**Supplementary materials**

**Изображение выглядит как снимок экрана, Прямоугольник, шов, прямоугольный

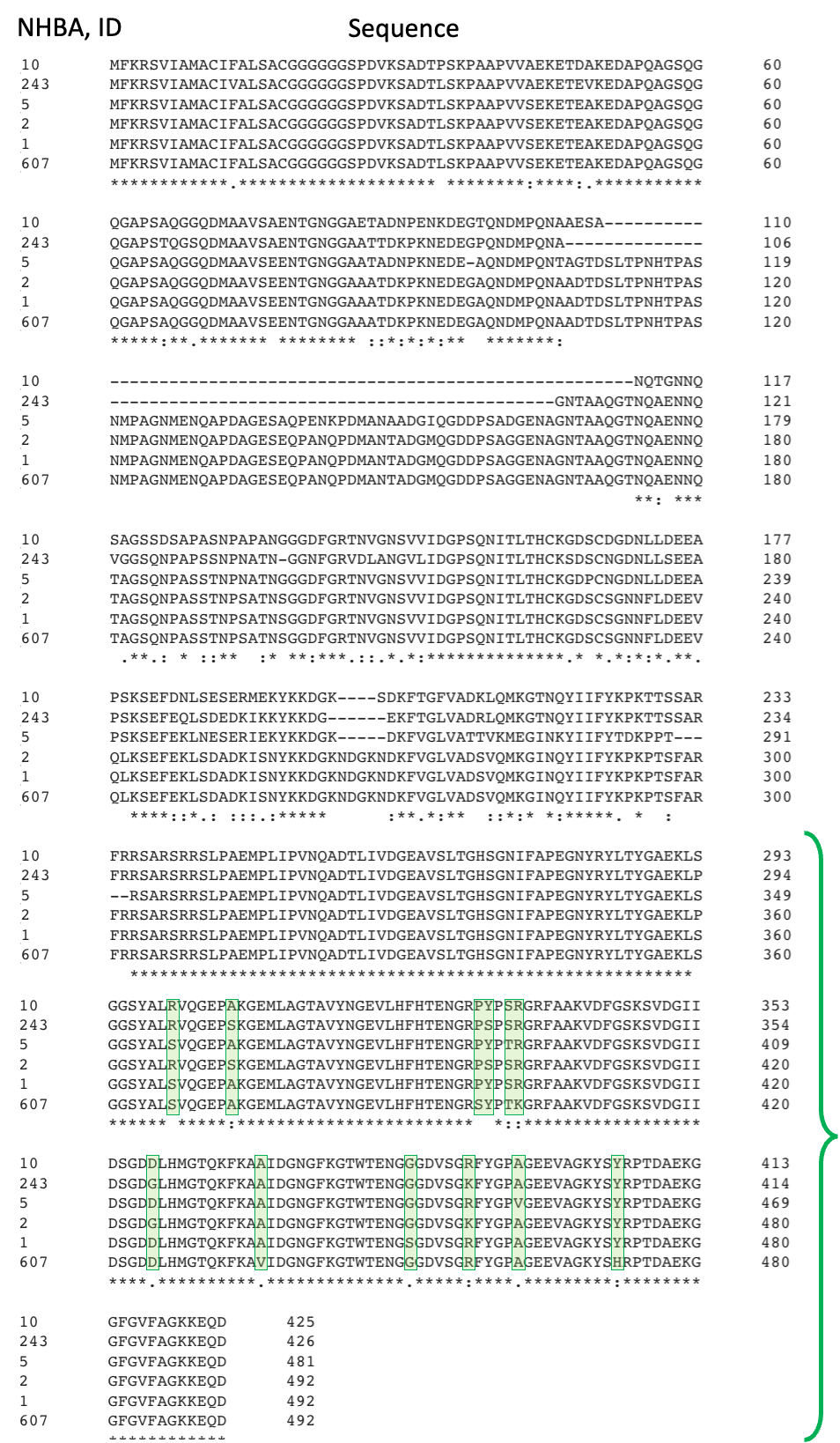
Автоматически созданное описание**

**Figure S1.** Alignment of amino acid sequences of cross-reactive variants of fHbp from isolates of MenB strains, 2013-2023, and fHbp peptide, ID 1, used in the 4CMenB vaccine (the sequence is marked with a green dot). Alignment was done in Clustal Omega service (https://www.kelleybioinfo.org/algorithms/tutorial/TAli2.pdf). Green color indicates polymorphic sites that occur more often than 30% of the sequences (i.e., at least 30% of the studied sequences of cross-reactive fHbp variants contain an amino acid substitution in this position). An asterisk “\*” indicates fully conserved amino acid position, two dots “:” indicate positions with conservation between amino acids with similar properties and one dot “.” indicates positions with conservation between amino acids with weakly similar properties. The number of last amino acid residue in line is indicated on right.

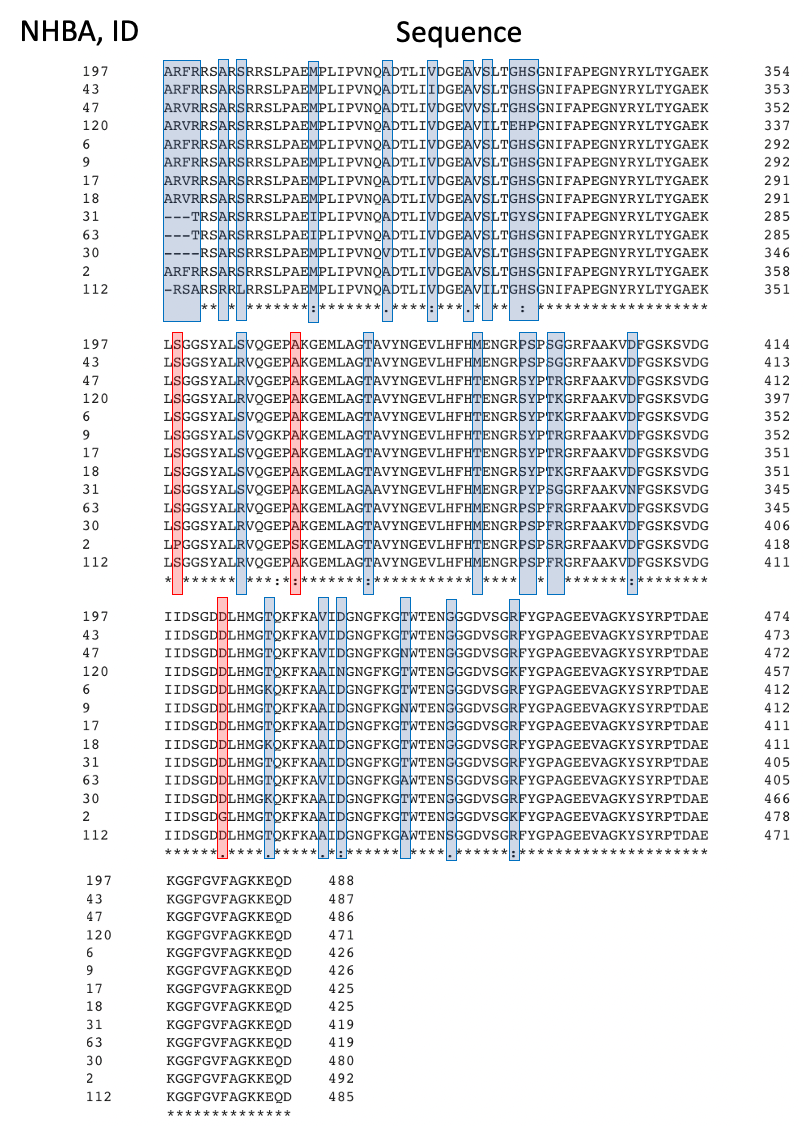
**Table S1.** Polymorphic sites in the C-terminal region that occur at different frequencies in fHbp sequences from the “Cross-reactive” and “None” groups\*.

| **Frequency of substitution, %** | **Cross-reactive fHbp** | **None fHbp** |
| --- | --- | --- |
| 100 | - | S140A  D142N  K143Q  G147-  **R149K**  T151E  R153H  G158S  S186T  D197N  T198L  P200A  G202E  R204S  S209L  S211D  V212T  I213R  **N215G**  G229D  K230R  G225A  I226L  S221T  S223H  T155K  I246V  R247H  H248E  L251I  T242I  N244E  G245K  A253G  V234I  E239T |
| 50-99 | **E146K**  **N178H**  G202E  R204H  A217D  K230Q  K241E  **V243A**  R247H | A162P  G163N  K165R  T167H  T169S  **N178H**  L189Q  D192E  A195S  Q217S  **V243R** |
| <50 | D142G  G147D  G148V  **R149S**  G158S  **N178Y**  D192E  D197Y  D197N  R200Q  G229E | **E146D**  **E146S**  **E146G**  G147D  A174K  A174N  A174S  **N178Y**  **K180R**  Q217G  **V243G** |

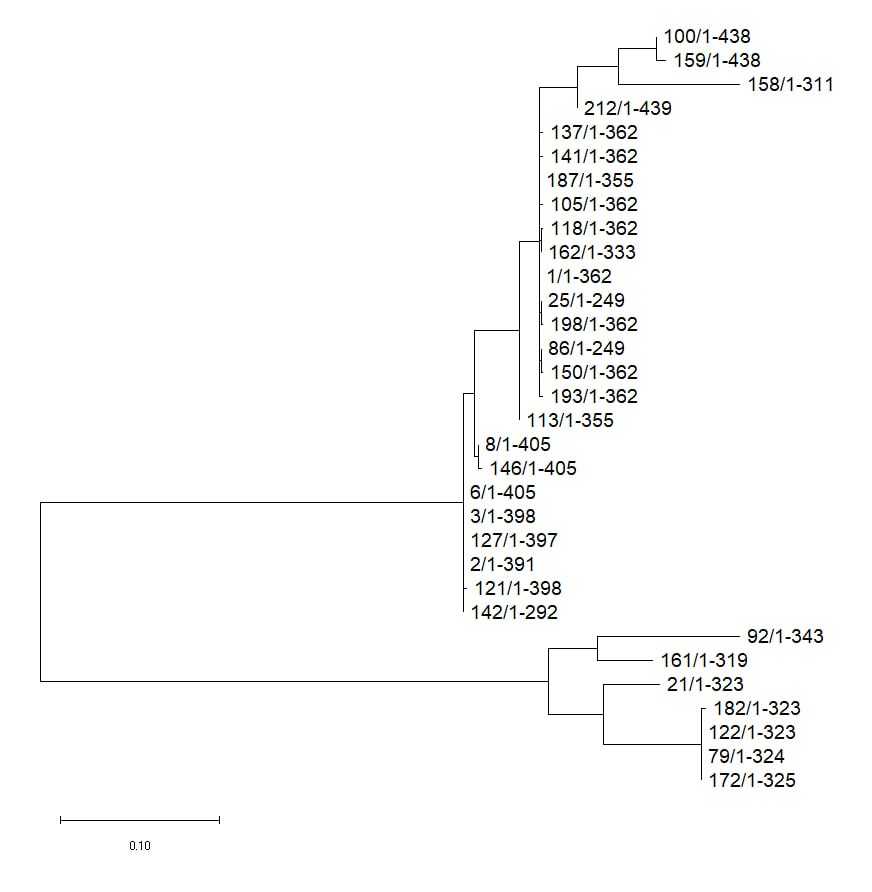
\*Underlined polymorphisms are common for sequences of fHbp from “Cross-reactive” and “None” groups. Polymorphisms in red were mentioned in literature earlier as potentially important for antigen-antibody interaction [13, 25, 26]. The fHbp vaccine antigen (ID 1) was used as a control.



**Figure S2.** Comparison of amino acid sequences of cross-reactive NHBA variants (full-length) with the sequence of NHBA peptide, ID 2, included in the 4CMenB vaccine. Polymorphic sites found in the C-terminal domain are indicated in green. Alignment of amino acid sequences constructed in Clustal Omega (https://www.kelleybioinfo.org/algorithms/tutorial/TAli2.pdf). An asterisk “\*” indicates fully conserved amino acid position, two dots “:” indicate positions with conservation between amino acids with similar properties and one dot “.” indicates positions with conservation between amino acids with weakly similar properties. The number of last amino acid residue in line is indicated on the right.



**Figure S3.** Comparison of the amino acid sequences of NHBA C-terminal domains from the “None” group with corresponding sequence of the NHBA peptide, ID 2, included in the 4CMenB vaccine. Red indicates the positions at which substitutions occur in the sequences of each NHBA of this group, and blue indicates the remaining polymorphic sites in the C-terminal domain. Alignment of amino acid sequences constructed in Clustal Omega (https://www.kelleybioinfo.org/algorithms/tutorial/TAli2.pdf). An asterisk “\*” indicates fully conserved amino acid position, two dots “:” indicate positions with conservation between amino acids with similar properties and one dot “.” indicates positions with conservation between amino acids with weakly similar properties. The number of last amino acid residue in line is indicated on the right.



**Figure S4.** The tree of the NadA. Multiple alignments of amino acids sequences produced by the MUSCLE algorithm to construct phylogenetic trees of the NadA antigen. The trees were constructed using the Maximum Likelihood algorithm and visualized in MEGA software.