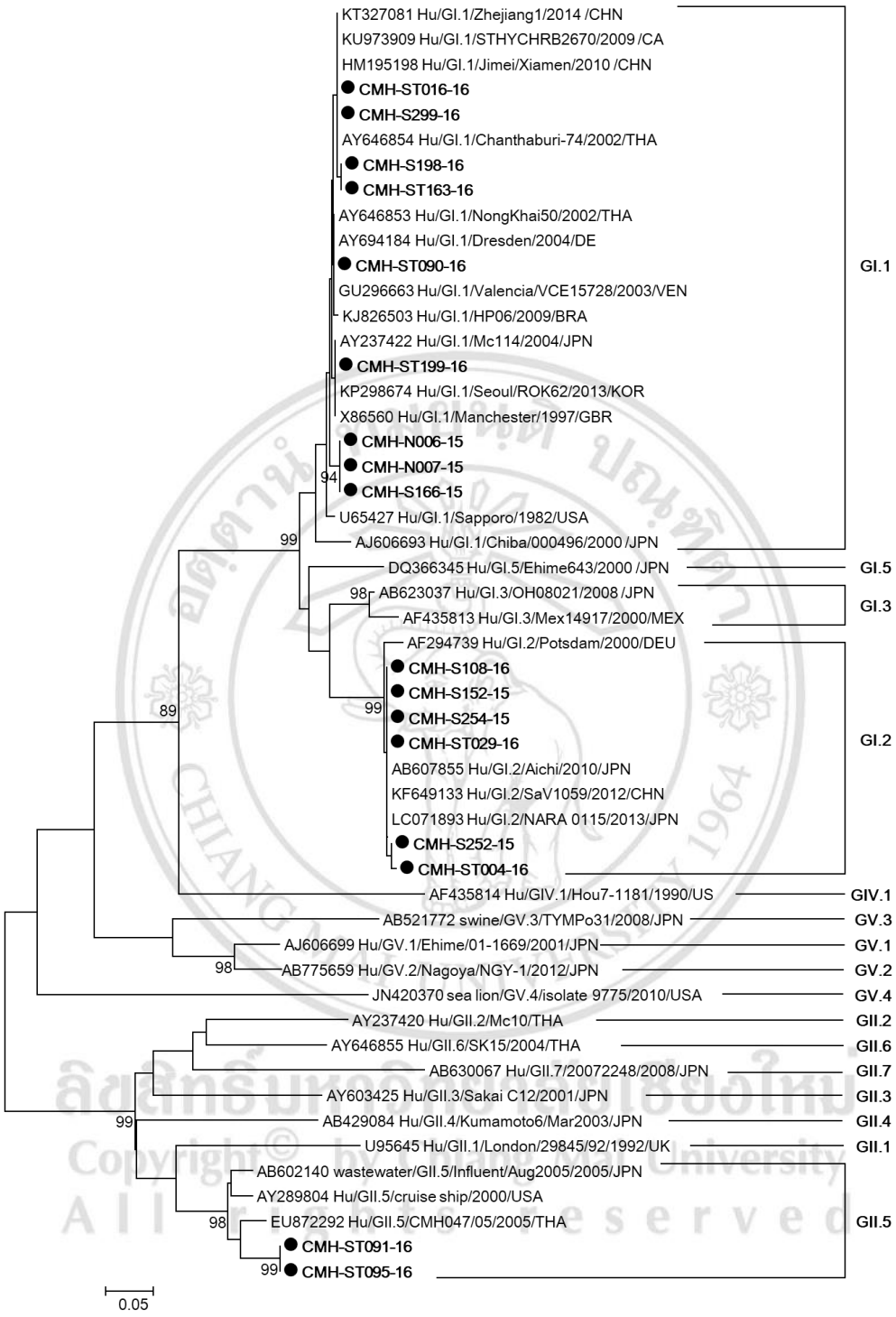


Figure 5.8 Phylogenetic tree of partial nucleotide VP1 gene of sapovirus. The phylogenetic tree was constructed by using the neighbor-joining method in MEGA7 program. The detected strains are presented in boldface with black dot symbol.

5.1.4 Genetic analysis of norovirus GII.2 recombinant strains

Norovirus genetic characterization demonstrated that GII.2 was detected as the second most common genotype following the GII.4 (Table 5.1). In this study, the NoV GII.2 was initially detected in January 2016 and reached the peak in December 2016, however, it was not detected in 2015. Among 120 NoV detected strains, 18 were identified as NoV GII.2. Thus, to focus on the genetic variation of the NoV GII.2 strains, the genetic characterization of the RdRp (ORF1) and VP1 (ORF2) regions of 18 detected GII.2 were conducted. In addition, NoV GII.2 strains from archival stool samples previously detected in 2007 and 2013 were included in this analysis. The RdRp and partial VP1 regions of 16 out of 18 GII.2 strains from 2016 together with one each from 2007 and 2013 were successfully amplified and sequenced (Figure 5.9). As shown in Figure 5.9A, based on the phylogenetic analysis on partial RdRp region, 8 of GII.2 strains detected in 2016 (CMH-S238-16, CMH-S241-16, CMH-S244-16, CMH-S246-16, CMH-S249-16, CMH-S251-16, CMH-ST197-16, and CMH-ST200-16) together with 2 GII.2 strains detected in 2007 (CMH-095-07) and 2013 (CMH-S099-13) were found to carry RdRp genotype as GII.P16. It was interesting to observed that GII.P16 was divided in 2 major lineages. The strains isolated recently in 2016 in this study were all clustered in the first lineage, while the strains isolated in 2007 and 2013 were located in the second lineage. When looking at the phylogenetic analysis of partial VP1 region of these 8 NoV strains and 2 strains of 2007 and 2013, all were clustered with NoV GII.2 (Figure 5.9B). Thus, all these 10 NoV strains were the GII.P16/GII.2 recombinant strains. In addition, the remaining other 8 GII.2 strains detected in 2016 (CMH-N010-16, CMH-S070-16, CMH-S117-16, CMH-ST071-16, CMH-ST090-16, CMH-ST123-16, CMH-ST149-16, and CMH-ST154-16) carried the same genotypes of both RdRp and VP1 regions as GII.P2/GII.2 (Figure 5.9A and B).

Furthermore, the detected GII.P16/GII.2 recombinant strains were analyzed for their potential recombination break points by using the SimPlot software

(v.3.5.1). In addition, the RDP4 program was also used to confirm the recombination event. The parental reference strains of NoV GII.16 genotype used for SimPlot analysis was Hu/GII.16/Neustrelitz260/2000/DE (AY772730) and for NoV GII.2 was Hu/GII.2/Melksham/1994/GB (X81879). The nucleotide recombination position of each strain was indicated based on the Lordsdale genome (X86557). In this study, the observed potential recombination points were at nucleotide positions 5,059 to 5,069 with the maximum-chi square values vary from 1.02×10^{-11} to 9.10×10^{-11} ($p < 0.01$) as shown in Table 5.2. Each recombinant strain showed the potential recombination points located within the downstream of RdRp region (Figure 5.10).

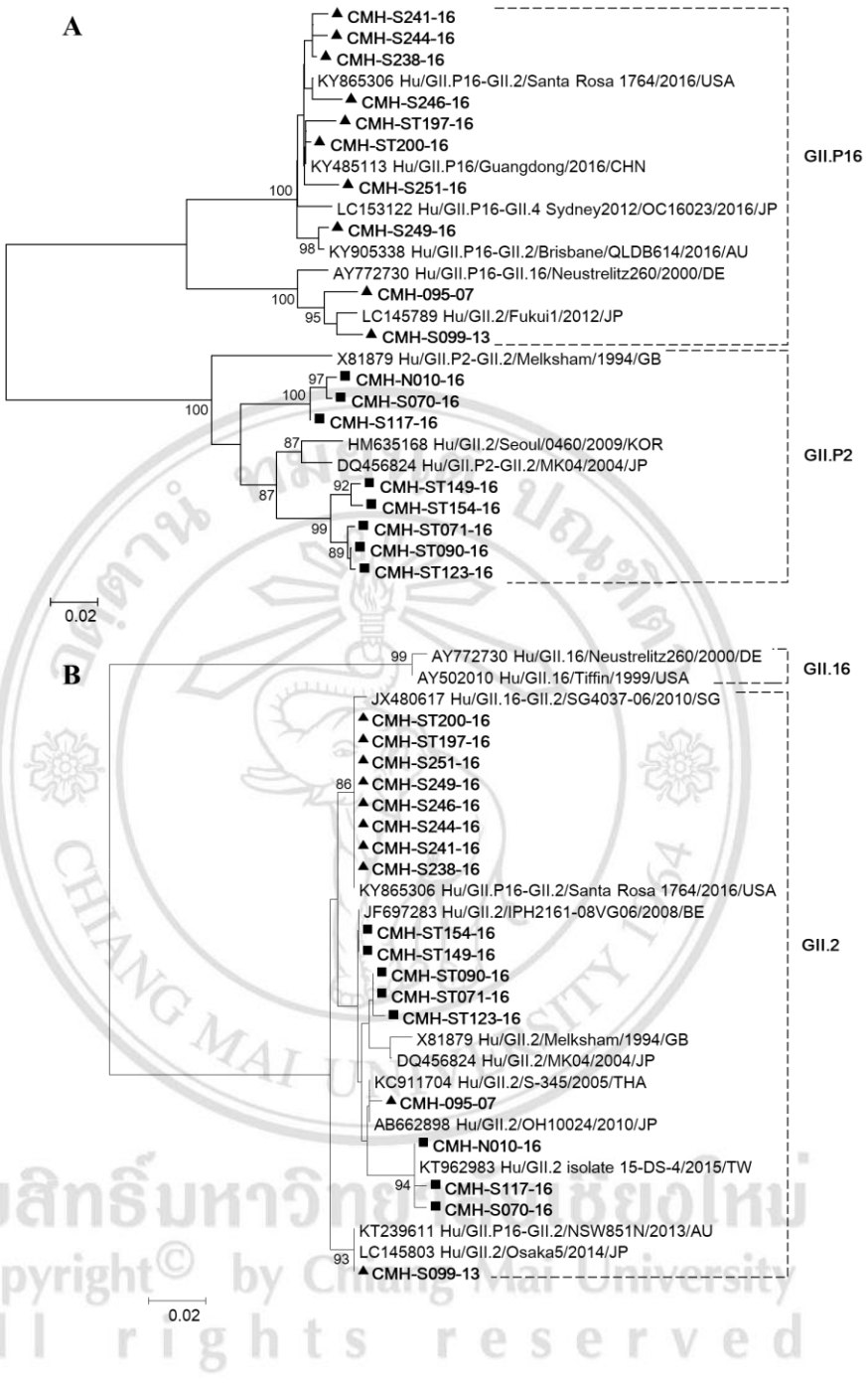


Figure 5.9 Phylogenetic analysis of A) the partial RdRp nucleotide sequences (ORF1 region) and B) the partial VP1 nucleotide sequences (ORF2 region) of norovirus GII.2 strains. The phylogenetic tree was constructed by the neighbor-joining method using the MEGA7 program. The detected GII.P16/GII.2 and GII.P2/GII.2 recombinant strains are indicated with black triangle and black square symbols, respectively.

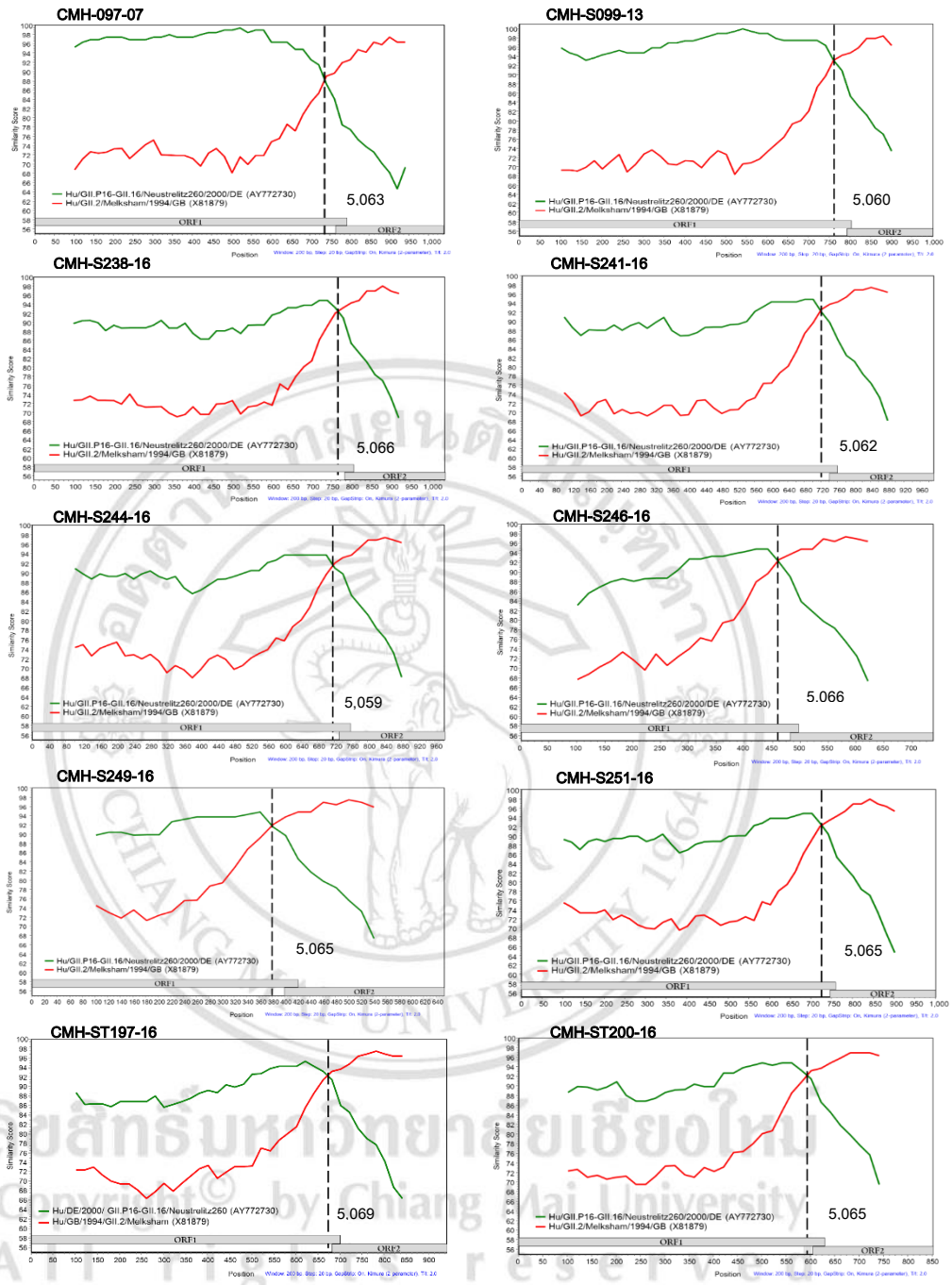


Figure 5.10 Similarity plots of the representative norovirus GII.2 recombinants detected in 2007, 2013, and 2016. The vertical axis indicates nucleotide similarity (%) between sequences of recombinant strains detected in this study and those of the reference strains. The line intersect defines the predicted recombination site based on the genomic nucleotide positions of Lordsdale reference strain (X86557) (vertical dashed line).

Table 5.2 Norovirus recombinant GII.P16/GII.2 strains associated with the sporadic cases of acute gastroenteritis detected in previous studies (2007, 2013) and this study (2016)

Year	Sample code	Collection date	Age	Gender	Predicted recombination point (nt)	Maximum-chi square values
2007	CMH-095-07	Jun-2007	NA	Female	5,063	2.70×10^{-14}
2013	CMH-S099-13	Dec-2013	1 Y 4 M	Female	5,060	2.90×10^{-11}
2016	CMH-S238-16	Dec-2016	1 Y 5 M	Female	5,066	1.02×10^{-11}
	CMH-S241-16	Dec-2016	3 Y 2 M	Male	5,062	1.71×10^{-11}
	CMH-S244-16	Dec-2016	1 Y 9 M	Female	5,059	9.10×10^{-11}
	CMH-S246-16	Dec-2016	1 Y 4 M	Female	5,066	4.66×10^{-11}
	CMH-S249-16	Dec-2016	1 Y 8 M	Male	5,065	1.07×10^{-11}
	CMH-S251-16	Dec-2016	2 Y	Male	5,065	2.47×10^{-11}
	CMH-ST197-16	Aug-2016	8 M	Female	5,069	1.63×10^{-11}
	CMH-ST200-16	Dec-2016	3 Y 9 M	Female	5,065	3.45×10^{-11}

NA = not available

5.2 Molecular characterization of norovirus recombinant strains circulating in Chiang Mai, Thailand during 2005 to 2015.

5.2.1 The norovirus GII recombinations

To identify the NoV recombinant strains circulating in Chiang Mai since 2005 to 2015, 298 of NoV GII capsid sequences obtained from pediatric patients with acute gastroenteritis in Chiang Mai during 2005 to 2015 were included in this analysis. Among these, 253 partial capsid sequences from 2005 to 2014 were obtained from archival database of our laboratory and 45 sequences were obtained in this study from the specimens collected in 2015. A total of 298 sequences were analyzed initially by performing nucleotide BLAST similarity using partial capsid sequences. The NoV GII capsid sequences that were similar to reference recombinant strains available in the GenBank databases were selected for further analysis. Nucleotide BLAST similarity analysis demonstrated that 51 out of 298 GII capsid sequences were similar to the NoV recombinant strains available in GenBank database.

Thus, all 51 NoV GII strains were selected for further analysis by amplification of partial RdRp (ORF1) and partial capsid VP1 (ORF2) in order to analyze for their genome recombination patterns. Nucleotide sequence analysis of partial RdRp/capsid VP1 revealed that 21 NoV GII strains were identified as NoV recombinant strains (Figure 5.11). Of these, 9 different recombination patterns were detected, including GII.Pe/GII.4 (n=4), GII.Pg/GII.1 (n=1), GII.Pg/GII.12 (n=1), GII.P7/GII.6 (n=3), GII.P7/ GII.14 (n=1), GII.P12/GII.4 (n=1), GII.P16/GII.2 (n=1), GII.P16/GII.13 (n=4), and GII.P21/GII.3 (n=5).

As shown in Table 5.3, wide variety of NoV recombination patterns were detected in pediatric patients admitted to the hospitals with acute gastroenteritis in Chiang Mai during 2005-2015. The GII.P12/GII.4 recombinant (CMH-076-05) was initially detected in 2005 while GII.P21/GII.3 was detected both in 2005 (CMH-145-05) and 2014 (CMH-N112-14, CMH-S026-14, CMH-S028-14, and CMH-S127-14). The GII.P7/GII.14 (CMH-047-07) was detected only in 2007, whereas GII.P7/ GII.6 was detected in 2007 (CMH-094-07), 2013 (CMH-N115-13), and 2014 (CMH-S101-14). In 2011, one recombinant strain, GII.Pg/GII.12 (CMH-N082-11), was detected. The GII.Pg/GII.1 (CMH-N009-12) and GII.P16/GII.13 (CMH-N180-12 and CMH-S063-12) recombinant strains were detected in 2012. The GII.P16/GII.13 recombinant (CMH-N070-1 and CMH-S003-13) was detected together with GII.P7/GII.6 (CMH-N115-13) and GII.P16/GII.2 (CMH-S099-13) in 2013. In 2014, four recombinant strains of GII.P21/GII.3 (CMH-N112-14, CMH-S026-14, CMH-S028-14, and CMH-S127-14) were detected together with GII.Pe/GII.4 (CMH-N052-14) and GII.P7/GII.6 (CMH-S101-14). In 2015, only GII.Pe/GII.4 recombinants (CMH-S123-15, CMH-S161-15, and CMH-ST023-15) were detected.

5.2.2 Recombination break point analysis

Among 9 different recombination patterns detected in this study, one strain from each pattern was selected for the putative recombination breakpoint prediction analysis using SimPlot software (v.3.5.1) and RDP4 program (Figure 5.12). The Lordsdale (X86557) strain was used as a reference strain for nucleotide position comparison. Among the total of 21 NoV recombinant strains detected in this study, 9 different recombination patterns were observed. The recombination breakpoints were located at the nt position encompassing nt positions 5,018-5,123. In this study, the recombination breakpoints were observed within ORF1 15 out of 21 strains, while the recombination breakpoints occurring within the ORF2 were found in 2 out of 21 strains. It was interesting to observe that nt position 5,070 of ORF1 was found as the most frequent nt position of the recombination occurring (Table 5.3). The recombination points determined by both Simplot and RDP4 programs were in concordance. Comparison the NoV sequences of all recombination patterns with the reference strain, the maximum-chi square values ranged from 4.7×10^{-12} to 5.4×10^{-1} ($p < 0.01$).

For the GII.P12/GII.4 recombination pattern, the CMH-076-05 detected in 2005 was selected as a representative strain for this analysis. The AB039775_GII.P12/2002/JP and KC013592_GII.4/2004/US were used as the parental reference strains for RdRp and VP1, respectively. The recombination breakpoint was observed at nt position 5,018, downstream of ORF1 within the C-terminal domain of RdRp, with a maximum-chi square value of 4.7×10^{-12} ($p < 0.01$) (Table 5.3, Figure 5.12A). For the GII.P7/ GII.14 recombination pattern, the CMH-047-07 strain detected in 2007 was selected. The GU017903_GII.P7/2008/JP and AB682735_GII.14/2011/JP were used as the parental reference strains. The recombination breakpoint was observed at nt position 5,112, upstream of ORF2 within the N-terminal domain of VP1, with maximum-chi square value of 5.4×10^{-1} ($p < 0.01$) (Table 5.3, Figure 5.12B). The GII.Pg/GII.12 recombination pattern, the CMH-N082-11 strain detected in 2011 was selected. The DQ379714_

GII.Pg/1983/US and KJ196299_GII.12/2001/JP were used as the parental reference strains. The recombination breakpoint was observed at the nt position 5,078, downstream of ORF1 within the C-terminal domain of RdRp, with a maximum-chi square value of 1.4 x 10^{-5} ($p < 0.01$) (Table 5.3, Figure 5.12C). For the GII.Pg/GII.1 recombination pattern, the CMH-N009-12 strain detected in 2012 was selected for this analysis. The JQ889815_ GII.Pg/2009/CHN and U07611_GII.1/1971/US were used as the parental reference strains. The recombination breakpoint was observed at the nt position 5,123, upstream of ORF2 within the N-terminal domain of VP1, with maximum-chi square value of 2.7 x 10^{-2} ($p < 0.01$) (Table 5.3, Figure 5.12D). For the GII.P16/GII.2 recombination pattern, the CMH-S099-13 detected in 2013 was selected as a representative strain for this analysis. The AY772730_GII.P16/2000/DE and X81879_GII.2/1994/GB were used as the parental reference strains. The recombination breakpoint was observed at the nt position 5,060, downstream of ORF1 within the C-terminal domain of RdRp, with a maximum-chi square value of 2.9 x 10^{-11} ($p < 0.01$) (Table 5.3, Figure 5.12E). For the GII.P16/GII.13 recombination pattern, the CMH-S003-13 strain detected in 2013 was selected for this analysis. The AY772730_GII.P16/2000/DE and EU921354_GII.13/2006/IN were used as parental reference strains. The recombination breakpoint was observed at nt position 5,090, within the overlapping region between ORF1 and ORF2, with a maximum-chi square value of 3.3 x 10^{-11} ($p < 0.01$) (Table 5.3, Figure 5.12F). For the GII.P7/GII.6 recombination pattern, the CMH-S101-14 strain detected in 2014 was selected. The HM635119_GII.P7/2008/KR and AB039778_GII.6/2002/JP were used as the parental reference strains. The recombination breakpoint was observed at the nt position 5,035, downstream of ORF1 within the C-terminal domain of RdRp, with a maximum-chi square value of 2.6 x 10^{-10} ($p < 0.01$) (Table 5.3, Figure 5.12G). For the GII.P21/GII.3 recombination pattern, the CMH-S127-14 strain detected in 2014 was selected as a representative strain. The JN899245_GII.P21/2011/US and U02030_GII.3/1997/US were used as the parental reference strains. The recombination breakpoint was observed at

the nt position 5,070, downstream of ORF1 within the C-terminal domain of RdRp, with a maximum-chi square value of 5.3×10^{-9} ($p < 0.01$) (Table 5.3, Figure 5.12H). For the GII.Pe/GII.4 recombination pattern, the CMH-ST023-15 strain detected in 2015 was selected. The GQ845369_GII.Pe /2008/AU and AY741811_GII.4/1997/GE were used as the parental reference strains. The result revealed that recombination breakpoint was observed at the nt position 5,070, downstream of ORF1 within the C-terminal domain of RdRp with a maximum-chi square value of 2.0×10^{-3} ($p < 0.01$) (Table 5.3, Figure 5.12I).

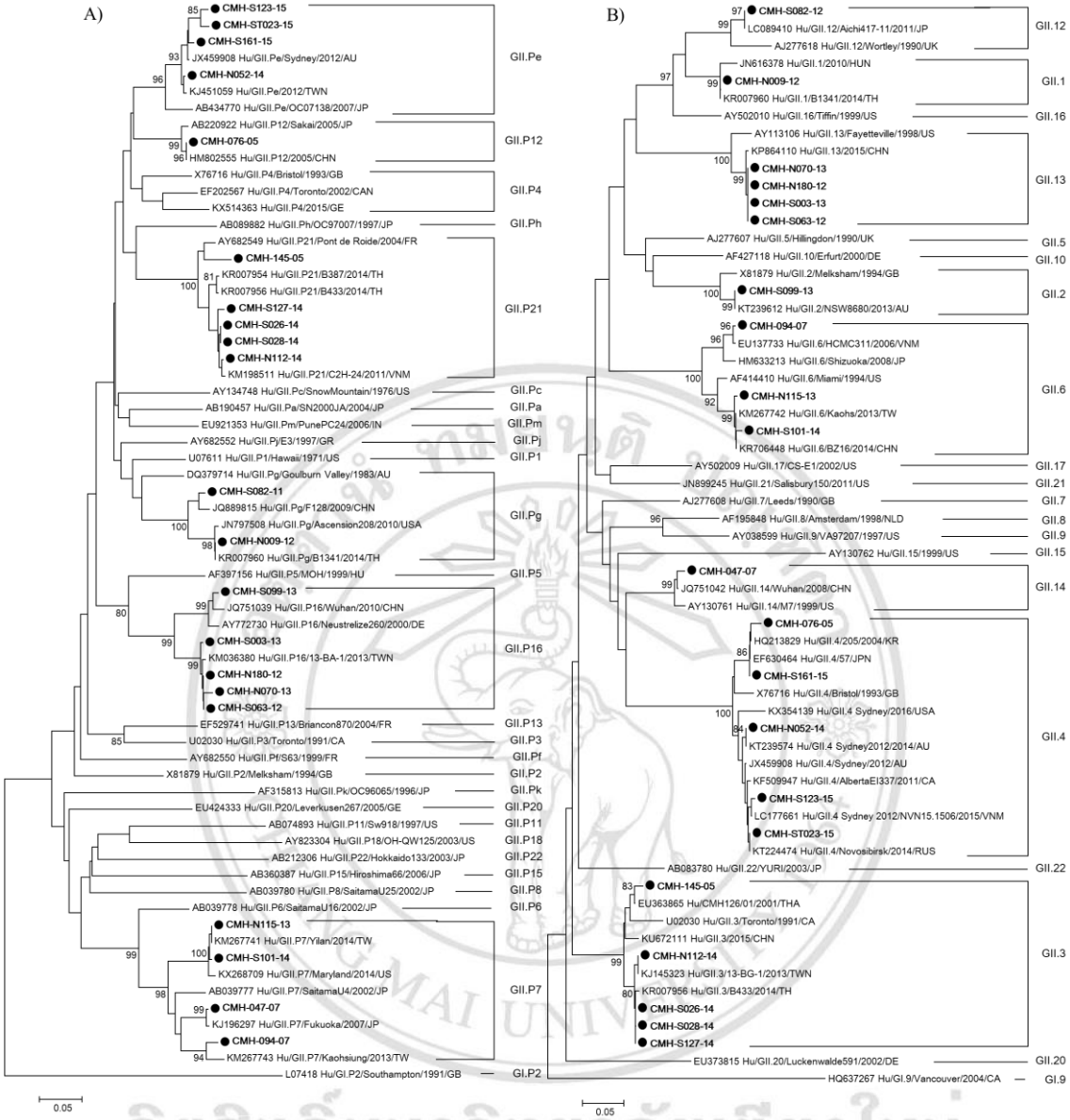
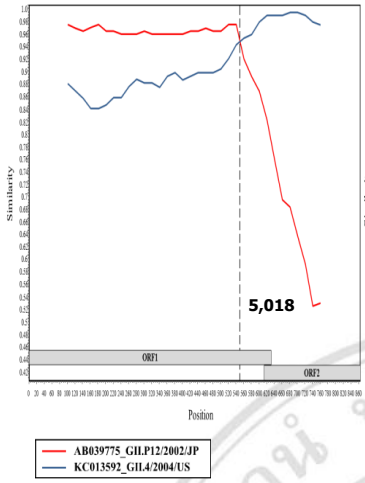


Figure 5.11 Phylogenetic analysis of norovirus GII based on: (A) partial ORF 1 region (RdRp sequences) and (B) partial ORF2 region (VP1 sequences). The phylogenetic trees were constructed using MEGA7 program based on the neighbor-joining method. The recombinant strains detected in this study are indicated by black dot symbol.

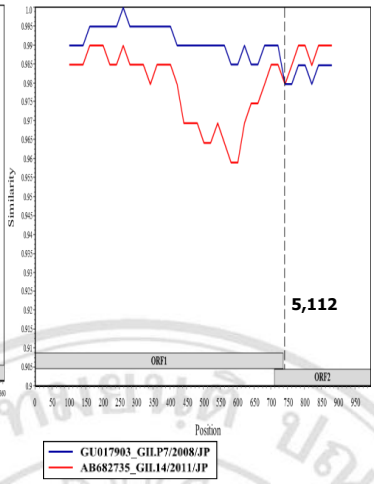
Table 5.3 Norovirus recombinant strains detected in pediatric patients with acute gastroenteritis from 2005 to 2015

Year	Sample code	Collection date (month-year)	Norovirus genotypes		Prototypes used for Simplot analysis		Predicted Recombination nt positions	Breakpoint on ORF	Maximum-chi square values
			RdRp	VP1	RdRp Genotype (Accession number)	VP1 Genotype (Accession number)			
2005	CMH-076-05	Jun-2005	GII.P12	GII.4	GII.P12_GII.12 (AB039775)	GII.P4_GII.4 (KC013592)	5,018	ORF 1	4.7×10^{-12}
	CMH-145-05	Dec-2005	GII.P21	GII.3	GII.P21_GII.21 (JN899245)	GII.P3_GII.3 (U02030)	5,064	ORF 1	3.1×10^{-12}
2007	CMH-047-07	Mar-2007	GII.P7	GII.14	GII.P7_GII.14 (GU017903)	GII.P7_GII.14 (AB682735)	5,112	ORF 2	5.4×10^{-1}
	CMH-094-07	Jun-2007	GII.P7	GII.6	GII.P7_GII.7 (HM635119)	GII.P6_GII.6 (AB039778)	5,033	ORF 1	1.6×10^{-4}
2011	CMH-N082-11	May-2011	GII.Pg	GII.12	GII.Pg_GII.13 (DQ379714)	GII.P12_GII.12 (KJ196299)	5,078	ORF 1	1.4×10^{-5}
2012	CMH-N009-12	Jan-2012	GII.Pg	GII.1	GII.Pg_GII.12 (JQ889815)	GII.P1_GII.1 (U07611)	5,123	ORF 2	2.7×10^{-2}
	CMH-N180-12	Dec-2012	GII.P16	GII.13	GII.P16_GII.16 (AY772730)	GII.P13_GII.13 (EU921354)	5,091	ORF 1/2	3.9×10^{-11}
	CMH-S063-12	Jun-2012	GII.P16	GII.13	GII.P16_GII.16 (AY772730)	GII.P13_GII.13 (EU921354)	5,093	ORF 1/2	1.2×10^{-11}
2013	CMH-N070-13	May-2013	GII.P16	GII.13	GII.P16_GII.16 (AY772730)	GII.P13_GII.13 (EU921354)	5,086	ORF 1/2	3.7×10^{-11}
	CMH-N115-13	Sep-2013	GII.P7	GII.6	GII.P7_GII.7 (HM635119)	GII.P6_GII.6 (AB039778)	5,032	ORF 1	7.3×10^{-4}
	CMH-S003-13	Jan-2013	GII.P16	GII.13	GII.P16_GII.16 (AY772730)	GII.P13_GII.13 (EU921354)	5,090	ORF 1/2	3.3×10^{-11}
	CMH-S099-13	Dec-2013	GII.P16	GII.2	GII.P16_GII.16 (AY772730)	GII.P2_GII.2 (X81879)	5,060	ORF 1	2.9×10^{-11}
2014	CMH-N052-14	Feb-2014	GII.Pe	GII.4	GII.Pe_GII.4 (GQ845369)	GII.P4_GII.4 (AY741811)	5,066	ORF 1	1.5×10^{-13}
	CMH-N112-14	Nov-2014	GII.P21	GII.3	GII.P21_GII.21 (JN899245)	GII.P3_GII.3 (U02030)	5,068	ORF 1	8.0×10^{-7}
	CMH-S026-14	Feb-2014	GII.P21	GII.3	GII.P21_GII.21 (JN899245)	GII.P3_GII.3 (U02030)	5,072	ORF 1	5.5×10^{-9}
	CMH-S028-14	Feb-2014	GII.P21	GII.3	GII.P21_GII.21 (JN899245)	GII.P3_GII.3 (U02030)	5,070	ORF 1	1.4×10^{-9}
	CMH-S101-14	Dec-2014	GII.P7	GII.6	GII.P7_GII.7 (HM635119)	GII.P6_GII.6 (AB039778)	5,035	ORF 1	2.6×10^{-10}
	CMH-S127-14	Jan-2014	GII.P21	GII.3	GII.P21_GII.21 (JN899245)	GII.P3_GII.3 (U02030)	5,070	ORF 1	5.3×10^{-9}
2015	CMH-S123-15	Jun-2015	GII.Pe	GII.4	GII.Pe_GII.4 (GQ845369)	GII.P4_GII.4 (AY741811)	5,069	ORF 1	2.5×10^{-3}
	CMH-S161-15	Aug-2015	GII.Pe	GII.4	GII.Pe_GII.4 (GQ845369)	GII.P4_GII.4 (AY741811)	5,070	ORF 1	3.9×10^{-2}
	CMH-ST023-15	Dec-2015	GII.Pe	GII.4	GII.Pe_GII.4 (GQ845369)	GII.P4_GII.4 (AY741811)	5,070	ORF 1	2.0×10^{-3}

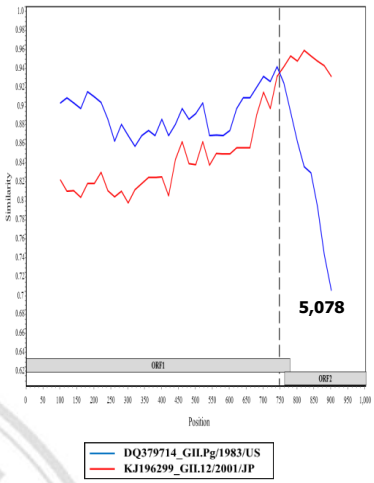
A) Query: CMH-076-05 (GII.P12/GII.4)



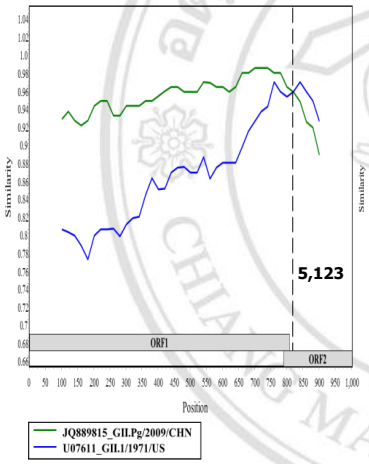
B) Query: CMH-047-07 (GII.P7/GII.14)



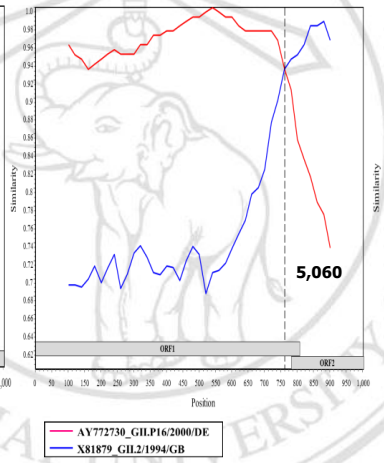
C) Query: CMH-N082-11 (GII.Pg/GII.12)



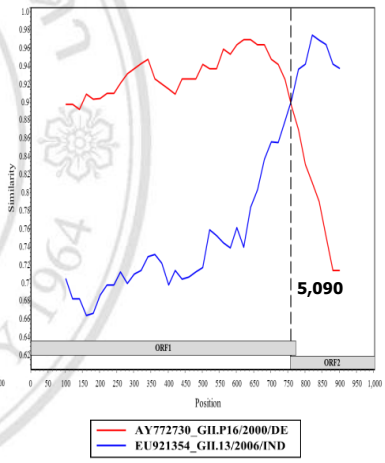
D) Query: CMH-N009-12 (GII.Pg/GII.1)



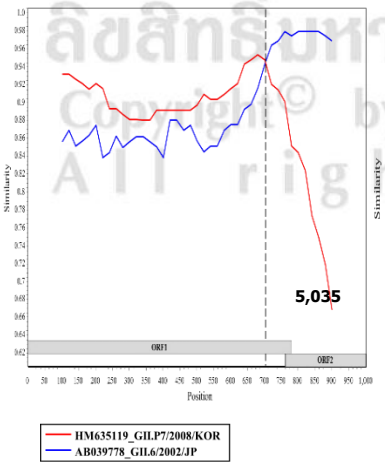
E) Query: CMH-S099-13 (GII.P16/GII.2)



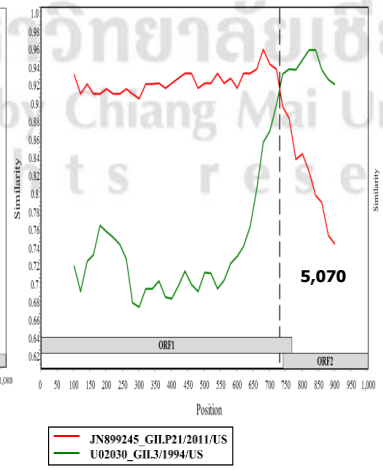
F) Query: CMH-S003-13 (GII.P16/GII.13)



G) Query: CMH-S101-14 (GII.P7/GII.6)



H) Query: CMH-S127-14 (GII.P21/GII.3)



I) Query: CMH-ST023-15 (GII.Pe/GII.4)

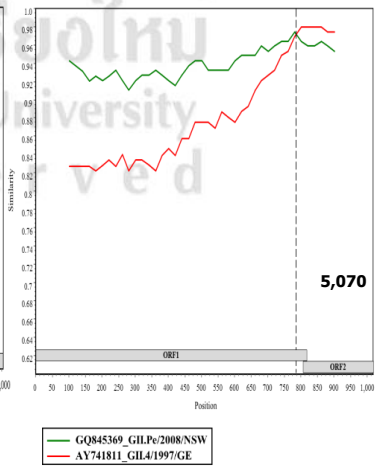


Figure 5.12 Similarity plot for norovirus GII recombinants detected in Chiang Mai, Thailand from 2005 to 2015. Representative sequences from each of nine recombinant patterns detected in this study (query strains) are: (A) CMH-076-05 (GII.P12/GII.4), (B) CMH-047-07 (GII.P7/GII.14), (C) CMH-N082-11 (GII.Pg/GII.12), (D) CMH-N009-12 (GII.Pg/GII.1), (E) CMH-S099-13 (GII.P16/GII.2), (F) CMH-S003-13 (GII.P16/GII.13), (G) CMH-S101-14 (GII.P7/GII.6), (H) CMH-S127-14 (GII.P21/GII.3), and (I) CMH-ST023-15 (GII.Pe/GII.4). By Simplot analysis with a slide window width of 200 bp and a step size of 20 bp, nucleotide sequences from nine NoV GII recombinant patterns were analyzed. The line intersect defines the predicted recombination site (vertical dashed line). The vertical axis indicates nucleotide similarity (%) between sequence from this study and those of the reference strains.