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L1 sequence of a new human papillomavirus type-58 variant associated with cervical intraepithelial neoplasia

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Abstract

The present study on molecular characterization of a human papillomavirus (HPV) isolated in Central Brazil describes the L1 gene sequence from a new variant of HPV-58, the isolate Bsb-02. The sample was from a smear obtained from a woman with cervical intraepithelial neoplasia grade II. The whole L1 gene from isolate Bsb-02 was sequenced automatically, showing 99.1% nucleotide identity with the gene from the HPV-58 reference. The clustering between Bsb-02 and HPV-58 reference sequence was also supported by phylogenetic analysis. Fourteen nucleotide substitutions were observed: eight were synonymous and six were associated with amino acid substitutions. A10V and V144I have not been previously described. At GenBank, the only complete L1 sequence from HPV-58 in addition to the HPV-58 reference one is that of Bsb-02. These data provide information that may be relevant to HPV diagnosis and to rational vaccine strategies. HPV variants may also be associated with host immune responses and with the risk of cervical neoplasia.

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The association of human papillomavirus (HPV) with benign and malignant neoplasias has led to intense research efforts to improve the understanding of the diversity of this virus group, so that diagnosis, treatment, and control of HPV infections may be optimized (1). HPV-related studies are mainly concentrated on types 16 and 18, which are the most prevalent worldwide. However, recent evidence has shown that the prevalence of some other types, such as HPV-58, has significant geographic, demographic, and clinical-pathological variations (2-7). Therefore, it is important to characterize other common HPV types, some of which are known to be associated with cervical lesions (2-9). Variations in sequence within HPVs may be related to host immune response, persistence, or risk of cervical intraepithelial neoplasia and invasive cancer and may also be relevant to the generation of rational vaccine strategies (10).

In the present paper, we describe the complete L1 gene sequence of one HPV-58 isolate, Bsb-02, which may be a novel variant. It was isolated in the Federal District of Brazil. Bsb-02 was isolated from a cervical scraping of a woman showing cytology com-

patible with cervical intraepithelial neoplasia grade II. The detection and partial characterization of the Bsb-02 L1 gene was previously performed by PCR using MY09/MY11 L1 consensus primers followed by automated sequencing (11,12).

In the present study, the complete L1 gene of isolate Bsb-02 was amplified using the DN6/DN7 primer set (DN6-5' GGGGA ATTCATGGTGCTGATTTTATGTTGCACC 3'; DN7-5' CCCAAGGTTTAGTGTAAG TACCACAAC 3') and subsequently cloned into a pGEM T easy vector (Promega). Internal primer sets were used for additional PCR amplifications: DN6/MY09 (positions 5565 to 7036), DN6/GP6+ (5565 to 6768), and GP5+/DN7 (6744 to 7159) (11). PCR was carried out using 2 U *Taq* polymerase (Gibco BRL, Rockville, MD, USA) and an MJ Research PTC-100 thermocycler (Watertown, MA, USA) as previously described (6). The amplified HPV segments were sequenced automatically by the *Taq* Dye-terminator method using a Megabace System (Amersham-Pharmacia, Carlsbad, CA, USA). Sense and antisense primers were used to sequence each sample and the sequences were aligned using the CLUSTAL W multiple-sequence alignment program (13). The aligned sequences were translated into amino acids and substitutions were defined as divergences from the HPV-58 reference sequence. The similarity of the generated nucleotide and amino acid sequences was determined using the Basic Local Alignment Search Tool (14). At least two independent PCR products and sequences from both orientations were generated for each pair of primers, including DN6/DN7. Sequence changes that were found at least twice were considered to be variants. Nucleotide and amino acid sequences of the complete L1 gene from Bsb-02 were deposited in GenBank under accession number AY101598. Phylogenetic analysis was performed by the neighbor-joining distance method with Kimura's two-parameter correction using the PHYLIP package (15). Reliability was verified by the bootstrap approach (1,000 replicates).

Nucleic acid similarity between the Bsb-02 L1 sequence and the HPV-58 reference sequence was 99.1% and the amino acid similarity was 98.8%. We identified 14 nucleotide changes in the L1 region. As shown in Figure 1, amino acid substitutions were predicted to result from six of these

	5	5	5	5	6	6	6	6	6	6	6	7	7	7	
	5	8	9	9	4	4	6	7	8	8	8	0	0	0	
	9	1	9	9	0	5	8	9	2	2	2	1	1	7	
	3	9	0	4	4	8	8	8	2	7	8	0	6	9	
HPV-58	C	A	T	G	G	G	C	A	G	C	A	A	A	A	Ref.
IS404	ND	ND	ND	ND	ND	ND	-	G	A	A	G	-	-	ND	Mali
IS417	ND	ND	ND	ND	ND	ND	-	G	A	A	G	-	G	ND	Mali
IS573	ND	ND	ND	ND	ND	ND	-	-	-	A	-	-	-	ND	Paraguay
IS1021	ND	ND	ND	ND	ND	ND	-	G	-	A	-	-	-	ND	Philippines
IS131	ND	ND	ND	ND	ND	ND	-	-	A	A	G	-	-	ND	Bolivia
Bsb-02	**T**	**G**	**A**	**A**	**A**	**A**	**A**	**G**	**A**	**A**	**G**	**G**	**G**	**C**	**Brazil**
	A	-	-	V	-	-	T	I	D	-	N	-	-	-	
	↓			↓			↓	↓	↓		↓				
	V			**I**			**N**	**V**	**N**		**D**				
	10			144			375	412	420		422				

Figure 1. Nucleotide and amino acid sequence alignment showing the variations in isolate Bsb-02. Nucleotide positions of polymorphic sites are given across the top of the figure. Numbering refers to the first nucleotide of the HPV-58 reference genome (accession number NC 001443), which is indicated as Ref. Each row indicates, from left to right, the specimen identification, the nucleotide sequence alignment in comparison to the reference and the origin of each specimen. The amino acid changes and their positions are indicated below the line, in comparison to the original amino acid. Non-conservative amino acid substitutions are underlined and Bsb-02 changes that had not been described previously are highlighted in gray. ND = not-determined.

mutations, whereas the other eight were synonymous. Nine nucleotides and two amino acids (A10V and V144I) of these substitutions have not been previously described by the analysis of the MY09/MY11 L1 fragment (Figure 1) (11). The clustering between the L1 sequences from the Bsb-02 isolate and the HPV-58 reference was supported by significant bootstrap values of more than 90% on phylogenetic analysis (Figure 2).

Analysis of the entire L1 gene of the Bsb-02 isolate and determination of the intratype nucleotide pairwise evolutionary distance in comparison to the closest HPV sequence was 0.9%. This suggests that Bsb-02 must be a novel HPV-58 variant. In addition to the reference sequence, the first complete HPV-58 L1 sequence deposited at GenBank is that of Bsb-02.

The determination of the role of intratype HPV variants in determining the risk to develop HPV-associated disease is becoming an active area of epidemiologic investigation. HPV protein sequence variation affects virus assembly and may also affect complex characteristics such as carcinogenic potential and host immunologic response (16).

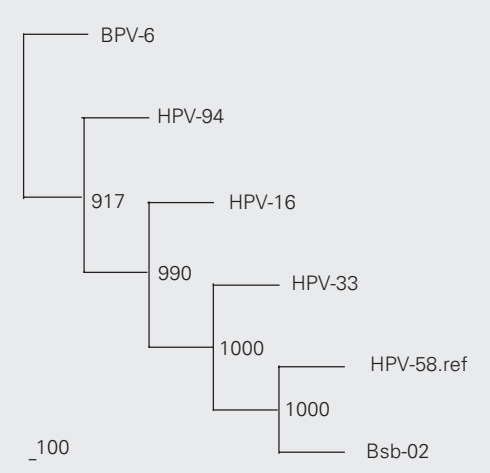

Figure 2. Phylogenetic neighbor-joining tree of L1 HPV sequences, including that of the Bsb-02 isolate. The tree was rooted with the BPV-6 L1 sequence. The numbers given at the branch points correspond to 1,000 bootstrap replicates.

Moreover, it is still unknown whether immunity to one HPV variant can protect against infection with another variant. Consequently, identification of HPV variants may prove to be important for the rational design of diagnostic, therapeutic, and vaccine strategies (10,17).

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