

DESCRIPTION

METHOD FOR DETERMINING HLA-A*24 GROUP

TECHNICAL FIELD

5 [0001]

The present invention relates to a method for determining whether an HLA type is an HLA-A*24 group or not.

Priority is claimed on Japanese Patent Application No. 2011-289336, filed on December 28, 2011, the content of which is incorporated herein by reference.

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BACKGROUND ART

[0002]

HLA (Human Leukocyte Antigen) is a gene product of a gene group encoded in an MHC (Major Histocompatibility Complex) region present in the short arm portion of the sixth chromosome. The HLA has highly sufficient diversity, and it is important to improve the suitability of the HLA, in particular, for the work in the medical field of hematopoietic stem cell transplantation, blood transfusion, or the like. In this regard, there is a demand to carry out accurate HLA typing with as little effort as possible in the medical field.

20 [0003]

HLA typing methods are roughly divided into a serological typing method and a DNA typing method. In the DNA typing method, it is possible to determine HLA types in detail, as compared to the serological typing method. Also with the DNA typing method, there sometimes occurs an ambiguity problem in that two or more kinds of alleles, which cannot be identified from the DNA typing results, exist and accordingly,

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the HLA types cannot be identified accurately.

[0004]

For example, PTL 1 discloses a method for typing a subtype (type) of an HLA-A antigen at a genetic level. Specifically, the HLA-A type is typed by classifying HLA-A allele genes (alleles) into specific groups, carrying out PCR using a primer capable of specifically amplifying the HLA-A alleles for each group, and subjecting the PCR products thus obtained to an RFLP method, a PCR-RFLP method, an SSOP method, a PCR-SSOP method, a PCR-SSP method, or a PCR-SSCP method. It is expected that the ambiguity can be eliminated and the HLA-A types can thus be typed in detail by a multi-stage assay in the same manner as this method, but there remains a problem that a great deal of efforts are made.

[0005]

Incidentally, the distribution of HLA types differ among races. According to the report from the HLA laboratory, a non-profit organization, the gene frequency of the HLA-A types in Japanese is the highest with the HLA-A*24:02 types, which account for 35.936%, as shown in Table 1, from the results of the typing of 21705 persons in 5538 Japanese families (as of December 19, 2011) (see, for example, NPL 1).

[0006]

[Table 1]

Allele type	Gene frequency	Rank	Allele type	Gene frequency	Rank	Allele type	Gene frequency	Rank
A*24:02	35.936%	1	A*02:07	3.488%	8	A*24:04	0.014%	27
A*02:01	11.532%	2	A*26:03	2.414%	9	A*24:07	0.014%	27
A*02:06	9.247%	3	A*26:02	1.857%	10	A*24:03	0.009%	31
A*11:01	9.15%	4	A*24:20	0.71%	11	A*24:88	0.005%	36
A*31:01	8.685%	5	A*24:08	0.041%	20			
A*26:01	7.528%	6	A*32:01	0.032%	22			
A*33:03	7.192%	7	A*24:25	0.018%	25			

PRIOR ART DOCUMENTS

PATENT DOCUMENTS

[0007]

PTL 1: Japanese Unexamined Patent Application, First Publication No.

H11-216000

5 Non-Patent Literature

[0008]

NPL 1: "Allele frequency search 1 seat/A search" [on line], Public interest incorporated foundation, HLA Laboratory, [search on December 19, 2013], Internet
<URL: <http://www.hla.or.jp/haplo/haplo-search.php?type=aril&loci=A&lang=ja>>

10 SUMMARY OF INVENTION

PROBLEMS TO BE SOLVED BY THE INVENTION

[0009]

It is an object of the present invention to provide a method for determining the typing of an HLA-A*24 group with less ambiguity in a simple manner.

15 MEANS FOR SOLVING THE PROBLEMS

[0010]

The present inventors have made extensive studies to solve the above-described problems, and as a result, they have found that the allele of an HLA-A*24 group can be determined with high precision by sufficiently reducing the ambiguity through a very
20 small number of steps, on the basis of the results of the typing of (a) a 211st base and (b) a 395th base, in which A (adenine) in the initiation codon for the HLA gene is defined as the 1st base, thereby completing the present invention.

[0011]

That is, the present invention provides:

25 (1) a method for determining an HLA-A*24 group, which includes

determining the HLA-A*24 group on the basis of the results of the typing of (a) a 211st base and (b) a 395th base in a genomic DNA of a subject, in which A (adenine) in the initiation codon for the HLA gene is defined as the 1st base in the genomic DNA of the test subject;

5 (2) the method for determining an HLA-A*24 group according to (1), whether the HLA-A*24 group is determined, further on the basis of the results of the typing of (c) at least one base selected from a 437th base, a 441st base, a 443rd base, a 444th base, a 447th base, and a 449th base;

 (3) the method for determining an HLA-A*24 group according to (2), wherein it
10 is determined whether or not the HLA type of the test subject is HLA-A*24:02;

 (4) the method for determining an HLA-A*24 group according to (3), wherein when the 211st base is C (cytosine), the 395th base is G (guanine), the 437th base is C, the 441st base is T (thymine), the 443rd base is G, the 444th base is C, the 447th base is T, and the 449th base is C in at least one allele, the HLA type of the test subject is
15 determined to be HLA-A*24:02;

 (5) the method for determining an HLA-A*24 group according to any one of (1) to (4), wherein the typing of the bases of (a) and (b) is carried out by a sequence analysis method or a single-base detection method;

 (6) the method for determining an HLA-A*24 group according to any one of (2)
20 to (5), wherein the typing of the bases of (c) is carried out by a sequence analysis method or a single-base detection method;

 (7) the method for determining an HLA-A*24 group according to any one of (2) to (5), wherein when an elongation product is obtained by an elongation reaction by a polymerase, using a primer containing a base sequence represented by SEQ NO: 7, the
25 bases of (c) are typed as follows; the 437th base is C, the 441st base is T, the 443rd base

is G, the 444th base is C, the 447th base is T, and the 449th base is C; and

(8) a kit for determining HLA-A*24:02, including a primer for determining HLA-A*24:02 containing a base sequence represented by SEQ NO: 7.

Effects of the Invention

5 [0012]

By the method for determining an HLA-A*24 group of the present invention, the typing of the HLA-A*24 group can be determined with high precision in a simpler manner. The method for determining an HLA-A*24 group of the present invention can determine the alleles of an HLA-A*24 group having high gene frequency, particularly in
10 the Japanese, and therefore, it is particularly suitable for determination when the test subject is Japanese.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013]

15 FIG. 1A is a view showing the -300th to 600th bases, in which A (adenine) in the initiation codon for the HLA gene is defined as the 1st base, in the base sequence of the genomic DNA of the HLA gene;

FIG. 1B is a view showing the 601st to 1500th bases, in which A (adenine) in the initiation codon for the HLA gene is defined as the 1st base, in the base sequence of
20 the genomic DNA of the HLA gene;

FIG. 1C is a view showing the 1501st to 2400th bases, in which A (adenine) in the initiation codon for the HLA gene is defined as the 1st base, in the base sequence of the genomic DNA of the HLA gene;

FIG. 1D is a view showing the 2401st to 3202nd bases, in which A (adenine) in
25 the initiation codon for the HLA gene is defined as the 1st base, in the base sequence of

the genomic DNA of the HLA gene;

FIG. 2 is a view showing the base sequence of each allele at the 211st base (“(a)” in the figure) and its vicinity, the 395th base (“(b)” in the figure) and its vicinity, and the 437th to 449th bases (“(c)” in the figure) and its vicinity in the second exon of the genomic DNA of the HLA gene.

FIG. 3 is a view schematically showing an embodiment of a method for typing the bases of (a) to (c); and

FIG. 4 is a view schematically showing an embodiment of a method for typing the bases of (a) to (c).

EMBODIMENTS FOR CARRYING OUT THE INVENTION

[0014]

In the present invention and the present specification, the expression “typing a base” is meant to determine the base among A (adenine), G (guanine), C (cytosine), and T (thymine).

[0015]

In the method for determining an HLA-A*24 group of the present invention (hereinafter sometimes referred to as “the determination method of the present invention”), the allele of the HLA-A*24 group is determined, wherein the allele of an HLA-A*24 group is determined on the basis of the results of the typing of (a) a 211st base and (b) a 395th base, in which A (adenine) in the initiation codon for the HLA gene is defined as the 1st base, in the genomic DNA of the test subject.

[0016]

Furthermore, the determination method of the present invention may be further based on the results of the typing of at least one selected from the group consisting of (c)

the 437th base, the 441st base, the 443rd base, the 444th base, the 447th base, and the 449th base, in addition to the results of the typing of the bases of (a) and (b). Further, since the 437th base, the 441st base, the 443rd base, the 444th base, the 447th base, and the 449th base are chained, at least one base of the 437th base, the 441st base, the 443rd base, the 444th base, the 447th base, and the 449th base may be typed. Further, the SNP (Single Nucleotide Polymorphism) characterizing the HLA-A*24:02 is not limited to the above, but a base distinct from an HLA-A group that is present with an allele frequency of 0.001% or more is preferred.

[0017]

FIG. 1 shows the base sequence (SEQ NO: 1) of the genomic DNA of the HLA gene. In FIGS. 1A to 1D, the region surrounded in a square is a coding region and the number of each base is assigned on the basis that A (adenine) in the initiation codon for the HLA gene is defined as the 1st base. The 211st base (C), the 395th base (G), the 437th base (C), the 441st base (T), the 443rd base (G), the 444th base (C), the 447th base (T), and the 449th base (C) are shaded. Further, the amino acid sequence corresponding to the base sequence of the coding region of the HLA gene is shown in SEQ NO: 2.

[0018]

These bases are all present in the second exons. In FIG. 2, the base sequence of each allele of the 211st base (“(a)” in the figure) and its vicinity, the 395th base (“(b)” in the figure) and its vicinity, and the 437th to 449th 6bases (“(c)” in the figure) and its vicinity in the second exon is shown. In the bases of each allele in the figures, “-” represents a base that is analogous to HLA-A*24:02.

[0019]

The gene frequency of each allele in the Japanese and the bases of (a) to (c)

above are shown in Table 2. Further, as the bases of (c), the 437th base was used. In addition, in Table 2, “others” means all the HLA-A types other than the HLA-A types specifically shown in Table 2.

[0020]

5 [Table 2]

Gene frequency (%)	Allele type	(a)	(b)	(c)
35.936	A*24:02	C	G	C
0.71	A*24:20	A	G	C
0.032	A*32:01	C	C	C
0.041	A*24:08	A	C	C
0.014	A*24:07	C	G	C
0.009	A*24:03	C	G	C
0.014	A*24:04	C	G	G
0.018	A*24:25	C	G	C
0.005	A*24:88	A	G	C
63.22	Others	C	C	G

[0021]

When the 211st base ((a)) is A or C, the 395th base ((b)) is G or C, and neither the 211st base nor the 395th base is C in at least one allele, the HLA type of the test
 10 subject is determined to be an HLA-A*24 group.

[0022]

The determination results in the case of typing the bases of (a) and (b) in the HLA gene included in the genomic DNA of the test subject, and determining the HLA-A type of the test subject on the basis of the results of the typing thus obtained, and the
 15 actual genetic types are shown in Table 3, together with the gene frequency. In Table 3, the column of “A” shows the allele types (HLA-A types) and the “others” have the same meanings as in Table 2. Further, the “+” in the column of “Type” represents a genomic DNA including the allele of an HLA-A*24 group, and the “-” represents a genomic DNA

including no allele of an HLA-A*24 group. Further, when at least one A or C exists in the column of “(a)” and at least one G or C exists in the column of “(b)” (provided that in the case where only C exists in the column of “(a)” (both of the alleles are C), and only C exists in the column of “(b)” is excluded), the genomic DNA is determined to include the allele of the HLA-A*24 group (that is, “+” is denoted in the column of “Determination”), and in the other cases, the genomic DNA is determined to include no allele of the HLA-A*24 group (that is, “-” is denoted in the column of “Determination”). Further, in the column of “Errata”, “○” denotes a case where the columns of “Type” and “Determination” are consistent with each other, whereas “×” denotes a case where the columns of “Type” and “Determination” are not consistent with each other. The “gene frequency” is defined as a product obtained by multiplying the gene frequencies of the respective alleles (for example, in the case of the homotype of A*24:02, $35.936\% \times 35.936\% = 12.91\%$). As shown in Table 3, in the determination method of the present invention, it can be determined whether the allele of the HLA-A*24 group is included or not in the genomic DNA of the test subject, on the basis of only the results of the typing of the bases of (a) and (b), with a higher precision.

[0023]

[Table 3]

A	A	(a)	(b)	Type	Determination	Errata	Frequency (%)
A*24:02	A*24:02	C/C	G/G	+	+	○	12.91
A*24:02	A*24:20	A/C	G/G	+	+	○	0.51
A*24:02	A*32:01	C/C	C/G	+	+	○	0.02
A*24:02	A*24:08	A/C	C/G	+	+	○	0.03
A*24:02	A*24:07/03/25	C/C	G/G	+	+	○	0.03
A*24:02	A*24:04	C/C	G/G	+	+	○	0.01
A*24:02	A*24:88	A/C	G/G	+	+	○	0.00
A*24:02	Others	C/C	C/G	+	+	○	45.44
A*24:20	A*24:20	A/A	G/G	+	+	○	0.01
A*24:20	A*32:01	A/C	C/G	+	+	○	0.00
A*24:20	A*24:08	A/A	C/G	+	+	○	0.00
A*24:20	A*24:07/03/25	A/C	G/G	+	+	○	0.00

A*24:20	A*24:04	A/C	G/G	+	+	○	0.00
A*24:20	A*24:88	A/A	G/G	+	+	○	0.00
A*24:20	Others	A/C	C/G	+	+	○	0.90
A*32:01	A*32:01	C/C	C/C	-	-	○	0.00
A*32:01	A*24:08	A/C	C/C	+	+	○	0.00
A*32:01	A*24:07/03/25	C/C	C/G	+	+	○	0.00
A*32:01	A*24:04	C/C	C/G	+	+	○	0.00
A*32:01	A*24:88	A/C	C/G	+	+	○	0.00
A*32:01	Others	C/C	C/C	-	-	○	0.04
A*24:08	A*24:08	A/A	C/C	+	+	○	0.00
A*24:08	A*24:07/03/25	A/C	C/G	+	+	○	0.00
A*24:08	A*24:04	A/C	C/G	+	+	○	0.00
A*24:08	A*24:88	A/A	C/G	+	+	○	0.00
A*24:08	Others	A/C	C/C	+	+	○	0.05
A*24:07/03/25	A*24:07/03/25	C/C	G/G	+	+	○	0.00
A*24:07/03/25	A*24:04	C/C	G/G	+	+	○	0.00
A*24:07/03/25	A*24:88	A/C	G/G	+	+	○	0.00
A*24:07/03/25	Others	C/C	C/G	+	+	○	0.05
A*24:04	A*24:04	C/C	G/G	+	+	○	0.00
A*24:04	A*24:88	A/C	G/G	+	+	○	0.00
A*24:04	Others	C/C	C/G	+	+	○	0.02
A*24:88	A*24:88	A/A	G/G	+	+	○	0.00
A*24:88	Others	A/C	C/G	+	+	○	0.01
Others	Others	C/C	C/C	-	-	○	39.97

[0024]

Moreover, as shown in Table 2, in the HLA-A*24 group except for the HLA-A*24:04, the bases of (c) are different from those of the alleles other than the HLA-A*24 group. Accordingly, the results of the typing of the bases of (c) may be used, in addition to the results of the typing of the bases of (a) and (b).

[0025]

For determining whether the allele of the HLA-A*24 group is included in the genomic DNA of the test subject, the use of only the results of the typing of the bases of (a) and (b) is sufficient, but the HLA type can be determined in more detail by using them in combination with the results of the typing of the other bases. For example, the HLA-A*24:02 in the HLA-A*24 group can be determined in more detail by using the results of the typing of the bases of (c) in addition to the results of the typing of the bases

of (a) and (b). Since the HLA-A*24:02 has the highest gene frequency in the Japanese, the embodiment is particularly useful for clinical tests, in which the test subjects are Japanese.

[0026]

5 Specifically, when these bases are all the same as HLA-A*24:02, that is, the 211st base is C, the 395th base is G, the 437th base is C, the 441st base is T, the 443rd base is G, the 444th base is C, the 447th base is T, and the 449th base is C in at least one allele, the HLA type of the test subject is determined to be HLA-A*24:02. Further, when the 437th base is C, then the 441st base is T, the 443rd base is G, the 444th base is C, the 447th base is T, and the 449th base is C in any case. That is, in the determination method of the present invention, it can be determined whether HLA-A*24:02 is included in a genomic DNA of a test subject, on the basis of the results of the typing of three bases.

[0027]

15 As shown in Table 2, the results of the typing of the bases of (a) to (c) of the HLA-A*24:07, the HLA-A*24:03, and the HLA-A*24:25 are the same as those of the HLA-A*24:02, but the alleles of the other HLA-A types are different from those of the HLA-A*24:02.

That is, from the results of the typing of the bases of (a) to (c), it is impossible to identify the HLA-A*24:02 from the HLA-A*24:07, the HLA-A*24:03, and the HLA-A*24:25, but it is possible to determine the HLA-A*24:02 from the alleles of the other types.

[0028]

The bases of (a) to (c) in the HLA gene included in the genomic DNA of the test subject is typed, then the determination results in the case of determining the HLA-A

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type of the test subject based on the typing results thus obtained and the determination criteria shown in Table 4 and the actual genetic types are shown in Table 5, together with the gene frequency. In Table 5, the column of “A” shows the allele types (HLA-A types) and the “others” has the same meaning as in Table 2. Further, in the column of “Type”, “+” denotes a genomic DNA including the HLA-A*24:02 and “-” denotes a genomic DNA not including the HLA-A*24:02. In Tables 4 and 5, in the column of “Determination”, “+” denotes that the genomic DNA of the test subject is determined to include the HLA-A*24:02 and “-” denotes that the genomic DNA of the test subject is determined to include no HLA-A*24:02. That is, in the case where there is at least one C in the column of “(a)”, there is at least one G in the column of “(b)”, and there is at least one C in the column of “(c)”, and also there is no G in the column of “(c)” if there is A in the column of “(a)”, the genomic DNA is determined to include the HLA-A*24:02 (that is, in the column of “Determination”, “+” is denoted). In the case where there is no C in the column of “(a)”; in the case where there is no G in the column of “(b)”; or in the case where there is no C in the column of “(c)”; and also or in the case where there is at least one A in the column of “(a)”, there is at least one G in the column of “(b)”, and there is at least one G in the column of “(c)”, the genomic DNA is determined not to include the HLA-A*24:02 (that is, “-” denotes such the cases in the column of “Determination”). In addition, in the column of “Errata”, “○” denotes a case where the columns of “Type” and “Determination” are consistent with each other, whereas “×” denotes a case where the columns of “Type” and “Determination” are not consistent with each other.

[0029]

[Table 4]

(a)	(b)	(c)	Determination
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C/C	G/G	C/C	+
A/C	G/G	C/C	+
C/C	C/G	C/C	+
A/C	C/G	C/C	+
C/C	G/G	C/G	+
C/C	C/G	C/G	+
A/A	G/G	C/C	-
A/A	C/G	C/C	-
A/A	C/C	C/C	-
A/C	G/G	C/G	-
A/C	C/G	C/G	-
A/C	C/C	C/G	-
A/C	C/C	C/C	-
C/C	G/G	G/G	-
C/C	C/G	G/G	-
C/C	C/C	G/G	-
C/C	C/C	C/G	-
C/C	C/C	C/C	-

[0030]

[Table 5]

A	A	(a)	(b)	(c)	Type	Determination	Errata	Frequency (%)
A*24:02	A*24:02	C/C	G/G	C/C	+	+	○	12.91
A*24:02	A*24:20	A/C	G/G	C/C	+	+	○	0.51
A*24:02	A*32:01	C/C	C/G	C/C	+	+	○	0.02
A*24:02	A*24:08	A/C	C/G	C/C	+	+	○	0.03
A*24:02	A*24:07/03/25	C/C	G/G	C/C	+	+	○	0.03
A*24:02	A*24:04	C/C	G/G	C/G	+	+	○	0.01
A*24:02	A*24:88	A/C	G/G	C/C	+	+	○	0.00
A*24:02	Others	C/C	C/G	C/G	+	+	○	45.44
A*24:20	A*24:20	A/A	G/G/	C/C	-	-	○	0.01
A*24:20	A*32:01	A/C	C/G	C/C	-	+	×	0.00
A*24:20	A*24:08	A/A	C/G	C/C	-	-	○	0.00
A*24:20	A*24:07/03/25	A/C	G/G	C/C	-	+	×	0.00
A*24:20	A*24:04	A/C	G/G	C/G	-	-	○	0.00
A*24:20	A*24:88	A/A	G/G	C/C	-	-	○	0.00
A*24:20	Others	A/C	C/G	C/G	-	-	○	0.90
A*32:01	A*32:01	C/C	C/C	C/C	-	-	○	0.00
A*32:01	A*24:08	A/C	C/C	C/C	-	-	○	0.00
A*32:01	A*24:07/03/25	C/C	C/G	C/C	-	+	×	0.00
A*32:01	A*24:04	C/C	C/G	C/G	-	+	×	0.00
A*32:01	A*24:88	A/C	C/G	C/C	-	+	×	0.00
A*32:01	Others	C/C	C/C	C/G	-	-	○	0.04
A*24:08	A*24:08	A/A	C/C	C/C	-	-	○	0.00
A*24:08	A*24:07/03/25	A/C	C/G	C/C	-	+	×	0.00
A*24:08	A*24:04	A/C	C/G	C/G	-	-	○	0.00

A*24:08	A*24:88	A/A	C/G	C/C	-	-	○	0.00
A*24:08	Others	A/C	C/C	C/G	-	-	○	0.05
A*24:07/03/25	A*24:07/03/25	C/C	G/G	C/C	-	+	×	0.00
A*24:07/03/25	A*24:04	C/C	G/G	C/G	-	+	×	0.00
A*24:07/03/25	A*24:88	A/C	G/G	C/C	-	+	×	0.00
A*24:07/03/25	Others	C/C	C/G	C/G	-	+	×	0.05
A*24:04	A*24:04	C/C	G/G	G/G	-	-	○	0.00
A*24:04	A*24:88	A/C	G/G	C/G	-	-	○	0.00
A*24:04	Others	C/C	C/G	G/G	-	-	○	0.02
A*24:88	A*24:88	A/A	G/G	C/C	-	-	○	0.00
A*24:88	Others	A/C	C/G	C/G	-	-	○	0.01
Others	Others	C/C	C/C	G/G	-	-	○	39.97

[0031]

From the results of the typing of the (a) to (c) bases, when the test subject has a genomic DNA represented by × in the column of “Errata” in Table 5, it is mistakenly

5 determined that the test subject has HLA-A*24:02 even though the genomic DNA does not actually include the HLA-A*24:02 (false-positive). However, for the Japanese, any of the gene frequency of the genomic DNA that is false-positive is extremely low, and accordingly, the proportion of the test subject determined to be false-positive is suppressed to only 0.05%. For example, when the method for determining whether or

10 not the HLA type of the test subject is HLA-A*24:02, using the results of the typing of the bases of (c), in addition to (a) and (b) (hereinafter sometimes referred to as the “method for determining HLA-A*24:02 of the present invention”) among the determination methods of the present invention is carried out for 1,000,000 Japanese persons, none is determined to be false-negative and 530 persons are determined to be

15 false-positives, of which the number is critically low in theory, as shown in Table 6, despite determining based on the results of the typing of only three bases.

[0032]

[Table 6]

	At a test for 1,000,000 persons	Frequency (%)
--	------------------------------------	---------------

	(persons)	
Positive	589,573	59
Negative	409,877	41
False-negative	0	0.00
False-positive	530	0.05

[0033]

Most of the alleles of HLA-A pertain to a combination of a plurality of polymorphisms, and therefore, when only a part of a site (polymorphic site) different from the other allele among the alleles is typed, it is difficult to determine the plurality of alleles from each other. When a test for the purpose of determining a specific allele is conducted, an allele, which is not a desired allele in fact, is mistakenly determined to be the desired allele (false-positive), if accompanied by an occurrence of ambiguity. The problems related to the false-positive determination can be solved by eliminating any ambiguity, but a large number of bases to be typed is required in order to eliminate any ambiguity, leading to an excessive increase in the test cost.

That is, in order to apply the method for determining HLA to medical fields of a clinical test or the like, it becomes difficult to achieve a balance between the test accuracy and cost.

15 [0034]

In the method for determining HLA-A*24:02 of the present invention, the bases to be used for the typing are identified by considering the gene frequency to minimize the probability of false-positives due to ambiguity as well as to minimize the number of bases to be typed in order to determine HLA-A*24:02. That is, since the method for determining HLA-A*24:02 of the present invention has a high determination accuracy of HLA-A*24:02 and is excellent in the test cost, it is suitable for a clinical test or the like.

[0035]

If the determination is carried out based on only the results of the typing of the bases of (a) and (b), as shown in Table 7, the test subjects that are “HLA-A*24:20 and others (alleles other than the alleles described in Table 2) having a high gene frequency” are mistakenly determined to include HLA-A*24:02. As a result, the probability of the false-positives is increased to 0.97%, and thus, when tested for 1,000,000 Japanese persons, 9,749 persons are determined to be false-positive in theory. From this calculation, it is apparent that the determination accuracy of the method for determining HLA-A*24:02 of the present invention is high.

[0036]

10

[Table 7]

A	A	(a)	(b)	Type	Determination	Errata	Frequency (%)
A*24:02	A*24:02	C/C	G/G	+	+	○	12.91
A*24:02	A*24:20	A/C	G/G	+	+	○	0.51
A*24:02	A*32:01	C/C	C/G	+	+	○	0.02
A*24:02	A*24:08	A/C	C/G	+	+	○	0.03
A*24:02	A*24:07/03/25	C/C	G/G	+	+	○	0.03
A*24:02	A*24:04	C/C	G/G	+	+	○	0.01
A*24:02	A*24:88	A/C	G/G	+	+	○	0.00
A*24:02	Others	C/C	C/G	+	+	○	45.44
A*24:20	A*24:20	A/A	G/G	-	-	○	0.01
A*24:20	A*32:01	A/C	C/G	-	+	×	0.00
A*24:20	A*24:08	A/A	C/G	-	-	○	0.00
A*24:20	A*24:07/03/25	A/C	G/G	-	+	×	0.00
A*24:20	A*24:04	A/C	G/G	-	+	×	0.00
A*24:20	A*24:88	A/A	G/G	-	-	○	0.00
A*24:20	Others	A/C	C/G	-	+	×	0.90
A*32:01	A*32:01	C/C	C/C	-	-	○	0.00
A*32:01	A*24:08	A/C	C/C	-	-	○	0.00
A*32:01	A*24:07/03/25	C/C	C/G	-	+	×	0.00
A*32:01	A*24:04	C/C	C/G	-	+	×	0.00
A*32:01	A*24:88	A/C	C/G	-	+	×	0.00
A*32:01	Others	C/C	C/C	-	-	○	0.04
A*24:08	A*24:08	A/A	C/C	-	-	○	0.00
A*24:08	A*24:07/03/25	A/C	C/G	-	+	×	0.00
A*24:08	A*24:04	A/C	C/G	-	+	×	0.00
A*24:08	A*24:88	A/A	C/G	-	-	○	0.00
A*24:08	Others	A/C	C/C	-	-	○	0.05
A*24:07/03/25	A*24:07/03/25	C/C	G/G	-	+	×	0.00
A*24:07/03/25	A*24:04	C/C	G/G	-	+	×	0.00
A*24:07/03/25	A*24:88	A/C	G/G	-	+	×	0.00
A*24:07/03/25	Others	C/C	C/G	-	+	×	0.05
A*24:04	A*24:04	C/C	G/G	-	+	×	0.00

A*24:04	A*24:88	A/C	G/G	-	+	×	0.00
A*24:04	Others	C/C	C/G	-	+	×	0.02
A*24:88	A*24:88	A/A	G/G	-	-	○	0.00
A*24:88	Others	A/C	C/G	-	+	×	0.01
Others	Others	C/C	C/C	-	-	○	39.97

[0037]

A nucleic acid sample provided for the determination method of the present invention may be one including nucleic acids having the base sequence of a region including the bases of (a) and (b) of the genomic DNA of the test subject (the bases of (a) to (c) of the genomic DNA of the test subject in the case of the method for determining HLA-A*24:02 of the present invention) reflected therein, and it may include either a genomic DNA or mRNA derived from the test subject.

For the typing of each base in an mRNA, the mRNA may be directly typed or may be typed using a cDNA synthesized by reverse transcription from the mRNA. The genomic DNA or mRNA derived from the test subject may be a nucleic acid that is extracted and purified from a biological sample taken from the test subject or may be a nucleic acid before purification. In addition, an amplification product obtained by amplifying a region including the bases of (a) and (b) or a region including the bases of (a) to (c) in the genomic DNA or mRNA derived from the test subject by PCR or the like may be used.

[0038]

In the determination method of the present invention, the method for typing the bases of (a) to (c) are not particularly limited and may be a sequence analysis method which is based on a Sanger or a single-base detection method. As the single-base detection method, various known methods used in the detection of mutation of a gene, detection of polymorphism, or the like, for example, a PCR-SSO (sequence specific

oligonucleotide) method, a PCR-SSP (sequence specific primer) method, or a modified method thereof may be used. The PCR-SSO method is a typing method according to the presence or absence for formation of an aggregate with the probe, using a probe that hybridizes specifically with a specific allele (allele-specific probe). The PCR-SSP

5 method is a method for typing according to the presence or absence for a PCR product when carrying out PCR using a primer that hybridizes specifically with a specific allele (allele-specific probe). Examples of the modified method thereof include an Invader method, a TaqMan probe method, a QProbe method, and a Scorpion-ARMS method. A detection method using fluorescence is preferred due to its superior detection sensitivity, and a method using a probe or primer that recognizes a specific allele, an Invader method, a TaqMan probe method, a QProbe method, and a Scorpion-ARMS method is preferred due to its superior detection sensitivity and accuracy. For example, the probe or primer used for the typing of the bases of (a) to (c) can be synthesized by designing by an ordinary method, for example, based on the base sequence 1 or 2.

15 [0039]

In the case of the typing by a sequence analysis method, the bases of (a) can be typed by carrying out PCR with a PCR primer (a primer-1 and a primer-2 in FIG. 3) for the amplification of a region having the bases of (a) interposed therein and analyzing the base sequence of the obtained amplification product, using a genomic DNA or mRNA

20 derived from a test subject (hereinafter sometimes simply referred to as a “genomic DNA”) as a template, for example, as shown in Figure 3. Similarly, the bases of (b) and (c) can be typed by carrying out PCR with a PCR primer (a primer-3 and a primer-4 in FIG. 3) for the amplification of a region having the bases of (b) and (c) interposed therein and analyzing the base sequence of the obtained amplification product. The six bases of (c) in FIG. 3 represent the 437th base, the 441st base, the 443rd base, the 444th base, the

25

447th base, and the 449th base from the upstream.

The primers-1 to -4 are not particularly limited as long as they are primers capable of amplifying a desired region including each of the bases of (a) to (c).

Examples thereof include a primer including the base sequence shown in Table 8. In

5 the case of typing only the bases of (a) and (b) by a sequence analysis method, PCR may be carried out using a PCR primer for the amplification of a region including the bases of (b), not including the bases of (c), and the base sequence of the amplification product thus obtained may be analyzed.

[0040]

10 [Table 8]

Primer	Base sequence (5' → 3')	SEQ NO:
Primer-1	AGGGTCGGGCGGGTCTCAGCCACT	3
Primer-2	AACCGCACGAACTGCGTGTCGTCCACGTA	4
Primer-3	TACGTGGACGACACGCAGTTCGTGCGGTT	5
Primer-4	TCACCGGCCTCGCTCTGGTTGTAGTAG	6

[0041]

Moreover, the typing of the bases may be carried out by a combination of a sequence analysis and a single-base detection method. For example, any of the bases of

15 (a) to (c) may be subjected to PCR using a primer that hybridizes specifically with an allele of an HLA-A*24 group and typed according to the presence or absence of PCR products, and the remaining bases may be typed by a sequence analysis method.

[0042]

Furthermore, the “primer that hybridizes specifically with a specific allele” is
 20 not limited to a primer having a base sequence fully complementary to a targeted allele, and it may be any primer that hybridizes preferentially with a targeted allele rather than other alleles or may include a mismatch base of one to several bases in a region that

hybridizes with a targeted allele.

[0043]

In the method for determining HLA-A*24:02 of the present invention, for example, any one of the bases of (a) to (c) may be subjected to PCR using a primer that hybridizes specifically with HLA-A*24:02 (HLA-A*24:02-specific primer) and typed according to the presence or absence of PCR products, and the remaining bases may be typed by a sequence analysis method.

[0044]

For example, as shown in FIG. 4, an elongation reaction using a polymerase such as PCR may be carried out, using a primer that hybridizes specifically with a region including the six bases of (c) (primer-5 in FIG. 4) and a primer that recognizes a region upstream of the bases of (b) (primer-3 in FIG. 4), with a genomic DNA derived from a test subject as a template. Further, the bases of (b) and (c) may be typed by investigating the presence or absence of PCR products and analyzing the base sequence of the PCR product. The typing of the bases of (a) may be carried out in the same manner as in FIG. 3.

[0045]

In the case of the typing method shown in Fig. 3, PCR products are obtained, using many alleles of an HLA gene included in the genomic DNA as a template. Therefore, when the HLA gene is a heterotype, the bases of (a) to (c) may be sometimes typed as two types of bases, depending on typing methods, or may be mistakenly determined even with the use of the results of the typing of (a) to (c). To the contrary, when a region including the six bases of (c) is identified with a primer and the bases of (b) are typed by a sequence analysis method or the like, as in the typing method shown in FIG. 4, the results from the combination of the bases of (b) and the bases of (c) are

obtained. That is, when the amplification is carried out using a primer which is specific to a group including HLA-A*24:02, the typing of the HLA-A*24:02 can be determined with high precision in a simpler manner than the typing method of FIG. 3, only by the determination of the bases of (a) and (b), even though the genomic DNA is a heterotype including HLA-A*24:02.

[0046]

The primer-5 is not particularly limited as long as it is a primer capable of hybridizing specifically to a region including at least one of the six bases of (c). Examples of the primer to be used as the HLA-A*24:02-specific primer include a primer-5 including the base sequence shown in Table 9. The primers-6 to -8 shown in Table 9 may also be used as the HLA-A*24:02-specific primer for typing the bases of (c) in the same manner as the primer-5.

[0047]

[Table 9]

Primer	Base sequence (5' → 3')	SEQ NO:
Primer-5	GGTTGTAGTAGCGGAGCGCGATCCG	7
Primer-6	TAGCGGAGCGCGATCCGCAGG	8
Primer-7	TAGCGGAGCGCGATCCGCAG	9
Primer-8	CCTCGCTCTGGTTGTAGTAGCGGAGCGCTA	10

[0048]

When the typing is carried out depending on the presence or absence of PCR products as shown in FIG. 4 and PCR products are failed to be obtained, it is hard to judge whether the cause of the failure was that the genomic DNA as a template did not include an HLA-A*24 group or that the reaction system itself had a problem. Therefore, it is preferable to carry out PCR with the genomic DNA as a template, using a primer designed to ensure that PCR products are obtained when the genomic DNA does not

include the HLA-A*24 group. Also, in the method for determining HLA-A*24:02 of the present invention, it is preferable to carry out PCR with the genomic DNA as a template, using a primer designed to ensure that PCR products are obtained when the genomic DNA does not include the HLA-A*24:02. Examples of the primer designed to ensure that PCR products are obtained when the PCR products are not obtained depending on the HLA-A*24:02-specific primers for typing the bases of (c) (that is, primers that hybridize specifically with alleles in which the 437th base is C, the 441st base is T, the 443rd base is G, the 444th base is C, the 447th base is T, and the 449th base is C) include the primers-9 to -12 shown in Table 10.

10 [0049]

[Table 10]

Primer	Base sequence (5' → 3')	SEQ NO:
Primer-9	GGTTGTAGTAGCCGCGCAGGGTCCC	11
Primer-10	TAGCCGCGCAGGGTCCCCAGG	12
Primer-11	TAGCCGCGCAGGGTCCCCAG	13
Primer-12	CTCGCTCTGGTTGTAGTAGCCGCGCAGTG	14

[0050]

The determination method of the present invention can be carried out in a simpler manner by making the probe or primer used for the typing of the bases of (a) to (c) into a kit form. For example, a combination of the primers-1 to -4 shown in FIG. 3 can be used as a kit for determining an HLA-A*24 group. In the present invention, a kit for determining an HLA-A*24 group including the primer-5 that is an HLA-A*24:02-specific primer is preferred. A kit including the HLA-A*24:02-specific primer is suitably used for determining the HLA-A*24:02.

20 [0051]

In addition, it is preferable that the kit for determining the HLA-A*24 group

include a reagent, an instrument, or the like for extracting and purifying nucleic acids from a biological sample collected from a test subject.

EXAMPLES

[0052]

5 Hereinafter, the present invention will be described specifically with reference to Examples, but the present invention is not limited to the following Examples.

[0053]

[Example 1]

PCR using the primers-1 and -2, and PCR using the primers-3 and -4, shown in
 10 Table 8, were carried out, using a genomic DNA extracted from a biological sample collected from a test subject, in which the HLA-A type is a [A*24:02/A*24:02] homotype, a heterotype of [A*24:02/A*24:08], a heterotype of [A*24:20/A*32:01], and a heterotype of [A*32:01/A*24:04] as a template, and each of the PCR products was subjected to sequence analysis.

15 The determination results on the basis of the sequence analysis results and the typing results thus obtained are shown in Table 11. In the Table, in the column of "Determination", "+" denotes a determination that the genomic DNA includes HLA-A*24:02 and "-" denotes a determination that the genomic DNA does not include HLA-A*24:02. As a result, the [A*24:02/A*24:02] homotype, the heterotype of
 20 [A*24:02/A*24:08], and the heterotype of [A*32:01/A*24:04] were all accurately determined. On the other hand, the heterotype of [A*24:20/A*32:01] was false-positive, as expected.

[0054]

[Table 11]

	(a)	(b)	(c)	Type	Determination	Errata
--	-----	-----	-----	------	---------------	--------

A*24:02/A*24:02	C	G	C	+	+	○
A*24:02/A*24:08	A/C	C/G	C	+	+	○
A*24:20/A*32:01	A/C	C/G	C	-	+	×
A*32:01/A*24:04	C	C/G	C/G	-	-	○

[0055]

[Example 2]

In the same manner as in Example 1 except that the primer-5 shown in Table 9

5 was used instead of the primer-4, PCR was carried out, using a genomic DNA extracted from a biological sample collected from a test subject, in which the HLA-A type is a [A*24:02/A*24:02] homotype, a heterotype of [A*24:02/A*24:08], a heterotype of [A*24:20/A*32:01], and a heterotype of [A*32:01/A*24:04], as a template. As a result, in all the cases where any genomic DNA was used as a template, PCR products were

10 obtained by PCR, using the primers-3 and -5. Therefore, the genomic DNA of all the test subjects was typed such that the 437th base was C, the 441st base was T, the 443rd base was G, the 444th base was C, the 447th base was T, and the 449th base was C. As a result of the sequence analysis of each PCR product, the same determination results as those of Example 8, as seen from Table 12, were obtained. However, when using a

15 genomic DNA derived from the heterotype of [A*32:01/A*24:04], only two types, C and G, of the bases of (b) were typed in Example 1, but in this Example, only one type, C, of the bases of (b) was typed.

[0056]

[Table 12]

	(a)	(b)	Type	Determination	Errata
A*24:02/A*24:02	C	G	+	+	○
A*24:02/A*24:08	A/C	C/G	+	+	○
A*24:20/A*32:01	A/C	C/G	-	+	×
A*32:01/A*24:04	C	C	-	-	○

Industrial Applicability

[0057]

Since the determination method of the present invention makes it possible to determine the typing of the HLA-A*24 group with high precision in a simpler manner, it
5 can be used in the fields of clinical genetic diagnostics, or the like, in particular, in the determination of an HLA type for cell or organ transplantation.

CLAIMS

1. A method for determining an HLA-A*24 group, comprising:
determining the HLA-A*24 group on the basis of the results of the typing of
5 (a) a 211st base and (b) a 395th base, in which A (adenine) in the initiation codon for an HLA gene is defined as the 1st base in the genomic DNA of a test subject.
2. The method for determining an HLA-A*24 group according to claim 1, wherein
the HLA-A*24 group is determined, further on the basis of the results of the typing of (c)
10 at least one base selected from the group consisting of a 437th base, a 441st base, a 443rd base, a 444th base, a 447th base, and a 449th base.
3. The method for determining an HLA-A*24 group according to claim 2, wherein
it is determined whether or not the HLA type of the test subject is HLA-A*24:02.
15
4. The method for determining an HLA-A*24 group according to claim 3, wherein
when the 211st base is C (cytosine), the 395th base is G (guanine), the 437th base is C,
the 441st base is T (thymine), the 443rd base is G, the 444th base is C, the 447th base is
T, and the 449th base is C in at least one allele, the HLA type of the test subject is
20 determined to be HLA-A*24:02.
5. The method for determining an HLA-A*24 group according to any one of claims 1
to 4, wherein
the typing of the bases of (a) and (b) is carried out by a sequence analysis method or a
25 single-base detection method.

6. The method for determining an HLA-A*24 group according to any one of claims 2 to 5, wherein the typing of the bases of (c) is carried out by a sequence analysis method or a single-base detection method.
7. The method for determining an HLA-A*24 group according to any one of claims 2 to 5, wherein when an elongation product is obtained by an elongation reaction by a polymerase using a primer containing a base sequence represented by SEQ NO: 7, the bases of (c) are typed as follows; the 437th base is C, the 441st base is T, the 443rd base is G, the 444th base is C, the 447th base is T, and the 449th base is C.
8. A kit for determining HLA-A*24:02, comprising a primer for determining HLA-A*24:02 containing a base sequence represented by SEQ NO: 7.