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Minireview

Classification of papillomaviruses

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Abstract

One hundred eighteen papillomavirus (PV) types have been completely described, and a yet higher number of presumed new types have been detected by preliminary data such as subgenomic amplicons. The classification of this diverse group of viruses, which include important human pathogens, has been debated for three decades. This article describes the higher-order PV taxonomy following the general criteria established by the International Committee on the Taxonomy of Viruses (ICTV), reviews the literature of the lower order taxa, lists all known "PV types", and interprets their phylogenetic relationship. PVs are a taxonomic family of their own, *Papillomaviridae*, unrelated to the polyomaviruses. Higher-order phylogenetic assemblages of PV types, such as the "genital human PVs", are considered a genus, the latter group, for example, the genus "Alpha-Papillomavirus". Lower-order assemblages of PV types within each genus are treated as species because they are phylogenetically closely related, but while they have distinct genomic sequences, they have identical or very similar biological or pathological properties. The taxonomic status of PV types, subtypes, and variants remains unchanged and is based on the traditional criteria that the sequence of their L1 genes should be at least 10%, 2–10%, and maximally 2% dissimilar from one another.

Keywords: Papillomaviruses; HPV type; Pathogen

Introduction

Papillomaviruses are highly diverse, and likely occur in most mammals and birds. Hundreds of "PV types" have been detected in humans, the only intensively studied host. It took three decades of research of a large number of specialists and sequences from thousands of PV isolates to establish a database that allows us to propose a classification system that will likely be stable while more PV types will be found. Here we review this database and its interpretation by phylogenetic criteria that led to the taxonomic levels "family", "genus", "species", "types", "subtypes", and "variants".

A large and diverse group of viruses

Papillomavirus (PV) isolates are traditionally described as "types". PV types have been detected in all carefully examined mammals and birds, with the possible exception of laboratory mice. In the only extensively studied host, humans, nearly 100 human PV (HPV) types were described based on the isolation of complete genomes, with a yet larger number presumed to exist based on the detection of subgenomic amplicons. Many of these HPV types have been shown to be ubiquitous and globally distributed.

PVs cause benign tumors (warts, papillomas) in their natural host and occasionally in related species. Papillomas are induced in the skin and mucosal epithelia, often at specific sites of the body. Some papillomatous proliferations induced by specific types of PVs bear a high risk for malignant progression (reviewed in Clifford et al., 2003;

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Munoz et al., 2003; Matsukura and Sugase, 2001; zur Hausen, 2002). The infection frequently leads to microlesions, which are barely or not at all visible without optical aid. PVs seem to coexist with their host over long periods of time. Many PVs appear to occur preferentially in a latent life cycle, because a wide variety of different types can be detected at random sites of healthy skin of humans and animals (Antonsson and Hansson, 2002; Antonsson et al., 2000, 2003). Conditions of immune suppression in humans lead to activation of latent infections or increased susceptibility to reinoculation from active infections resulting in overt lesions (reviewed in de Villiers, 1998; Forslund et al., 2003a, 2003b; Jablonska and Majewski, 1994). Immunodeficiency may also predispose animals to develop papillomas, as PV infections in domestic cats have been reported in an animal already infected with feline immunodeficiency virus or suffering from other immune deficient conditions (reviewed in Sundberg et al., 2000). Animals held in captivity may be prone to immune suppression, as suggested by the case of Florida manatees developing multiple cutaneous papillomas (Bossart et al., 2002), and eight putative new PV types identified in six cat species either free living or from zoos (Schulman et al., 2003; Sundberg et al., 2000). Alternatively, close physical proximity may be a prerequisite for PV types to occasionally cross host–species barriers, as suggested by the detection of various bovine PVs in other hoofed domestic animals. Most of the animal papillomaviruses found in natural conditions have been reviewed (Bernard and Chan, 1997).

Genomic properties and taxonomy

PVs have circular double-stranded DNA genomes with sizes close to 8 kb. In spite of their small size, their molecular biology is very complex. In short, three oncogenes, E5, E6, and E7, modulate the transformation process, two regulatory proteins, E1 and E2, modulate transcription and replication, and two structural proteins, L1 and L2, compose the viral capsid (for a review, see Munger and Howley, 2002). The E1, E2, L1, and L2 ORFs are particularly well conserved among all members of the family. Most *cis*-responsive elements are in the long control region (LCR) between L1 and E6, a segment with little sequence conservation. The last 30 years have seen a development of PV taxonomy initially based on genomic cross-hybridizations and restriction patterns to a system based on phylogenetic algorithms comparing either whole PV genome sequences or subgenomic segments. This scientific progress has led to a refinement, but never to contradictions of previous taxonomies. There is also strong evidence that PV genomes are very static, and sequence changes by mutation or recombination are very rare events indeed. Mutational changes apparently occur at frequencies not very different from those of the DNA genomes of the infected host organism.

The history of the PV type identification

Although the first PV types were isolated as early as 30 years ago (Orth et al., 1977; Coggin and zur Hausen, 1979), the difficulty to find appropriate cell culture systems (terminally differentiating epithelia) to propagate these viruses has hampered progress in studying viral functions and limited the establishment of a taxonomy based on biological properties. Some powerful raft culture and xenograft models (Hummel et al., 1992; Kreider et al., 1985) have been developed over the last few years but did not have a significant impact on the functional and taxonomic comparison of PV types.

In the early phase of research on PV taxonomy, common warts and lesions from *Epidermodysplasia verruciformis* (EV) patients were used to isolate large quantities of viral particles for isolation of the DNA genomes. These DNA preparations were classified by comparing panels of restriction digests, cross-wise Southern blot hybridizations under nonstringent conditions, and liquid hybridizations. Among the few researchers active in this field in the late 1970s, it was agreed to use a numbering system to identify a type, i.e., HPV 1, an abbreviation of human papillomavirus type 1.

Toward a nucleotide sequence based classification, the first full genomes to become available were those of BPV 1 (Chen et al., 1982), HPV 1 (Danos et al., 1982), HPV 6 (Schwarz et al., 1983), and HPV 16 (Seedorf et al., 1985). Presently, the complete genomes of almost all isolated PV genomes have been fully sequenced. Among these, the majority were published by Delius and Hofmann (1994). Aside from the general GenBank and EMBL databases, most PV sequences are available with annotations in a website established by Myers et al. (1994) (http://hpv-web.lanl.gov/stdgen/virus/hpv/compendium/htdocs/) as described by Farmer et al., 1995.

The family Papillomaviridae

The papillomaviruses had been originally lumped together with the polyomaviruses in one family, the *Papovaviridae*. This was based on similar, nonenveloped capsids and the common circular double-stranded DNA genomes. As it was later recognized that the two virus groups have different genome sizes, completely different genome organizations, and no major nucleotide or amino acid sequence similarities, they are now officially recognized by the International Committee on the Taxonomy of Viruses (ICTV) as two separate families, *Papillomaviridae* and *Polyomaviridae*. As one considers the lack of any overall homology among the viral genomes in the two families, one should take note, however, of a helicase motif of the PV E1 protein, a domain stretching greater than about 230 amino acids, which has some sequence similarity with the SV40 T-antigen, the parvovirus NS1 protein, and a planarian virus-like element (Rebrikov et al., 2002). While there is no doubt that the respective helicase

domains of these viruses are homologous, there is no evidence that this establishes a monophyletic origin of these four different groups of viruses.

PV types: a traditional and future taxonomic term in PV classification

The L1 ORF is the most conserved gene within the genome and has therefore been used for the identification of new PV types over the past 15 years. A new PV isolate is recognized as such if the complete genome has been cloned and the DNA sequence of the L1 ORF differs by more than 10% from the closest known PV type. Differences between 2% and 10% homology define a subtype and less than 2% a variant. This definition was agreed upon between all PV scientists working on PV taxonomy and diagnosis at the International Papillomavirus Workshop held in Quebec in 1995.

A few hundred putative new PV types have been identified after the advent of PCR and application of degenerate or consensus primers. Amplification of conserved regions, mostly within the L1 ORF, has been used. These partial fragments are usually labeled by using the initials of an individual or laboratory, followed by a laboratory number (see, e.g., Chow and Leong, 1999). A number of these short fragments constitute partial sequences of later defined HPV types (Table 1). The designation, e.g., HPV (number), is only given after isolation and characterization of the complete genome. This full-length genome is deposited at the Reference Center for Papillomaviruses (Heidelberg) where the genome organization and sequence is verified as a new PV type.

Conventional cloning of complete genomes has been hampered lately either by the limited amounts of sample available or by sequences of the PV genome in question proving toxic for various vector systems (de Villiers, 2001). This has led to the increased use of PCR amplification of overlapping fragments in obtaining the full-length genome. It was decided at a Workshop on the Classification of Papillomaviruses held at the 18th International Papillomavirus Conference in Barcelona (July 2000) to distinguish between isolates cloned by conventional techniques and those generated using PCR amplification by using the term HPV cand(number) for the latter (see, e.g., Forslund et al., 2003a, 2003b; Menzo et al., 2001; Terai and Burk, 2001, 2002a).

The rapid increase in the number of PV isolates has clearly demonstrated a need for a taxonomic classification within the family *Papillomaviridae*. Such a classification should have at least three objectives: (i) It should establish the relationship between PV types; (ii) it should compare the term PV type against the taxonomic terms "species" and "genus", which are used for the systematics of all biological organisms and frequently applied in virology; and (iii) it should investigate the relationship between the taxonomic classification and biological and pathological properties of the virus.

Table 1
Partial PCR fragments of characterized HPV types

PCR product	HPV type
CP141	HPV 70
LVX160	
CP8061	HPV 71
CP4173	HPV 72
LVX100	
MM9	HPV 73
PAP238a	
CP8304	HPV 81
MM4	HPV 82
W13B	
IS39	Subtype of HPV 82
AE2	
MM7	HPV 83
LVX82	
PAP219	
MM8	HPV 84
PAP155	
HLT7474	candHPV85
CP6108	candHPV89
AE6	
X06/X08	
AE11	candHPV90
Han831	
Han1353	
JC9710	
AE13	candHPV91
JC9813	
FAIMVS2	candHPV92
FAIMVS6	candHPV93
DL40	HPV 94
FA47	candHPV96

This table lists HPV types or candidate HPV types that were originally isolated as partial fragments of HPV genomes, generated by PCR, and frequently referred to in the literature under these laboratory designations.

An understanding of the relationship between PV types based on nucleotide sequence comparison began to emerge more than 10 years ago (Chan et al., 1992a, 1992b, 1995; van Ranst et al., 1992a, 1992b). Continued research based on these principles has led to the taxonomic groupings, which are shown in this paper, and whose relationship is today widely accepted. It was much more problematic, however, to correlate these phylogenetic groups with traditional taxonomic terms and with viral properties.

Toward this, several attempts have been made over the years, but led only to limited breakthroughs. One reason for this is that systematic functional analyses of specific genes of the majority of all PVs have never been performed. Also, biological data available on individual PV types are sporadic, but nevertheless indicate an enormous spectrum of variation in biological activities (de Villiers, 1989, 1994, 1997, 2001). Specifically, phylogenetic assemblages sometimes coincide with biological and pathological properties, but often diverge. The closely related HPV types HPV-2 and 27, HPV-6 and 11, and HPV-16 and 31, which cause

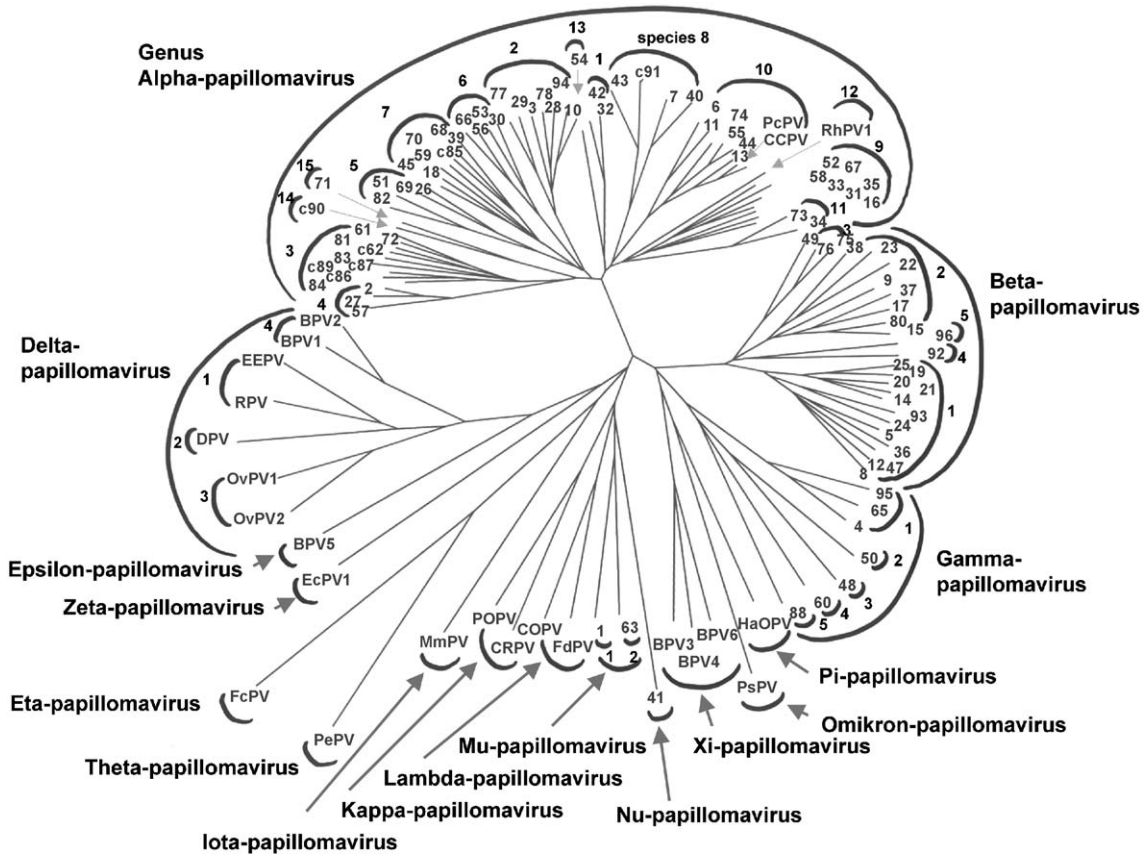

Fig. 1. Phylogenetic tree containing the sequences of 118 papillomavirus types. The L1 ORF sequences were used in a modified version of the Phylip version 3.572 and based on a weighted version of the neighbor-joining analysis. The tree was constructed using the Treeview program of the University of Glasgow. The numbers at the ends of each of the branches identify an HPV type; c-numbers refer to candidate HPV types. All other abbreviations refer to animal papillomavirus types. For the meaning of each abbreviation, please refer to Table 3. The outermost semicircular symbols identify papillomavirus genera, e.g. the genus alpha-papillomavirus. The number at the inner semicircular symbol refers to papillomavirus species. To give an example taken from the upper part of the figure, the HPV types 7, 40, 43, and cand91 together form the HPV species 8 in the genus alpha-papillomavirus.

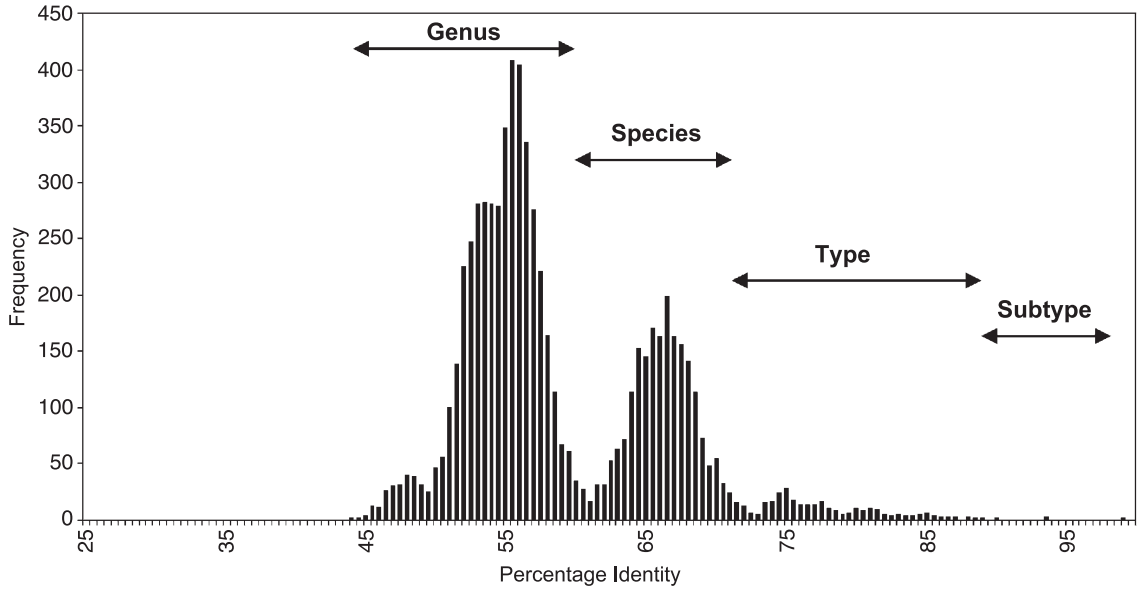

Fig. 2. Frequency distribution of pairwise identity percentages from nucleotide sequence comparison of the L1 ORFs of 118 papillomavirus types.

common warts, genital warts, and cervical cancer, respectively, are three excellent cases of the numerous consistencies between phylogeny and pathology. Examples of discrepancy include the example that the phylogenetic group of genital HPV types, which incorporates all HPV types found in genital lesions, also contains some HPV types mostly found in cutaneous lesions, such as HPV-2. Also, highly unrelated viruses, such as HPV-2 and HPV-4, can cause similar cutaneous papillomas.

Comparison of PV types

The evolution of PVs has often been debated (Chan et al., 1992a, 1992b, 1995, 1997a, 1997b, 1997c; Halpern, 2000; Ho et al., 1993; Myers et al., 1996; Ong et al., 1993; Salmon et al., 1997). Comparative studies using the E6, L1, or the combined E6–E7–L1 ORFs (Chan et al., 1995; Myers et al., 1994; van Ranst et al., 1992a, 1992b), however, have resulted in phylogenetic trees establishing

Table 2
Biological properties and genome organization characteristics for each genus

Genus	Biological properties	Genome organization
Alpha-papillomavirus	Mucosal and cutaneous lesions in humans and primates High- and low-risk classification based on molecular biological data—high-risk types (pre- and malignant lesions) immortalize human keratinocytes; low-risk types (benign lesions) do not. Recent compilations of epidemiological data demonstrate more frequent association of specific species as high-risk types.	Conserved with an E5 ORF within the ELR[a] (ca. 300–500 bp) ORFs in ELR from different species may be divided into three groups: –classical E5 ORF –closer related to the ungulate E5 ORF –putative ORF with distinct conserved motives[b]
Beta-papillomavirus	Cutaneous lesions in humans Infections exist in latent form in general population, activated under conditions of immune suppression Also referred to EV-HPV types due to close association with disease *Epidermodysplasia verruciformis* (EV)	ELR is generally less than 100nt in length E5 ORF absent
Gamma-papillomavirus	Cutaneous lesions in humans—histologically distinguishable by intracytoplasmic inclusion bodies specific for type species	ELR is generally less than 100 nt in length. E5 ORF absent
Delta-papillomavirus	Lesions in ungulates Induces fibropapillomas in the respective host Trans-species transmission occurs inducing sarcoids	ORFs located in ELR have transforming properties
Epsilon-papillomavirus	Bovine papillomavirus cutaneous papillomas in cattle	
Zeta-papillomavirus	Cutaneous lesions in horses	Undefined ORF overlapping with L2 ORF
Eta-papillomavirus	Avian papillomaviruses Cutaneous lesions in host	E4 and E5 ORFs absent No typical E6 ORF, but an ancestral E7 ORF with partial E6 characteristics
Theta-papillomavirus	Avian papillomaviruses Cutaneous lesions in host	E4 and E5 ORFs absent No typical E6 ORF, but an ancestral E7 ORF with partial E6 characteristics
Iota-papillomavirus	Rodent papillomaviruses Cutaneous lesions	E5 ORF absent E2 ORF considerably larger then in other genera
Kappa-papillomavirus	Isolated from rabbits Cutaneous and mucosal lesions	E6 ORF larger than in other papillomaviruses. Harbors an uncharacterized E8 ORF within the E6 ORF region
Lambda-papillomavirus	Animal papillomaviruses Benign mucosal and cutaneous lesions	ELR region exceptionally large (1500 bp and 1271 bp in 2 known species)
Mu-papillomavirus	Human papillomaviruses Cutaneous lesions—histologically distinguishable by intracytoplasmic inclusion bodies specific for type species	URR relatively large (982 bp and 558 bp for two known species)
Nu-papillomavirus	Human papillomavirus Benign and malignant cutaneous lesions	Several larger uncharacterized ORFs scattered throughout genome. E2 binding sites in URR all modified
Xi-papillomavirus	Bovine papillomaviruses Induce true papillomas in host. Cutaneous or mucosal lesions	Characteristic E6 ORF absent E8 ORF (located in E6 ORF region) with properties similar to E5 ORF of BPV 1
Omikron-papillomavirus	Isolated from genital warts in cetaceans	E7 ORF absent Several larger unidentified ORFs located in L1 ORF region
Pi-papillomavirus	Isolated from hamsters Mucosal lesions	ELR absent with E2 and L2 ORFs partially overlapping

[a] ELR = region between the early and late genes of the papillomavirus genome.
[b] Bravo and Alonso (submitted for publication).

Table 3
Characteristics of species within specific genera

Genus	Species	Type species	Other papillomavirus types	Comments
Alpha-papillomavirus	1	HPV 32 (X74475)	HPV 42 (M73236)	More frequently in benign lesions (low-risk). Oral or genital mucosa. Third ORF in ELR
	2	HPV 10 (X74465)	HPV 3 (X74462) HPV 28 (U31783) HPV 29 (U31784) HPV 78 HPV 94[a] (AJ620211)	More frequently cause cutaneous than mucosal lesions. Low-risk. E5 biologically different
	3	HPV 61 (U31793)	HPV 72 (X94164) HPV 81 (AJ620209) HPV 83 (AF151983) HPV 84 (AF293960) candHPV 62 candHPV 86 (AF349909) candHPV 87 (AJ400628) candHPV 89 (AF436128)	Mucosal lesions. Lower risk
	4	HPV 2 (X55964)	HPV 27 (X73373) HPV 57 (X55965)	Common skin warts. Frequently in benign genital lesions in children. Several larger uncharacterized ORFs scattered throughout genome. E5 ORF biologically different
	5	HPV 26 (X74472)	HPV 51 (M62877) HPV 69 (AB027020) HPV 82 (AB027021)	High-risk mucosal lesions, also in benign lesions
	6	HPV 53 (X74482)	HPV 30 (X74474) HPV 56 (X74483) HPV 66 (U31794)	High-risk mucosal, but also in benign lesions
	7	HPV 18 (X05015)	HPV 39 (M62849) HPV 45 (X74479) HPV 59 (X77858) HPV 68 (X67161) HPV 70 (U21941) candHPV85(AF131950)	High-risk mucosal lesion
	8	HPV 7 (X74463)	HPV 40 (X74478) HPV 43 (AJ620205) candHPV 91 (AF131950)	Low-risk mucosal and cutaneous lesions. HPV 7 also known as butcher's wart virus—often in mucosal and skin lesions in HIV-infected patients
	9	HPV 16 (K02718)	HPV 31 (J04353) HPV 33 (M12732) HPV 35 (X74476) HPV 52 (X74481) HPV 58 (D90400) HPV 67 (D21208)	High-risk—malignant mucosal lesions
	10	HPV 6 (X00203)	HPV 11 (M14119) HPV 13 (X62843) HPV 44 (U31788) HPV 74 (U40822) PcPV (X62844)	Mostly associated with benign mucosal lesions. Low risk. Reports of HPV 6 in verrucous carcinoma
	11	HPV 34 (X74476)	HPV 73 (X94165)	Mucosal lesions—high-risk
	12	RhPV 1 (M60184)	–	Mucosal genital lesions in Rhesus monkeys
	13	HPV 54 (U37488)	–	Low-risk mucosal
	14	candHPV 90 (AY057438)	–	Low-risk mucosal
	15	HPV 71 (AB040456)	–	Low-risk mucosal
Beta-papillomavirus	1	HPV 5 (M17463)	HPV 8 (M12737) HPV 12 (X74466) HPV 14 (X74467) HPV 19 (X74470) HPV 20 (U31778) HPV 21 (U31779) HPV 25 (U74471)	Most frequently causing cutaneous lesions, but reports of DNA in mucosa. Commonly associated with lesions in EV or immune-suppressed patients. Mostly benign lesions, but reported in malignant lesions, also in immune-competent patients

Table 3 (*continued*)

Genus	Species	Type species	Other papillomavirus types	Comments
Beta-papillomavirus	1	HPV 5 (M17463)	HPV 36 (U31785) HPV 47 (M32305) HPV 93[b] (AY382778)	
	2	HPV 9 (X74464)	HPV 15 (X74468) HPV 17 (X74469) HPV 22 (U31780) HPV 23 (U31781) HPV 37 (U31786) HPV 38 (U31787) HPV 80 (Y15176)	Most frequently causing cutaneous lesions, but reports of DNA in mucosa. Commonly associated with lesions in EV or immune suppressed patients. Mostly benign lesions, but reported in malignant lesions, also in immune-competent patients
	3	HPV 49 (X74480)	HPV 75 (Y15173) HPV 76 (Y15174)	Benign cutaneous lesions
	4	HPVcand92(AF531420)	–	Pre-and malignant cutaneous lesions
	5	HPVcand96[b] (AY382779)		Pre-and malignant cutaneous lesions
Gamma-papillomavirus	1	HPV 4 (X70827)	HPV 65 (X70829) HPV 95[c] (AJ620210)	Cutaneous lesions. Histologically distinct homogenous intracytoplasmic inclusion bodies
	2	HPV 48 (U31790)	–	Cutaneous lesions
	3	HPV 50 (U31790)	–	Cutaneous lesions
	4	HPV 60 (U31792)	–	Cutaneous lesions
	5	HPV 88[d]	–	Cutaneous lesions
Delta-papillomavirus	1	European elk papillomavirus (EEPV) (M15953)	Reindeer papillomavirus (RPV) (AF443292)	E9 gene within ELR with transforming properties
	2	Deer papillomavirus (DPV) (M11910)	–	E9 gene within ELR with transforming properties
	3	Ovine papillomavirus 1 (OvPV1) (U83594)	OvPV2 (U83585)	
	4	Bovine papillomavirus 1 (BPV 1) (X02346)	BPV 2 (M20219)	E5 gene in ELR with transforming properties. Trans-species infection—causing sarcoids in horses
Epsilon-papillomavirus	1	Bovine papillomavirus type 5 (BPV 5) (AF457465)	–	(see text)
Zeta-papillomavirus	1	*Equus caballus papillomavirus* (EcPV, horse papillomavirus) (AF498323)	–	(see text)
Eta-papillomavirus	1	*Fringilla coelebs papillomavirus* (FcPV, chaffinch papillomavirus) (AY957109)	–	(see text)
Theta-papillomavirus	1	*Psittacus erithacus timneh papillomavirus* (PePV, parrot papillomavirus) (AF420235)	–	(see text)
Iota-papillomavirus	1	*Mastomys natalensis papillomavirus* (MnPV) (U01834)	–	(see text)[e]
Kappa-papillomavirus	1	Cottontail rabbit papillomavirus (CRPV) (K02708)		High divergence within the E6 and E7 ORFs described for different isolates Associated with cutaneous lesions
	2	Rabbit oral papillomavirus (ROPV) (AF227240)		Associated with oral lesions
Lambda-papillomavirus	1	Canine oral papillomavirus (COPV) (L22695)	–	ELR is 1500 bp in length
	2	*Felis domesticus* (cat) papillomavirus (FdPV) (AF377865)	–	ELR is 1271 bp in length[f]
Mu-papillomavirus	1	Human papillomavirus type 1 (HPV 1) (V01116)	–	Histologically distinct heterogenous intracytoplasmic inclusion bodies URR is 982 bp in length

(continued on next page)

Table 3 (*continued*)

Genus	Species	Type species	Other papillomavirus types	Comments
Mu-papillomavirus	2	HPV 63 (X70828)	–	Histologically distinct filamentous intracytoplasmic inclusion bodies URR is 558 bp in length
Nu-papillomavirus	1	Human papillomavirus 41 (HPV 41) (X56147)	–	Several larger uncharacterized ORFs scattered throughout the genome. ELR only 17 nt. All E2 binding sites in URR modified[g]
Xi-papillomavirus	1	Bovine papillomavirus type 3 (BPV 3) (AF486184)	BPV 4 (X05817) BPV 6 (AJ620208)	E8 gene within E6 region of BPV4 has transforming properties—similar to E5 of BPV1[h]
Omikron-papillomavirus	1	*Phocoena spinipinnis* papillomavirus (PsPV) (AJ238373)		E7 ORF absent. Several larger ORFs in L1 ORF region
Pi-papillomavirus	1	Hamster oral papillomavirus (HaOPV) (E15110)	–	No ELR—partial overlap between E2 and L2 ORFs

The table shows division of the papillomaviridae into genera and species, following the phylogenetic tree shown in Fig. 1. For each species, the table lists a type species, other papillomavirus types belonging to these species, and biological and pathological properties of each species.

[a] Rahn and de Villiers, personal communication.
[b] Forslund, Ly, Higgins, Hunziker and de Villiers, personal communication.
[c] Egawa, Cop and de Villiers, personal communication.
[d] Kitasato and Egawa, personal communication.
[e] Tan et al., 1994.
[f] Terai and Burk, 2002b.
[g] Hirt et al., 1991.
[h] Jackson et al., 1991.

similar or often even identical relationships. As a matter of fact, a frequently used 291-bp amplicon, a small segment of the L1 gene, suffices as a foundation to generate highly informative phylogenetic comparisons (Bernard et al., 1994b). Sequence comparisons between the complete genomes of 118 PVs reveal a high diversity between viruses, but a distribution similar to that found when comparing the L1 ORF sequences. A cladogram based on the complete L1 ORF of 96 HPV types and 22 animal papillomavirus types is presented in Fig. 1. The frequency distribution of pairwise identity percentages from sequence comparisons of the L1 ORF demonstrates three taxonomic levels, both when complete genomes are compared (data not shown), and based on the comparison of L1 genes, namely genera, species, and types (Fig. 2). The observations of our working group were integrated with the classification standards established by ICTV and led to an official interpretation of phylogenetic clusters of PV types as "genera" and "species", respectively.

Criteria for genera and species

Extensive sequence comparisons using the L1 ORF of 96 human papillomavirus types and 22 animal papillomaviruses led to the establishment of the following classifications:

1. Higher-order clusters of HPV types (e.g., the "genital PVs") had previously been called "supergroups" or "major branches" (Myers et al., 1994; Chan et al., 1995). For these taxa, we now introduce the term "genus". Different genera share less than 60% nucleotide sequence identity in the L1 ORF. Full-length sequences of complete genomes have more than 23%, but less than 43% nucleotide sequence identity when comparing genera of the *Papillomaviridae*.
2. Lower-order clusters of HPV types (e.g., HPV-6, 11, 44, 55) had been called "groups", "subgroups", or "minor branches". For these taxa, we now introduce the term "species". Such species within a genus share between 60% and 70% nucleotide identity.
3. The traditional PV types within a species share between 71% and 89% nucleotide identity within the complete L1 ORF.

The introduction of the term "genus" is useful, as this concise term will now replace the somewhat vague expressions, "major branches" or "supergroups". Throughout biology, including virology, specific genera typically unite species, which are clearly phylogenetically related, but often biologically quite diverse. The same applies to PV genera. A summary of the biological properties known for each genus is presented in Table 2, together with specific characteristics of its genome organization in cases where this differs from the typical pattern.

The introduction of the term "species" is biologically useful, as these are natural taxa based on close phylogenetic relationship of certain types and because such species typically lump PV types, which have common biological

and pathological properties, a requirement of ICTV guidelines. To give examples, all HPV types that form a species together with HPV-2 are typically found in common skin warts, and all HPV types that form a species together with HPV-16 are "high-risk" HPV types found in cervical cancer and its precursor lesions. More detailed information about each species and PV types within a genus is presented in Table 3. The type species have been chosen either because they are the most comprehensively investigated type, or because they represent best the species, or because there is only one type in that taxon. Table 3 is an important reference that lumps with the type species in many type-rich taxons all those HPV types that belong to the same species, and will presumably have properties similar or identical to the type species, but cannot be studied (for purposes of basic research, drug development, and vaccination) as intensely as the type species. To give an example, species No. 9 lumps with the type species HPV-16, the HPV types HPV-31, 33, 35, 52, 58, and 67, which are little studied (with the exception of HPV-31), but are likely to have similar biological and medical properties as HPV-16.

Several hundred PV types have been partially identified in the form of short DNA fragments. Unfortunately, interest in isolating full-length genomes has been declining. The number of HPV types isolated and fully characterized has reached 96 and will soon exceed 100. Several reports have recently appeared on several animal PVs, often describing the same isolates in different publications, a process that clearly needs closer regulation. A regulated taxonomic description of nonhuman PVs is particularly necessary because it is extremely likely that only a tiny fraction of all animal PV types have been identified or isolated. The present methodology used for the detection of PV types is very limiting, as it is based on the information available from known types. Hopefully, future efforts will be directed toward identifying additional PV types very distantly related to the known genera. An example of the large diversity of animal PVs are the only two recently described PV types from birds, both of which lack traditional E6 and E7 ORFs (Tachezy et al., 2002; Terai et al., 2002), and are less related to any mammalian PV type than any two of these are among one another. Several of the PV types that presently appear as single species within a genus have in the past been identified only because of the availability of lesions harboring many viral particles or from which substantial amounts of circular double-stranded DNA could be purified.

Subtypes of PV types

As written above, subtypes of PV types are defined as being genomically 2–10% different from any PV type. This term originally had a different meaning, and was used when different isolates of the same type differed partially in their restriction enzyme cleavage patterns, such as HPV 2a, HPV 2b, and HPV 2c (Favre et al., 1975; Gissmann et al, 1977; Heilmann et al., 1980; Orth et al., 1978). It later became clear that these subtypes would rather fall under the category "variants" (see below) (Chan et al., 1997c; Heinzel et al., 1995).

Other misclassifications happened to PVs, which were originally based on hybridization data, classified as PV types, but fall now under the subtype classification. The HPV 55 genome shares 95% homology to that of HPV 44 and therefore constitutes a subtype of HPV 44. The same applies to HPV 64 being a subtype of HPV 34 and HPV 46 a subtype of HPV 20. The numbers of HPV 46, HPV 55, and HPV 64 will remain vacant to avoid any future confusion with published data. Also, comparing published data of the L1 ORF between the pygmy chimpanzee PV (van Ranst et al., 1991) and the common chimpanzee PV (Scinicariello et al., 1997) showed only 93% similarity. The latter is therefore a subtype of the pygmy chimpanzee PV. As the search for new PVs identified so few genomes that diverged 2–10% from defined types one can conclude PV types are clearly natural taxa. It is unclear why genomes intermediate to closely related PV types are so rare.

Variants of PV types

Most HPV types have been isolated repeatedly in a large number of clinical studies, and the sequences of these isolates have been compared. As one may expect, most of these isolates differ from one another. It should be stressed, however, that there is no rapid diversification as in certain RNA viruses, as most HPV types could be reisolated in the form of only 10–100 different genomic variants that normally showed about 1–2% sequence diversity. The phylogenetic implications of this, namely the slow linked evolution of host and virus, have been extensively discussed (Bernard, 1994; Bernard et al., 1994a,b; Chan et al., 1997c; Heinzel et al., 1995; Ho et al., 1993; Ong et al., 1993), while the clinical implications, i.e., pathological diversity within individual HPV types, are still under investigation.

Acknowledgments

The space available for this article did not permit to cite all publications relevant for the taxonomy of papillomaviruses. Several review articles referred to in the text cover the majority of published data. More recent references are included in the reference list. We apologize to colleagues who may not have been cited.

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