

CLAIMS

1. A method of determining whether a subject has impaired glucose tolerance or the likelihood that a subject has impaired glucose tolerance, comprising forming a biomarker panel having N biomarker proteins, and detecting the level of each of the N biomarker proteins in a sample from the subject, wherein N is at least 3, and wherein at least one of the N biomarker proteins is selected from ACY1, COL1A1, RTN4R, CRLF1:CLCF1 complex, CBX7, KIN, SERPINA11, PELI2, TFF3, FABP12, GAD1, SVEP1, SOCS7, F9, STC1, MYOC, WFDC11, CALB1, CCL16, SMCO2, CCL23, OSTM1, RNASE10, ITIH1, ZNF134, CFAP45, and SFTPD.

2. A method of determining whether a subject has pre-diabetes or is likely to develop pre-diabetes or diabetes, comprising forming a biomarker panel having N biomarker proteins, and detecting the level of each of the N biomarker proteins in a sample from the subject, wherein N is at least 3, and wherein at least one of the N biomarker proteins is selected from ACY1, COL1A1, RTN4R, CRLF1:CLCF1 complex, CBX7, KIN, SERPINA11, PELI2, TFF3, FABP12, GAD1, SVEP1, SOCS7, F9, STC1, MYOC, WFDC11, CALB1, CCL16, SMCO2, CCL23, OSTM1, RNASE10, ITIH1, ZNF134, CFAP45, and SFTPD.

3. A method of detecting the levels of N biomarker proteins in a sample, comprising obtaining the sample from the subject, forming a biomarker panel having N biomarker proteins, and detecting the level of each of the N biomarker proteins in the sample from the subject, wherein N is at least 3, and wherein at least one of the N biomarker proteins is selected from ACY1, COL1A1, RTN4R, CRLF1:CLCF1 complex, CBX7, KIN, SERPINA11, PELI2, TFF3, FABP12, GAD1, SVEP1, SOCS7, F9, STC1, MYOC, WFDC11, CALB1, CCL16, SMCO2, CCL23, OSTM1, RNASE10, ITIH1, ZNF134, CFAP45, and SFTPD.

4. The method of any one of the preceding claims, wherein N is 3 to 41, or N is 4 to 41, or N is 5 to 41, or N is 6 to 41, or N is 7 to 41, or N is 8 to 41, or N is 9 to 41, or N is 10 to 41, or N is 11 to 41, or N is 12 to 41, or N is 13 to 41, or N is 14 to 41, or N is 15 to 41, or N is 16 to 41.

5. The method of any one of the preceding claims, wherein N is 3, or N is 4, or N is 5, or N is 6, or N is 7, or N is 8, or N is 9, or N is 10, or N is 11, or N is 12, or N is 13, or N is 14, or N is 15, or N is 16, or N is 17, or N is 18, or N is 19, or N is 20, or N is 21, or N is 22, or N is 23, or N is 24, or N is 25, or N is 26, or N is 27, or N is 28, or N is 29, or N

is 30, or N is 31, or N is 32, or N is 33, or N is 34, or N is 35, or N is 36, or N is 37, or N is 38, or N is 39, or N is 40, or N is 41.

6. The method of any one of the preceding claims, wherein each of the N biomarker proteins is selected from Table 1.

7. The method of any one of the preceding claims, wherein at least one of the N biomarker proteins is selected from ACY1, COL1A1, RTN4R, CRLF1:CLCF1 complex, CBX7, and KIN.

8. The method of any one of the preceding claims, wherein one or two of the N biomarker proteins are INHBC and/or SHBG.

9. The method of any one of the preceding claims, wherein at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 of the N protein biomarkers are selected from ACY1, COL1A1, RTN4R, CRLF1:CLCF1 complex, CBX7, KIN, SERPINA11, PELI2, TFF3, FABP12, GAD1, SVEP1, SOCS7, F9, STC1, MYOC, WFDC11, CALB1, CCL16, SMC02, CCL23, OSTM1, RNASE10, ITIH1, ZNF134, CFAP45, and SFTPD.

10. The method of any one of the preceding claims, wherein at least one, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 of the N biomarker proteins is selected from FAM20B, COL15A1, MARCKSL1, HTRA1, CHAD, CPM, DLK1, HERC1, IL20RB, MAP2K4, GPX2, and FGFR4.

11. The method of any one of the preceding claims, wherein two of the N biomarker proteins are INHBC and ACY1, or two of the N biomarker proteins are SHBG and ACY1, or three of the N biomarker proteins are INHBC, SHBG, and ACY1.

12. The method of any one of the preceding claims, wherein two of the N biomarker proteins are INHBC and COL1A1, or two of the N biomarker proteins are SHBG and COL1A1, or wherein three of the N biomarker proteins are INHBC, SHBG, and COL1A1.

13. The method of any one of the preceding claims, wherein two of the N biomarker proteins are INHBC and RTN4R, or two of the N biomarker proteins are SHBG and RTN4R, or wherein three of the N biomarker proteins are INHBC, SHBG, and RTN4R.

14. The method of any one of the preceding claims, wherein two of the N biomarker proteins are INHBC and CRLF1:CLCF1 complex, or two of the N biomarker proteins are SHBG and CRLF1:CLCF1 complex, or wherein three of the N biomarker proteins are INHBC, SHBG, and CRLF1:CLCF1 complex.

15. The method of any one of the preceding claims, wherein two of the N biomarker proteins are INHBC and CBX7, or two of the N biomarker proteins are SHBG and CBX7, or wherein three of the N biomarker proteins are INHBC, SHBG, and CBX7.
16. The method of any one of the preceding claims, wherein two of the N biomarker proteins are INHBC and KIN, or two of the N biomarker proteins are SHBG and KIN, or wherein three of the N biomarker proteins are INHBC, SHBG, and KIN.
17. The method of any one of the preceding claims, wherein N is at least five and five of the N biomarker proteins are INHBC, SHBG, ACY1, COL1A1, and RTN4R.
18. The method of any one of the preceding claims, wherein N is at least 16, and wherein 16 of the N biomarker proteins are ACY1, COL1A1, RTN4R, CRLF1, CBX7, KIN, SERPINA11, PELI2, TFF3, FABP12, INHBC, SHBG, FAM20B, COL15A1, MARCKSL1, and HTRA1.
19. The method of any one of the preceding claims, wherein the sample is a blood sample, a plasma sample, or a serum sample.
20. The method of any one of the preceding claims, wherein the subject continues eating a typical diet and a glucose solution is not administered to the subject before the sample is obtained.
21. The method of any one of the preceding claims, wherein a level of at least one biomarker selected from SHBG, COL1A1, CRLF1:CLCF1 complex, FAM20B, COL15A1, KIN, SERPINA11, PELI2, MARCKSL1, CHAD, IL20RB, MYOC, WFDC11, MAP2K4, CALB1, FGFR4, OSTM1, ITIH1, CFAP45, and SFTPD that is higher than a control level of the respective biomarker, indicates that the subject has or likely has impaired glucose tolerance, has or is likely to develop pre-diabetes, and/or is likely to develop diabetes.
22. The method of any one of the preceding claims, wherein a level of at least one biomarker selected from INHBC, ACY1, RTN4R, CBX7, TFF3, HTRA1, FABP12, GAD1, CPM, SVEP1, SOCS7, F9, DLK1, HERC1, STC1, CCL16, SMC02, GPX2, CCL23, RNASE10, and ZNF134 that is lower than a control level of the respective biomarker, indicates that the subject has or likely has impaired glucose tolerance, has or is likely to develop pre-diabetes, and/or is likely to develop diabetes.
23. The method of any one of the preceding claims, wherein the subject is at risk of developing diabetes.
24. The method of any one of the preceding claims, comprising determining whether the subject has or likely has impaired glucose tolerance.

25. The method of claim 24, wherein the subject likely has impaired glucose tolerance.
26. The method of claim 24, wherein the subject has impaired glucose tolerance.
27. The method of any one of claims 2 to 26, comprising determining whether the subject is likely to develop or has pre-diabetes.
28. The method of claim 27, wherein the subject is likely to develop pre-diabetes.
29. The method of claim 27, wherein the subject has pre-diabetes.
30. The method of any one of claims 25, 26, 28, and 29, wherein the subject is likely to develop diabetes.
31. The method of claim 30, wherein the diabetes is type 2 diabetes.
32. The method of any one of claims 21-23, 25, 26, and 28-31, comprising administering a treatment to the subject.
33. The method of claim 32, wherein the treatment comprises insulin, metformin, implementing a weight loss program, implementing dietary restrictions, implementing caloric restrictions and/or implementing an exercise program.
34. The method of any one of the preceding claims, wherein the method comprises contacting biomarker proteins of the sample or samples with a set of biomarker capture reagents, wherein each biomarker capture reagent of the set of biomarker capture reagents specifically binds to a different biomarker protein being detected.
35. The method of claim 34, wherein each biomarker capture reagent is an antibody or an aptamer.
36. The method of claim 35, wherein each biomarker capture reagent is an aptamer.
37. The method of claim 36, wherein at least one aptamer is a slow off-rate aptamer.
38. The method of claim 37, wherein at least one slow off-rate aptamer comprises at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, or at least 10 nucleotides with modifications.
39. The method of claim 37 or claim 38, wherein each slow off-rate aptamer binds to its target protein with an off rate ($t_{1/2}$) of ≥ 30 minutes, ≥ 60 minutes, ≥ 90 minutes, ≥ 120 minutes, ≥ 150 minutes, ≥ 180 minutes, ≥ 210 minutes, or ≥ 240 minutes.
40. The method of any one of the preceding claims, wherein if the subject has or likely has impaired glucose tolerance, has pre-diabetes, or is likely to develop pre-diabetes

or diabetes, the subject is recommended a regimen comprising weight loss, blood sugar control, and/or drug treatment.

41. The method of any one of the preceding claims, wherein the determining comprises analyzing the levels of the N biomarker protein levels using a classification model or an elastic net logistic regression model.

42. The method of any one of the preceding claims, wherein the method comprises determining whether a subject has or likely has impaired glucose tolerance, has pre-diabetes, or is likely to develop pre-diabetes or diabetes for the purpose of determining a medical insurance premium or life insurance premium.

43. The method of any one of the preceding claims, wherein the method further comprises determining a medical insurance premium or life insurance premium.

44. The method of any one of the preceding claims, wherein the method further comprises using information resulting from the method to predict and/or manage the utilization of medical resources.

45. A kit comprising N biomarker protein capture reagents, wherein N is at least 3, and wherein at least one of the N biomarker protein capture reagents specifically binds to a biomarker protein selected from ACY1, COL1A1, RTN4R, CRLF1:CLCF1 complex, CBX7, KIN, SERPINA11, PELI2, TFF3, FABP12, GAD1, SVEP1, SOCS7, F9, STC1, MYOC, WFDC11, CALB1, CCL16, SMCO2, CCL23, OSTM1, RNASE10, ITIH1, ZNF134, CFAP45, and SFTPD.

46. The kit of claim 45, wherein N is 3 to 41, or N is 4 to 41, or N is 5 to 41, or N is 6 to 41, or N is 7 to 41, or N is 8 to 41, or N is 9 to 41, or N is 10 to 41, or N is 11 to 41, or N is 12 to 41, or N is 13 to 41, or N is 14 to 41, or N is 15 to 41, or N is 16 to 41.

47. The kit claim 45 or 46, wherein N is 3, or N is 4, or N is 5, or N is 6, or N is 7, or N is 8, or N is 9, or N is 10, or N is 11, or N is 12, or N is 13, or N is 14, or N is 15, or N is 16, or N is 17, or N is 18, or N is 19, or N is 20, or N is 21, or N is 22, or N is 23, or N is 24, or N is 25, or N is 26, or N is 27, or N is 28, or N is 29, or N is 30, or N is 31, or N is 32, or N is 33, or N is 34, or N is 35, or N is 36, or N is 37, or N is 38, or N is 39, or N is 40, or N is 41.

48. The kit of any one of claims 45 to 47, wherein each of the N biomarker protein capture reagents specifically binds to a different biomarker protein.

49. The kit of any one of claims 45 to 48, wherein each of the N biomarker protein capture reagents specifically binds to a biomarker protein selected from Table 1.

50. The kit of any one of claims 45 to 49, wherein at least one of the N biomarker protein capture reagents specifically binds to a biomarker protein selected from ACY1, COL1A1, RTN4R, CRLF1:CLCF1 complex, CBX7, and KIN.
51. The kit of any one of claims 45 to 50, wherein one or two of the N biomarker protein capture reagents specifically binds to INHBC and/or SHBG.
52. The kit of any one of claims 45 to 51, wherein at least 2 or at least 3 of the N protein biomarker capture reagents each specifically bind to a protein selected from ACY1, COL1A1, RTN4R, CRLF1:CLCF1 complex, CBX7, KIN, SERPINA11, PELI2, TFF3, FABP12, GAD1, SVEP1, SOCS7, F9, STC1, MYOC, WFDC11, CALB1, CCL16, SMCO2, CCL23, OSTM1, RNASE10, ITIH1, ZNF134, CFAP45, and SFTPD.
53. The kit of any one of claims 45 to 52, wherein at least one of the N biomarker protein capture reagents specifically binds to a protein selected from FAM20B, COL15A1, MARCKSL1, HTRA1, CHAD, CPM, DLK1, HERC1, IL20RB, MAP2K4, GPX2, and FGFR4.
54. The kit of any one of claims 45 to 53, wherein each of two of the N biomarker protein capture reagents specifically binds to INHBC or ACY1, or each of two of the N biomarker protein capture reagents specifically binds to SHBG or ACY1, or each of three of the N biomarker protein capture reagents specifically binds to a biomarker protein selected from INHBC, SHBG, and ACY1.
55. The kit of any one of claims 45 to 54, wherein each of two of the N biomarker protein capture reagents specifically binds to INHBC or COL1A1, each of two of the N biomarker protein capture reagents specifically binds to SHBG or COL1A1, or each of three of the N biomarker protein capture reagents specifically binds to a biomarker protein selected from INHBC, SHBG, and COL1A1.
56. The kit of any one of claims 45 to 55, wherein each of two of the N biomarker protein capture reagents specifically binds to INHBC or RTN4R, each of two of the N biomarker protein capture reagents specifically binds to SHBG or RTN4R, or each of three of the N biomarker protein capture reagents specifically binds to a biomarker protein selected from INHBC, SHBG, and RTN4R.
57. The kit of any one of claims 45 to 56, wherein each of two of the N biomarker protein capture reagents specifically binds to INHBC or CRLF1:CLCF1 complex, each of two of the N biomarker protein capture reagents specifically binds to SHBG or CRLF1:CLCF1 complex, or each of three of the N biomarker protein capture reagents

specifically binds to a biomarker protein selected from INHBC, SHBG, and CRLF1:CLCF1 complex.

58. The kit of any one of claims 45 to 57, wherein each of two of the N biomarker protein capture reagents specifically binds to INHBC or CBX7, each of two of the N biomarker protein capture reagents specifically binds to SHBG or CBX7, or each of three of the N biomarker protein capture reagents specifically binds to a biomarker protein selected from INHBC, SHBG, and CBX7.

59. The kit of any one of claims 45 to 58, wherein each of two of the N biomarker protein capture reagents specifically binds to INHBC or KIN, each of two of the N biomarker protein capture reagents specifically binds to SHBG or KIN, or each of three of the N biomarker protein capture reagents specifically binds to a biomarker protein selected from INHBC, SHBG, and KIN.

60. The kit of any one of claims 45 to 59, wherein N is at least five and each of five of the N biomarker protein capture reagents specifically binds to a biomarker protein selected from INHBC, SHBG, ACY1, COL1A1, and RTN4R.

61. The kit of any one of claims 45 to 60, wherein N is at least 16, and wherein each of 16 of the N biomarker protein capture reagents specifically binds to a biomarker protein selected from ACY1, COL1A1, RTN4R, CRLF1, CBX7, KIN, SERPINA, PELI2, TFF3, FABP12, INHBC, SHBG, FAM20B, COL15A1, MARCKSL1, and HTRA1.

62. The kit of any one of claims 45 to 61, wherein each of the N biomarker capture reagents is an antibody or an aptamer.

63. The kit of claim 62, wherein each biomarker capture reagent is an aptamer.

64. The kit of claim 63, wherein at least one aptamer is a slow off-rate aptamer.

65. The kit of claim 64, wherein at least one slow off-rate aptamer comprises at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, or at least 10 nucleotides with modifications.

66. The kit of claim 64 or claim 65, wherein each slow off-rate aptamer binds to its target protein with an off rate ($t_{1/2}$) of ≥ 30 minutes, ≥ 60 minutes, ≥ 90 minutes, ≥ 120 minutes, ≥ 150 minutes, ≥ 180 minutes, ≥ 210 minutes, or ≥ 240 minutes.

67. The kit of any one of claims 45 to 66, for use in detecting the N biomarker proteins in a sample from a subject.

68. The kit of claim 67, for use in determining whether the subject has or likely has impaired glucose tolerance or has pre-diabetes, or is likely to develop pre-diabetes or diabetes.