Original Research Article

Improvement of Multiple-Locus VNTR Analysis Typing Scheme for Helicobacter pylori

ABSTRACT

Aims: To improve a multiple-locus variable-number tandem-repeat (VNTR) analysis (MLVA) assay for Helicobacter pylori typing.

Materials and Methods: Polymorphic VNTRs were searched by Gene Expert. The distribution and polymorphism of each VNTR locus were analyzed in 18 H. pylori genomes from the NCBI genome database by BLAST and were compared with a collection of 15 clinical H. pylori strains. The MLVA assay was compared with MLST-typing for discriminating H. pylori isolates.

Results: Twelve VNTR loci were identified by bioinformatic screening of H.pylori genomes, and five of them were highly polymorphic. Therefore, an MLVA assay composed of five VNTR loci was developed with greatest discriminatory power.

Conclusion: MLVA typing is a faster and more standardized method for studying the genetic relatedness of H.pylori isolates. At preliminary stage it is sufficient to use only 3 VNTR loci for the differentiation of H.pylori strains.

Keywords: H.pylori; MLVA typing; VNTR; Cluster Analysis

1. INTRODUCTION

Stomach infection with Helicobacter pylori (H. pylori) is one of the most common infectious disease of humans. H. pylori infection may cause gastric and duodenal ulcer disease, the development of gastric mucosal atrophy, gastric carcinoma. H. pylori infections cause very high morbidity and mortality and are of particular concern in developing countries, where H. pylori prevalence as high as 90% have been reported. The population of H. pylori shows a high genomic variability among isolates. Few molecular typing tools have been described to reflect genetic relatedness in H. pylori isolates. These include pulsed-field gel electrophoresis [1], random fragment length polymorphism [2], randomly amplified polymorphic DNA [3,4], amplified fragment length polymorphism [5,6], and PCR-based genotyping of repetitive sequences, namely, repetitive extragenic palindromes [7,8] and enterobacterial repetitive intergenic consensus elements [9]. Phylogenetic analysis based on multi-locus sequence typing (MLST) of several genes revealed geographical differentiation since H.pylori left Africa together with Homo sapiens [10]. All these techniques indicate that the H. pylori population genetic structure is panmictic, and a high level of DNA diversity is found within strains. Not so far, two different methods of MLVA typing of H.pylori were described [11,12]. Aim of this study was to improve MLVA assay for H. pylori typing suggested earlier [13].

2. MATERIAL AND METHODS

DNA isolation and VNTR primer design were described earlier [13]. PCR conditions were as described earlier [13]. PCR reaction mixture (30 µL) containing 5 µL of DNA template, 10

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pmol of each primer, 1 unit of Taq DNA polymerase, 200 µM of dNTPs and 10 × PCR buffer (500 mM KCl, 100 mM TrisHCl (pH 8.3) 25 mM MgCl2) was utilized. Amplification was carried out in a DNA thermocycler Tercyc (DNA-Technology, Russia) with denaturation at 94°C for 5min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 57°C (55°C for HpD*) for 45 s and elongation at 72°C for 45 s. A 5-min elongation at 72°C was performed after the last cycle. Each PCR product (5 µL) was resolved by 5-8% polyacrylamide gel electrophoresis. Allelic sizes were estimated using a pBlueScript DNA / MspI (MBI Fermentas, Vilnius, Lithuania) as a size marker. Gels were visualised using UV transillumination and the images captured using the ChemiDoc System (BioRad). The polymorphism index for individual or combined VNTR loci was calculated using the Hunter-Gaston diversity index (HGDI) [14]. All calculations were done by the free online tool Comparing Partitions (available at www.comparingpartitions.info). A dendrogram was created with the Mega4 software by using the unweighted-pair group method using average linkages (UPGMA).

3. RESULTS AND DISCUSSION

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As mentioned above, Chinese scientists have proposed their method for multi - locus VNTR-typing of H. pylori strains based on 12 VNTR-loci with repeat length from 12 to 138 bp and detection of fragments in agarose gel [11]. Later, when trying to use this method for typing Brazilian strains, it was shown that in silico analysis four loci out of 12 have a significant drawback: DNA of many H. pylori strains from the NCBI database has two or more positions for hybridization with the corresponding primers [15]. Another locus has insufficient incidence among Brazilian strains (60%), and three loci have no correlation between the number of repeats and the size of the amplified fragment. Thus, to characterize the population of Brazilian strains of H. pylori only 4 loci out of 12 were used, and one of them was monomorphic for this population. As a result, only 13 genotypes were found in 90 studied strains [15]. The method we offer allowed to reveal 48 strains from Russian population with 48 genotypes, which means 100% discrimination ability of the method [13]. However, in the study of H. pylori clinical isolates by HpF locus, sometimes we encountered two or more fragments in a single sample and defined this as the presence of a mixed culture with the exclusion of the sample from further consideration. With the increasing number of DNA sequences of H. pylori in the database NCBI, strains appeared with two or more positions for priming the primers to the HpF locus. Accordingly, it was decided to replace the HpF locus with a new one – HpG. Detailed characteristics of this locus are given in Table 1 and 2. Also, to increase the accuracy of determining the size of fragments at the HpD locus, new primers are proposed, reducing the size of amplified fragments by about 100 bp (Table1).

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Table 1. Oligonucleotide Primers of VNTRs Analyzed in This Study

Primer name	Direction	Sequence (5'-3')
HpA	Forward	TGGGGAACAAAACGAAGTTAAAAGG
	Reverse	TCTTATTCGCCCATTTTCCAACG
HpD	Forward	CGTTTCTATCAACGCCCCTATTTC
	Reverse	AAAAGGCGAAATACTGGGATAGCTT
HpE	Forward	ACCGCTCAAATCCCACCAACC
	Reverse	ATGATGCTATAATCACTAATCACT
HpF	Forward	GGTAATATTCATATTGCTTTTTGCGCG
	Reverse	AGATCGTTAAGATTTTGGACGCTTTC
HpG	Forward	CCAAAATAGCTTGGTTGAACAATCC
	Reverse	TGGGGGTGGGTATGTTATACATTTT
HpD*	Forward	TTGGATATTCAATCTATTTTGGTATAATAG

	Reverse	**CTGGGATAGCTTATAGCGAGTTAGC**

** new primers for **HpD***

Table 2. HGDI and Characters for Individual VNTR Locus

Locus	Repeat	Repeat Size (bp)	N*	HGDI
HpA	**TTTTGATGA**	**9**	**9**	**0.836**
HpD	**AAATACAT**	**8**	**23**	**0.979**
HpE	**TAATCAC**	**7**	**13**	**0.866**
HpF	**AATTCTGTGTTT**	**12**	**6**	**0.727**
HpG	**GCCAAGTA**	**8**	**7**	**0.795**

***number of different repeats*

Dendrogram was generated using MLVA types (MTs) of 15 regional strains *H. pylori* and 18 strains from the NCBI database with known geographical origin [16]. MTs were determined for all 5 proposed VNTR loci (Fig.1 and Fig.2).

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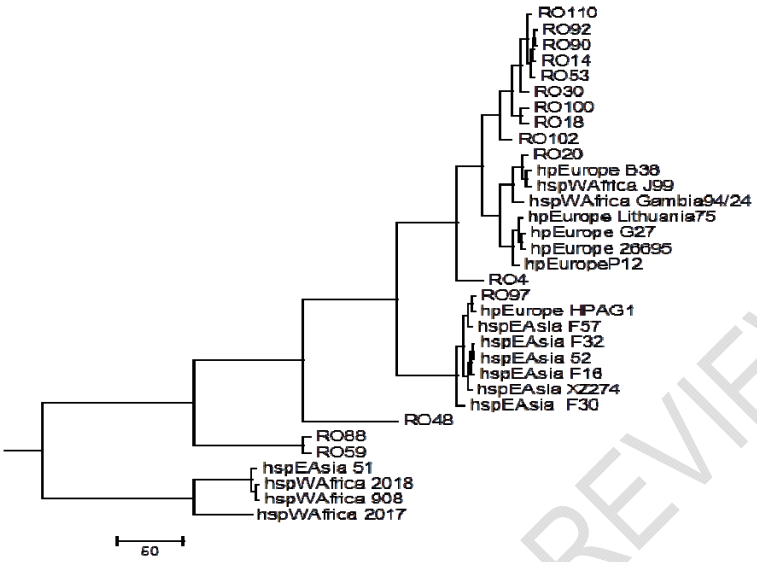

Fig. 1. Dendrogram of genetic relationships of *H. pylori* strains at the loci of HpA, HpD, HpE, HpF

RO – regional strains

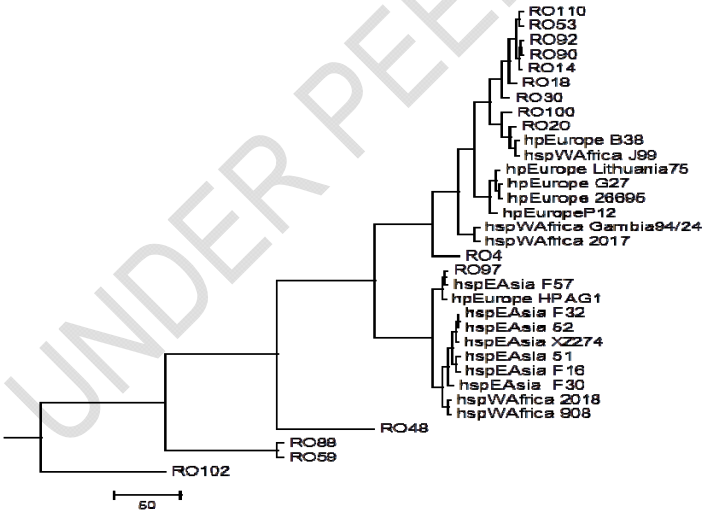

Fig. 2. Dendrogram of genetic relationships of *H. pylori* strains at the loci of HpA, HpD, HpE, HpG

RO – regional strains

When comparing Figs. 1 and 2 it is obvious that the replacement of the HpF locus on HpG changed the location of some strains, but did not affect the discriminating ability of the method.

Due to the hypervariable locus HpD, at the preliminary stage of the study, it is possible to reduce the number of loci studied to three, which practically does not reduce the discrimination ability of the method for a given sample of strains (Fig. 3 and 4).

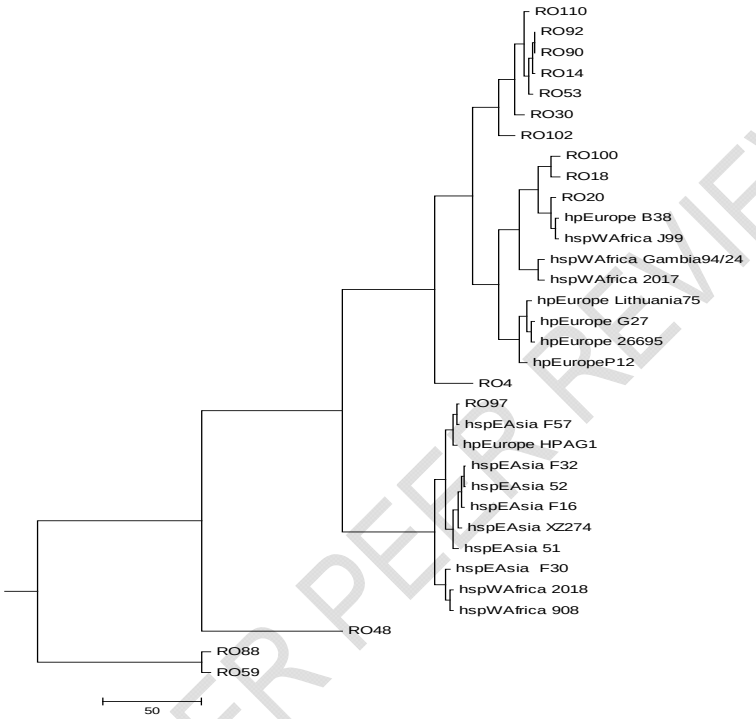

Figure 3. Dendrogram of genetic relationships of 33 strains of *H.pylori* at the loci of HpA, HpD and HpE

RO – regional strains

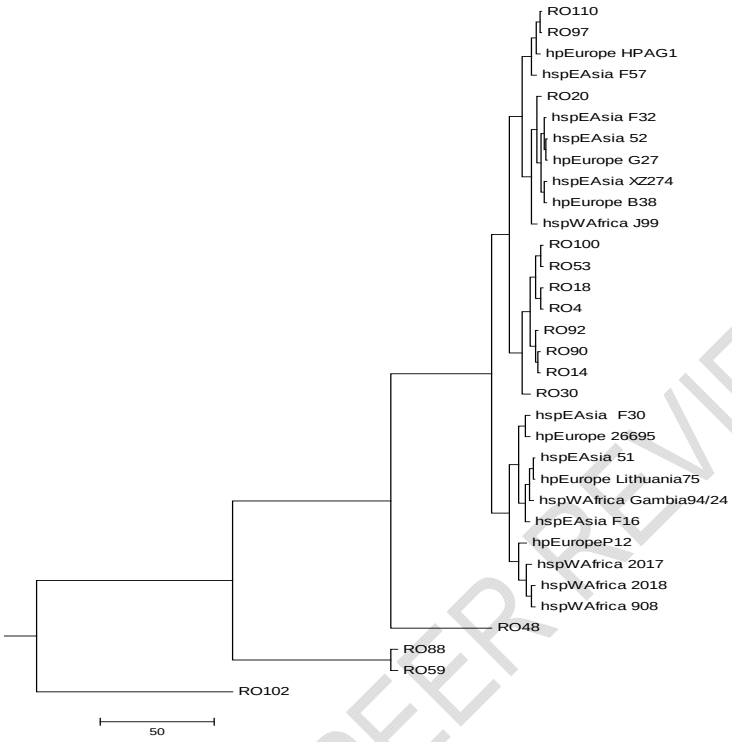

FIGURE 4. DENDROGRAM OF GENETIC RELATIONSHIPS OF 33 STRAINS OF *H.PYLORI* AT THE LOCI OF HPA, HPD AND HPG
RO – regional strains

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The first of a set of loci (HpA, HpD and HpE) allows for a more clear grouping of strains according to geographic features and the assumption that most regional strains belong to a subpopulation of hpEurope, which agrees with the previously obtained data [17].

4. CONCLUSION

MLVA typing is a faster and more standardized method for studying the genetic relatedness of *H.pylori* isolates. At preliminary stage it is sufficient to use only 3 VNTR loci for the differentiation of *H.pylori* strains.

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