

Claims

1. A set of biomarkers comprising at least:
 - i) the subset of 6 mRNA listed in the following Table:

Name	Ensemble ID
Calpain 2 (CAPN2)	ENSG00000162909
Dynactin Subunit 2 (DCTN2)	ENSG00000175203
Diacylglycerol Kinase Delta (DGKD)	ENSG00000077044
Major Histocompatibility Complex, Class II, DP Alpha 1 (HLA-DPA1)	ENSG00000231389
Major Histocompatibility Complex, Class II, DP Beta 1 (HLA-DPB1)	ENSG00000223865
WD Repeat Domain 86 (WDR86)	ENSG00000187260

5

- ii) the subset of the following 7 metabolites:

- 2-aminooctanoate
- 4-methoxyphenol sulfate
- hypotaurine
- lysine
- N-acetyl-2-aminooctanoate
- phenol sulfate
- γ -glutamylleucine;

10

and

iii) the lipid dococenedioate (C12:1-DC);

for the diagnosis of Brugada Syndrome in a human being.

2. A set of biomarkers according to claim 1, further comprising one or more of the

5 following additional biomarkers:

i) RNA selected from the group consisting of:

Name	Ensemble ID	Type
Acyl-CoA Synthetase Family Member 4 (AASDH)	ENSG00000157426	mRNA
Ankyrin Repeat And Death Domain Containing 1A (ANKDD1A)	ENSG00000166839	mRNA
Ankyrin Repeat Domain 49 (ANKRD49)	ENSG00000168876	mRNA
Rho/Rac Guanine Nucleotide Exchange Factor 18 (ARHGEF18)	ENSG00000104880	mRNA
BRICHOS Domain Containing 5 (BRICD5)	ENSG00000182685	mRNA
BRCA1 Interacting Protein C-Terminal Helicase 1 (BRIP1)	ENSG00000136492	mRNA
BRO1 Domain And CAAX Motif Containing (BROX)	ENSG00000162819	mRNA
Glucocorticoid Receptor AF-1 Coactivator-1 (BTBD6)	ENSG00000184887	mRNA

Chromosome 19 Open Reading Frame 79 (C12orf79)	ENSG00000257242	Long non-coding RNA
Chromosome 19 Open Reading Frame 38 (C19orf38)	ENSG00000214212	mRNA
Calcium/Calmodulin Dependent Protein Kinase Kinase 2 (CAMKK2)	ENSG00000110931	mRNA
Chimerin I (CHN1)	ENSG00000128656	mRNA
Cyclin-M3 (CNNM3)	ENSG00000168763	mRNA
Cytochrome P450 Family 2 Subfamily S Member 1 (CYP2S1)	ENSG00000167600	mRNA
ERBB Receptor Feedback Inhibitor 1 (ERRFI1)	ENSG00000116285	mRNA
Retrotransposon Gag Like 8C (FAM127A)	ENSG00000134590	mRNA
Glycoprotein, Alpha- Galactosyltransferase (GGTA1P)	ENSG00000204136	mRNA
Glutathione-Disulfide Reductase (GSR)	ENSG00000104687	mRNA
HLA Complex Group 18 (HCG18)	ENSG00000231074	mRNA
Histone Cluster 1 H1 Family Member C (HIST1H1C)	ENSG00000187837	mRNA
Histone Cluster 1 H2B	ENSG00000197903	mRNA

Family Member K (HIST1H2BK)		
Insulin Like Growth Factor Binding Protein 3 (IGFBP3)	ENSG00000146674	mRNA
Ras GTPase-Activating-Like Protein (IQGAP2)	ENSG00000145703	mRNA
Lymphotoxin Beta (LTB)	ENSG00000227507	mRNA
Methionine Adenosyltransferase 2A (MAT2A)	ENSG00000168906	mRNA
Mitochondrial Ribosomal Protein L22 (MRPS22)	ENSG00000175110	mRNA
Nucleosome Assembly Protein 1 Like 2 (NAP1L2)	ENSG00000186462	mRNA
Peptidyl Arginine Deiminase 4 (PADI4)	ENSG00000159339	mRNA
PDZ And LIM Domain 7 (PDLIM7)	ENSG00000196923	mRNA
Platelet Activating Factor Receptor (PTAFR)	ENSG00000169403	mRNA
RAN Binding Protein 6 (RANBP6)	ENSG00000137040	mRNA
RNA, 7SL, Cytoplasmic 473, Pseudogene (RN7SL473P)	ENSG00000277452	mRNA
RP11-429E11.3	ENSG00000179253.3	mRNA
RP11-4B16.1	ENSG00000279433	microRNA
Ribosomal Protein S20 (RPS20)	ENSG00000008988	mRNA

Syntaxin 10 (STX10)	ENSG00000104915	mRNA
T Cell Receptor Beta Variable 11-2 (TRBV11-2)	ENSG00000241657	mRNA
Zwilch Kinetochore Protein (ZWILCH)	ENSG00000174442	mRNA

ii) metabolite selected from the group consisting of:

- 5-methyluridine (ribothymidine)
- fibrinopeptide A, phosphono-ser(3)
- glyco-alpha-muricholate;
- 3-(4-hydroxyphenyl)lactate
- glucuronate
- Trans-4-hydroxyproline;

iii) phosphatidylinositol (16:0/18:1); or a combination thereof.

3. A set of biomarkers according to claim 2, comprising:

i) the subset of 44 RNAs listed in the following Table:

Name	Ensemble ID	Type
Acyl-CoA Synthetase Family Member 4 (AASDH)	ENSG00000157426	mRNA
Ankyrin Repeat And Death Domain Containing 1A (ANKDD1A)	ENSG00000166839	mRNA
Ankyrin Repeat Domain 49 (ANKRD49)	ENSG00000168876	mRNA
Rho/Rac Guanine Nucleotide Exchange Factor 18 (ARHGEF18)	ENSG00000104880	mRNA

BRICHOS Domain Containing 5 (BRICD5)	ENSG00000182685	mRNA
BRCA1 Interacting Protein C-Terminal Helicase 1 (BRIP1)	ENSG00000136492	mRNA
BRO1 Domain And CAAX Motif Containing (BROX)	ENSG00000162819	mRNA
Glucocorticoid Receptor AF-1 Coactivator-1 (BTBD6)	ENSG00000184887	mRNA
Chromosome 19 Open Reading Frame 79 (C12orf79)	ENSG00000257242	Long non-coding RNA
Chromosome 19 Open Reading Frame 38 (C19orf38)	ENSG00000214212	mRNA
Calcium/Calmodulin Dependent Protein Kinase Kinase 2 (CAMKK2)	ENSG00000110931	mRNA
Calpain 2 (CAPN2)	ENSG00000162909	mRNA
Chimerin I (CHN1)	ENSG00000128656	mRNA
Cyclin-M3 (CNNM3)	ENSG00000168763	mRNA
Cytochrome P450 Family 2 Subfamily S Member 1 (CYP2S1)	ENSG00000167600	mRNA
Dynactin Subunit 2 (DCTN2)	ENSG00000175203	mRNA
Diacylglycerol Kinase Delta (DGKD)	ENSG00000077044	mRNA
ERBB Receptor Feedback Inhibitor 1 (ERRFI1)	ENSG00000116285	mRNA
Retrotransposon Gag Like 8C (FAM127A)	ENSG00000134590	mRNA

Glycoprotein, Alpha-Galactosyltransferase (GGTA1P)	ENSG00000204136	mRNA
Glutathione-Disulfide Reductase (GSR)	ENSG00000104687	mRNA
HLA Complex Group 18 (HCG18)	ENSG00000231074	mRNA
Histone Cluster 1 H1 Family Member C (HIST1H1C)	ENSG00000187837	mRNA
Histone Cluster 1 H2B Family Member K (HIST1H2BK)	ENSG00000197903	mRNA
Major Histocompatibility Complex, Class II, DP Alpha 1 (HLA-DPA1)	ENSG00000231389	mRNA
Major Histocompatibility Complex, Class II, DP Beta 1 (HLA-DPB1)	ENSG00000223865	mRNA
Insulin Like Growth Factor Binding Protein 3 (IGFBP3)	ENSG00000146674	mRNA
Ras GTPase-Activating-Like Protein (IQGAP2)	ENSG00000145703	mRNA
Lymphotoxin Beta (LTB)	ENSG00000227507	mRNA
Methionine Adenosyltransferase 2A (MAT2A)	ENSG00000168906	mRNA
Mitochondrial Ribosomal Protein L22 (MRPS22)	ENSG00000175110	mRNA
Nucleosome Assembly Protein 1 Like 2 (NAP1L2)	ENSG00000186462	mRNA
Peptidyl Arginine Deiminase 4 (PADI4)	ENSG00000159339	mRNA

PDZ And LIM Domain 7 (PDLIM7)	ENSG00000196923	mRNA
Platelet Activating Factor Receptor (PTAFR)	ENSG00000169403	mRNA
RAN Binding Protein 6 (RANBP6)	ENSG00000137040	mRNA
RNA, 7SL, Cytoplasmic 473, Pseudogene (RN7SL473P)	ENSG00000277452	mRNA
RP11-429E11.3	ENSG00000179253.3	mRNA
RP11-4B16.1	ENSG00000279433	microRNA
Ribosomal Protein S20 (RPS20)	ENSG00000008988	mRNA
Syntaxin 10 (STX10)	ENSG00000104915	mRNA
T Cell Receptor Beta Variable 11-2 (TRBV11-2)	ENSG00000241657	mRNA
WD Repeat Domain 86 (WDR86)	ENSG00000187260	mRNA
Zwilch Kinetochores Protein (ZWILCH)	ENSG00000174442	mRNA

ii) the subset of the following 13 metabolites:

- 2-aminooctanoate
- 3-(4-hydroxyphenyl)lactate
- 4-methoxyphenol sulfate
- 5 - hypotaurine
- lysine
- glucuronate
- Trans-4-hydroxyproline
- N-acetyl-2-aminooctanoate
- 10 - phenol sulfate

- γ -glutamylleucine
- 5-methyluridine (ribothymidine)
- fibrinopeptide A, phosphono-ser(3)
- glyco-alpha-muricholate;

5 and iii) the subset of the following lipids:

- dodecenedioate (C12:1-DC)
- phosphatidylinositol (16:0/18:1);

for the diagnosis of Brugada Syndrome in a human being.

4. An in vitro method for detecting the presence or measuring concentration of the
10 occurrence of one of the set of biomarkers according to anyone of claim 1-3 in a biological sample of a human being.

5. An in vitro method for detecting the presence of one of the set of biomarkers according to claim 4, which comprises the following steps:

a) detecting the presence of at least 6 up to 44 RNAs of subset i) by an assay
15 selected from the group comprising mRNA microarray detection, Real-Time PCR, Droplet Digital PCR or hybridization-based assays, and branched DNA (bDNA) technology (i.e. QuantiGene® assay), or a combination thereof;

b) detection of at least 7 up to 13 of the metabolites of subset ii) and at least one or both the lipids of subset iii) by an assay such as one selected from the
20 group comprising MS, GC-MS, fluorimetric or colorimetric assays, NMR, spectroscopy with nanoprobe, Enzyme-Linked oligonucleotide assays, or a combination thereof;

wherein the positive detection of all the biomarkers compared to a normal control allows the identification of patients affected by Brugada Syndrome.

25 6. An in vitro method for measuring the concentration of one of the set of biomarkers according to claim 4, comprising the following steps:

- a) quantitative determination of at least 6 up to 44 RNAs of subset i) by a quantitative technique such as quantitative PCR, preferably by Real Time-PCR, Droplet Digital PCR, Quantigene assay, or a combination thereof;
- b) quantitative determination of at least 7 up to 13 of the metabolites of subset ii) by a technique selected between HPLC, MS, NMR analysis, ELONA, or a combination thereof;
- c) quantitative determination of at least or both the lipids of subset iii) by a technique selected between HPLC, MS, NMR analysis, or a combination thereof; wherein an increase of the concentration of the biomarkers compared to a normal control indicates a diagnosis of Brugada Syndrome.
7. An in vitro method according to anyone of the claims 4-6, wherein the biological sample is selected from the group consisting of whole blood, plasma, serum, peripheral blood and PBMCs.
8. An in vitro method according to anyone of the claims 4-7, wherein the human being is asymptomatic.
9. An in vitro method according to anyone of the claims 4-8, wherein the human being is at high risk for Brugada Syndrome due to family history, previous events of heart atrial and/or ventricular fibrillation, diabetes or obesity.
10. An in vitro method according to anyone of the claims 4-9, wherein the human being is about 40 years old or younger.
11. A kit comprising oligonucleotides that are at least 85, 90, 95, 96, 97, 98, 99 or 100% complementary to each of the RNAs of subset i) according to anyone of claim 1-3, wherein said oligonucleotides are primers or probes optionally attached to a solid support.
12. A computer-implemented method for indicating a diagnosis of Brugada syndrome, comprising the following steps:

a) retrieving on a computer biomarker information for a human being, wherein the biomarker information comprises biomarker values for each of the biomarkers of the set according to claim 1-3;

b) performing with the computer a classification of said biomarker values.

- 5 13. The computer-implemented method of claim 12, wherein the indication of a diagnosis of Brugada syndrome comprises indicating the likelihood of a Brugada syndrome diagnosis, determined from a plurality of classifications.