[0005] In addition, blood glucose levels may also be used as early indicators associated with diseases as varied as Cushing syndrome, hyperthyroidism, pancreatic cancer, pancreatitis, pre-diabetes, and diabetes.

[0006] As described above, diagnostic tests are currently available and performed on a regular basis to detect the presence or absence of a normal state in an individual. These tests, however, do not address the diversity of the immunorepertoire in a patient. The ability to detect the presence or absence of a diverse immunorepertoire, relative to a standard, in an individual may assist with an assessment of the health of such individual.

SUMMARY OF INVENTION

[0007] The present disclosure relates to a method for determining the immunorepertoire diversity in a patient. The method comprises: quantifying the expression of third complementarity-determining region ("CDR3") in a population or sub-population of immune system cells in a patient sample; and identifying the percentage of pCDR3 represented by such CDR3s within that population or sub-population, wherein normal immunorepertoire diversity is characterized by the presence of a minimum percentage of pCDR3 and an abnormal immunorepertoire diversity status is characterized by the absence of a minimum percentage of pCDR3.

[0007a] In one aspect, the present invention provides for a method for using immunorepertoire diversity to assess a normal or abnormal status of an individual patient's immunorepertoire, the method comprising the steps of:

(a) determining the pCDR3 sequences, the commonly-shared CDR3 sequences identified from a reference group of individuals, comprising comparing each sample from the reference group and identifying those CDR3 sequences that are most-frequently shared within said reference group;

(b) using primers directed to pCDR3 sequences to detect the presence in the patient's immunorepertoire of individual CDR3s within that shared group of pCDR3 sequences; and

(c) calculating the normality index, comprising dividing the number of pCDR3 sequences from the reference group by the number of pCDR3 sequences present in the patient's immunorepertoire; and

(d) assessing the status of the patient's immunorepertoire, wherein the normality index is compared with a predetermined minimum percentage of

shared pCDR3 sequences, such that the immunorepertoire of the patient is considered normal when the normality index meets or exceeds the minimum percentage of shared pCDR3 sequences and the immunorepertoire of the patient is considered abnormal when the normality index is below the minimum percentage of shared pCDR3 sequences.

[0007b] In another aspect, the present invention provides a method for using immunorepertoire diversity to assess a normal or abnormal status of an individual patient's immunorepertoire, the method comprising:

(a) amplifying polynucleotides from a population of white blood cells from a human or animal subject in a reaction mix comprising target-specific nested primers to produce a set of first amplicons, at least a portion of the target-specific nested primers comprising additional nucleotides which, during amplification, serve as a template for incorporating into the first amplicons a binding site for at least one common primer;

(b) transferring a portion of the first reaction mix containing the first amplicons to a second reaction mix comprising at least one common primer;

(c) amplifying, using the at least one common primer, the first amplicons to produce a set of second amplicons;

(d) sequencing the second amplicons to identify CDR3 sequences in the subpopulation of white blood cells;

(e) determining the pCDR3 sequences, the commonly-shared CDR3 sequences identified from a reference group of individuals, comprising comparing each sample from the reference group and identifying those CDR3 sequences that are most-frequently shared within said reference group;

(f) using the identified CDR3 sequences to calculate the normality index, comprising dividing the number of pCDR3 sequences from the reference group by the number of pCDR3 sequences present in the patient's identified CDR3 sequences; and

(g) assessing the status of the patient's immunorepertoire, wherein the normality index is compared with a predetermined minimum percentage of shared pCDR3 sequences, such that the immunorepertoire of the patient is considered normal when the normality index meets or exceeds the minimum percentage of shared pCDR3 sequences and the immunorepertoire of the patient

is considered abnormal when the normality index is below the minimum percentage of shared pCDR3 sequences.

[0008] In certain examples, the minimum percentage of pCDR3 in such method is a percentage number selected from about 25 percent to about 75 percent. In other examples, the minimum percentage of pCDR3 is about 25 percent. In other examples, the pCDR3 is composed of about the 1000 most-frequently-shared CDR3. In other examples, the pCDR3 is composed of more than 1000 of the most-frequently-shared CDR3. In yet other examples, the pCDR3 is composed of fewer than 1000 of the most-frequently-shared CDR3. In other examples, the pCDR3 is composed of CDR3 shared by at least 25 percent of a pool of individuals.

[0009] In certain examples, the pCDR3 is composed of CDR3 shared by at least 50 percent of a pool of individuals. In certain examples, such pool contains at least 100 individuals. In other examples, such pool contains about 1000 individuals.

[0010] The present disclosure also relates to a method for determining the immunorepertoire diversity in a patient. The method comprises: amplifying polynucleotides from a population of white blood cells from a human or animal subject in a reaction mix comprising target-specific nested primers to produce a set of first

amplicons, at least a portion of the target-specific nested primers comprising additional nucleotides which, during amplification, serve as a template for incorporating into the first amplicons a binding site for at least one common primer; transferring a portion of the first reaction mix containing the first amplicons to a second reaction mix comprising at least one common primer; amplifying, using the at least one common primer, the first amplicons to produce a set of second amplicons; sequencing the second amplicons to identify CDR3 sequences in the subpopulation of white blood cells; using the identified CDR3 sequences to quantify the percentage of pCDR3 represented by the sample to provide a normality index; and identifying whether the normality index is normal or abnormal, wherein a normal state is characterized by the presence of a minimum percentage of pCDR3 and an abnormal state is characterized by the absence of a minimum percentage of pCDR3.

[0011] In certain examples, such second method measures a population of white blood cells, wherein the population includes about 100,000 randomly-selected cells. In certain examples, the method is repeated about 10 to 100 times, each time with a random selection of cells, to determine the average percentage of pCDR3 expressed by the patient. In certain examples, the minimum percentage of pCDR3 is a percentage number from about 25 to about 75. In certainexamples, the pCDR3 is composed of at least the 1000 most-frequently-shared CDR3. In certainexamples, the pCDR3 includes SEQ ID NO:1 through SEQ ID NO: 4014 as described herein. For example, the normality index of a patient may be determined based on the percentage of pCDR3 sequences, represented by SEQ ID NO: 1 through SEQ ID NO: 4014, present in a predetermined number of reads of a patient sample. In certainexamples, the pCDR3 is composed of CDR3 shared by at least 50 percent of a pool of individuals.

[0012] The reference pool of individuals may be different sizes. In certain examples, the reference pool includes at least 100 individuals. In certain embodiments, the pool contains about 1000 individuals. In certainexamples, the pool is composed of healthy control individuals, whereas in otherexamples, the pool is composed of individuals with one or more specific disease states. The reference pool may further be controlled to account for certain variables of a given patient. In certainexamples, the pool of individuals is approximately age-matched to the patient. In otherexamples, the pool of individuals is gender-matched to the patient.

[0013] In various aspects of the present disclosure, the immune system cells that are analyzed, under any method described herein, may include, for example, all T cells

CLAIMS

Now, therefore, the following is claimed:

1. A method for using immunorepertoire diversity to assess a normal or abnormal status of an individual patient's immunorepertoire, the method comprising the steps of:

(a) determining the pCDR3 sequences, the commonly-shared CDR3 sequences identified from a reference group of individuals, comprising comparing each sample from the reference group and identifying those CDR3 sequences that are most-frequently shared within said reference group;

(b) using primers directed to pCDR3 sequences to detect the presence in the patient's immunorepertoire of individual CDR3s within that shared group of pCDR3 sequences; and

(c) calculating the normality index, comprising dividing the number of pCDR3 sequences from the reference group by the number of pCDR3 sequences present in the patient's immunorepertoire; and

(d) assessing the status of the patient's immunorepertoire, wherein the normality index is compared with a predetermined minimum percentage of shared pCDR3 sequences, such that the immunorepertoire of the patient is considered normal when the normality index meets or exceeds the minimum percentage of shared pCDR3 sequences and the immunorepertoire of the patient is considered abnormal when the normality index is below the minimum percentage of shared pCDR3 sequences.

2. The method of claim 1 wherein the population of cells is selected from the group consisting of all T cells [panT], CD8+ T cells [cytotoxic T (Ta)], CD4+ T cells and their subsets [TH1, TH2, TH17, regulatory T (Treg), follicular T (TFH)], developmental subsets of T cells, such as naive T (Tn), activated T (Ta), memory T (Tm), all B cells (panB), naive B (Br), activated B (Ba), memory B (Bm), plasma and plasmablast B cells.

3. The method of claim 1, wherein the minimum percentage of pCDR3 is a percentage number selected from about 25 percent to about 75 percent.

4. The method of claim 1, wherein the minimum percentage of pCDR3 is about 25 percent.

5. The method of claim 1, where the pCDR3 is composed of about the 1000 most-frequently-shared CDR3.

6. The method of claim 1, where the pCDR3 is composed of more than 1000 of the most-frequently-shared CDR3.

7. The method of claim 1, where the pCDR3 is composed of fewer than 1000 of the most-frequently-shared CDR3.

8. The method of claim 1, wherein the pCDR3 is composed of CDR3 shared by at least 25 percent of a pool of individuals.

9. The method of claim 1, wherein the pCDR3 is composed of CDR3 shared by at least 50 percent of a pool of individuals.

10. The method of claim 9, wherein the pool contains at least 100 individuals.

11. The method of claim 9, wherein the pool contains about 1000 individuals.

12. A method for using immunorepertoire diversity to assess a normal or abnormal status of an individual patient's immunorepertoire, the method comprising:

(a) amplifying polynucleotides from a population of white blood cells from a human or animal subject in a reaction mix comprising target-specific nested primers to produce a set of first amplicons, at least a portion of the target-specific nested primers comprising additional nucleotides which, during amplification, serve as a template for incorporating into the first amplicons a binding site for at least one common primer;

(b) transferring a portion of the first reaction mix containing the first amplicons to a second reaction mix comprising at least one common primer;

(c) amplifying, using the at least one common primer, the first amplicons to produce a set of second amplicons;

(d) sequencing the second amplicons to identify CDR3 sequences in the subpopulation of white blood cells;

(e) determining the pCDR3 sequences, the commonly-shared CDR3 sequences identified from a reference group of individuals, comprising comparing each sample from the reference group and identifying those CDR3 sequences that are most-frequently shared within said reference group;

(f) using the identified CDR3 sequences to calculate the normality index, comprising dividing the number of pCDR3 sequences from the reference group by the number of pCDR3 sequences present in the patient's identified CDR3 sequences; and

(g) assessing the status of the patient's immunorepertoire, wherein the normality index is compared with a predetermined minimum percentage of shared pCDR3 sequences, such that the immunorepertoire of the patient is considered normal when the normality index meets or exceeds the minimum percentage of shared pCDR3 sequences and the immunorepertoire of the patient is considered abnormal when the normality index is below the minimum percentage of shared pCDR3 sequences.