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Prevaccination genomic diversity of human papillomavirus genotype 6 (HPV 6)

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ABSTRACT

Prevaccination genomic diversity of human papillomavirus genotype 6 (HPV 6) was established by sequencing 3798 bp of 77 clinically important HPV 6 isolates obtained from 45 and 32 patients with genital warts and laryngeal papillomas, respectively. By analyzing pooled L1, LCR, E6, E2, and E5 nucleotide data of an individual isolate, a total of 36 different genomic variants were identified, of which six (12 isolates), one (one isolate) and 29 (64 isolates) corresponded to HPV 6b, HPV 6a, and HPV 6vc genetic lineages, respectively. Several novel, potentially important mutations were identified. Non-prototypic HPV 6vc genomic variants were found in the majority of genital warts and laryngeal papillomas included in the study. The presence of serious HPV 6 genome sequence errors was confirmed and novel sequence errors were identified in sequence repositories.

Introduction

Human papillomavirus genotype 6 (HPV 6) is one of the most important HPV genotypes. Next to HPV 11, it is the major causative agent of genital warts and laryngeal papillomas, the most frequent benign tumours in the anogenital region and lower respiratory tract, respectively (Gissmann et al., 1983; Gale et al., 1994; Brown et al., 1999; Gale, 2005; Gale and Zidar, 2006; Potočnik et al., 2007). According to the latest papillomavirus classification criteria, HPV 6 is placed in the alpha genus – species 10, together with HPV 11, HPV 13, HPV 44, and HPV 74 (de Villiers et al., 2004). Based on its clinical association mainly with benign lesions, HPV 6 is regarded as a "low-risk" HPV genotype (reviewed in Grassmann et al. (1996)). HPV 6 is one of the four primary targets of the recently introduced quadrivalent HPV vaccine (Barr and Tamms, 2007; Garland et al., 2007).

HPV 6 was discovered by Southern blot hybridization in a tissue specimen of condyloma acuminata (Gissmann and zur Hausen, 1980). The complete genome, later designated HPV 6b, was cloned in 1981 (de Villiers et al., 1981) and fully sequenced and completely characterized two years later (Schwarz et al., 1983). Molecular studies performed in the years following the discovery of this virus demonstrated that HPV 6 is polymorphic and consists of several genomic variants. The focus of these studies has been on certain portions of the HPV 6 genome, particularly the long-control region (LCR), which contains regulatory elements for viral transcription and replication, and the coding regions for L1, L2, E6, E7, and E2 proteins (Rando et al., 1986; Kasher and Roman, 1988; Farr et al., 1991; Icenogle et al., 1991; Yaegashi et al., 1993; Kitasato et al., 1994; Heinzel et al., 1995; Roman and Brown, 1995; Grassmann et al., 1996; Suzuki et al., 1997; Caparros-Wanderley et al., 1999; Kovelman et al., 1999). In addition to HPV 6b, complete genomes of two closely related HPV 6 isolates, designated HPV 6a and HPV 6vc, have been cloned and fully characterized (Hofmann et al., 1995; Kovelman et al., 1999). On the basis of nucleotide sequence comparisons, HPV 6 isolates are usually grouped into prototype HPV 6b-related (prototypic) and HPV 6a/6vc-related (non-prototypic) genomic variants (Heinzel et al., 1995; Grassmann et al., 1996; Caparros-Wanderley et al., 1999; Kovelman et al., 1999). Non-prototypic HPV 6 genomic variants seem to predominate in genital warts (Gissmann et al., 1983; Rubben et al., 1992; Brown et al., 1993; Krige et al., 1997; Suzuki et al., 1997; Caparros-Wanderley et al., 1999).

Knowledge about the natural genetic diversity of HPV 6 is fairly limited, since the majority of HPV 6 genomic diversity studies performed so far have only investigated a limited number of isolates, which were almost exclusively from genital warts, and studies have focused on a single HPV 6 genomic region or even its short part only. Thus, in the present study we further investigated genomic diversity

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of HPV 6 in the prevaccination era, in order to provide important data for future epidemiological, functional and molecular assay development and vaccination studies. To achieve this, approximately half of the genome (3798 bp) of 77 clinically important HPV 6 isolates (e.g., those causing disease) was sequenced. The nucleotide sequence alignments were used to identify HPV 6 genomic variants and to investigate the linkage of these variants across the L1, LCR, E6, E2, E5a and E5b genomic regions. In addition, the coding regions were examined in detail to determine the prevalence, extent and distribution of amino acid changes that might have important biological properties. The LCR genomic region was used to determine the relationship between genomic variants described in our study and those described previously (Heinzel et al., 1995). This study, carried out on the largest number of HPV 6 isolates to date, is believed to be the first extensive work on the genetic diversity of the HPV 6 genotype.

Results

All 77 HPV 6 isolates included in the study were successfully amplified and sequenced across the L1 (nt 5700–7495), LCR-E6 (nt 7233–650) and E2–E5 (nt 3142–4538) genomic regions. Genomic sequences were established for 541 bp of E2 ORF (nt 3289–3829), for the entire LCR genomic region and for the entire L1, E6, E5a, and E5b ORFs. HPV 6 L1, E6, E2 and E5 genomic variants were identified using the prototype HPV 6b genome (GenBank accession no. X00203) as a standard for comparison and nucleotide position numbering. For the determination of HPV 6 LCR genomic variants, an LCR sequence amended by inclusion of a 94 bp segment at position 7350 in the prototype HPV 6b genome (Heinzel et al., 1995) was used.

HPV 6 L1 genomic variants

A total of fifteen L1 genomic variants were identified among 77 HPV 6 isolates (Fig. 1). Prototypic and non-prototypic HPV 6 genomic variants were determined in 12/77 (15.6%) and in 65/77 (84.4%) isolates, respectively. The prototype L1 sequence was identified in only 6/77 (7.8%) isolates. Sequence analysis of the entire *L1* gene revealed 25 nucleotide exchanges between the variants and the prototype HPV 6 sequence. The maximum distance between the variants and the prototype sequence was 11 nucleotides (0.7% of the entire L1 ORF). Nucleotide substitutions altered the L1 amino acid sequence of one prototypic (6-L1-3, represented by two isolates) and two non-prototypic (6-L1-13 and 6-L1-14, represented by ten and one isolate, respectively) HPV 6 variants; up to two amino acids (0.4% of the L1 protein) were exchanged (Fig. 1).

HPV 6 LCR genomic variants

A total of fifteen LCR genomic variants were identified among 77 HPV 6 isolates (Fig. 1). Prototypic and non-prototypic HPV 6 genomic variants were determined in 12/77 (15.6%) and in 65/77 (84.4%) isolates, respectively. None of the isolates corresponded to the prototype LCR sequence. Mutations were observed in 39 genomic positions: 30 single nucleotide exchanges, one 6 bp and one 1 bp deletion, and two 20 bp, one 14 bp and one 1 bp insertions. The maximum genomic distance between the variants and the prototype sequence was 20 mutations, thereby affecting 2.5% (20/806) of the entire LCR genomic region.

HPV 6 E6 genomic variants

A total of ten E6 genomic variants were identified among 77 HPV 6 isolates (Fig. 1). Prototypic and non-prototypic HPV 6 genomic variants were determined in 12/77 (15.6%) and in 65/77 (84.4%) isolates, respectively. None of the isolates corresponded to the reference E6 sequence. Molecular analysis of the entire E6 ORF revealed 13 point mutations observed in 12 genomic positions. The maximum distance between the variants and the prototype sequence was 7 nucleotides (1.5% of the entire *E6* gene). Nucleotide substitutions altered the E6 amino acid sequence of all non-prototypic HPV 6 variants (65 isolates); up to two amino acids (1.3% of the E6 protein) were exchanged (Fig. 1).

HPV 6 E2 genomic variants

A total of nine E2 genomic variants were identified among 77 HPV-6 isolates (Fig. 2). Prototypic and non-prototypic HPV 6 genomic variants were determined in 12/77 (15.6%) and in 65/77 (84.4%) isolates, respectively. The reference E2 sequence was identified in 9/77 (11.8%) isolates. Sequence analysis of the 541 bp segment of E2 ORF revealed 23 nucleotide exchanges between the variants and the prototype E2 sequence. The maximum variant divergence from the prototype sequence was 16 nucleotides, thereby affecting 2.9% of the evaluated E2 nucleotide sequence. Point mutations altered the E2 amino acid sequence of one prototypic (6-E2-2, represented by one isolate) and all non-prototypic (65 isolates) HPV 6 variants. As shown in Fig. 2, up to 10 amino acids (5.6% of the analyzed part of the E2 protein) were exchanged.

HPV 6 E5a and E5b genomic variants

A total of sixteen E5a genomic variants were identified among 77 HPV 6 isolates (Fig. 2). Prototypic and non-prototypic HPV 6 genomic variants were determined in 12/77 (15.6%) and in 65/77 (84.4%) isolates, respectively. The reference E5a sequence was identified in 12/77 (15.6%) isolates. There were 22 point mutations observed in 19 genomic positions. The maximum genomic distance between the variants and the prototype sequence was 14 nucleotides, amounting to a diversity of 5.1% of the entire E5a ORF. Nucleotide substitutions altered the E5a amino acid sequence of all non-prototypic HPV 6 variants (65 isolates); up to seven amino acids (7.7% of the E5a protein) were exchanged (Fig. 2).

A total of nine E5b genomic variants were identified among 77 HPV 6 isolates (Fig. 2). Prototypic and non-prototypic HPV 6 genomic variants were determined in 12/77 (15.6%) and in 65/77 (84.4%) isolates, respectively. The prototype E5b sequence was identified in 10/77 (13.0%) isolates. Sixteen point mutations were observed in 15 positions. The maximum distance between the variants and the prototype sequence was 11 nucleotides, thereby affecting 5.0% of the entire E5b ORF. Point mutations altered the E5b amino acid sequence of all non-prototypic HPV 6 variants (65 isolates) — up to seven amino acids (9.7% of the E5b protein) were exchanged (Fig. 2).

HPV 6 genomic variants

The nucleotide data of L1, LCR, E6, E2, and E5 genomic regions were combined in individual isolates and HPV 6 genomic variants were determined. Thus, among 77 HPV 6 analyzed isolates, 36 different genomic variants were identified (Fig. 3). As shown in Fig. 3, strong intergenomic co-variation between LCR, late (*L1*), and early (*E6*, *E2*, *E5a*, and *E5b*) viral genes was observed in all analyzed isolates. Twelve (15.6%) HPV 6 isolates represented genomic variants of the prototype HPV 6 isolate (six genomic variants) while 65 (84.4%) isolates represented closely related genomic variants of HPV 6a (one genomic variant, represented by one isolate) and HPV 6vc (29 genomic variants) (Fig. 3).

Distribution of HPV 6 genomic variants in genital warts vs. laryngeal papillomas

A total of 22 different HPV 6 genomic variants were identified among 45 HPV 6 isolates from genital warts. Prototypic HPV 6 genomic