VARIABILITY OF THE *VAA* CYTOADHESIN GENES IN CLINICAL ISOLATES OF *MYCOPLASMA HOMINIS*

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Abstract.

The *Mycoplasma hominis vaa* gene encodes a highly variable, surface antigen involved in the adhesion to host cells. We studied 15 clinical isolates of *Mycoplasma hominis* with three types of the *vaa* gene. These *vaa* versions determine various forms of Vaa protein, which are characterized by different quantity and structure of homologous replaceable cassettes. Each cassette contains heptad (please check this words!) repeats and sites for adherence. The differences on single nucleotides were observed in the primary sequences of the homologous modules of the *vaa* gene. A high frequency of nucleotide replacements in V module of the *vaa* gene (first and/or second position in codon) was determined. This region with various clusters of direct and indirect repeats of nucleotide sequences is incorporated into the area of the *vaa* gene. Amino-acid sequences corresponded to the hyper-variable region of the *vaa* gene are associated with the sections of coiled-coils and loops of Vaa. These bacterial regions involved into interaction with the host cell membranes could yield useful indications to a better knowledge concerning the mechanism for mycoplasma persistence in humans.

Keywords: *Mycoplasma hominis,* clinical isolates, *vaa* genes, variability

Peculiar properties as the absence of the cell wall, the genome reduction, and the limited biosynthetic and regulatory capabilities are not the principle for overcoming various protective systems of the higher host organisms. Instead, adaptation to the unfavorable conditions, persistence in various eukaryotes and circulation in the nature is provided by genetic variability of these bacteria (Borkhsenius et al., 2001 and 2002 ; Chernov et al., 2004; Razin et al., 2002). In this regard, genetically determined variability of the mycoplasma surface immuno-dominant proteins involved into persistence seems to be very important (Chambaud et al., 1999).

Mycoplasma hominis is the opportunist human pathogen capable of colonizing epithelial cells and causing the UTI and extragenital pathologies (Borkhsenius et al., 2002; Razin et al., 2002).

Adherence of these microorganisms to the host epithelial cells is mediated by the immuno-dominant Vaa protein (Razin et al., 2002). Variability of the Vaa cytoadhesin may be responsible for the mycoplasma persistence in various individuals.

The aim of our study is focused to the analysis of the primary structure of the *vaa* genes in clinical isolates of *M. hominis* to obtain more information on Vaa cytoadhesin structure.

M. hominis was isolated from cervical and/or urethral epithelium of patients in clinic of Kazan Medical Academy. The presence of the *mycoplasma* was detected by PCR method, with 16S rDNA primers (Litech, Russia).

Mycoplasmal cells were grown in medium with heart infusion broth (2.2 %, w/v) using 10%, horse serum (15%, v/v), fresh yeast extract (10%, w/v), arginine (0.5%, w/v), benzylpenicillin (1000 IU/ml), phenol red (0.1 %, w/v) at 37 C, for 3-7 days. DNA was isolated using "DNA-express" according to the manufacturer's instructions (Litech, Russia). Amplification of *M. hominis* the *vaa* gene was performed with oligonucleotide primers (Litech, Russia) in terms of nucleotide sequence flanking the following genes: F1: 5' - CCC CGG AGA TTA TTA AGT CTC - 3'; R1: 5' - GTG CCC ATT AGT AGC ACT ATT TTT TG - 3' according to [6]. PCR was performed with a programmable thermal cycler "Tercyc" (DNA-technology, Russia) with the following program: 94 0C - 30s (1 cycle), 93 0C - 20s, 58 0C - 40s, 72 0C - 60 s (30 cycles), 72 0C - 10 min (1 cycle). DNA enzymic hydrolysis of *vaa*-amplicons was made using *Hin*dIII and *Hin*61 restrictase (Fermentas, Lithuania). DNA electrophoregraphy was done in 2% agarose gel with Tris-acetate buffer (pH 8.0-8.2). GeneRules DNA Ladder fragments were used as molecular mass markers (Fermentas, Lithuania). Estimation of amplicon's and restriction fragment's sizes were performed using DNASIS software (version 3.0), DNA sequence was determined according to (Sanger et al., 1977), and data were analyzed with Vector NTI 7.1 program (InforMax, Inc.). NPSA (Network Protein Sequence Analysis, http://npsa-pbil.ibcp.fr) program package with Self-Optimized Prediction Method (SOPM) (Geourjon et al., 1994; Lupas et al., 1991) and coiled-coil prediction as well as the data of Boesen *et al* (10) were used for prediction and analysis of protein's secondary structure. Estimation of α-helixes (h), β-sheets (e) and super-helixes (c) were made at window values of 14, 21 and 28.

We identified 15 *M. hominis* isolates containing various versions of the mycoplasma *vaa* gene (Fig 1) according to its modular structure (Boesen et al., 1998).

The versions of the *vaa* gene encode the distinct Vaa types characterized by different amounts as well as by configurations of homologous replaceable cassettes with the chained modules of nucleotide sequences (Boesen et al., 2001).

In general, each Vaa cassette consists of 110 amino acids and contains the sites of adherence and heptad repeats determining the coiled-coils regions. Such structures mediate the pathogenic reactions of the extra-cellular proteins in a number of viruses and bacteria, ensuring the penetration of microorganisms into the host cells (Lupas et al., 1996)

Formation of the rod-like structures is the general feature of the coiled-coils structures in most microbial surface proteins. In this case, the rod-like structure exposed at some distance from the surface membrane promotes the binding with the host cell membrane. Reiteration of domains with the protein adherence sites typical for *M. hominis* Vaa (Fig 2, 3) may significantly increase avidity.

As a result of our comparative analysis the differences associated with the single-nucleotide replacements were revealed in the primary sequences of the homologous modules of the *vaa* genes in clinical isolates of *M. hominis*. The proportion of GC:AT in the replacements proved to be 1:1 despite the low content (~23%) of GC base pairs in *M. hominis* genome as a whole and in the *vaa* gene, in particular (Borkhsenius et al., 2002; Razin et al., 2002).

A region with a high frequency of the nucleotide replacements was revealed in the module V of the *vaa* gene (the regions corresponding to 272-282 and 297-317 amino-acid's location). Localization of the nucleotide replacements in codons (first and/or second position) may lead to reorganizing the appropriate protein zone. This region incorporated into zone of the *vaa* gene, proved to be presented by clusters of direct and indirect repeats homologous to recurrent sequences dispersed in human chromosomes. The amino-acid sequences of the *vaa* hyper-variable region were associated with coiled-coils and loops of the mycoplasma Vaa (Fig 2). These regions determine the interaction of viruses and bacteria with the host cell membranes (Boesen et al., 1998) and may be crucial for persistence of the mycoplasma in various individuals.

Thus, in our study a variability of the *vaa* genes in clinical isolates of *M. hominis* was revealed. We have determined a hyper-variable region of the *vaa* gene connected with an area encoding the immuno-significant part of the mycoplasma Vaa protein. This region is essential for immune recognition, persistence of mycoplasma in humans, and colonizing host cells. A more detailed spatial pattern for the Vaa surface adhesin as well as a new model system for the study of pathogenesis of these microorganisms have been proposed.

Acknowledgement

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Legends

Figure 1. Scheme of the modular structure of the *vaa* gene in clinical isolates of *M. hominis*

I - signal fragment;

II-VIII - modular sequences of the *vaa* gene;

M. hominis 132, *M. hominis* 4195, *M. hominis* FBG - two- and three-cassette version of the *vaa* gene in the mycoplasma strains (132, 4195 and FBG) revealed in 15 (7:1:7) clinical isolates.

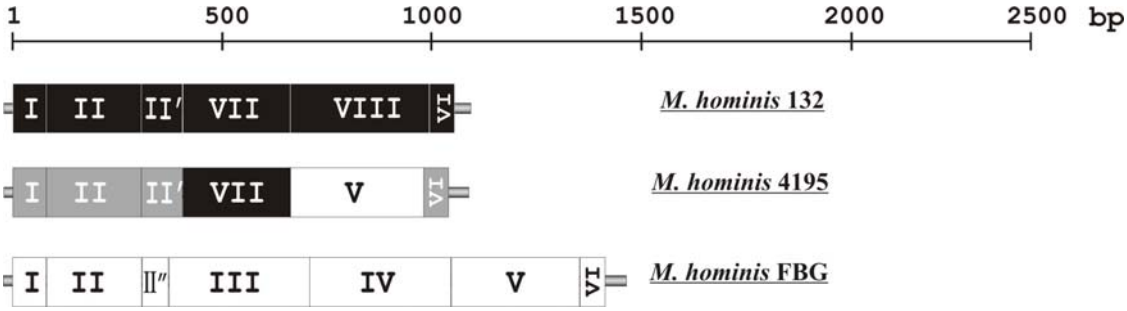

Figure 2. Peculiarities of the secondary structure of two-cassette version of the Vaa with modules II, II", V и VI according to NPSA data with the protein secondary structure prediction using SOPM and coiled-coils.

- modular sequences of the *vaa* gene (II, II", VII, V and VI, respectively);

abcdefg - structural positions of amino-acid residues taking part in the formation of the coiled-coils structures.

p - probability (at window's value 21 and 28);

h, e, c - □-helixes, □-sheets and super-helixes, respectively;

- location of □-sheets;

SFKE (ELESFKE) - adherence sequence (according to the data of Boesen *et al.*, 1998);

T..E..DDK.. - regions of amino-acid replacements;

- the hyper-variable region of the *vaa* gene.

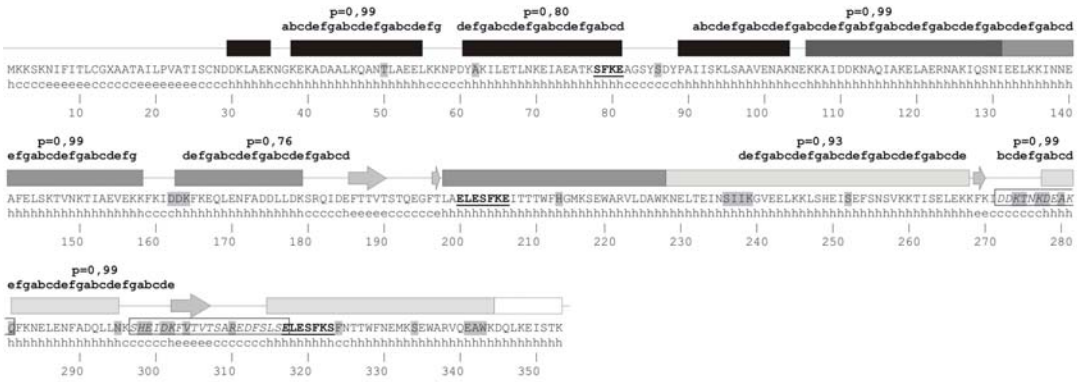

Figure 3. Model of the two-cassette Vaa protein of *M. hominis*.

II, V, VI и VII - modules of Vaa;

N and C - terminal fragments;

▮ - membrane lipid anchor;

⇨ - □-sheet;

⬭- sites for adherence.