CHAPTER V

DISCUSSION AND CONCLUSION

1. Population study

The patients that used to study the prevalence of HCV genotype in this study were the patients from King Chulalongkorn Memorial Hospital. Then, the data of this study could not represent a picture of the situation of HCV infection or the distribution of HCV genotype in Thailand.

All of samples are from chronically infected and candidate for interferon therapy. The genotypes and progression of liver disease are not available except the 26 specimens from Ramathibodi Hospital who has been genotype by TRUGENE HCV 5' NC Genotyping kit.

2. RT-PCR amplification

The one hundred and twenty-six HCV RNA specimens were subjected to amplification. There were detectable levels of amplified products in the first round reaction of some samples. This may due to the concentration of virus in the circulation. However, amplified products were presence in all specimens in the nested round of the in-house PCR. These results suggested the limited of first round PCR to amplify the HCV RNA in the original specimen. The specimen with low quantities of viral RNA must be performed the nested PCR amplification. Although the nested PCR would add delays, expense and the risk of contamination to the procedure, the products of nested PCR amplification were generated the sufficient products for sequencing in the next step.

3. The reliable results of in-house HCV 5' NCR direct DNA sequencing assay.

The comparison between the in-house 5'NCR direct DNA sequencing assay and TRUEGENE HCV 5'NC Genotyping Kit showed the concordance genotype in 80.77% and the concordance subtype in 65.38 %.

All specimens that TRUEGENE assay identified as genotype 3 could be identified as genotype 3 by in-house 5' NCR genotyping assay. For the subtype in the genotype 3, the specimens that identified as subtype 3a by TRUEGENE HCV 5'NC Genotyping kit were identified as subtype 3a in all specimens by in-house 5'NCR direct DNA sequencing assay. The one case of subtype 3b showed the concordance subtype. However, the one case of subtype 3d was classified into subtype 3a by in-house 5'NCR direct DNA sequencing assay. The discordance in this case was a lack of subtype 3d reference database in the in-house 5'NCR direct DNA sequencing assay. Then, this case must be classified subtype in genotype 3a that sequence was the most similar with existing database. According to the therapeutic recommendation, the treatment of patients who infected with subtype 3a and subtype 3d would not different. However, the reference database of subtype 3d will be update.

For the typing of genotype 1, the result is 64.29% (9 of 14) concordance. Of the discordance genotype, genotype 6 was identified in 3 specimens and two specimens were genotype 3 by in-house 5' NCR genotyping assay. The identification of different genotype between two assays may be because of the database that used to compare the genotype. The database of the TRUEGENE HCV 5'NC Genotyping kit had a small number of genotype 6 reference. Moreover, the reference sequence in 5' NCR of genotype 1 and genotype 6 that reported from Thailand differ in 5 nt at position as followed. The sequence of sample has similar nucleotides in all 5 positions of genotype 6. Then, this sample was assigned as genotype 6 variants by this method.

Figure 13. The sequence alignment of HCV 5' NCR between genotype 1b, genotype 6 variants (6f.TH.TH97) and the specimen no. rama26. This specimen was assigned genotype 1b by TRUGENE while the genotype 6 was identified by this method.
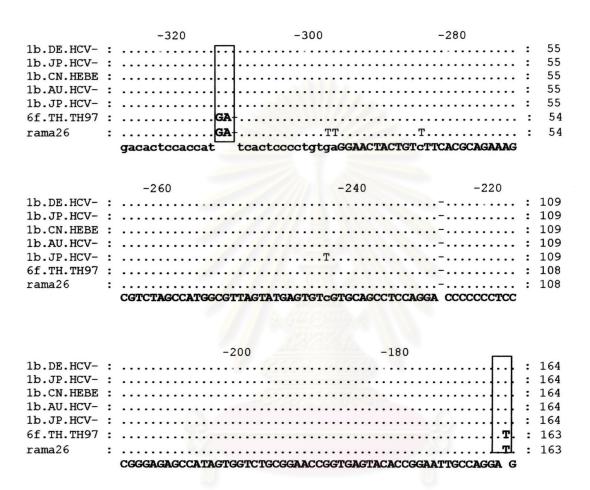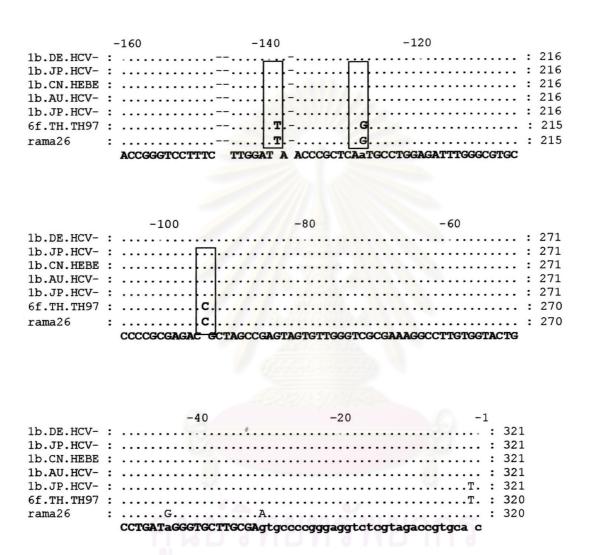


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                        -320           -300           -280
1b.DE.HCV- : ............... ..  .................................. :  55
1b.JP.HCV- : ............... ..  .................................. :  55
1b.CN.HEBE : ............... ..  .................................. :  55
1b.AU.HCV- : ............... ..  .................................. :  55
1b.JP.HCV- : ............... ..  .................................. :  55
6f.TH.TH97 : ...............GA ................................... :  54
rama26     : ...............GA .............TT.........T........... :  54
             gacactccaccat    tcactcccctgtgaGGAACTACTGTcTTCACGCAGAAAG


                        -260           -240           -220
1b.DE.HCV- : .......................................-.......... : 109
1b.JP.HCV- : .......................................-.......... : 109
1b.CN.HEBE : .......................................-.......... : 109
1b.AU.HCV- : .......................................-.......... : 109
1b.JP.HCV- : ...........................T...........-.......... : 109
6f.TH.TH97 : .......................................-.......... : 108
rama26     : .......................................-.......... : 108
             CGTCTAGCCATGGCGTTAGTATGAGTGTcGTGCAGCCTCCAGGA CCCCCCCTCC


                        -200           -180
1b.DE.HCV- : .................................................. : 164
1b.JP.HCV- : .................................................. : 164
1b.CN.HEBE : .................................................. : 164
1b.AU.HCV- : .................................................. : 164
1b.JP.HCV- : .................................................. : 164
6f.TH.TH97 : ................................................T. : 163
rama26     : ................................................T. : 163
             CGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACACCGGAATTGCCAGGA G
```

Figure13. The sequence alignment of HCV 5' NCR between genotype 1b, genotype 6 variants (6f.TH.TH97) and the specimen no. rama26. This specimen was assigned genotype 1b by TRUGENE while the genotype 6 was identified by this method (continued).

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              -160           -140            -120
1b.DE.HCV- : ............--.....|..|.-........|....|.............. : 216
1b.JP.HCV- : ............--.....|..|.-........|....|.............. : 216
1b.CN.HEBE : ............--.....|..|.-........|....|.............. : 216
1b.AU.HCV- : ............--.....|..|.-........|....|.............. : 216
1b.JP.HCV- : ............--.....|..|.-........|....|.............. : 216
6f.TH.TH97 : ............--.....|.T|.-........|.G.|.............. : 215
rama26     : ............--.....|.T|.-........|.G.|.............. : 215
             ACCGGGTCCTTTC   TTGGAT A ACCCGCTCAaTGCCTGGAGATTTGGGCGTGC
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```
              -100            -80             -60
1b.DE.HCV- : ....................................................... : 271
1b.JP.HCV- : .........|..|......................................... : 271
1b.CN.HEBE : .........|  |......................................... : 271
1b.AU.HCV- : .........|  |......................................... : 271
1b.JP.HCV- : .........|  |......................................... : 271
6f.TH.TH97 : .........|C |......................................... : 270
rama26     : .........|C |......................................... : 270
             CCCCGCGAGAC GCTAGCCGAGTAGTGTTGGGTCGCGAAAGGCCTTGTGGTACTG
```

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              -40             -20             -1
1b.DE.HCV- : .......................................'............... : 321
1b.JP.HCV- : ...................................................... : 321
1b.CN.HEBE : ...................................................... : 321
1b.AU.HCV- : ...................................................... : 321
1b.JP.HCV- : ...................................................T. : 321
6f.TH.TH97 : ...................................................T. : 320
rama26     : ......G...........A.................................. : 320
             CCTGATaGGGTGCTTGCGAgtgccccgggaggtctcgtagaccgtgca c
```

The genotype 6 variants were identified in Thailand and neighboring countries such as Vietnam and Hong Kong. The previous study demonstrated that the prevalence of genotype 6 variants was found at least 15% in Thai population. The significant contribution of these variants in this region of the world needed further investigation and the importance of genotype 6 is not clearly known.

For the classification of subtype, all specimens of subtype 1a were concordance subtype. Only four out of nine of genotype 1b are typing as 1b by our method. The discordance genotypes were assigned as genotype 6 variants in 3 cases and 2 cases were identified as genotype 3a and 3b. The sequences of these two cases were analyzed in detail by aligned with the reference sequences of genotype 1b and 3a, 1b and 3b. The result showed as followed.

Figure **14**. The sequence alignment of HCV 5' NCR between genotype 1b and genotype 3a. The specimen, rama3, was assigned genotype 1b by TRUGENE while the genotype 3a was identified by this method.

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                -320            -300            -280
3a.CB      : GACACTCCACCATGGATCACTCCCCTGTGAGGAACTTCTGTCT-TCACGC :  49
3a.QC8     : -----------ATGGATCACTCCCCTGTGAGGAACTTCTGTCT-TCACGC :  38
3a.QC10    : -----------ATGGATCACTCCCCTGTGAGGAACTTCTGTCT-TCACGC :  38
3a.NZL1    : GACACTCCACCATGGATCACTCCCCTGTGAGGAACTTCTGTCT-TCACGC :  49
3a.FR.HPCS : --------ACCATGGATCACTCCCCTGTGAGGAACTTCTGTCT-TCACGC :  41
rama3      : GACACTCCACCATGGATCACTCCCCTGTTTGGAACTACTGTTTCTCACGC :  50
1b.AU.HCV- : GACACTCCACCATAGATCACTCCCCTGTGAGGAACTACTGTCT-TCACGC :  49
1b.CN.HEBE : GACACTCCACCATAGATCACTCCCCTGTGAGGAACTACTGTCT-TCACGC :  49
1b.DE.HCV- : GACACTCCACCATAGATCACTCCCCTGTGAGGAACTACTGTCT-TCACGC :  49
1b.JP.HCV- : GACACTCCACCATAGATCACTCCCCTGTGAGGAACTACTGTCT-TCACGC :  49
1b.JP.HCV- : GACACTCCACCATAGATCACTCCCCTGTGAGGAACTACTGTCT-TCACGC :  49
             accAT GATCACTCCCCTGTgaGGAACT CTGTcT TCACGC

                  -260            -240
3a.CB      : GGAAAGCGCCTAGCCATGGCGTTAGTACGAGTGTCGTGCAGCCTCCAGGC :  99
3a.QC8     : GGAAAGCGCCTAGCCATGGCGTTAGTACGAGTGTCGTGCAGCCTCCAGGA :  88
3a.QC10    : GGAAAGCGCCTAGCCATGGCGTTAGTACGAGTGTCGTGCAGCCTCCAGGA :  88
3a.NZL1    : GGAAAGCGCCTAGCCATGGCGTTAGTACGAGTGTCGTGCAGCCTCCAGGA :  99
3a.FR.HPCS : GGAAAGCGCCTAGCCATGGCGTTAGTACGAGTGTCGTGCAGCCTCCAGGA :  91
rama3      : AGAAAGCGCCTAGCCATGGCGTTAGTACGAGTGTCGTGCAGCCTCCAGGA : 100
1b.AU.HCV- : AGAAAGCGTCTAGCCATGGCGTTAGTATGAGTGTCGTGCAGCCTCCAGGA :  99
1b.CN.HEBE : AGAAAGCGTCTAGCCATGGCGTTAGTATGAGTGTCGTGCAGCCTCCAGGA :  99
1b.DE.HCV- : AGAAAGCGTCTAGCCATGGCGTTAGTATGAGTGTCGTGCAGCCTCCAGGA :  99
1b.JP.HCV- : AGAAAGCGTCTAGCCATGGCGTTAGTATGAGTGTCGTGCAGCCTCCAGGA :  99
1b.JP.HCV- : AGAAAGCGTCTAGCCATGGCGTTAGTATGAGTGTTGTGCAGCCTCCAGGA :  99
             GAAAGCG CTAGCCATGGCGTTAGTA GAGTGTcGTGCAGCCTCCAGGa

                -220            -200            -180
3a.CB      : -CCCCCCTCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACAC : 148
3a.QC8     : -CCCCCCCTCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACAC : 137
3a.QC10    : -CCCCCCCTCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACAC : 137
3a.NZL1    : -CCCCCCCTCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACAC : 148
3a.FR.HPCS : -CCCCCCCTCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACAC : 140
rama3      : -CCCCCCCTCCCGGGAGA-CCATAGTGGTCTGCGGAAC-GGTGAGTACAC : 147
1b.AU.HCV- : -CCCCCCCTCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACAC : 148
1b.CN.HEBE : -CCCCCCCTCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACAC : 148
1b.DE.HCV- : -CCCCCCCTCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACAC : 148
1b.JP.HCV- : -CCCCCCCTCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACAC : 148
1b.JP.HCV- : -CCCCCCCTCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACAC : 148
             CCCCCCCTCCCGGGAGAgCCATAGTGGTCTGCGGAACcGGTGAGTACAC
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                      -160                -140
3a.CB      : CGGAATCGCTGGGGTGACCGGGTCCTTTC--TTGGAACA-ACCCGCTCAA : 195
3a.QC8     : CGGAATCGCTGGGGTGACCGGGTCCTTTC--TTGGAACA-ACCCGCTCAA : 184
3a.QC10    : CGGAATCGCTGGGGTGACCGGGTCCTTTC--TTGGAACA-ACCCGCTCAA : 184
3a.NZL1    : CGGAATCGCTGGGGTGACCGGGTCCTTTC--TTGGAGCA-ACCCGCTCAA : 195
3a.FR.HPCS : CGGAATCGCTGGGGTGACCGGGTCCTTTC--TTGGAGCA-ACCCGCTCAA : 187
rama3      : CGGAATCGCTGGGGTGACCGGGTCCTTTC--TTGGAGTA-ACCCGCTCAA : 194
1b.AU.HCV- : CGGAATTGCCAGGACGACCGGGTCCTTTC--TTGGATCA-ACCCGCTCAA : 195
1b.CN.HEBE : CGGAATTGCCAGGACGACCGGGTCCTTTC--TTGGATCA-ACCCGCTCAA : 195
1b.DE.HCV- : CGGAATTGCCAGGACGACCGGGTCCTTTC--TTGGATCA-ACCCGCTCAA : 195
1b.JP.HCV- : CGGAATTGCCAGGACGACCGGGTCCTTTC--TTGGATCA-ACCCGCTCAA : 195
1b.JP.HCV- : CGGAATTGCCAGGACGACCGGGTCCTTTC--TTGGATCA-ACCCGCTCAA : 195
             CGGAAT GC  GG  GACCGGGTCCTTTC  TTGGA cA ACCCGCTCAA


                      -120              -100               -80
3a.CB      : TACCCAGAAATTTGGGCGTGCCCCCGCGAGATCACTAGCCGAGTAGTGTT : 245
3a.QC8     : TACCCAGAAATTTGGGCGTGCCCCCGCGAGATCACTAGCCGAGTAGTGTT : 234
3a.QC10    : TACCCGGAAATTTGGGCGTGCCCCCGCGAGATCACTAGCCGAGTAGTGTT : 234
3a.NZL1    : TACCCAGAAATTTGGGCGTGCCCCCGCGAGATCACTAGCCGAGTAGTGTT : 245
3a.FR.HPCS : TACCCAGAAATTTGGGCGTGCCCCCGCGAGATCACTAGCCGAGTAGTGTT : 237
rama3      : TACCCAGAAATTTGGGCGTGCCCCCGCGAGATCACTAGCCGAGTANTGTT : 244
1b.AU.HCV- : TGCCTGGAGATTTGGGCGTGCCCCCGCGAGACTGCTAGCCGAGTAGTGTT : 245
1b.CN.HEBE : TGCCTGGAGATTTGGGCGTGCCCCCGCGAGACTGCTAGCCGAGTAGTGTT : 245
1b.DE.HCV- : TGCCTGGAGATTTGGGCGTGCCCCCGCGAGACTGCTAGCCGAGTAGTGTT : 245
1b.JP.HCV- : TGCCTGGAGATTTGGGCGTGCCCCCGCGAGACTGCTAGCCGAGTAGTGTT : 245
1b.JP.HCV- : TGCCTGGAGATTTGGGCGTGCCCCCGCGAGACTGCTAGCCGAGTAGTGTT : 245
             T CC  GA ATTTGGGCGTGCCCCCGCGAGA   CTAGCCGAGTAGTGTT


                      -60                -40
3a.CB      : GGGTCGCGAAAGGCCTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGCC : 295
3a.QC8     : GGGTCGCGAAAGGCCTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGCC : 284
3a.QC10    : GGGTCGCGAAAGGCCTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGCC : 284
3a.NZL1    : GGGTCGCGAAAGGCCTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGCC : 295
3a.FR.HPCS : GGGTCGCGAAAGGCCTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGCC : 287
rama3      : GGGTCGCGAAAGGCCTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGCC : 294
1b.AU.HCV- : GGGTCGCGAAAGGCCTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGCC : 295
1b.CN.HEBE : GGGTCGCGAAAGGCCTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGCC : 295
1b.DE.HCV- : GGGTCGCGAAAGGCCTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGCC : 295
1b.JP.HCV- : GGGTCGCGAAAGGCCTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGCC : 295
1b.JP.HCV- : GGGTCGCGAAAGGCCTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGCC : 295
             GGGTCGCGAAAGGCCTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGCC


                      -20                -1
3a.CB      : CCGGGAGGTCTCGTAGACCGTGCAAC : 321
3a.QC8     : CCGGGAGGTCTCGTAGACCGTGCAAC : 310
3a.QC10    : CCGGGAGGTCTCGTAGACCGTGCAAC : 310
3a.NZL1    : CCGGGAGGTCTCGTAGACCGTGCAAC : 321
3a.FR.HPCS : CCGGGAGGTCTCGTAGACCGTGCACC : 313
rama3      : CCGGGAGGTCTCGTAGACCGTGCAAC : 320
1b.AU.HCV- : CCGGGAGGTCTCGTAGACCGTGCACC : 321
1b.CN.HEBE : CCGGGAGGTCTCGTAGACCGTGCACC : 321
1b.DE.HCV- : CCGGGAGGTCTCGTAGACCGTGCACC : 321
1b.JP.HCV- : CCGGGAGGTCTCGTAGACCGTGCACC : 321
1b.JP.HCV- : CCGGGAGGTCTCGTAGACCGTGCATC : 321
             CCGGGAGGTCTCGTAGACCGTGCA C
```

The variation sequences of specimen rama3 was similar to genotype 3a in 16 nt while similar to genotype 1 only 2 nt. The sample assigned as 1b by TRUGENE while only 2 positions similar to genotype 1b might be because of the incorrectly labeling of samples.

The other discordance genotype was the convergent between genotype 1b by TRUGENE and genotype 3b by in-house 5' NCR genotyping assay. The specimen was aligned with the reference sequences of genotype 1b and 3b. The result showed as followed.

Figure 15. The sequence alignment of HCV 5' NCR between genotype 1b and genotype 3b. This specimen was assigned genotype 1b by TRUGENE while the genotype 3b was identified by this method.

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                            *         20        *         40        *
1b.DE.HCV-  : GACACTCCACCATAGATCACTCCCCTGTGAGGAACTACTGTCTTC-ACGC :  49
1b.JP.HCV-  : GACACTCCACCATAGATCACTCCCCTGTGAGGAACTACTGTCTTC-ACGC :  49
1b.CN.HEBE  : GACACTCCACCATAGATCACTCCCCTGTGAGGAACTACTGTCTTC-ACGC :  49
1b.AU.HCV-  : GACACTCCACCATAGATCACTCCCCTGTGAGGAACTACTGTCTTC-ACGC :  49
1b.JP.HCV-  : GACACTCCACCATAGATCACTCCCCTGTGAGGAACTACTGTCTTC-ACGC :  49
3b.JP.HCV-  : GACACTCCACCATGAATCACTCCCCTGTGAGGAACTTCTGTCTTC-ACGC :  49
3b.TH.TH52  : GACACTCCACCATGAGTCACTCCCCTGTGAGGAACTTCTGTCTTC-ACGC :  49
3b.TH.TH57  : GACACTCCACCATGAATCACTCCCCTGTGAGGAACTTCTGTCTTC-ACGC :  49
rama19      : GACACTCCACCATGAATCACTCCCCTGTTTGGAACTACTTTTTCCCACGC :  50
              GACACTCCACCAT  aTCACTCCCCTGTgaGGAACT CTgTcTtc ACGC

                           60        *         80        *        100
1b.DE.HCV-  : AGAAAGCGTCTAGCCATGGCGTTAGTATGAGTGTCGTGCAGCCTCCAGGA :  99
1b.JP.HCV-  : AGAAAGCGTCTAGCCATGGCGTTAGTATGAGTGTCGTGCAGCCTCCAGGA :  99
1b.CN.HEBE  : AGAAAGCGTCTAGCCATGGCGTTAGTATGAGTGTCGTGCAGCCTCCAGGA :  99
1b.AU.HCV-  : AGAAAGCGTCTAGCCATGGCGTTAGTATGAGTGTCGTGCAGCCTCCAGGA :  99
1b.JP.HCV-  : AGAAAGCGTCTAGCCATGGCGTTAGTATGAGTGTTGTGCAGCCTCCAGGA :  99
3b.JP.HCV-  : GGAAAGCGTCTAGCCATGGCGTTAGTACGAGTGTCGTGCAGCCTCCAGGC :  99
3b.TH.TH52  : GGAAAGCGTCTAGCCATGGCGTTAGTACGAGTGTCGTGCAGCCTCCAGGC :  99
3b.TH.TH57  : GGAAAGCGTCTAGCCATGGCGTTAGTACGAGTGTCGTGCAGCCTCCAGGC :  99
rama19      : GGAAAGCGTCTAGCCATGGCGTTAGTACGAGTGTCGTGCAGCCTCCAGGA : 100
              GAAAGCGTCTAGCCATGGCGTTAGTA GAGTGTcGTGCAGCCTCCAGG
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                      *         120         *        140          *
1b.DE.HCV-  : -CCCCCCCTCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACAC : 148
1b.JP.HCV-  : -CCCCCCCTCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACAC : 148
1b.CN.HEBE  : -CCCCCCCTCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACAC : 148
1b.AU.HCV-  : -CCCCCCCTCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACAC : 148
1b.JP.HCV-  : -CCCCCCCTCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACAC : 148
3b.JP.HCV-  : -CCCCCCCTTCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACAC : 148
3b.TH.TH52  : -CCCCCCCTCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACAC : 148
3b.TH.TH57  : -CCCCCCCTCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACAC : 148
rama19      : -CCCCCCCTCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACAC : 149
              CCCCCCCTcCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACAC

                      *         160         *        180          *        200
1b.DE.HCV-  : CGGAATTGCCAGGACGACCGGGTCCTTTC--TTGGATCA-ACCCGCTCAA : 195
1b.JP.HCV-  : CGGAATTGCCAGGACGACCGGGTCCTTTC--TTGGATCA-ACCCGCTCAA : 195
1b.CN.HEBE  : CGGAATTGCCAGGACGACCGGGTCCTTTC--TTGGATCA-ACCCGCTCAA : 195
1b.AU.HCV-  : CGGAATTGCCAGGACGACCGGGTCCTTTC--TTGGATCA-ACCCGCTCAA : 195
1b.JP.HCV-  : CGGAATTGCCAGGACGACCGGGTCCTTTC--TTGGATCA-ACCCGCTCAA : 195
3b.JP.HCV-  : CGGAATCGCCGGGATGACCGGGTCCTTTC--TTGGAACA-ACCCGCTCAA : 195
3b.TH.TH52  : CGGAATCGCCGGGATGACCGGGTCCTTTC--TTGGAACA-ACCCGCTCAA : 195
3b.TH.TH57  : CGGAATCGCCGGGATGACCGGGTCCTTTC--TTGGAACA-ACCCGCTCAA : 195
rama19      : CGGAATCGCCAGGATGACCGGGTCCTTTC--TTGGAACA-ACCCGCTCAA : 196
              CGGAAT GCC GGA GACCGGGTCCTTTC  TTGGA CA ACCCGCTCAA

                      *         220         *        240          *
1b.DE.HCV-  : TGCCTGGAGATTTGGGCGTGCCCCCGCGAGACTGCTAGCCGAGTAGTGTT : 245
1b.JP.HCV-  : TGCCTGGAGATTTGGGCGTGCCCCCGCGAGACTGCTAGCCGAGTAGTGTT : 245
1b.CN.HEBE  : TGCCTGGAGATTTGGGCGTGCCCCCGCGAGACTGCTAGCCGAGTAGTGTT : 245
1b.AU.HCV-  : TGCCTGGAGATTTGGGCGTGCCCCCGCGAGACTGCTAGCCGAGTAGTGTT : 245
1b.JP.HCV-  : TGCCTGGAGATTTGGGCGTGCCCCCGCGAGACTGCTAGCCGAGTAGTGTT : 245
3b.JP.HCV-  : TGCCCGGAAATTTGGGCGTGCCCCCGCGAGATCACTAGCCGAGTAGTGTT : 245
3b.TH.TH52  : TGCCCGGAAATTTGGGCGTGCCCCCGCGAGATCACTAGCCGAGTAGTGTT : 245
3b.TH.TH57  : TGCCTGGAAATTTGGGCGTGCCCCCGCGAGATCACTAGCCGAGTAGTGTT : 245
rama19      : TGCCTGGAAATTTGGGCGTGCCCCCGCGAGATCACTAGCCGAGTAGTGTT : 246
              TGCCtGGA ATTTGGGCGTGCCCCCGCGAGA   CTAGCCGAGTAGTGTT

                      *         260         *        280          *        300
1b.DE.HCV-  : GGGTCGCGAAAGGCCTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGCC : 295
1b.JP.HCV-  : GGGTCGCGAAAGGCCTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGCC : 295
1b.CN.HEBE  : GGGTCGCGAAAGGCCTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGCC : 295
1b.AU.HCV-  : GGGTCGCGAAAGGCCTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGCC : 295
1b.JP.HCV-  : GGGTCGCGAAAGGCCTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGCC : 295
3b.JP.HCV-  : GGGTCGCGAAAGGCCTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGCC : 295
3b.TH.TH52  : GGGTCGCGAAAGGCCTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGCC : 295
3b.TH.TH57  : GGGTCGCGAAAGGCCTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGCC : 295
rama19      : GGGTCGCGAAAGGCCTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGCC : 296
              GGGTCGCGAAAGGCCTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGCC

                      *         320
1b.DE.HCV-  : CCGGGAGGTCTCGTAGACCGTGCACC : 321
1b.JP.HCV-  : CCGGGAGGTCTCGTAGACCGTGCACC : 321
1b.CN.HEBE  : CCGGGAGGTCTCGTAGACCGTGCACC : 321
1b.AU.HCV-  : CCGGGAGGTCTCGTAGACCGTGCACC : 321
1b.JP.HCV-  : CCGGGAGGTCTCGTAGACCGTGCATC : 321
3b.JP.HCV-  : CCGGGAGGTCTCGTAGACCGTGCAAC : 321
3b.TH.TH52  : CCGGGAGGTCTCGTAGACCGTGCAAC : 321
3b.TH.TH57  : CCGGGAGGTCTCGTAGACCGTGCATC : 321
rama19      : CCGGGAGGTCTCGTAGACCGTGCATC : 322
              CCGGGAGGTCTCGTAGACCGTGCA C
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The sample assigned genotype 3b by in-house 5' NCR genotyping assay presented 2 positions similar to genotype 1b but similar to genotype 3b in 11 nt. These two discordance sequences were blasted and the most similarity sequence were HCV genotype 3a [gi:7288533] and HCV genotype 3a [gi:7288531], respectively.

The failure of these two cases may be because of the length of sequence that used to determine the genotype. The sequences of 183 bp fragment at nucleotides 96 to 278 were used to determine the genotype by TRUGENE assay. This assay was not able to completely resolve all existing HCV genotypes and subtypes such as 1a, 1b and some case of 1b and 3a. (80).

4. The prevalence of the HCV genotypes in patients from King Chulalongkorn Memorial Hospital.

The distribution pattern of HCV genotypes was slightly different from those found in other studies of Thai samples. The discrepancy may be due to the choice of the subjects and genotyping method. The samples in this study were subjected from Bangkok, which is in central Thailand. The prevalence of HCV genotype in different regions of Thailand may differ from another. However, HCV genotype 3a was also the most common genotype. According to this assay, HCV genotype 3a/3b and genotype 1a/1b/6 variants were equally prevalent. These data suggested that the genotyping of HCV genotype was necessary and importance in management of therapy.

5. Increasing the efficiency of genotyping of 5' NCR by sequencing longer template.

Although the high degree of sequence conservation was found within the 5' NCR of the HCV genome but the sequences variation between genotypes were demonstrated. The various genotyping methods were used the previous variable regions within position –240 to position – 80 to identify the genotype. Most of them could not differentiate between subtype in genotype1 and genotype 6 variants. The other 3 variable regions were present within the primer range of this study.

At the position –317 to –306, an AG or GA at position –312 to-311 could ascribed the subtype in genotype 1 as subtype 1b or 1a respectively. A G and a T at position –317 and – 312 could be found in genotype 6a variants while genotype 2a were a G and a C at position –317 and –309. Moreover, genotype 4a was presented a specific pattern at position –309 as a C and a G at position –307. Genotype 3 was the only genotype that presented a T and a G at position –289 and –276 respectively. However, the variation at position –5 to –1 may not present the pattern that specific for each genotype as same as other two variable region. These variable data suggested that longer of sequence can increase the efficiency of HCV genotyping of 5' NCR. The discordance results in genotyping of HCV genotype 6 variants may be because of elimination of database in TRUGENE HCV 5 ' NC Genotyping kit and the length of sequence that used to identify the HCV genotype was only 183 bp fragment. Moreover, the position of their primers could not correctly differentiate between subtypes of genotype 1 and 2 in some specimen.

Because differences in geographical distribution, disease outcome, and response to therapy among HCV genotype have been suggested, reliable methods for determining the HCV genotype may become an important clinical test. This genotyping method is reliable compare to commercial kit. This method is a modification of commercial kit with the improve efficiency for genotyping. All specimens can be genotyped and the genotype 6 variants can be identified. The genotype 6 variants and genotype 1b have similar sequence at the 183 bp

region of 5' NCR. The longer sequences of 5' NCR can be useful in the differentiation this two genotypes. Moreover, this assay is less expensive because the in-house PCR product from diagnostic assay can be used for genotyping. The Free ware was used to analyze the results with the large number of references database that can be update. The prevalence study reported the concordance data with the previous study. The genotype data will be useful in the clinical management and treatment. This study will help clinician addressed the role of genotype in liver disease progression or response to interferon therapy. Also the clinical significance of HCV genotype that are not common in United States, Europe, Japan which has received minimal attention because most scientific investigation are being conducted in these countries. These genotypes, which include genotype 6 has been found mostly in Southeast Asia and clinical significance of these genotypes in severity of liver disease or response to interferon therapy have never been reported due to the lack of scientific investigation.