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(54)Title: **METHODS FOR JUDGING THE POSSIBILITY OF THE ONSET OF BOVINE LEUKEMIA AND THE RESISTANCE THERETO**

(54)発明の名称 ウシ白血病の発症可能性及び抵抗性の判定方法

(57) **Abstract**

A method for judging the possibility of the onset of bovine leukemia caused by bovine leukemia virus (BLV) wherein an individual carrying the amino acid sequence Val-Asp-Thr-Tyr as the one specified by the amino acid numbers 75 to 78 in the β1 domain of the bovine MHC Class II DRβ chain is judged to have a fear of the onset of this disease; and a method for judging the resistance to the onset of bovine leukemia caused by BLV wherein an individual carrying the amino acid Val as the one specified by the amino acid number 78 in the β1 domain of the bovine MHC Class II DRβ chain is judged to be resistant thereto.

（５７）要約

ウシ白血病ウイルスBLV に対するウシの白血病発症の可能性を判定する方法であって、ウシ個体のウシMHC ClassII DRβ鎖のβ１ドメインのアミノ酸番号75から78で特定されるアミノ酸配列がVal-Asp-Thr-Tyr であるウシ個体を白血病の発症可能性ありと判定する方法；並びに、ウシ白血病ウイルスBLV に対するウシの白血病発症の抵抗性を判定する方法であって、ウシ個体のウシMHC ClassII DRβ鎖のβ１ドメインのアミノ酸番号78で特定されるアミノ酸がVal であるウシ個体を白血病の発症抵抗性ありと判定する方法。

Abstract

A method for judging a possibility of the onset of bovine leukemia caused by bovine leukemia virus BLV, wherein a bovine individual, in which an amino acid sequence defined by the amino acid numbers 75 to 78 of the β 1 domain of the bovine MHC Class II DRβ chain is Val-Asp-Thr-Tyr, is judged to have a possibility of the onset of the leukemia; and a method for judging a resistance to the onset of bovine leukemia caused by the bovine leukemia virus BLV, wherein a bovine individual, in which an amino acid defined by the amino acid number 78 of the β 1 domain of the bovine MHC Class II DRβ chain is Val, is judged to have a resistance to the onset of the leukemia.

Specification

Methods for judging a possibility of the onset of bovine leukemia and a resistance thereto

Technical Field

The present invention relates to a method for judging a possibility of the onset of bovine leukemia caused by bovine leukemia virus BLV and a resistance to the onset of the leukemia.

Background Art

The major histocompatibility antigens (MHC antigens) are molecules involved in self-nonself differentiation in the defense mechanism of the living body against infection. They are classified into Class I molecule composed of α chain and β2M, and class II molecule composed of α chain and β chain. A groove for trapping an antigen peptide is present on the α1 and α2 domains, and also on the α1 and β1 domains. They are featured to have the T cell receptor recognize only a fragmented peptide trapped in the groove, thereby achieve cell death (cellular immunity) by CD8+ cells which have recognized the class I antigens, as well as induce mainly antibody production (humoral immunity) by CD4+ cells which have recognized the class II antigens.

The MHC genes constitute a gene group most full of polymorphism, and the locations of pockets, shapes, sizes and properties of the peptide trapping grooves are different among haplotypes. It is considered that association conditions of the trapped fragment peptides may vary depending on these differences, which decide immune response and disease sensitivity of each individual. The correlation between the MHC haplotypes and a resistance to a disease (disease insusceptibility) or a possibility of the onset of a disease (disease susceptibility) has been reported, for example, as to human immune deficiency virus (HIV), human T cell leukemia virus (HTLV) and malaria.

As for the bovine MHC (BoLA) class II genes, existence of DQA, DQB, DRA, DRB, DNA, DOB, DYA, and DYB genes has been estimated. DRB3, inter alia, which

is one of the three genes (DRB1 to B3) identified on the DRB genetic locus, has been known to encode a functional protein, and existence of 73 alleles has been revealed so far. However, there is almost no report about correlation between bovine infectious diseases and the bovine MHC (BoLA) haplotypes.

In particular, as to the bovine leukemia virus (BLV), which has the gene PX that regulates virus proliferation in the same manner as the human immunodeficiency virus (HIV) and is a retrovirus most related to HTLV-I, a research group in the United States has reported its relationship with the bovine MHC (BoLA) haplotypes mainly focusing disease resistance; however, its relationship with possibility of onset of the leukemia has not been reported. The ratio of cattle infected by this virus (infection rate in Japan) is 10-20%, and 1-2% of the infected cattle develops extremely malignant endemic bovine leukemia to die after a long latent period of 10-15 years. Therefore, economic loss of stockbreeders caused by the virus is very serious. If a possibility of the onset of a cattle after BLV infection can be evaluated by the analysis of bovine MHC (BoLA) haplotypes, it becomes possible to preliminarily select disease resistant cattle for bleeding, and it is expected that extremely safe cattle breeding can be continued.

Accordingly, an object of the present invention is to elucidate the relationship between the bovine leukemia virus (BLV) and the bovine MHC (BoLA) haplotypes, and to provide a method for convenient judgement of a possibility of the onset of leukemia of a cattle caused by the bovine leukemia virus (BLV) and a resistance to the onset of the leukemia by means of genetic engineering techniques. Another object of the present invention is to provide a primer set useful for the aforementioned method for judgement.

Disclosure of the Invention

The inventors of the present invention previously analyzed the structure of DRB gene locus among the bovine MHC (BoLA) class II genes, and reported the structures of DRB3 gene (BoLA-DRB3) and the gene product thereof (Biochem. Biophys. Res. Commun., 209, pp.981-988, 1995). The inventors further studied the function of the gene and found that a portion is present, whose amino acid sequence is distinctly different between a cattle developing the leukemia and a cattle not

developing the disease, in the gene product from the second exon (β 1 domain) of BoLA-DRB3 showing particularly noticeable polymorphism. They also found that the amino acid substitutions directly correlated with disease susceptibility to BLV and disease resistance. The present invention was achieved on the basis of these findings.

The present invention thus provides a method for judging a possibility of the onset of bovine leukemia caused by bovine leukemia virus BLV, wherein a bovine individual, in which an amino acid sequence defined by the amino acid number from 75 to 78 of the β 1 domain of the bovine MHC Class II DRβ chain is Val-Asp-Thr-Tyr, is judged to have a possibility of the onset of the leukemia. As preferred embodiments of the method of the present invention, there are provided the aforementioned method which is applied to a cattle infected by the bovine leukemia virus BLV; and the aforementioned method wherein a bovine individual, in which an amino acid sequence defined by the amino acid numbers 75-78 of the β 1 domain of the bovine MHC Class II DRβ chain is Val-Asp-Thr-Tyr in both of the alleles, is judged to have a risk of the onset.

According to another embodiment of the method of the present invention, there is provided a method for judging a possibility of the onset of bovine leukemia caused by the bovine leukemia virus BLV, which comprises the steps of:

(1) amplifying genomic DNA isolated from a bovine individual by the polymerase chain reaction (PCR) to prepare a PCR product containing a DNA coding for a part or full length of the β 1 domain of the bovine MHC Class II DRβ chain, and

(2) judging that the bovine individual, in which an amino acid sequence corresponding to the amino acid number from 75 to 78 of the β 1 domain of the bovine MHC Class II DRβ chain is Val-Asp-Thr-Tyr in the amino acid sequence encoded by the DNA contained in the PCR product, has a possibility of the onset of the leukemia. A preferred embodiment of the aforementioned method comprises a step of digesting the PCR product by using PstI.

According to another aspect of the present invention, there is provided a method for judging a resistance to the onset of bovine leukemia caused by the bovine leukemia virus BLV, wherein a bovine individual, in which an amino acid defined by the amino acid number 78 of the β 1 domain of the bovine MHC Class II DRβ chain is Val, is judged to have resistant to the onset of the leukemia. As preferred

embodiments of the method of the present invention, there are provided the aforementioned method which is applied to a cattle infected by the bovine leukemia virus BLV; the aforementioned method wherein the bovine individual, in which the amino acid specified by the amino acid number 78 of the β1 domain of the bovine MHC Class II DRβ chain is Val in at least one of the alleles, is judged to have a resistance to the onset; and the aforementioned method wherein the bovine individual, in which the amino acid specified by the amino acid number 78 of the β1 domain of the bovine MHC Class II DRβ chain is Val in both of the alleles, is judged to have a high resistance to the onset.

According to another embodiment of the method of the present invention, there is provided a method for judging a resistance to the onset of bovine leukemia caused by the bovine leukemia virus BLV, which comprises the steps of:

(1) amplifying genome DNA isolated from a bovine individual by the polymerase chain reaction (PCR) to prepare a PCR product containing a DNA coding for a part or full length of the β1 domain of the bovine MHC Class II DRβ chain, and

(2) judging that the bovine individual, in which an amino acid corresponding to amino acid number 78 of the β1 domain of the bovine MHC Class II DRβ chain is Val in the amino acid sequence encoded by the DNA contained in the PCR product, has a resistance to the onset of the leukemia. A preferred embodiment of the aforementioned method comprises a step of digesting the PCR product by using PstI.

According to preferred embodiments of these inventions, there are provided each of the primer sets set out below, and the aforementioned methods wherein said primer set is used, preferably in those applied to cattle infected by the bovine leukemia virus BLV. The present invention further provides the following primer sets (1) to (3) each consisting of A primer and B primer, which are used for judging a possibility of the onset of bovine leukemia caused by the bovine leukemia virus BLV:

Primer set (1)

A primer: 5'-TGTAAAACGACGGCCAGTCTCTCTCTGCAGCACATTTCCT-3'

B primer: 5'-CAGGAAACAGCTATGACCCGCCGCTGCACAGTGAAACTC-3'

Primer set (2)

A primer: 5'-GGAATTCCTCTCTCTGCAGCACATTTCCT-3'

B primer: 5'-AAGTCGACCGCTGCACAGTGAAACTC-3'

Primer set (3)

A primer: a primer which is selected from the group consisting of

5'-GAGTGTCATTTCTTCAACGGGAC-3',

5'-GGAGAAGAGTTCGTGCGCTTCGA-3', and

5'-GGAATTCCTCTCTCTGCAGCACATTTCCT-3'

B primer: 5'-AAGTCGACCGCTGCACAGTGAAACTC-3'

Brief Description of the Drawings

Figure 1 depicts the structure of the bovine MHC Class II DRβ chain. In the figure, (A) shows the structure of bovine MHC Class II DRβ chain mRNA, and (B) shows the full length cDNA coding for the bovine MHC Class II DRβ chain and the amino acid sequence of the gene product. The β1 domain is a portion defined by the amino acid sequence of the amino acid number from 1 to 94.

Figures 2(A) to (C) show the results of comparison of amino acids of the β1 domain of the bovine MHC Class II DRβ chain (amino acid sequences defined by the amino acid number from 9 to 86) derived from cattle infected by the bovine leukemia virus BLV but not developing the disease ((A): 7 cattle developing lymphocytosis, and (B) and (C): antibody positive 24 healthy cattle not developing the disease). The numbers at the left end are ID numbers of bovine individuals, and amino acids indicated as one letter symbols in the figure.

Figures 3 (A) and (B) show the results of comparison of amino acids of the β1 domain of the bovine MHC Class II DRβ chain (amino acid sequences defined by the amino acid number from 9 to 86) derived from cattle developing leukemia (24 cattle). The numbers at the left end are ID numbers of bovine individuals, and amino acids are indicated as one letter symbols in the figure.

Best Mode for Carrying Out the Invention

The method of the present invention is applied to bovine individuals, including cattle infected with the bovine leukemia virus BLV and cattle not infected with the virus, in order to judge a possibility of the onset of the leukemia of the individuals. Another method of the present invention is applied to bovine individuals, including cattle infected with the bovine leukemia virus BLV and cattle not infected

with the virus, in order to judge a resistance to the onset of the leukemia of the individuals.

According to a preferred embodiment of the present invention, genomic DNA of a bovine individual is isolated, and a gene coding for a part or the full length of the β 1 domain of DR β chain of the bovine MHC Class II (the second exon of DRB3 gene) is amplified by the PCR method, and then the resulting PCR product is subjected to a sequencing to deduce the amino acid sequence defined by the amino acid number from 75 to 78 of the β 1 domain. When a bovine individual, in which the amino acid sequence (amino acid numbers 75 to 78) is Val-Asp-Thr-Tyr (indicated as VDTY in the one letter symbols), is already with infection by the bovine leukemia virus BLV, or when the individual will suffer from infection by the bovine leukemia virus BLV, the bovine individual has a possibility of the onset of the leukemia. Whether or not a bovine individual is infected by the bovine leukemia virus BLV can be readily verified by a test using an antibody recognizing the bovine leukemia virus BLV.

In order to carry out more accurate judgement, it is preferred to compare the aforementioned amino acid sequences in the alleles (haplotypes). When the amino acid sequence (amino acid number 75 to 78) is Val-Asp-Thr-Tyr in both of the alleles (i.e., VDTY homozygote), the bovine individual has a high risk of the onset of the leukemia when the individual is already infected by the bovine leukemia virus BLV, or will suffer from the infection by the virus. On the other hand, when the amino acid sequences in the alleles are heterozygote of Val-Asp-Thr-Tyr (VDTY) and Val-Asp-Thr-Val (VDTV); heterozygote of Val-Asp-Thr-Tyr (VDTY) and Val-Asp-Arg-Val (VDRV); homozygote of Val-Asp-Thr-Val (VDTV); homozygote of Val-Asp-Arg-Val (VDRV); heterozygote of Val-Asp-Arg-Val (VDRV) and Val-Asp-Thr-Val (VDTV) or the like, the bovine individual has a very low possibility of the onset of the leukemia even if the bovine individual is already infected by the bovine leukemia virus BLV.or will suffer from the infection by the virus.

Furthermore, from a viewpoint of a resistance to the onset of he leukemia, the amino acid defined by the amino acid number 78 of the β 1 domain may be deduced. When a bovine individual having Val (represented as V in the one letter symbol) as the amino acid (i.e., amino acid number 78) is already infected by the bovine leukemia virus BLV, or will suffer from infection by the virus, the bovine individual is resistant

to the onset of the leukemia. Also for the judgement of the resistance, it is preferred to compare the aforementioned amino acid in the alleles (haplotypes). When the amino acid, defined by the amino acid number 78 of the β 1 domain, is Val in at least one of the alleles, the individual has a resistance to the onset of the leukemia, and when the above amino acid is Val in both of the alleles, the individual has a high resistance to the onset of the leukemia.

The amino acid sequence of the β 1 domain of the bovine MHC Class II DRβ chain was reported by Aida et al. (Aida, Y., et al., Biochem. Biophys. Res. Commun., 209, pp.981-988, 1995). The structure of mRNA of the bovine MHC Class II DRβ chain (A), and the full length cDNA and the amino acid sequence of the gene product (B) are shown in Figure 1. In the figure, the β 1 domain is a portion defined by the amino acid sequence of amino acid number from 1 to 94, and the nucleotide sequence and the amino acid sequence are shown where the peptide sequence of the amino acid number from 75 to 78 is "Val-Asp-Thr-Tyr (VDTY)".

Cattle to be judged by the method according to the present invention are not particularly limited. The method may be applied to any sorts of cattle including dairy cattle, dairy and beef cattle, beef cattle, working cattle, working and beef cattle and the like, so long as they may be infected by the bovine leukemia virus BLV and have a possibility of developing the leukemia owing to the infection. More specifically, examples include Japanese cattle such as Japanese Black and Japanese Shorthorn, or breeds such as Holstein, Jersey, Hereford, Aberdeen Angus, and Friesian. However, breeds are not limited to these examples.

As a sample for preparing genomic DNA from bovine individuals, peripheral blood, organ and the like can be utilized. For example, a tissue section of the lymph node and other may be used as the organ. As methods for preparing genomic DNA from the sample mentioned above, any methods available to those skilled in the art can be employed. When peripheral blood leucocytes or peripheral blood lymphocytes are used as a sample, for example, the method of Hughes et al. (Hughes, S.H., Cell, 15, pp.1397-1410, 1978) may be applied. When an organ is used, for example, a frozen tissue section may be sliced by using scissors, and then treated by the sodium dodecylsulfate and phenol-chloroform method (Mcknight, G.S., Cell, 14, pp.403-413, 1978) to obtain genomic DNA. The simplified extraction of genomic DNA from cells

may also be used, whose details are described in the examples.

As primers used for amplifying the resulting genomic DNA by the PCR method, any primers may be used so long as they can amplify a DNA containing a gene coding for a partial amino acid sequence of amino acid number from 75 to 78 of the β 1 domain of the DRβ chain of the bovine MHC Class II or the full length of the β 1 domain.

An example of a primer set most suitably used for the methods of the present invention includes primer set (1):

A primer: 5'-TGTAAAACGACGGCCAGTCTCTCTCTGCAGCACATTTCCT-3'; and

B primer: 5'-CAGGAAACAGCTATGACCCGCCGCTGCACAGTGAAACTC-3'

which enables direct sequencing methods such as the cycle sequencing and the Dynabeads DNA direct sequencing. As primer sets introduced with a restriction endonuclease cleavage site, primer set (2):

A primer: 5'-GGAATTCCTCTCTCTGCAGCACATTTCCT-3'; and

B primer: 5'-AAGTCGACCGCTGCACAGTGAAACTC-3',

or primer set (3):

A primer: a primer selected from the group consisting of:

5'-GAGTGTCATTTCTTCAACGGGAC-3',

5'-GGAGAAGAGTTCGTGCGCTTCGA-3', and

5'-GGAATTCCTCTCTCTGCAGCACATTTCCT-3'; and

B primer: 5'-AAGTCGACCGCTGCACAGTGAAACTC-3'

may be utilized. In particular, by digesting PCR alleles with PstI that are amplified by using the primer set (3), and then observing differences in the resulting cleavage patterns, it can easily judge whether or not the bovine individual is resistant to the leukemia, or whether or not the individual has a possibility of the onset of the leukemia. However, primers and primer sets which may be used for the methods of the present invention are not limited to the forgoing examples.

An amount of DNA used for the PCR method can be appropriately chosen. For example, the amount may be about 0.1-0.5 μg when peripheral blood leucocytes or peripheral lymphocytes are used. As sequencing methods applied to the DNA amplified as descried above (the PCR product), any methods available to those skilled in the art may be utilized. For example, the direct sequencing may preferably be used,

whose specific examples are described in the examples. Most of cattle are heterozygotes, and when alleles derived from father and mother cattle may have different nucleotide sequences, the direct sequencing may fail to determine which of the alleles corresponds to the target sequence. In that case, the PCR product amplified by using the above primer set (2) may be digested with restriction endonuclease EcoRI and Sal I, and then subcloned into a vector to carry out the sequencing of only one of the alleles, and the results may be referred to for comparison to enable a definite sequencing of the other allele. To obtain more precise genetic information, it is preferred that both of the alleles from the PCR product are subcloned and each of the nucleotide sequences is determined. The specific method and applicable primers are detailed in the following examples.

Examples

The present invention will be explained more specifically by referring to examples. However, the scope of the present invention is not limited to the examples set out below.

Example 1: Examination of a possibility of the onset of the leukemia

Peripheral blood was collected as a sample from a bovine individual by using a syringe containing an anticoagulant, and centrifuged under conditions of 4℃ and 3,000 rpm for 20 minutes to obtain a leucocyte layer. The separated leucocyte layer was washed with phosphate buffered saline (PBS) and centrifuged to obtain a pellet, which was used as a sample of peripheral blood leucocyte. Peripheral blood lymphocytes were also obtained by the method of Miyasaka et al. (Miyasaka, M. and Trnka, Z., Immunological Methods, Vol.3, pp.403-423, 1985, Academic Press, NY) from peripheral blood obtained in the same manner as described above, and a sample of peripheral lymphocyte was prepared by obtaining a pellet as described above. A BLV infected cell suspension was centrifuged under conditions of 4℃ and 1,100 rpm for 5 minutes to remove a culture medium, and the cells were washed with PBS and centrifuged to obtain a pellet as a sample. In addition, tissue sections were isolated from the lymph node and a tumor tissue of a cattle which developed BLV infection lymphosarcoma, and rapidly frozen in liquid nitrogen without immobilization, and

then stored at -80℃ as samples of the tissue sections.

Each of the above sample cells were washed twice with PBS in a 1.5 ml-microcentrifugal tube, and the precipitated cells were suspended again in PBS by using a vortex mixer. To 1×10^6 cells, 200 μl of 1×PCR buffer [10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.5% Tween-20] and 1 μl of Proteinase K (20 mg/ml) were added, and the cells were suspended again by a vortex mixer and incubated at 56℃ for 45-60 minutes. The mixture was further treated at 95℃ for ten minutes, and cooled on ice for 5 minutes or more. About 5-10 μl of the reaction mixture was used for amplification by PCR.

The genome DNA was dissolved in 50 μl of 1×PCR buffer [10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% (w/v) gelatin] containing 200 μM of each dNTP, 0.2-0.4 μM of primers, and 2.5 units of Taq polymerase (Gene Amp Kit; Perkin-Elmer Cetus), and then subjected to amplification by 25 cycles, each cycle consisting of treatments at 94℃ for 1 minute, at 61℃ for 1 minute, and at 72℃ for 1 minute, and then further treated at 72℃ for 5 minutes. As the primers, the following primers were used:

A primer: 5'-TGTAAAACGACGGCCAGTCTCTCTCTGCAGCACATTTCCT-3'

B primer: 5'-CAGGAAACAGCTATGACCCGCCGCTGCACAGTGAAACTC-3'

which can specifically amplify the β1 domain of the bovine MHC Class II DRβ chain (β1 domain of BoLA-DR β: the second exon of DRB3 gene) by the PCR method. Specific biotinylation was introduced into the 5' end of the B primer. These primers can be suitably used for the cycle sequencing.

20 μl of DYNABEADS M-280 Streptoavidin (Dynal A.S, N-0212, Oslo, Norway) was washed with 100 μl of 2×binding-washing buffer (B&W buffer: 10 mM Tris-HCl (pH 7.5), 1.0 mM EDTA, 2M NaCl, 0.1% Tween-20), and the beads were suspended again in 80 μl of 2×B&W buffer. The above PCR product (50 μl) was added to the bead suspension and gently mixed by pipetting, and then incubated at room temperature for 15 minutes with slow rotation using a wheel rotator. The tube containing the immobilized PCR product was put on a magnet (Dynal MPC) and the supernatant was removed by using a pipet, and then 100 μl of 2×B&W buffer was added to wash the beads. The supernatant was removed again by using a magnet, and the residue was suspended in 50 μl of 0.1 M NaOH prepared just before use.

The beads immobilizing the biotinylated chains were gathered on the tube wall by using a magnet and the supernatant was removed, and then the beads were washed once with 50 μl of 0.1 M NaOH and three times with 100 μl of 1×B&W buffer, and once with 50 μl of TE buffer. In every operation, the beads were resuspended with smooth strokes. After washing with 100 μl of distilled water, the supernatant was removed, and distilled water was added to the residue to adjust the volume for the use in a sequencing. The sequencing was performed by using BcaBEST Dideoxy Sequencing Kit (Takara Biomedicals) and according to the conditions described in the attached instructions. The following primers were used as sequencing primers.

Forward primer: 5'-TGTAAAACGACGGCCAGT-3'

Reverse primer: 5'-CAGGAAACAGCTATGACC-3'

The results are shown in Figs. 2 and 3 (in the figures, amino acids of number 9 to 86 of the β1 domains of the bovine MHC Class II DRβ are shown, and the numbers at the left end are ID numbers of bovine individuals). By comparing the amino acids of the β1 domain of bovine MHC Class II DRβ derived from cattle infected by the bovine leukemia virus but not developing the leukemia [7 cattle with lymphocytosis (pre-cancer state), and 24 cattle not developing the leukemia (antibody positive healthy cattle not developing the disease), top and bottom of Figure 2, respectively], and cattle already developing the leukemia (24 cattle, Figure 3), a markedly characteristic result was obtained that the cattle with the developed leukemia had Val-Asp-Thr-Tyr (VDTY) motif as the sequence of amino acid number from 75 to 78 in both of the alleles. The portion of the amino acids from 75 to 78 is located on an α-helix of the β1 domain, and may have a function as a T cell recognition site. Furthermore, as a result of an analysis using a computer, it was revealed that this motif exists only in pol protein in the bovine leukemia virus BLV.

The above results are summarized in Table 1. The representation of the genotype such as VDTY/VDTY in the table indicates amino acid sequences of the both allele (amino acid number 75 to 78 of the β1 domain of the bovine MHC Class II DR β chain) described as the one letter symbols. The infection status of the BLV infected cattle were classified according to the criteria of Levy et al. (Levy, D., et al., Int. J. Cancer, 19, pp.822-827, 1977) and Aida et al. (Aida, Y., et al., Cancer Res., 52,

pp.6463-6470, 1992).

Table 1

Genotype	BLV infection status (positive rate)		
	Development of leukemia	Lymphocytosis	Healthy
VDTY/VDTY	19/24	5/7	4/24
VDTY/VDTV	2/24	2/7	2/24
VDTY/VDRV	2/24	0/7	14/24
VDRV/VDRV	0/24	0/7	1/24
VDTV/VDTV	1/24	0/7	0/24
VDTV/VDRV	0/24	0/7	3/24

Example 2: Study on resistance to the onset of leukemia

It the same manner as in Example 1, kinds of the amino acid at number 78 of the β 1 domains of bovine MHC Class II DRβ was determined for cattle developing the leukemia (24 cattle), cattle not developing the leukemia (cattle with lymphocytosis and healthy cattle, 31 individuals in total), and the results are shown in Table 2. The kind of the amino acids at numbers 71 and 74 were also determined (in the table, amino acids are indicated as one letter symbols, Y: Tyr; V: Val; R: Arg; E: Glu; K: Lys; and N: Asn). As a result, it was revealed that individuals where the 78th amino acid was heterozygote of valine and tyrosine, and individuals where the 78th amino acid was homozygote of valine were resistant to the onset of the leukemia, and in particular, the individuals where the 78th amino acid was homozygote of valine were highly resistant to the onset of the leukemia. Furthermore, because all of the 74th amino acids of cattle not developing the leukemia were Gln or Asn, and the 71st amino acid residues were Lys or Arg, it was suggested that individuals having the allele where the 71st amino acid is lysine or arginine, the 74th amino acid is glutamic acid or asparagine, and the 78th amino acid is valine have high resistant to the onset of the leukemia.

Table 2

Genotype	BLV infection status	Positive rate
Y^{78}/Y^{78}	Cattle developing leukemia	19/24
V^{78}/Y^{78}	Cattle developing leukemia	4/24
	(R^{71}-E^{74}-V^{78}/Y^{78}:	3/24)
	(K^{71}-E^{74}-V^{78}/Y^{78}:	1/24)
	(K^{71}-N^{74}-V^{78}/Y^{78}:	0/24)
V^{78}/V^{78}	Cattle developing leukemia	1/24
	(R^{71}-E^{74}-V^{78}/R^{71}-E^{74}-V^{78}:	1/24)
	(K^{71}-E^{74}-V^{78}/K^{71}-E^{74}-V^{78}:	0/24)
	(K^{71}-N^{74}-V^{78}/K^{71}-N^{74}-V^{78}:	0/24)
Y^{78}/Y^{78}	Cattle not developing leukemia	9/31
V^{78}/Y^{78}	Cattle not developing leukemia	18/31
	(R^{71}-E^{74}-V^{78}/Y^{78}:	11/31)
	(K^{71}-E^{74}-V^{78}/Y^{78}:	4/31)
	(K^{71}-N^{74}-V^{78}/Y^{78}:	3/31)
V^{78}/V^{78}	Cattle not developing leukemia	4/31
	(R^{71}-E^{74}-V^{78}/R^{71}-E^{74}-V^{78}:	3/31)
	(K^{71}-E^{74}-V^{78}/K^{71}-E^{74}-V^{78}:	1/31)
	(K^{71}-N^{74}-V^{78}/K^{71}-N^{74}-V^{78}:	0/31)

Example 3: Method for quick judgement of possibility and resistance to the onset

As described above, individuals having the gene coding for Val as the amino acid at number 78 of the β1 domain of the bovine MHC Class II DRβ chain are resistant to the leukemia caused by the bovine leukemia virus, whereas individuals where the 78th amino acid is Tyr in both of the alleles have a possibility of the onset of the leukemia. Therefore, whether or not an bovine individual is leukemia resistant, or whether or not an individual has a possibility of the onset of the leukemia is easily judged by utilizing restriction endonuclease PstI cleavage site which is present in a gene where the 78th allele is Val but absent in a gene where the 78th allele is Tyr, i.e., by digesting PCR amplified alleles and differentiating the cleavage pattern.

The following primers were used as PCR primers.

A primer

DRB40: 5'-GAGTGTCATTTCTTCAACGGGAC-3'

DRB100: 5'-GGAGAAGAGTTCGTGCGCTTCGA-3'

ERB3: 5'-GGAATTCCTCTCTCTGCAGCACATTTCCT-3'

B primer

SRB3: 5'-AAGTCGACCGCTGCACAGTGAAACTC-3'

The conditions of the PCR were similar to the conditions of Example 1. Specifically, amplification was performed by 35 cycles, each cycle consisting of the following steps depending on the combination of the primers, followed by a treatment at 72℃ for 10 minutes. The genomic DNA was used in an amount of 100 ng for 100 μl of the PCR system.

DRB40/SRB3: 94℃ for 1 minute, 63℃ for 2 minutes, 72℃ for 2 minutes

DRB100/SRB3: 94℃ for 1 minute, 66℃ for 2 minutes, 72℃ for 2 minutes

ERB3/SRB3: 94℃ for 1 minute, 61℃ for 2 minutes, 72℃ for 2 minutes

The PCR product was subjected to 2% agarose gel electrophoresis, and then cleaved by using restriction endonuclease PstI (1.2 μl of 10×restriction endonuclease buffer, 6-7 μl of DNA after amplification, 2 units of restriction endonuclease PstI, and H_2O in the total volume of 12 μl). After completion of the reaction with the restriction endonuclease, each specimen was examined by 3% agarose gel electrophoresis for judgement. The results are shown in Table 3.

Table 3

Primer	PCR product	Size (bp) of PstI fragment			
	(bp)	Allele for Y	Allele for V	Y/Y	Y/V
DRB40/SRB3	247	199, 48	247	199, 48	247, 199, 48
DRB100/SRB3	187	139, 48	187	139, 48	139, 187, 48
ERB3/SRB3*	292	226, 48	274	226, 48	226, 274, 48

*PstI cleavage site is present in the ERB3 primer, and hence a 18 bp fragment was contained in each reaction mixture.

Industrial Applicability

A possibility of the onset of the leukemia caused by the bovine leukemia virus (BLV) and a resistance thereto of a bovine individual can be surely estimated by the methods of the present invention. Therefore, the invention enables safe cattle breeding and achieves prevention of economic loss of stockbreeders.

What is claimed is:

1. A method for judging a possibility of the onset of bovine leukemia caused by bovine leukemia virus BLV, wherein a bovine individual, in which an amino acid sequence defined by the amino acid numbers 75 to 78 of the β1 domain of the bovine MHC Class II DRβ chain is Val-Asp-Thr-Tyr, is judged to have a possibility of the onset of the leukemia.

2. The method according to claim 1 wherein a bovine individual, in which an amino acid sequence defined by the amino acid numbers 75-78 of the β1 domain of the bovine MHC Class II DRβ chain is Val-Asp-Thr-Tyr in both of the alleles, is judged to have a risk of the onset.

3. A method for judging a possibility of the onset of bovine leukemia caused by bovine leukemia virus BLV, which comprises the steps of:
(1) amplifying genome DNA isolated from a bovine individual by the polymerase chain reaction (PCR) to prepare a PCR product containing a DNA coding for a part or full length of the β1 domain of the bovine MHC Class II DRβ chain, and
(2) judging that the bovine individual, in which an amino acid sequence corresponding to the amino acid numbers 75 to 78 of the β1 domain of the bovine MHC Class II DR β chain is Val-Asp-Thr-Tyr in the amino acid sequence encoded by the DNA contained in the PCR product, has a possibility of onset of the leukemia.

4. The method according to claim 3 which comprises a step of digesting the PCR product by using PstI.

5. A method for judging a resistance to the onset of bovine leukemia caused by the bovine leukemia virus BLV, wherein a bovine individual, in which an amino acid defined by the amino acid number 78 of the β1 domain of the bovine MHC Class II DR β chain is Val, is judged to have resistance to the onset of the leukemia.

6. The method according to claim 5, the bovine individual, in which the amino acid specified by the amino acid number 78 of the β1 domain of the bovine MHC Class II DRβ chain is Val in at least one of the alleles, is judged to have resistance to the onset.

7. The method according to claim 5 wherein the bovine individual, in which the amino acid specified by the amino acid number 78 of the β1 domain of the bovine

MHC Class II DRβ chain is Val in both of the alleles, is judged to have high resistance to the onset.

8. A method for judging a resistance to the onset of bovine leukemia caused by bovine leukemia virus BLV, which comprises the steps of:

(1) amplifying genome DNA isolated from a bovine individual by the polymerase chain reaction to prepare a PCR product containing a DNA coding for a part or full length of the β 1 domain of the bovine MHC Class II DRβ chain, and

(2) judging that the bovine individual, in which an amino acid corresponding to the amino acid number 78 of the β 1 domain of the bovine MHC Class II DRβ chain is Val in the amino acid sequence encoded by the DNA contained in the PCR product, has a resistance to the onset of the leukemia.

9. The method according to claim 8 which comprises a step of digesting the PCR product by using PstI.

10. A primer set used for judgement of a possibility of onset of bovine leukemia caused by bovine leukemia virus BLV or a resistance thereto, which comprises:

(a) A primer: 5'-TGTAAAACGACGGCCAGTCTCTCTCTGCAGCACATTTCCT-3' and

(b) B primer: 5'-CAGGAAACAGCTATGACCCGCCGCTGCACAGTGAAACTC-3'.

11. A primer set used for judgement of a possibility of onset of bovine leukemia caused by bovine leukemia virus BLV or a resistance thereto, which comprises:

(a) A primer: 5'-GGAATTCCTCTCTCTGCAGCACATTTCCT-3' and

(b) B primer: 5'-AAGTCGACCGCTGCACAGTGAAACTC-3'.

12. A primer set used for judgement of a possibility of onset of bovine leukemia caused by bovine leukemia virus BLV or a resistance thereto, which comprises:

(a) A primer which is selected from the group consisting of

5'-GAGTGTCATTTCTTCAACGGGAC-3',

5'-GGAGAAGAGTTCGTGCGCTTCGA-3', and

5'-GGAATTCCTCTCTCTGCAGCACATTTCCT-3', and

(b) B primer: 5'-AAGTCGACCGCTGCACAGTGAAACTC-3'.

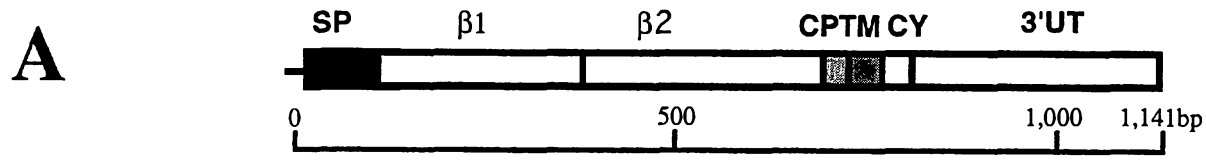

Fig. 1

B

```
     5'UT                SP
-29                    MetValCysLeuTyrPheSerGlyGlySerTrpMetAlaAlaLeuIleValMetLeuMetValLeuCysPro
  0  CTCTGCTGTTCTCCGGCATGGTGTGCCTGTATTTCTCTGGAGGCTCCTGGATGGCAGCTCTGATAGTGATGCTGATGGTGCTGTGCCCT

                  β1
 -5  ProLeuAlaTrpAlaArgGluIleGlnProHisPheLeuGluTyrThrLysLysGluCysHisPhePheAsnGlyThrGluArgValArg
 90  CCCCTGGCCTGGGCCAGGGAGATCCAACCACATTTCCTGGAGTATACCAAGAAAGAGTGTCATTTCTTCAACGGGACCGAGCGGGTGCGG

 26  PheLeuAspArgTyrPheHisAsnGlyGluGluPheValArgPheAspSerAspTrpGlyGluTyrArgAlaValThrGluLeuGlyArg
180  TTCCTGGACAGATACTTCCATAATGGAGAAGAGTTCGTGCGCTTCGATAGCGACTGGGGCGAGTACCGGGCGGTGACCGAGCTAGGGCGG

 56  ProAspAlaLysTyrTrpAsnSerGlnLysAspPheLeuGluGluLysArgAlaAlaValAspThrTyrCysArgHisAsnTyrGlyVal
270  CCGGACGCCAAGTACTGGAACAGCCAGAAGGACTTCCTGGAGGAGAAGCGGGCCGCGGTGGACACGTACTGCAGACACAACTACGGGGTC

                  β2
 86  GlyGluSerPheThrValGlnArgArgValGluProIleValThrValTyrProAlaLysThrGlnProLeuGlnHisHisAsnLeuLeu
360  GGTGAGAGTTTCACTGTGCAGCGGCGAGTGGAACCTATAGTGACTGTGTATCCTGCAAAGACCCAGCCCCTGCAGCACCACAACCTCCTG

116  ValCysSerValAsnGlyPheTyrProGlyAsnIleGluValArgTrpPheArgAsnGlyHisGluGluGluAlaGlyValIleSerThr
450  GTCTGCTCTGTGAACGGTTTCTACCCAGGCAACATTGAAGTCAGGTGGTTCCGGAATGGCCATGAAGAGGAGGCTGGGGTGATCTCCACA

146  GlyLeuIleGlnAsnGlyAspTrpThrPheGlnThrMetValMetLeuGluThrValProGlnSerGlyGluValTyrThrCysGlnVal
540  GGCCTGATCCAGAATGGAGACTGGACCTTCCAGACCATGGTGATGCTTGAAACAGTTCCTCAGAGTGGAGAGGTCTACACCTGCCAAGTG

                                  CP/TM/CY
176  GluHisProSerGlnThrSerProIleThrValGluTrpArgAlaArgSerAspSerAlaGlnSerLysMetMetSerGlyValGlyGly
630  GAGCACCCCAGCCAGACAAGCCCTATCACAGTAGAATGGAGGGCACGGTCTGACTCTGCTCAGAGCAAGATGATGAGTGGAGTCGGGGGC

206  PheValLeuGlyLeuPhePheLeuAlaValGlyLeuPheIleTyrPheArgAsnGlnLysGlyArgProThrLeuGlnProThrGlyLeu
720  TTCGTTCTGGGTCTGTTCTTCCTTGCCGTGGGGCTCTTCATCTACTTCAGGAATCAGAAAGGACGCCCTACACTTCAGCCAACAGGGCTC

          3'UT
236  LeuSerEnd
810  CTGAGCTGAAGTGAAGATGGTCACACTCAAGGAAGAACCTTCTGTCCCAGCTTCTTCACAGCATGGAAAGGTTTCCTGCTTAGTGCTAAC

900  TCTTCCACAATGAAGTACTTTCTCAGGATCTCATTTGCTCCTGGCTCAGTGACCCCTTAAAAACTGTCCTCGATGGTTTTCTCAGTCACC
990  TCCACCCTGCTGCCCTCAGCCTTTGACCTGGAAGTTCTCAATATTGATTCCAGTACCTTATGTTCTTTCTTCCTTGGTTCCCTTTCTTTT
1080 CAACTTCTGTTTCCTGTGCATCTGAGCTCATCTGTTCATTTTACTTTATAATGTGTTCTCTC
```

Cattle with lymphocytosis

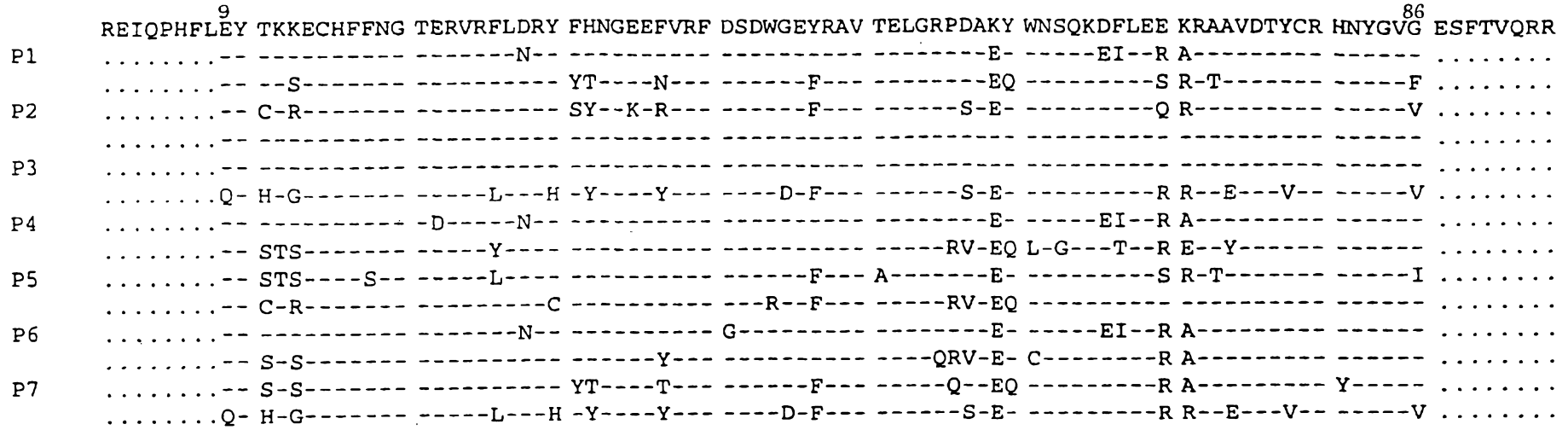

Fig. 2 (A)

Antibody positive healthy cattle not developing leukemia

```
              9                                                                                        86
       REIQPHFLEY TKKECHFFNG TERVRFLDRY FHNGEEFVRF DSDWGEYRAV TELGRPDAKY WNSQKDFLEE KRAAVDTYCR HNYGVG ESFTVQRR
H1     ........-- S-S------- -------E-S -Y----Y--- ---------- --------E- -----EI--- R--E--RV-- ------ ........
       ........-- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ------ ........
H2     ........-- S-S------- -------E-S -Y----N--- ---------- --------E- -----EI--R ---N--RV-- ------ ........
       ........-- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ------ ........
H3     ........-- ---------- -------N-- ---------- ---------- --------E- -----EI--R A--------- ------ ........
       ........-- S-S------- -------E-S -Y----N--- ---------- --------E- -----EY--- R--E--RV-- ------ ........
H4     ........-- S-S------- -------E-S -Y----N--- ---------- --------E- -----EI--R ---N--RV-- ------ ........
       ........-- ---------- -------N-- ---------- ---------- --------E- -----EI--R A--------- -----I ........
H5     ........-- C-R------- ---------C ---------- ------F--- -----RV-EQ ---------- R--E--RV-- -----V ........
       ........-- ---------- -------N-- ---------- ---------- --------E- -----EI--R A--------- ------ ........
H6     ........-- C-S------- -------E-S -Y----N--- ---------- --------E- -----EI--- R--E--RV-- ------ ........
       ........Q- H-G------- -----L---H -Y----Y--- ----D-F--- ------S-E- ---------R R--E---V-- -----V ........
H7     ........-- L-S------- -------E-S -Y----N--- ---------- ---------- ------L--R ---N------ -----V ........
       ........-- C-R------- ---------C ---------- ------F--- -----RV-EQ ---------- R--E--RV-- -----V ........
H8     ........Q- H-G------- -----L---H -Y-------- ----D-F--- ------A-EQ ---------Q ---E--RV-- ------ ........
       ........Q- H-G----L-- -----L---H -Y----Y--- ----D-F--- ------S-E- ---------R R--E---V-- ------ ........
H9     ........-- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ------ ........
       ........-- S-S------- ---------- YT----T--- ------F--- -----Q--E- ---------- ---E--RV-- ----GM ........
H10    ........-- S-S------- -------E-S -Y----N--- ---------- --------E- -----EI--R ---N--RV-- ------ ........
       ........-- ---------- -------N-- ---------- ---------- --------E- -----EI--R A--------- ------ ........
H11    ........-- S-S------- ---------S -Y----N--- ---------- --------E- -----EI--- R--E--RV-- ------ ........
       ........-- ---------- ---------- ---------- ---------- --------E- -----EI--- R--E--RV-- ------ ........
H12    ........-- ---------- -------N-- ---------- ---------- --------E- -----EI--R A--------- ------ ........
       ........-- S-S------- ---------- YT----N--- ------F--- --------EQ ---------S R-T------- -----F ........
```

Fig. 2 (B)

Antibody positive healthy cattle not developing leukemia

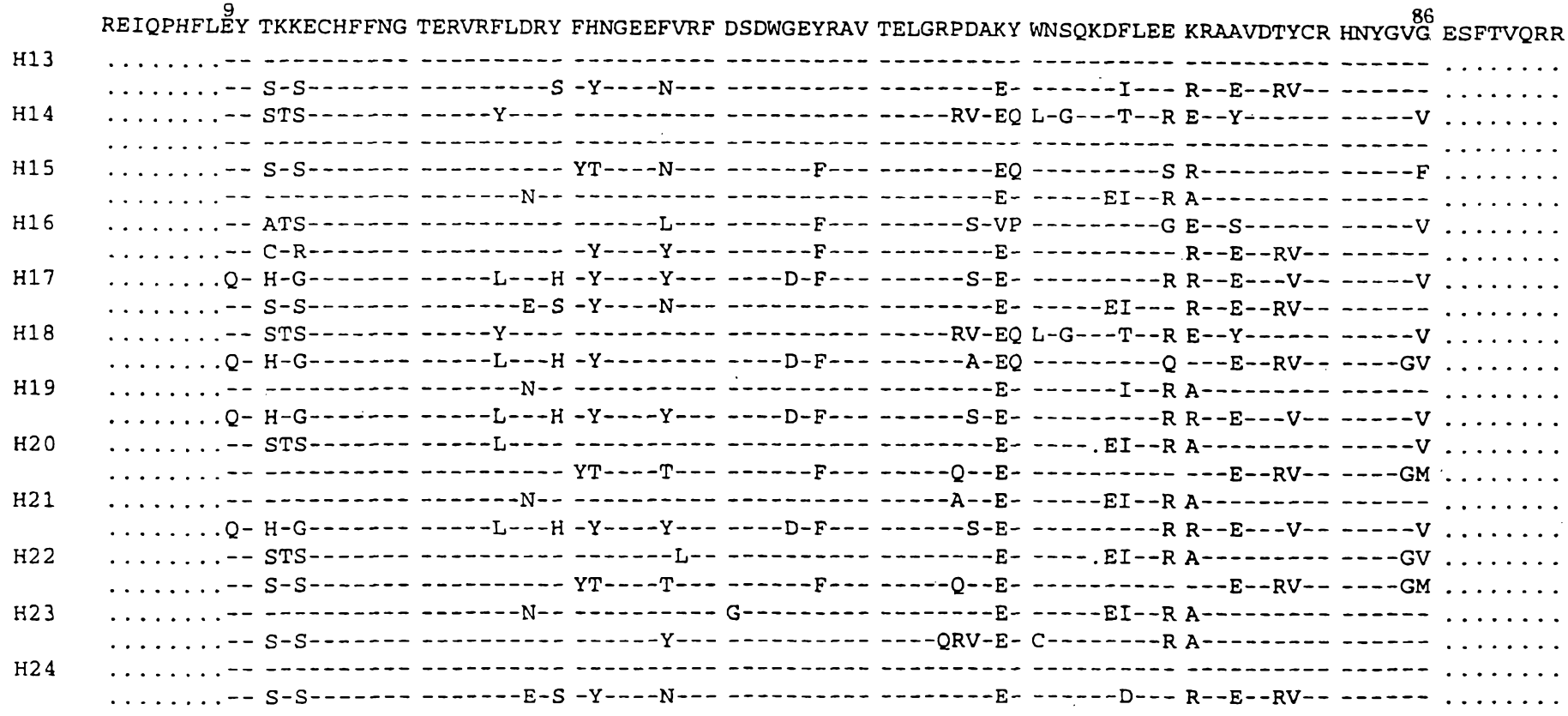

Fig. 2 (C)

Fig. 3 (A)

Cattle developing leukemia

	9					86	
	REIQPHFLEY	TKKECHFFNG	TERVRFLDRY	FHNGEEFVRF	DSDWGEYRAV	TELGRPDAKY	WNSQKDFLEE	KRAAVDTYCR	HNYGVG	ESFTVQRR
L1	.....--	STS-------	----------	----------	----------	--------E-	----.EI--R	A---------	-----V	.....
	.....--	C-R-------	----------	----------	----------	------A--Q	----------	----------	-----V	.....
L2	.....--	S-S-------	-------E-S	-Y----N---	----------	-----RV-EQ	L--------Q	---N------	------	.....
	.....--	----------	----------	----------	----------	----------	R---------	---Q------	------	.....
L3	.....Q-	H-G-------	-----L---H	-Y----Y---	----D-F---	------S-E-	---------R	R--E---V--	-----V	.....
	.....--	----------	----------	----------	----------	----------	----------	----------	------	.....
L4	.....--	----------	-------N--	----------	----------	--------E-	------I--R	A---------	------	.....
	.....--	S-S-------	----------	------Y---	----------	----QRV-E-	C--------R	A---------	------	.....
L5	.....--	S-S-------	----------	------Y---	----------	----QRV-E-	S--------R	A---------	------	.....
	.....--	S-S-------	----------	------Y---	----------	----QRV-E-	C--------R	A---------	------	.....
L6	.....--	----------	----------	----------	----------	----------	----------	----------	------	.....
	.....--	S-S-------	----------	------Y---	----------	----QRV-E-	C--------R	A---------	------	.....
L7	.....--	S-S-------	----------	YT----N---	------F---	--------EQ	---------S	R-T-------	-----F	.....
	.....--	S-S-------	-------E-S	-Y----N---	----------	--------E-	-----EI---	R--E--RV--	------	.....
L8	.....--	STS-------	-----Y----	----------	----------	-----RV-EQ	L-G---T--R	E--Y------	-----V	.....
	.....--	STS-------	-----Y----	----------	----------	-----RV-RQ	L-G---T--R	E--Y------	-----V	.....
L9	.....Q-	H-G----L--	-----L---H	------Y---	----D-F---	------S-E-	---------R	R--E---V--	-----V	.....
	.....--	H-G-------	-----L----	-Y----Y---	----D-F---	------S-E-	---------R	R--E---V--	------	.....
L10	.....--	H-S--R--H-	-----Y----	-Y----Y---	-N----F---	A-------E-	---------R	---E------	-----I	.....
	.....--	S-S-------	----------	YT----N---	------F---	--------EQ	---------S	R-T-------	-----F	.....
L11	.....--	S-S-------	----------	-Y----Y---	------F---	A-----E---	------I--R	----------	-----I	.....
	.....--	Y---------	----------	YT----T---	------F--L	-----Q--EQ	---------R	A---------	Y-----	.....
L12	.....--	STR-------	----------	----------	----------	--------E-	-H--.EI--R	A---------	----GV	.....
	.....--	Y-S-------	----------	YT--G-T---	------F--L	-----Q--GQ	---------R	A---------	Y-----	.....

Fig. 3 (B)

Cattle developing leukemia