Expanded classification of hepatitis C Virus into 7 genotypes and 67 Subtypes

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The 2005 consensus proposal for the classification of hepatitis C virus (HCV) presented an agreed and uniform nomenclature for HCV variants and the criteria for their assignment into genotypes and subtypes. Since its publication, the available dataset of HCV sequences has vastly expanded through advancement in nucleotide sequencing technologies and an increasing focus on the role of HCV genetic variation in disease and treatment outcomes. The current study represents a major update to the previous consensus HCV classification, incorporating additional sequence information derived from over 1,300 (near-)complete genome sequences of HCV available on public databases in May 2013. Analysis resolved several nomenclature conflicts between genotype designations and using consensus criteria created a classification of HCV into seven confirmed genotypes and 67 subtypes. There are 21 additional complete coding region sequences of unassigned subtype. The study additionally describes the development of a Web resource hosted by the International Committee for Taxonomy of Viruses (ICTV) that maintains and regularly updates tables of reference isolates, accession numbers, and annotated alignments (http://talk.ictvonline.org/links/hcv/hcv-classification.htm). The Flaviviridae Study Group urges those who need to check or propose new genotypes or subtypes of HCV to contact the Study Group in advance of publication to avoid nomenclature conflicts appearing in the literature. While the criteria for assigning genotypes and subtypes remain unchanged from previous consensus proposals, changes are proposed in the assignment of provisional subtypes, subtype numbering beyond “w,” and the nomenclature of intergenotypic recombinant. Conclusion: This study represents an important reference point for the consensus classification of HCV variants that will be of value to researchers working in clinical and basic science fields. (HEPATOLOGY 2014;59:318-327)
The application of these criteria confirmed the assignment of six distinct genotypes, comprising 18 subtypes. In addition, 58 subtypes were provisionally assigned pending the availability of a complete coding region sequence or additional isolates. This agreement on nomenclature was mirrored by the establishment of several curated databases that organized HCV sequences as they became available and indicated which genotypes and subtypes were confirmed or provisionally assigned (Los Alamos HCV Sequence Database,3 euHCVdb,4 Hepatitis Virus Database: http://s2as02.genes.nig.ac.jp/). Concurrently, a proposal was made to unify the numbering of HCV with reference to the genotype 1a isolate H77 (AF009606).5

Recently, this remarkable agreement and cooperation in HC-V nomenclature has been complicated by several developments. None of the HCV sequence databases are now actively curated and responsibility for naming new genotypes and subtypes has reverted de facto to individual researchers. This, combined with publication delays, has created new contradictions in which isolates assigned to the same subtype (4b: FJ462435, FJ025855, FJ025856, and FJ025854; 6k: DQ278891 and DQ278893; 6u: EU408330, EU408331, and EU408332) belong to different subtypes according to the consensus criteria. Another challenge is that the number of complete coding region sequences has increased from 238 in 2005 to more than 1,300. Similarly, the number of variants matching the criteria for assignment as confirmed genotypes/subtypes has expanded from 18 to 67; several recent publications contain figures that are illegible with regard to isolate name and/or accession number,6–10 complicating subsequent comparisons.

Finally, advances in sequencing technology have accelerated the rate at which HCV sequences are generated. Recent articles have reported the partial sequences of 282 isolates from Vietnam11 and 393 isolates from China,10 in each case identifying additional subtypes of genotype 6. Technological advances have also made it easier to obtain HCV complete coding region sequences through both dideoxysequencing and pyrosequencing. The latter technique was recently used to obtain 31 complete coding region sequences belonging to 13 different subtypes.8 More than 225,000 HCV sequences are now available on GenBank and about 30,000 added every year. This volume of sequence information and the diversity of known HCV variants make it increasingly important for researchers to have a single curated resource to refer to for accurate subtype designations, reference genomes and alignments.

This article updates the genotype and subtype assignments2,7 and the nomenclature rules, and describes the establishment of a reference Website hosted by the International Committee for the Taxonomy of Viruses (ICTV) to validate new genotype and subtype assignments, and provide updated reference alignments.

Revision of Confirmed Genotypes and Subtypes

Unique HCV complete or nearly complete coding region sequences available on NCBI Genome (969 sequences, http://www.ncbi.nlm.nih.gov/genome) and the Los Alamos HCV sequence database (1,364 sequences >8,000 nt from http://hcv.lanl.gov/content/index) were aligned within SSEv1.12 using Muscle v3.8.3113 and refined manually. Phylogenetic analysis of sequences containing >95% of the coding region reveals seven major phylogenetic groupings corresponding to genotypes 1-7 (Fig. 1). Within these genotypes, grouping of the constituent subtypes is supported by 100% of bootstrap replications.

Based on the consensus criteria,2 confirmed subtypes (indicated by a letter following the genotype) require a complete or nearly complete coding region sequence differing from other sequences by at least 15% of nucleotide positions and sequence information from at least two other isolates in core/E1 (>90% of the sequence corresponding to positions 869 to 1,292 of the H77 reference sequence [accession number AF009606] numbered according to reference5) and NS5B (>90% of positions 8,276 to 8,615) (Table 1). The use of a 15% threshold over the complete coding region is supported by analysis of the large number of potential subtypes now sequenced (Fig. 2). This reveals major and consistently placed gaps in the distribution of pairwise distances between and within subtypes of
each genotype as follows: genotype 1: 12.9%-17.0%, genotype 2: 13.1%-17.6%, genotype 3: 12.5%-19.6%, genotype 4: 12.7%-15.3% (except distances of 14% and 14.2% between JX227963 and two subtype 4g sequences), and genotype 6: 9.9%-14.9% (except distances of 13.1%-13.7% between EU246931 and three subtype 6e sequences). Hence, for all genotypes and with remarkably few exceptions, a clear division can be made between isolates that differ by <13% over their complete coding region sequences (members of the same subtype) and those that differ by >15% (different genotypes or subtypes). This analysis includes sequences distinct from any of the confirmed HCV subtypes but not currently represented by three or more independent isolates that remain unclassified subtypes (Table 2). Whether the exceptions noted are due to technical problems or to differing epidemiological histories is unknown.

The seven confirmed genotypes (discussed below) comprise 67 confirmed subtypes, 20 provisionally
assigned subtypes, and 21 unassigned subtypes. These tables have been posted on the ICTV Website at http://talk.ictvonline.org/links/hcv/hcv-classification.htm and will be updated regularly by the authors with information shared across existing HCV databases (http://hcv.lanl.gov/; http://euhcvdb.ibcp.fr/euHCVdb/), typing tools, and other resources (e.g., http://www.bioafrica.net/rega-genotype/html/subtypinghcv.html; http://comet.retrovirology.lu/; http://hcv.lanl.gov/content/sequence/phyloplace/; http://s2as02.genes.nig.ac.jp/; http://www.viprbrc.org/). Alignments including representatives of these subtypes are available on the

Table 1. Confirmed HCV Genotypes/Subtypes

| Genotype* | Locus/Isolate(s) | Accession number(s) | Reference(s) |
|-----------|------------------|---------------------|--------------|
| Genotype 1 |                  |                     |              |
| 1a        | HPCPUPRE, HPCGGAA | M62321, M67463      | 29,30        |
| 1b        | HPCJCO, HPCJUMR  | D90208, M58335      | 31,32        |
| 1c        | HPCGO5, AY051292 | D14863, AY051292    | 33           |
| 1a        | 146656           | KC248194            | 34           |
| 1b        | 1804             | AM910652            | 9            |
| 1a        | EY443, EW9       | KC248198, KC248199  | 9            |
| 1a        | 136142, EW424     | KC248193, KC248197  | 9            |
| Genotype 2 |                  |                     |              |
| 2a        | HPCPOLP, FHJ-1   | D00944, AB047639    | 35,36        |
| 2b        | HPCIC86, JPT971017 | D10988, AB030907    | 37,38        |
| 2c        | BEBE1            | D50409              | 39           |
| 2d        | QC259            | JF735114            | 40           |
| 2e        | QC64             | JF735120            | 40           |
| 2i        | D54              | DQ155561            | 41           |
| 2j        | C1799, QC232     | HM777358, JF735113  | 42           |
| 2k        | VAT96            | AB031663            | 42           |
| 2m        | QC178, BID-G134  | JF735111, JX227967  | 42,43        |
| 2q        | 963, 852         | FN666424, FN666429  | 42           |
| 2r        | QC283            | JF735115            | 40           |
| Genotype 3 |                  |                     |              |
| 3a        | HPCCEGS, HPCK3A  | D17763, D28917      | 44,45        |
| 3b        | HPCFG            | D49374              | 46           |
| 3g        | BID-G1243, QC260 | JX227954, JF735123  | 47           |
| 3h        | QC29             | JF735121            | 48           |
| 3l        | HCJ-CHV, BID-G1244 | FJ407092, JX227955 | 48           |
| 3k        | HPCKJ049E1, QC105 | D63821, JF735122    | 47,48        |
| Genotype 4 |                  |                     |              |
| 4a        | ED43             | Y11604              | 49           |
| 4b        | QC264            | FJ462435            | 50           |
| 4c        | QC381            | FJ462436            | 51           |
| 4d        | 03-18, QC382     | DQ418786, FJ462437  | 52           |
| 4f        | IFBT88, PS6      | EF589161, EU392175  | 53           |
| 4g        | QC193            | FJ462432            | 54           |
| 4k        | PS3, QC383       | EU392173, FJ462438  | 55           |
| 4l        | QC274            | FJ439870            | 56           |
| 4m        | QC249            | FJ462433            | 57           |
| 4n        | QC97             | FJ462441            | 58           |
| 4p        | QC93             | FJ462440            | 59           |
| 4q        | QC139            | FJ462431            | 60           |
| 4r        | QC262            | FJ462434            | 61           |
| 4t        | QC384            | FJ462439            | 62           |
| 4u        | QC155            | FJ439899            | 63           |
| 4v        | CYCJ20V3, BID-G1248 | HQ537400, JX227959 | 64           |
| 4w        | P212, P245       | FJ025855, FJ025856  | 65           |
| Genotype 5 |                  |                     |              |
| 5a        | EUH1480, SA13     | Y13184, A064490     | 66           |
| Genotype 6 |                  |                     |              |
| 6a        | EUHK2, 6a33      | Y12083, AY859526    | 67           |
| 6b        | ThS80            | FJ462438            | 68           |
| 6c        | ThS86            | FJ462439            | 69           |
| 6d        | PN135            | FJ462440            | 70           |
| 6e        | GQ004            | FJ462441            | 71           |
| 6f        | C-0044           | FJ462442            | 72           |
| 6g        | HPCKJ046E2       | FJ462443            | 73           |
| 6h        | VN004            | FJ462444            | 74           |
| 6i        | Th602            | FJ462445            | 75           |
| 6j        | ThS53            | FJ462446            | 76           |
| 6k        | VN405            | FJ462447            | 77           |
| 6l        | S57795           | FJ462448            | 78           |
| 6m        | B4, 92           | FJ462449            | 79           |
| 6n        | KM42, D68/93     | DQ778994, DQ835768  | 80           |
| 6o        | QC227            | FJ839869            | 81           |

Table 1. Continued

| Genotype* | Locus/Isolate(s) | Accession number(s) | Reference(s) |
|-----------|------------------|---------------------|--------------|
| 6p        | QC216            | EF424626            | 82           |
| 6q        | QC399            | EF424625            | 83           |
| 6r        | QC245            | EU408328            | 84           |
| 6s        | QC66             | EU408329            | 85           |
| 6t        | VT21, D49        | EF632071, EU246939  | 86           |
| 6u        | D83              | EU246940            | 87           |
| 6v        | NK46, KMN-02     | EU158186, EU798760  | 88           |
| 6w        | GZ52957, D140    | DQ778992, EU643834  | 89           |
| 6a         | DH012, DH028     | EU408330, EU408332  | 90           |
| Genotype 7 |                  |                     |              |
| 7a        | QC69             | EF108306            | 91           |

Additions and changes from assignments proposed in \(^2\) shown in bold.

*Consensus proposed genotype/subtype names. Where multiple sequences of a HCV genotype are available, two sequences have been listed, prioritized by (a) publication date or (b) submission date when unpublished.

†Locus (or isolate name if locus is the same as the accession number).
‡Previously described as 4b.\(^7,\)\(^14\)
§Sequence obtained from acute phase plasma of a chimpanzee experimentally infected with (human-derived) isolate SA13.
\(^k\)Previously described as 6u.\(^18\)

Fig. 2. Distribution of p-distances between complete coding region sequences. The frequency of p-distances was calculated within and between genotypes using SSE.\(^12\) Intra-genotype pairwise distances were calculated for all available complete coding region sequences except for subtypes 1a, 1b, and 2b where 20 random sequences were used. For p-distances >0.15 (equivalent to a percent difference of 15%), frequencies were scaled to reduce the maximum frequency to less than 300. Distances between genotypes were calculated using one or two representatives of each confirmed and unassigned subtype, with the frequencies scaled as above.
ICTV Website and at http://hcv.lanl.gov/content/sequence/NEWALIGN/align.html/. The process of producing these tables has detected a small number of variants with conflicting assignments. Isolates P026, P212, P245, (FJ025854-6) are described as subtype 4b, 14 but these complete coding region sequences show <85% identity to the core/E1 of isolate Z1 (U10235, L16677), provisionally assigned as 4b15 that is more closely related to core/E1 of the complete coding region sequence of isolate QC264 (FJ46243516). P212 and P245 belong to the same, novel subtype for which NS5B sequence is available from a third isolate (P213, GU049362), so this becomes confirmed subtype 4w. Isolate P026 differs from all other genotype 4 sequences by >17.5% but being represented by a single sequence remains currently unassigned (Table 2).

Similarly, isolates KM45 and KM41 (DQ278891,3) have been assigned to subtype 6k, 17 but differ by >17% in complete coding region sequence from the subtype 6k isolate VN405 (D84264) and 6.7% from each other, and so remain an unclassified subtype of genotype 6. Two distinct groups of isolates have been assigned to subtype 6u; EU408330-218 and EU246940.19 The latter was submitted first to GenBank and is represented by NS5B sequences from two additional isolates and so is assigned subtype 6u, while EU408330, EU408331, and EU408332 are designated subtype 6xa (see below).

Finally, our analysis of both phylogenetic groupings and sequence distances suggests that a number of isolates20 described in their GenBank accessions as “subtype k-related” (QC273, TV257, TV476, KM35), “subtype l-related” (TV533, L349), “intermediate between subtypes 6m and 6n” (DH027), or “intermediate between subtypes 6j and 6i” (QC271) should be considered as unassigned novel subtypes.

### Additional Taxonomic Levels

In making this taxonomic distinction into virus genotypes and subtypes we are aware of the difficulties of imposing a discrete classification scheme on a complex taxonomy. In particular, for genotypes 3 and 6 there are undoubtedly several hierarchies of taxonomic relationships. For example, subtypes 6k and 6l form a clade along with several unassigned genotype 6 isolates.20 A higher-level clade includes these sequences and subtypes 6m and 6n, while a further grouping consists of these subtypes and subtypes 6i and 6j (Fig. 1). These phylogenetic hierarchies are reflected in the discontinuous distribution of p-distances between complete coding region sequences (Fig. 2), which comprises three almost merging distributions (roughly 15% to 20%, 20% to 25%, and 25% to 30%). Three distributions of intersubtype distances were also observed for genotype 3 (20% to 25%, 25% to 27%, and 27% to 30%), two distributions for genotype 2 (18% to 22.5%, 23% to 26.5%), and uniform distributions for genotype 1 (17.7% to 25.4%) and genotype 4 (15.3% to 23.1%). However, the internal divisions defined by the multiple distributions of distances within genotypes 2, 3, and 6 have not been shown to correspond with geographical or epidemiological differences. The higher-level grouping of subtypes 3b, 3g, and 3i does not reflect a common geographical origin distinct from that of 3h and 3k.21 There is also no geographical correlation with the groupings of subtypes 6k, 6l, and various unassigned isolates; for 6m, 6n, and an unassigned isolate; for 6h, 6i, 6j, and an unassigned isolate; for 6a and 6b; for 6f and 6r; or for 6r and 6e.22 Similarly, there are currently no known virological or clinical reasons to recognize these higher-level groupings.

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### Table 2. Unassigned Complete Coding Region Sequences

| Genotype* | Locus/Isolate(s) | Accession no(s) | Reference |
|-----------|-----------------|-----------------|-----------|
| Genotype 1 |                 |                 |           |
| 1_A851228 | A851228         | A851228         | 65        |
| 1_K248195 | 160526          | NC248195        | 9         |
| 1_HQ537007 | CYNC025 | HQ537007       | 52        |
| Genotype 2 |                 |                 |           |
| 2_JF735119 | QC331          | JF735119        | 40        |
| 2_JF735112 | QC182          | JF735112        | 40        |
| 2_JF735110 | QC114          | JF735110        | 40        |
| 2_JF735117 | QC297          | JF735117        | 40        |
| 2_JF735116 | QC289          | JF735116        | 40        |
| 2_JF735118 | QC302          | JF735118        | 40        |
| Genotype 3 |                 |                 |           |
| 3_JF735124 | QC115          | JF735124        | 21        |
| Genotype 4 |                 |                 |           |
| 4_JX27964 | BID-G1253      | JX27964         | 8         |
| 4_F025854 | F025854        | F025854         | 14        |
| Genotype 6 |                 |                 |           |
| 6_DQ278891 | KM45,M41       | DQ278891,DQ278893 | 17     |
| 6_JX183550 | QC273          | JX183550        | 20        |
| 6_JX183552 | TV476          | JX183552        | 20        |
| 6_JX183549 | KM35           | JX183549        | 20        |
| 6_JX183551 | TV257          | JX183551        | 20        |
| 6_JX183553 | TV539          | JX183553        | 20        |
| 6_JX183554 | L349           | JX183554        | 20        |
| 6_JX183557 | DH027          | JX183557        | 20        |
| 6_JX183558 | QC271          | JX183558        | 20        |

*Classification of sequences into genotypes but without subtype assignments using the format “genotype_Accession number.”
1Locus (or isolate name if locus is the same as the accession number).
‡Previously described as 4b.14
§ Previously described as 6k.17

*Classification of sequences into genotypes but without subtype assignments using the format “genotype_Accession number.”
1Locus (or isolate name if locus is the same as the accession number).
‡Previously described as 4b.14
§ Previously described as 6k.17
utility, we therefore propose that the observed within-genotype hierarchies are not given any formal recognition in their nomenclature.

Proposed Updates and Changes to Rules for Genotype/Subtype Assignments

**Subtype Names.** By definition, subtype name assignments would be limited to a maximum of 26 if designated by a single letter suffix (e.g., 2a-2z). We therefore suggest that subtypes are assigned up to the letter “w” and subsequent designations follow the eXtended form xa, xb, … xz, in turn followed by ya, … yz, za, … zz, potentially giving a total of 101 subtypes of each genotype. This avoids potentially ambiguous terms such as “subtype 6x,” which could be interpreted as “genotype 6 of unknown subtype,” or designations such as “subtype 3aa,” which might suggest a relationship with 3a.

**Provisional Genotypes.** According to the 2005 consensus classification protocol, new genotypes could be provisionally assigned from a single complete coding region sequence, but partial or complete coding region sequences from additional isolates would be required to confirm these assignments. Since there is only one confirmed genotype has been identified (7a) represented by a single isolate (QC69, EF108306). Thus, in contrast to subtype assignments, the number of genotypes appears relatively limited and the requirement to sequence multiple isolates now seems onerous. We propose that only a single complete coding region sequence is needed to confirm a new genotype assignment; QC69 is therefore confirmed as genotype 7a.

**Provisional Subtypes.** The 2005 consensus protocol also proposed that provisional subtypes could be assigned on the basis of sequence comparisons in the core/E1 and NS5B regions for at least three independent isolates, requiring in addition a complete coding region sequence before being confirmed. Of the 58 subtypes provisionally assigned in the 2005 article, 38 have now been confirmed (Table 1). However, it is now much easier to obtain complete coding region sequences and very few additional provisional subtypes have been proposed. Instead, some authors have inconsistently labeled unusual isolates with the suffix “?,” “unassigned group I” or “subtype 1(I).” We propose that provisional subtype designations should no longer be provided for variants where complete genome sequences are lacking. The 20 remaining provisionally assigned subtypes will be maintained (Table 3), since they already exist in the literature. Future subtype assignments will only be made (as confirmed assignments) when sequence data from three or more isolates including at least one complete or nearly complete coding region is provided. Where a complete coding region sequence is available but there are fewer than three isolates, we propose that these remain unassigned. In Table 2 these are labeled using the form “Genotype_Accession number,” e.g., 1_AJ851228.

**Recombinant and Other Forms.** One issue that was not addressed in the 2005 consensus protocol was the naming of the newly discovered recombinant forms of HCV, their importance being unknown. Nine different recombinant forms of HCV have now been described (Table 4), of which only one (2k/1b) is represented by multiple isolates; no multiple recombinants have been reported (reviewed in reference25). In this context it does not seem necessary to revise the nomenclature generally used in the literature in which “RF” (recombinant form) is followed by the contributory subtypes separated by “/” in the order in which they appear in the complete genome sequence. We suggest that recombinant forms with the same genotypic structure but with different breakpoints or where the component genomic sections are unrelated are numbered consecutively with a numerical suffix (for example, RF2b/1b_1).

**Proposals for New Genotype/Subtype Assignments.** The ICTV Flaviviridae Study Group is willing to take a coordinating role in the assignment of newly described variants of HCV. We urge researchers who have characterized new HCV variants that potentially qualify as new types or subtypes to contact Donald Smith (D.B.Smith@ed.ac.uk) or any member of the Study group (listed on http://ictvonline.org/subcommittee.asp?committee=25&sec=5) in confidence before publication so that naming conflicts can be avoided and appropriate assignments made.

Future Developments

Despite the increasing number and diversity of HCV sequences, the system of classification of variants into genotypes and subtypes has proven surprisingly robust. The seven confirmed genotypes have strong bootstrap support (Fig. 1), and the partition of these genotypes into subtypes that differ over a complete coding region sequence by >15% reflects a natural hiatus in the distribution of sequence distances (Fig. 2). We welcome any comments or suggestions for the proposed classification guidelines. Areas of uncertainty remain with respect to the region of endemicity of genotype 5, represented by a single subtype
isolated in Europe, Brazil, North Africa, and South Africa, and genotype 7, isolated from an emigrant from the Congo. We might also anticipate the further discovery of other HCV-like viruses in the genus Hepacivirus, and variants closer genetically to HCV than the nonprimate hepacivirus that appears to be an endemic infection of horses worldwide. As more is learned about the host-specificity and diversity of hepaciviruses, the genotype classification of HCV may be logically incorporated within a unified classification of hepaciviruses at the species and potentially subspecies and subgenus levels.

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