Title: COMPOSITIONS AND METHODS RELATING TO BLOOD-BASED BIOMARKERS OF BREAST CANCER

Abstract: The present invention relates generally to the field of breast cancer. More specifically, the invention concerns methods and compositions useful for diagnosing treating breast cancer.

FIG. 1

GATK variants (called from 35 samples)

1,579,855 SNVs  216,488 Indels

Remove "common" variants (1000 Genomes, ESP, TCGA, local)

57,451 SNVs  15,270 Indels

Remove variants that do not fall within Entrez Gene exons

22,608 SNVs  2,884 Indels

Remove variants by conservation and protein-coding effect

4,737 SNVs  485 Indels

Collapse variants = gene/pathway is "mutated" if any variant is present in a given sample

3,986 genes

Exclude genes mutated frequently in TCGA germline samples

2,107 genes

Compare gene and pathway mutation frequencies between HBC cases and controls.

Genes and pathways of potential interest

[Continued on next page]
Published: 17 April 2014

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))
INTERNATIONAL SEARCH REPORT

PCT/US13/56201

A.  CLASSIFICATION OF SUBJECT MATTER

IPC (8) - C40B 30/04; C07K 16/18; G01N 33/567 (2014.01)

USPC - 506/7; 435/6; 514/314

According to International Patent Classification (IPC) or to both national classification and IPC

B.  FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8) Classification(s): C40B 30/04; C07K 16/18; G01N 33/567 (2014.01)

USPC Classification(s): 506/7; 16; 424/134.1; 435/6; 514/314

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)


C.  DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>WO 2012/078365 A2 (MURACA, P) June 14, 2012; page 2, lines 4-10; page 4, lines 5-13, 21-24; page 7, lines 24-31; page 26, lines 16-27; page 31, lines 14-15; page 32, lines 10-15; page 37, lines 5-7; Claim 15</td>
<td>1, 2, 6-17, 21, 23-30, 34, 36-41, 45</td>
</tr>
<tr>
<td>A</td>
<td>WO 2009/158143 A1 (PEROU, C et al.) December 30, 2009; entire document</td>
<td>1, 2, 6-17, 21, 23-30, 34, 36-41, 45</td>
</tr>
</tbody>
</table>

* Special categories of cited documents:
  "A" document defining the general state of the art which is not considered to be of particular relevance
  "E" earlier application or patent but published on or after the international filing date
  "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
  "O" document referring to an oral disclosure, use, exhibition or other means
  "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"K" document member of the same patent family

Date of the actual completion of the international search
11 February 2014 (11.02.2014)

Date of mailing of the international search report
24 FEB 2014

Name and mailing address of the ISA/US
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[Diagram of Patent Search Result]
Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

-""""Please See Supplemental Page.""""

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

   Group 1-1, 2, 6-17, 21, 23-30, 34, 36-41, 45, RLP7AP50 gene

Remark on Protest

The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

No protest accompanied the payment of additional search fees.
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I: Claims 1-53 and RPL7AP50 are directed toward a gene expression panel or array indicative of the risk of developing breast cancer, said panel or array consisting of primers or probes capable of detecting at least 1 gene selected from: (i) the genes of Table 1; (ii) the genes of Table 2; and (iii) genes of Table 3; or (iv) the genes of Table 4; a diagnostic kit containing probes or primers for measuring the expression of one or more of genes of Table 1, Table 2, Table 3, and Table 4; a method of assessing a subject's susceptibility to develop cancer, wherein said cancer is a breast cancer comprising: a. obtaining a nucleic acid for variant detection and/or deregulation of genes or gene expression products, wherein said genes or gene expression products are selected from: (i) the genes of Table 1; (ii) the genes of Table 2; and (iii) genes of Table 3; or (iv) the genes of Table 4; and wherein 1-300 genes or gene expression products are selected. b. obtaining a profile of the expression levels of the selected genes or gene expression products in said sample; and c. assessing a subject's susceptibility to develop breast cancer based upon genetic variants and/or a variance in the obtained profile of expression levels of said selected genes or gene expression products in said subject's sample from the same selected genes or gene expression products of a control gene expression profile from a similar biological sample of a healthy subject, or assessing a subject's susceptibility to develop breast cancer based upon a similarity in the obtained profile of expression levels of said selected genes or gene expression products in said subject's sample to the same selected genes or gene expression products in said subject's sample from the same selected genes or gene expression products from a control sample.

Groups II: Claims 1-53 and RPL7AP50 are directed toward a gene expression panel or array indicative of the risk of developing breast cancer, said panel or array consisting of primers or probes capable of detecting at least 1 gene selected from: (i) the genes of Table 1; (ii) the genes of Table 2; and (iii) genes of Table 3; or (iv) the genes of Table 4; and wherein at least 5 genes or gene expression products are selected. b. obtaining a profile of the expression levels of the selected genes or gene expression products in said sample; and c. diagnosing breast cancer based upon a pattern of obtained expression levels of the said genes or gene expression products that form a gene expression profile characteristic of breast cancer in said subject's sample; a method of assessing a subject's susceptibility to develop breast cancer, wherein said cancer is a breast cancer comprising: a. obtaining a nucleic acid for analysis of genes or gene expression products, wherein said genes or gene expression products are selected from: (i) the genes of Table 1; (ii) the genes of Table 2; and (iii) genes of Table 3; or (iv) the genes of Table 4; and wherein at least 20 genes or gene expression products are selected. b. obtaining a profile of the expression levels of the selected genes or gene expression products in said sample; and c. normalizing said expression level to obtain a normalized expression level of the genes of; (b) d. assessing a subject's susceptibility to develop breast cancer based upon a variance in the obtained profile of expression levels of the said selected genes or gene expression products in said subject's sample from the same selected genes or gene expression products of a control gene expression profile from a similar biological sample of a healthy subject, or assessing a subject's susceptibility to develop breast cancer based upon a similarity in the obtained profile of expression levels of said selected genes or gene expression products in said subject's sample to the same selected genes or gene expression products in a gene expression profile characteristic of a subject with breast cancer or a subject who has a proven susceptibility to develop breast cancer; a method for diagnosing breast cancer in a mammalian subject comprising: a. obtaining a nucleic acid for analysis of genes or gene expression products, wherein said genes or gene expression products are selected from: (i) the genes of Table 1; (ii) the genes of Table 2; and (iii) genes of Table 3; or (iv) the genes of Table 4; and wherein at least 20 genes or gene expression products are selected. b. obtaining a profile of the expression levels of the selected genes or gene expression products in said sample; and c. normalizing said expression level to obtain a normalized expression level of the genes of; (a) and d. diagnosing breast cancer based upon a pattern of obtained expression levels of the said genes or gene expression products that form a gene expression profile characteristic of breast cancer in said subject's sample; a method of preparing a personalized genomics profile for a breast cancer subject, comprising: a. obtaining a nucleic acid for analysis of genes or gene expression products, wherein said genes or gene expression products are selected from: (i) the genes of Table 1; (ii) the genes of Table 2; and (iii) genes of Table 3; or (iv) the genes of Table 4; and wherein at least 20 genes or gene expression products are selected. b. obtaining a profile of the expression levels of the selected genes or gene expression products in said sample; and c. normalizing said expression level to obtain a normalized expression level of the genes of; (a) and d. diagnosing breast cancer based upon a pattern of obtained expression levels of the said genes or gene expression products that form a gene expression profile characteristic of breast cancer in said subject's sample; a method of assessing a subject's susceptibility to develop breast cancer, wherein said cancer is a breast cancer comprising: a. obtaining a nucleic acid for analysis of genes or gene expression products, wherein said genes or gene expression products are selected from: (i) the genes of Table 1; (ii) the genes of Table 2; and (iii) genes of Table 3; or (iv) the genes of Table 4; and wherein at least 1 gene or gene expression products are selected. b. obtaining a profile of the expression levels of the selected genes or gene expression products in said sample; and c. assessing a subject's susceptibility to develop breast cancer based upon a variance in the obtained profile of expression levels of the said selected genes or gene expression products in said subject's sample from the same selected genes or gene expression products in a gene expression profile characteristic of a subject with breast cancer or a subject who has a proven susceptibility to develop breast cancer.
***-Continued from Previous Supplemental Box***

The gene expression panel or array indicative of the risk of developing breast cancer, said panel or array consisting of primers or probes capable of detecting at least 1 gene, a diagnostic kit containing probes or primers for measuring the expression of one or more genes, and a method of assessing a subject's susceptibility to develop cancer, wherein said cancer is a breast cancer comprising:

a. obtaining a nucleic acid for variant detection and/or deregulation of genes or gene expression products will be searched to the extent that they encompass RPL7AP50, the first gene of Table 1 of the priority document of the instant PCT application. It is believed that Claims 1, 2, 6-17, 21, 22 and 45 (in-part) encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass RPL7AP50, the first gene of Table 1 of the priority document of the instant PCT application. Applicants must indicate, if applicable, the claims which encompass the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. Additional genes can be searched upon the payment of additional fees. An Exemplary Election would be: Gene VDACP1 P9.

Groups I- share the technical features including a gene expression panel or array indicative of the risk of developing breast cancer, said panel or array consisting of primers or probes capable of detecting at least 1 gene; a diagnostic kit containing probes or primers for measuring the expression of one or more genes; a method of assessing a subject's susceptibility to develop cancer, wherein said cancer is a breast cancer comprising: a. obtaining a nucleic acid for variant detection and/or deregulation of genes or gene expression products, and wherein 1-300 genes or gene expression products are selected, b. obtaining a profile of the expression levels of the selected genes or gene expression products in said sample, and c. assessing a subject's susceptibility to develop breast cancer based upon genetic variants and/or a variance in the obtained profile of expression levels of the said selected genes or gene expression products in said subject's sample from the same selected genes or gene expression products of a control gene expression profile from a similar biological sample of a healthy subject, or assessing a subject's susceptibility to develop breast cancer based upon a similarity in the obtained profile of expression levels of said selected genes or gene expression products in said subject's sample to the same selected genes or gene expression products in a gene expression profile characteristic of a subject with breast cancer or a subject who has a proven susceptibility to develop breast cancer; a method for diagnosing breast cancer in a mammalian subject comprising: a. obtaining a nucleic acid for analysis of genes or gene expression products, and wherein at least 5 genes or gene expression products are selected, b. obtaining a profile of the expression levels of the selected genes or gene expression products in said sample, and c. diagnosing breast cancer based upon a pattern of obtained expression levels of the said genes or gene expression products that form a gene expression profile characteristic of breast cancer in said subject's sample; a method of assessing a subject's susceptibility to develop cancer, wherein said cancer is a breast cancer comprising: a. obtaining a nucleic acid for analysis of genes or gene expression products, and wherein at least 20 genes or gene expression products are selected, b. obtaining a profile of the expression levels of the selected genes or gene expression products in said sample, c. normalizing said expression level to obtain a normalized expression level of the genes of (b); and d. assessing a subject's susceptibility to develop breast cancer based upon a variance in the obtained profile of expression levels of the said selected genes or gene expression products in said subject's sample from the same selected genes or gene expression products of a control gene expression profile from a similar biological sample of a healthy subject, or assessing a subject's susceptibility to develop breast cancer based upon a similarity in the obtained profile of expression levels of said selected genes or gene expression products in said subject's sample to the same selected genes or gene expression products in a gene expression profile characteristic of a subject with breast cancer or a subject who has a proven susceptibility to develop breast cancer; a method for diagnosing breast cancer in a mammalian subject comprising: a. obtaining a nucleic acid for analysis of genes or gene expression products, and wherein at least 20 genes or gene expression products are selected, b. obtaining a profile of the expression levels of the selected genes or gene expression products in said sample, c. normalizing said expression level to obtain a normalized expression level of the genes of (a); and d. diagnosing breast cancer based upon a pattern of obtained expression levels of the said genes or gene expression products that form a gene expression profile characteristic of breast cancer in said subject's sample; a method of preparing a personalized genomics profile for a breast cancer subject, comprising: a. obtaining a nucleic acid for analysis of genes or gene expression products, wherein at least 20 genes or gene expression products are selected, b. obtaining the expression levels of said genes or gene expression products in said sample; wherein the expression level is normalized against the expression level of at least one reference gene to obtain normalized data or the expression levels in a breast cancer reference tissue set, and c. creating a report summarizing the normalized data obtained by said gene expression analysis, wherein said report includes a prediction of a subject's increased likelihood to develop breast cancer; and a gene expression panel or array indicative of the risk of developing breast cancer, said panel or array consisting of primers or probes capable of detecting at least 20 genes. .... Continued on Next Supplemental Page ...
However, these shared technical features are previously disclosed by WO 2012/078365 A2 (MURACA). Muraca discloses a gene expression panel or array (a gene expression panel or array: page 5, lines 28-31) indicative of the risk of developing breast cancer (indicative of the risk of developing breast cancer: page 2, lines 4-10), said panel or array consisting of primers or probes (panel or array consisting of primers or probes: page 7, lines 24-31) capable of detecting at least 1 gene (capable of detecting at least 1 gene: page 4, lines 20-24); a diagnostic (diagnostic: lines 3-11) kit (kit: page 37, lines 5-7) containing probes or primers (containing probes or primers: page 7, lines 24-31) for measuring the expression of one or more genes (for measuring the expression of one or more genes: page 4, lines 21-24); a method of assessing a subject's susceptibility to develop cancer (a method of assessing a subject's susceptibility to develop cancer: page 2, lines 4-10), wherein said cancer is a breast cancer (wherein the cancer is breast cancer: page 2, lines 4-10) comprising: a obtaining a nucleic acid for variant detection and/or deregulation of genes or gene expression products, and wherein 1-300 genes or gene expression products are selected (a obtaining a nucleic acid for variant detection and/or deregulation of genes or gene expression products, and wherein 1-300 genes or gene expression products are selected: page 4, lines 3-17), b obtaining a profile of the expression levels of the selected genes or gene expression products in said sample (obtaining a profile of the expression levels of the selected genes or gene expression products in said sample: page 4, lines 3-17), and c assessing a subject's susceptibility to develop breast cancer based upon genetic variants and/or a variance in the obtained profile of expression levels of the said selected genes or gene expression products in said subject's sample from the same selected genes or gene expression products of a control gene expression profile from a similar biological sample of a healthy subject, or assessing a subject's susceptibility to develop breast cancer (assessing a subject's susceptibility to develop breast cancer: page 4, lines 3-4) based upon a similarity in the obtained profile of expression levels of said selected genes or gene expression products in said subject's sample to the same selected genes or gene expression products in a gene expression profile characteristic of a subject with breast cancer or a subject who has a proven susceptibility to develop breast cancer (based upon a similarity in the obtained profile of expression levels of said selected genes or gene expression products in said subject's sample to the same selected genes or gene expression products in a gene expression profile characteristic of a subject with breast cancer or a subject who has a proven susceptibility to develop breast cancer: page 3, lines 1-14, page 25, line 19 to page 25, line 7); a method for diagnosing (method for diagnosing: page 8, lines 3-11) breast cancer in a mammalian subject (method for diagnosing: page 8, lines 3-11) comprising: a obtaining a nucleic acid for analysis of genes or gene expression products, and wherein at least 5 genes or gene expression products are selected (a obtaining a nucleic acid for analysis of genes or gene expression products, and wherein at least 5 genes or gene expression products are selected: page 4, lines 1-14); b obtaining a profile of the expression levels of the selected genes or gene expression products in said sample (a obtaining a profile of the expression levels of the selected genes or gene expression products in said sample: page 4, lines 3-17), and c diagnosing (diagnosing: page 8, lines 3-11) breast cancer (breast cancer: page 2, lines 4-10) based upon a pattern of obtained expression levels of the said selected genes or gene expression products that form a gene expression profile characteristic of breast cancer in said subject's sample (diagnosing: page 2, lines 4-10) comprising: a obtaining a nucleic acid for analysis of genes or gene expression products, and wherein at least 20 genes or gene expression products are selected (a obtaining a nucleic acid for analysis of genes or gene expression products, and wherein at least 20 genes or gene expression products are selected: page 4, lines 3-17), b obtaining a profile of the expression levels of the selected genes or gene expression products in said sample (obtaining a profile of the expression levels of the selected genes or gene expression products in said sample: page 4, lines 3-17), and c normalizing (normalizing: page 8, lines 3-11) said sample to obtain a normalized expression level of the genes of (a) (normalizing said sample to obtain a normalized expression level of the genes of (a): page 25, line 27 to page 26, line 7) and d assessing a subject's susceptibility to develop breast cancer based upon a variance in the obtained profile of expression levels of the said selected genes or gene expression products in said subject's sample from the same selected genes or gene expression products of a control gene expression profile from a similar biological sample of a healthy subject, or assessing a subject's susceptibility to develop breast cancer (assessing a subject's susceptibility to develop breast cancer: page 4, lines 3-4) based upon a similarity in the obtained profile of expression levels of said selected genes or gene expression products in said subject's sample to the same selected genes or gene expression products in a gene expression profile characteristic of a subject with breast cancer or a subject who has a proven susceptibility to develop breast cancer (based upon a similarity in the obtained profile of expression levels of said selected genes or gene expression products in said subject's sample to the same selected genes or gene expression products in a gene expression profile characteristic of a subject with breast cancer or a subject who has a proven susceptibility to develop breast cancer: page 3, lines 1-14, page 25, line 19 to page 25, line 7); a method for diagnosing (method for diagnosing: page 8, lines 3-11) breast cancer in a mammalian subject (method for diagnosing: page 8, lines 3-11) comprising: a obtaining a nucleic acid for analysis of genes or gene expression products, and wherein at least 20 genes or gene expression products are selected (a obtaining a nucleic acid for analysis of genes or gene expression products, and wherein at least 20 genes or gene expression products are selected: page 4, lines 3-17), b obtaining a profile of the expression levels of the selected genes or gene expression products in said sample (obtaining a profile of the expression levels of the selected genes or gene expression products in said sample: page 4, lines 3-17), c normalizing (normalizing: page 8, lines 3-11) said sample to obtain a normalized expression level of the genes of (b) (normalizing said sample to obtain a normalized expression level of the genes of (b): page 25, line 27 to page 26, line 7); and d assessing a subject's susceptibility to develop breast cancer based upon a pattern of obtained expression levels of the said selected genes or gene expression products in said subject's sample from the same selected genes or gene expression products of a control gene expression profile from a similar biological sample of a healthy subject, or assessing a subject's susceptibility to develop breast cancer (assessing a subject's susceptibility to develop breast cancer: page 4, lines 3-4) based upon a similarity in the obtained profile of expression levels of said selected genes or gene expression products in said subject's sample to the same selected genes or gene expression products in a gene expression profile characteristic of a subject with breast cancer or a subject who has a proven susceptibility to develop breast cancer (based upon a similarity in the obtained profile of expression levels of said selected genes or gene expression products in said subject's sample to the same selected genes or gene expression products in a gene expression profile characteristic of a subject with breast cancer or a subject who has a proven susceptibility to develop breast cancer: page 3, lines 1-14, page 25, line 19 to page 25, line 7); a method for preparing a personalized genomics profile (a method for preparing a personalized genomics profile: page 7, lines 24-26) for a breast cancer subject (for a breast cancer subject: page 2, lines 4-10), comprising: a) obtaining a nucleic acid for analysis of genes or gene expression products, wherein at least 20 genes or gene expression products are selected (obtaining a nucleic acid for analysis of genes or gene expression products, wherein at least 20 genes or gene expression products are selected: page 4, lines 3-17), b) obtaining the expression levels of said genes or gene expression products in said sample (b) obtaining the expression levels of said genes or gene expression products in said sample: page 4, lines 3-17); and c continuing on Next Supplemental Page...
Continued from Previous Supplemental Page:

wherein the expression level is normalized against the expression level of at least one reference gene to obtain normalized data or the expression levels in a breast cancer reference tissue set (wherein the expression level is normalized against the expression level of at least one reference gene to obtain normalized data or the expression levels in a breast cancer reference tissue set; page 25, line 27 to page 26, line 7); and a gene expression panel or array (a gene expression panel or array; page 5, lines 28-31) indicative of the risk of developing breast cancer (indicative of the risk of developing breast cancer; page 2, lines 4-10), said panel or array consisting of primers or probes capable of detecting at least 20 genes (said panel or array consisting of primers or probes capable of detecting at least 20 genes; page 4, lines 3-17).

Muraca does not disclose creating a report summarizing the normalized data obtained by said gene expression analysis, wherein said report includes a prediction of a subject's increased likelihood to develop breast cancer.

However, provided the previous disclosure of Muraca, regarding the generation of a personalized gene expression analysis for the prediction of a subject's likelihood to develop breast cancer based on normalized data, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have created a report of the results summarizing the data for communication to a subject, or a medical practitioner designated by a subject, for enabling the subject or the practitioner to make decisions regarding the course or care or therapy for the subject, without undue experimentation or testing.

Since none of the special technical features of the Groups 1+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Muraca reference, unity of invention is lacking.