genic diversity of these proteins among different *H. influenzae* strains. An ideal antigen must therefore be both exposed on the bacterial surface and antigenically well conserved. In this laboratory, a 42-kDa membrane protein (protein D) that is widely distributed and antigenically conserved among both Hib and NTHi strains has been isolated, cloned, sequenced, and shown to be a pathogenicity factor and a possible vaccine candidate (1-5).

Two decades ago, *Haemophilus influenzae* and *M. catarrhalis* were shown to display a strong affinity for both soluble and surface-bound human IgD (6). The IgD-binding seems to be paralleled by a similar interaction with surface-bound IgD at the cellular level, a phenomenon that explains the strong mitogenic effects on human lymphocytes by *H. influenzae* and *M. catarrhalis* (7-9). An IgD-binding outer membrane protein from *H. influenzae* (protein D) was isolated and cloned, and shown to be an important pathogenicity factor (1-5). However, protein D does not bind universally to all IgD myelomas(10).

Summary

In view of the fact that *H. influenzae* has been found to be such a leading cause of infections in the upper and lower airways, there is a current need to develop vaccines that can be used against *H. influenzae.*

The aim of the present disclosure has therefore been to find out in which way *H. influenzae* interacts with cells in the body and interacts with the immune system, and be able to provide a new type of vaccine.

In one aspect, the present disclosure refers to an immunogenic composition comprising a surface exposed protein, which can be detected in Haemophilus influenzae having an amino acid sequence as described in Sequence ID No. 1, or a immunogenic fragment thereof, wherein the immunogenic fragment comprises the amino acids in position 22-160 (SEQ ID NO: 2), 22-95 (SEQ ID NO: 5),22-

125 (SEQ ID NO: 6), 56-125 (SEQ ID NO: 7), 56-160 (SEQ ID NO: 8) or 86-160 (SEQ ID NO: 9) of the amino acid sequence of SEQ ID NO: 1 which fragment (if necessary when coupled to a carrier) is capable of raising an immune response which recognizes the polypeptide of SEQ ID NO: 1. In another aspect, the present disclosure refers to an immunogenic composition comprising a polypeptide consisting of the amino acid sequence which lacks a signal peptide (amino acids 1-20) of SEQ ID NO: 1. In another aspect, the present disclosure refers to an immunogenic composition comprising a polypeptide consisting of the amino acid sequence which has Thr instead of Ile at position 20 of SEQ ID NO: 1. In yet another aspect, the present disclosure refers to an immunogenic composition comprising an isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO: 1, obtainable by screening an appropriate library under stringent hybridization conditions with a labeled probe having the sequence of SEQ ID NO: 11 or a fragment thereof. In yet another aspect, the present disclosure refers to a process for producing a polypeptide or an immunogenic fragment as described herein, comprising culturing a host cell as described herein under conditions sufficient for the production of said polypeptide or said immunogenic fragment and recovering the polypeptide from the culture medium. In yet another aspect, the present disclosure refers to a process for expressing a polynucleotide as described herein comprising transforming a host cell with an expression vector comprising at least one of said polynucleotides and culturing said host cell under conditions sufficient for expression of any one of said polynucleotides.

In a further aspect, the present disclosure refers to the use of an immunogenic composition as described herein in the manufacture of a medicament for the prophylaxis or treatment of an infection. In a further aspect, the present disclosure refers to a medicament comprising at least one protein, fragment or peptide as described herein and one or more pharmaceutically acceptable adjuvants, vehicles, excipients, binders, carriers, preservatives, buffering agents, emulsifying agents, wetting agents, or transfection facilitating compounds. In a further aspect, the present disclosure refers to a method of isolation of a protein, fragment or peptide as described herein, said method comprising the steps: a) growing Haemophilus influenzae or E.coli comprising the DNA coding for said protein, fragment or peptide, harvesting the bacteria and isolating outer membranes or inclusion bodies; b) solubilizing the inclusion bodies with a strong solvatising agent; c) adding a renaturating agent; and d) dialyzing the resulting suspension against a buffer with a pH of from 8 to 10.

In yet another aspect, the present disclosure refers to an immunogenic composition comprising a polypeptide, wherein the polypeptide comprises an amino acid sequence that varies from SEQ ID NO: 1 at one or more positions, wherein the variation(s) are selected from the group consisting of a deletion of a signal peptide (amino acids 1-20) of SEQ ID NO: 1; a Thr substitution of Ile at position 20 of SEQ ID NO: 1, a Val substitution of Ala at position 23 of SEQ ID NO: 1, a Glu substitution of Lys at position 24 of SEQ ID NO: 1, a Met substitution of Val at position 28 of SEQ ID NO: 1, a Thr substitution of Ala at

position 31 of SEQ ID NO: 1, a Val substitution of Ile at position 41 of SEQ ID NO: 1, an Ala substitution of Val at position 47 of SEQ ID NO: 1, a Lys substitution of Gly at position 152 of SEQ ID NO: 1, a Val substitution of Ala at position 154 of SEQ ID NO: 1, a Ser-Ala-Pro addition at the C-terminus of SEQ ID NO: 1, a Val substitution of Pro at position 32 of SEQ ID NO: 1, and an Ala substitution of Pro at position 32 of SEQ ID NO: 1.

In yet another aspect, the present disclosure refers to an immunogenic composition comprising a polypeptide, wherein the polypeptide comprises an amino acid sequence that varies from SEQ ID NO: 1 in at least three positions, wherein the variation(s) are selected from the group consisting of a deletion of a signal peptide (amino acids 1-20) of SEQ ID NO: 1; a Thr substitution of Ile at position 20 of SEQ ID NO: 1, a Val substitution of Ala at position 23 of SEQ ID NO: 1, a Glu substitution of Lys at position 24 of SEQ ID NO: 1, a Met substitution of Val at position 28 of SEQ ID NO: 1, a Thr substitution of Ala at position 31 of SEQ ID NO: 1, a Val substitution of Ile at position 41 of SEQ ID NO: 1, an Ala substitution of Val at position 47 of SEQ ID NO: 1, a Lys substitution of Arg at position 76 of SEQ ID NO: 1, a Val substitution of Ile at position 107 of SEQ ID NO: 1, a Lys substitution of Gly at position 152 of SEQ ID NO: 1, a Val substitution of Ala at position 154 of SEQ ID NO: 1, a Ser-Ala-Pro addition at the C-terminus of SEQ ID NO: 1, a Val substitution of Pro at position 32 of SEQ ID NO: 1, and an Ala substitution of Pro at position 32 of SEQ ID NO: 1.

According to one example, the present disclosure provides a surface exposed protein, which can be detected

in *Haemophilus influenzae*, having an amino acid sequence according to Sequence ID No. 1, or a fragment, homologue, functional equivalent, derivative, degenerate or hydroxylation, sulphonation or glycosylation product or other secondary processing product thereof.

According to another example the present disclosure provides an immunogenic fragment of said surface exposed protein, which fragment can be detected in *Haemophilus influenzae*, or naturally occurring or artificially modified variants thereof.

According to a further example the present disclosure provides a recombinant immunogenic protein based on the surface exposed protein mentioned above, wherein the amino acids in position 1 to 21 of Sequence ID No. 1 have been deleted or replaced by one or more amino acids. In one example the amino acids in position 1 to 21 of Sequence ID No. 1 have been replaced by a sequence of 0 to 21 optional amino acids. In another example the recombinant immunogenic protein have an amino acid sequence according to Sequence ID No 2, or a fragment, homologue, functional equivalent, derivative, degenerate or hydroxylation, sulphonation or glycosylation product or other secondary processing product thereof.

According to a further example the present disclosure provides a peptide having an amino acid sequence according to Sequence ID No. 3, or a fragment, homologue, functional equivalent, derivative, degenerate or hydroxylation, sulphonation or glycosylation product or other secondary processing product thereof.

According to still a further example the present disclosure provides a peptide having an amino acid sequence according to Sequence ID No. 4, or a fragment, homologue, functional equivalent, derivative, degenerate or hydroxylation, sulphonation or glycosylation product or other secondary processing product thereof.

According to yet a further example the present disclosure provides a peptide having an amino acid sequence according to Sequence ID No. 5, or a fragment, homologue, functional equivalent, derivative, degenerate or hydroxylation, sulphonation or glycosylation product or other secondary processing product thereof.

According to a further example the present disclosure provides a peptide having an amino acid sequence according to Sequence ID No. 6, or a fragment, homologue, functional equivalent, derivative, degenerate or hydroxylation, sulphonation or glycosylation product or other secondary processing product thereof.

According to still a further example the present disclosure provides a peptide having an amino acid sequence according to Sequence ID No. 7, or a fragment, homologue, functional equivalent, derivative, degenerate or hydroxylation, sulphonation or glycosylation product or other secondary processing product thereof.

According to yet a further example the present disclosure provides a peptide having an amino acid sequence according to Sequence ID No. 8, or a fragment, homologue, functional equivalent, derivative, degenerate or hydroxylation, sulphonation or glycosylation product or other secondary processing product thereof.

According to a further example the present disclosure provides a peptide having an amino acid sequence according to Sequence ID No. 9, or a fragment, homologue, functional equivalent, derivative, degenerate or hydroxylation, sulphonation or glycosylation product or other secondary processing product thereof.

According to another example the present disclosure provides a peptide having an amino acid sequence according to Sequence ID No. 10, or a fragment, homologue, functional equivalent, derivative, degenerate or hydroxy

lation, sulphonation or glycosylation product or other secondary processing product thereof.

According to another example the present disclosure provides the use of at least one protein, fragment or peptide as described above for the manufacturing of a medicament for the prophylaxis or treatment of an infection. In one example the infection is caused by *Haemophilus influenzae*, and in another example the *Haemophilus influenzae* is encapsulated or non-typable. In still another example, said use is for the prophylaxis or treatment of otitis media, sinusitis or lower respiratory tract infections, in children as well as adults with, for example, chronic obstructive pulmonary disease (COPD).

According to one example, the present disclosure provides a medicament comprising at least one protein, fragment or peptide as described above and one or more pharmaceutically acceptable adjuvants, vehicles, excipients, binders, carriers, preservatives, buffering agents, emulsifying agents, wetting agents, or transfection facilitating compounds.

According to another example, the present disclosure provides a vaccine composition comprising at least one protein, fragment or peptide as described above. In one example, said vaccine composition comprises at least one di-, tri- or multimer of said protein, fragment or peptide. In another example, the vaccine composition, further comprises one or more pharmaceutically acceptable adjuvants, vehicles, excipients, binders, carriers, preservatives, buffering agents, emulsifying agents, wetting agents, or transfection facilitating compounds. In still another example, said vaccine composition comprises at least one further vaccine, and in yet another example it comprises an immunogenic portion of another molecule, wherein the immunogenic portion of another molecule can be chosen from the group comprising Protein D of *H. influenzae* (EP 594 610), MID of *Moraxella catarrhalis* (WO

03/004651, WO 97/41731 and WO96/34960), UspA1 or UspA2 of *Moraxella catarrhalis* (WO93/03761), and outer membrane protein or carbohydrate capsule, of any respiratory tract pathogen, or DNA oligonucleotides, such as CpG motif.

In one example the present disclosure relates to a nucleic acid sequence encoding a protein, fragment or peptide as described above, as well as homologues, polymorphisms, degenerates and splice variants thereof. In one example, said nucleic acid sequence is fused to at least another gene.

In another example the present disclosure relates to a plasmid or phage comprising a nucleic acid sequence as described above.

In yet another example the present disclosure relates to a non human host comprising at least one plasmid as described above and capable of producing a protein, fragment or peptide as discussed above, as well as homologues, polymorphisms, degenerates and splice variants thereof, which host is chosen among bacteria, yeast and plants. In one example, said host is *E. coli*.

In still another example, the present disclosure provides a fusion protein or polypeptide, in which a protein, fragment or peptide as described above is combined with at least another protein by the use of a recombinant nucleic acid sequence as discussed above. In one example, said fusion protein is a di-, tri or multimer of a protein, fragment or peptide as discussed above.

In one example, the present disclosure relates to a fusion product, in which a protein, fragment or peptide as described above is covalently, or by any other means, bound to a protein, carbohydrate or matrix.

In yet another example the present disclosure relates to a method of isolation of a protein, fragment or peptide as described above, said method comprising the steps:

a) growing *Haemophilus influenzae* or *E. coli* comprising the DNA coding for said protein, fragment or

peptide, harvesting the bacteria and isolating outer membranes or inclusion bodies;

b) solubilizing the inclusion bodies with a strong solvatising agent;

c) adding a renaturating agent; and

d) dialyzing the resulting suspension against a buffer with a pH of from 8 to 10.

In one example of said method the solvalising agent is guanidium hydrochloride, and in another example the renaturating agent is arginin.

In another example the present disclosure relates to a medicament or a vaccine composition as discussed above, comprising said fusion protein or polypeptide, or said fusion product.

In one example the present disclosure relates to a method of preventing or treating an infection in an individual comprising administering a pharmaceutically effective amount of a medicament or a vaccine composition as described above. In one example said infection is caused by *Haemophilus influenzae*, both encapsulated or non-typable, and in yet another example the infection is chosen from the group consisting of otitis media, sinusitis or lower respiratory tract infections.

The present disclosure relates to Protein E, in particular Protein E polypeptides and Protein E polynucleotides, recombinant materials and methods for their production. In another example, the disclosure relates to methods for using such polypeptides and polynucleotides, including prevention and treatment of microbial diseases, amongst others. In a further example, the disclosure relates to diagnostic assays for detecting diseases associated with microbial infections and conditions associated with such infections, such as assays for detecting expression or activity of Protein E polynucleotides or polypeptides.

Various changes and modifications within the spirit and scope of the disclosed disclosure will become readily

apparent to those skilled in the art from reading the following descriptions and from reading the other parts of the present disclosure.

Description of the figures

Fig. 1. The 16.3 kDa *Haemophilus influenzae* protein E is detected by an IgD(□) myeloma protein. In *A*, flow cytometry analysis of pE expression in *H. influenzae* 772 is demonstrated. SDS-PAGE and Western blot (*B*) and 2-

Claims

1. An immunogenic composition comprising a surface exposed protein, which can be detected in Haemophilus influenzae having an amino acid sequence as described in Sequence ID No. 1, or a immunogenic fragment thereof, wherein the immunogenic fragment comprises the amino acids in position 22-160 (SEQ ID NO: 2), 22-95 (SEQ ID NO: 5),22-125 (SEQ ID NO: 6), 56-125 (SEQ ID NO: 7), 56-160 (SEQ ID NO: 8) or 86-160 (SEQ ID NO: 9) of the amino acid sequence of SEQ ID NO: 1 which fragment (if necessary when coupled to a carrier) is capable of raising an immune response which recognizes the polypeptide of SEQ ID NO: 1.

2. An immunogenic composition comprising an immunogenic fragment of the surface exposed protein according to claim 1, which fragment can be detected in Haemophilus influenzae.

3. An immunogenic composition according to claim 1 or 2, further comprising one or more pharmaceutically acceptable adjuvants, vehicles, excipients, binders, carriers, preservatives, buffering agents, emulsifying agents, wetting agents, or transfection facilitating compounds.

4. An immunogenic composition according to any one of claims 1-3, comprising at least one further vaccine.

5. An immunogenic composition according to any one of claims 1-4, comprising an immunogenic portion of another molecule.

6. An immunogenic composition according to claim 5, wherein the immunogenic portion of another molecule is chosen from the group comprising Protein D of H. influenzae, MID of Moraxella

catarrhalis, UspA1 or UspA2 of Moraxella catarrhalis, and outer membrane protein of any respiratory tract pathogen.

7. An immunogenic composition comprising a nucleic acid sequence encoding a protein, fragment or peptide thereof according to claim 1 or 2.

8. An immunogenic composition comprising a recombinant nucleic acid sequence comprising a nucleic acid sequence according to claim 7, which is fused to at least another gene.

9. An immunogenic composition comprising a plasmid or phage comprising a nucleic acid sequence according to claim 7 or 8.

10. An immunogenic composition comprising a non-human host comprising at least one plasmid according to claim 9 and capable of producing a protein, fragment or peptide according to claim 1 or 2, which host is chosen among bacteria, yeast and plants.

11. An immunogenic composition comprising a host according to claim 10 which is E. coli.

12. An immunogenic composition comprising a fusion protein or polypeptide, in which a protein, fragment or peptide according to claim 1 or 2 is combined with at least another protein by the use of a recombinant nucleic acid sequence according to claim 8.

13. An immunogenic composition comprising a polypeptide consisting of the amino acid sequence which lacks a signal peptide (amino acids 1-20) of SEQ ID NO: 1.

14. An immunogenic composition comprising a polypeptide consisting of the amino acid sequence which has Thr instead of Ile at position 20 of SEQ ID NO: 1.

15. The immunogenic composition of claim 13 or 14 wherein the polypeptide has Val instead of Ala at position 23 of SEQ ID NO: 1.

16. The immunogenic composition of any one of claims 13-15 wherein the polypeptide has Gln instead of Lys at position 24 of SEQ ID NO: 1.

17. The immunogenic composition of any one of claims 13-16 wherein the polypeptide has Met instead of Val at position 28 of SEQ ID NO: 1.

18. The immunogenic composition of any one of claims 13-17, wherein the polypeptide has Thr instead of Ala at position 31 of SEQ ID NO: 1.

19. The immunogenic composition of any one of claims 13-18, wherein the polypeptide has Val instead of Ile at position 41 of SEQ ID NO: 1.

20. The immunogenic composition of any one of claims 13-19 wherein the polypeptide has Ala instead of Val at position 47 of SEQ ID NO: 1.

21. The immunogenic composition of any one of claims 13-20 wherein the polypeptide has Lys instead of Arg at position 76 of SEQ ID NO: 1.

22. The immunogenic composition of any one of claims 13-21 wherein the polypeptide has Val instead of Ile at position 107 of SEQ ID NO: 1.

23. The immunogenic composition of any one of claims 13-22 wherein the polypeptide has Lys instead of Gly at position 152 of SEQ ID NO: 1.

24. The immunogenic composition of any one of claims 13-23 wherein the polypeptide has Val instead of Ala at position 154 of SEQ ID NO: 1.

25. The immunogenic composition of any one of claims 13-24 wherein the polypeptide has the additional amino acid sequence Ser Ala Pro at its C-terminus of SEQ ID NO: 1.

26. The immunogenic composition of any one of claims 13-25 wherein the polypeptide has Val instead of Pro at position 32 of SEQ ID NO: 1.

27. The immunogenic composition of any one of claims 13-26 wherein the polypeptide has Ala instead of Pro at position 32 of SEQ ID NO: 1.

28. An immunogenic composition comprising a polypeptide or an immunogenic fragment as claimed in any one of claims 13-27 wherein said polypeptide or said immunogenic fragment is part of a larger fusion protein.

29. An immunogenic composition comprising an isolated polynucleotide encoding a polypeptide or an immunogenic fragment as claimed in any one of claims 13-28.

30. An immunogenic composition comprising an isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO: 1, obtainable by screening an appropriate library under stringent hybridization conditions with a labeled probe having the sequence of SEQ ID NO: 11 or a fragment thereof.

31. An immunogenic composition comprising an expression vector or a recombinant live microorganism comprising an isolated polynucleotide according to claim 29.

32. An immunogenic composition comprising a recombinant live microorganism comprising an expression vector according to claim 31.

33. An immunogenic composition comprising a host cell comprising the expression vector of claim 31.

34. A process for producing a polypeptide or an immunogenic fragment of claims 13-28, comprising culturing a host cell of claim 33 under conditions sufficient for the production of said polypeptide or said immunogenic fragment and recovering the polypeptide from the culture medium.

35. A process for expressing a polynucleotide of claim 29 comprising transforming a host cell with an expression vector comprising at least one of said polynucleotides and culturing said host cell under conditions sufficient for expression of any one of said polynucleotides.

36. An immunogenic composition comprising an effective amount of the polypeptide or the immunogenic fragment of any one of claims 13-28 and a pharmaceutically acceptable excipient.

37. An immunogenic composition comprising an effective amount of the polynucleotide of claim 29 or 30 and a pharmaceutically acceptable excipient.

38. The immunogenic composition according to claim 36 or 37 wherein said composition comprises at least one other Haemophilus influenzae antigen.

39. The immunogenic composition according to any one of claims 1-30 formulated with pneumolysin from Streptococcus pneumoniae.

40. The immunogenic composition according to any one of claims 1-30 formulated with Omp106 from Moraxella catarrhalis.

41. The immunogenic composition according to any one of claims 1-30 formulated with UspA1 and/or UspA2 from Moraxella catarrhalis.

42. The immunogenic composition according to any one of claims 1-30, formulated with Hly 3 from Moraxella catarrhalis.

43. The immunogenic composition according to any one of claims 1-30, formulated with OmpCD from Moraxella catarrhalis.

44. The immunogenic composition according to any one of claims 1-30, formulated with D15 from Moraxella catarrhalis.

45. The immunogenic composition according to any one of claims 1-30, formulated with Omp 26 from Haemophilus influenzae.

46. The immunogenic composition according to any one of claims 1-30, formulated with P6 from Haemophilus influenzae.

47. The immunogenic composition according to any one of claims 1-30, formulated with Protein D from Haemophilus influenzae.

48. The immunogenic composition according to any one of claims 1-30, formulated with NlpC2 from Haemophilus influenzae.

49. The immunogenic composition according to any one of claims 1-30, formulated with Slp from Haemophilus influenzae.

50. Use of an immunogenic composition according to claim 1 or 2 in the manufacture of a medicament for the prophylaxis or treatment of an infection.

51. Use according to claim 50, wherein the infection is caused by Haemophilus influenzae.

52. Use according to claim 51, wherein the Haemophilus influenzae is encapsulated or non-typable.

53. Use according to any one of claims 50-52, for the prophylaxis or treatment of otitis media, sinusitis or lower respiratory tract infection.

54. A medicament comprising at least one protein, fragment or peptide according to claim 1 or 2 and one or more pharmaceutically acceptable adjuvants, vehicles, excipients, binders, carriers, preservatives, buffering agents, emulsifying agents, wetting agents, or transfection facilitating compounds.

55. A method of isolation of a protein, fragment or peptide according to claim 1 or 2, said method comprising the steps:

a) growing Haemophilus influenzae or E.coli comprising the DNA coding for said protein, fragment or peptide, harvesting the bacteria and isolating outer membranes or inclusion bodies;

b) solubilizing the inclusion bodies with a strong solvatising agent;

c) adding a renaturating agent; and

d) dialyzing the resulting suspension against a buffer with a pH of from 8 to 10.

56. A method according to claim 55, wherein the solvatising agent is guanidium hydrochloride.

57. A method according to claim 55 or 56, wherein the renaturating agent is arginine.

58. A method of making an immunogenic composition comprising the steps of claims 55-57, wherein the protein, fragment or peptide is formulated with an excipient.

59. An immunogenic composition comprising a polypeptide, wherein the polypeptide comprises an amino acid sequence that varies from SEQ ID NO: 1 at one or more positions, wherein the variation(s) are selected from the group consisting of a deletion of a signal peptide (amino acids 1-20) of SEQ ID NO: 1; a Thr substitution of Ile at position 20 of SEQ ID NO: 1, a Val substitution of Ala at
position 23 of SEQ ID NO: 1, a Glu substitution of Lys at position 24 of SEQ ID NO: 1, a Met substitution of Val at position 28 of SEQ ID NO: 1, a Thr substitution of Ala at position 31 of SEQ ID NO: 1, a Val substitution of Ile at

position 41 of SEQ ID NO: 1, an Ala substitution of Val at position 47 of SEQ ID NO: 1, a Lys substitution of Gly at position 152 of SEQ ID NO: 1, a Val substitution of Ala at position 154 of SEQ ID NO: 1, a Ser-Ala-Pro addition at the C-terminus of SEQ ID NO: 1, a Val substitution of Pro at position 32 of SEQ ID NO: 1, and an Ala substitution of Pro at position 32 of SEQ ID NO: 1.

60. An immunogenic composition comprising a polypeptide, wherein the polypeptide comprises an amino acid sequence that varies from SEQ ID NO: 1 in at least three positions, wherein the variation(s) are selected from the group consisting of a deletion of a signal peptide (amino acids 1-20) of SEQ ID NO: 1; a Thr substitution of Ile at position 20 of SEQ ID NO: 1, a Val substitution of Ala at position 23 of SEQ ID NO: 1, a Glu substitution of Lys at position 24 of SEQ ID NO: 1, a Met substitution of Val at position 28 of SEQ ID NO: 1, a Thr substitution of Ala at position 31 of SEQ ID NO: 1, a Val substitution of Ile at position 41 of SEQ ID NO: 1, an Ala substitution of Val at position 47 of SEQ ID NO: 1, a Lys substitution of Arg at position 76 of SEQ ID NO: 1, a Val substitution of Ile at position 107 of SEQ ID NO: 1, a Lys substitution of Gly at position 152 of SEQ ID NO: 1, a Val substitution of Ala at position 154 of SEQ ID NO: 1, a Ser-Ala-Pro addition at the C-terminus of SEQ ID NO: 1, a Val substitution of Pro at position 32 of SEQ ID NO: 1, and an Ala substitution of Pro at position 32 of SEQ ID NO: 1.