US 20160042121A1

(19) **United States**

(12) **Patent Application Publication**

GOZAL et al.

(10) **Pub. No.: US 2016/0042121 A1**

(43) **Pub. Date: Feb. 11, 2016**

(54) **COMPOSITIONS AND METHODS RELATED TO OBSTRUCTIVE SLEEP APNEA**

(71) Applicant: **THE UNIVERSITY OF CHICAGO**, Chicago, IL (US)

(72) Inventors: **David GOZAL**, Chicago, IL (US); **Lev BECKER**, Chicago, IL (US)

(73) Assignee: **THE UNIVERSITY OF CHICAGO**, Chicago, IL (US)

(21) Appl. No.: **14/771,875**

(22) PCT Filed: **Mar. 7, 2014**

(86) PCT No.: **PCT/US14/21750**

§ 371 (c)(1),
(2) Date: **Sep. 1, 2015**

Related U.S. Application Data

(60) Provisional application No. 61/773,936, filed on Mar. 7, 2013.

Publication Classification

(51) **Int. Cl.**

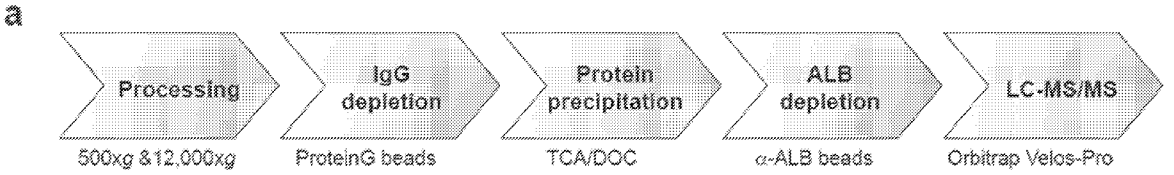
G06F 19/24	(2006.01)
G01N 33/68	(2006.01)
G01N 30/72	(2006.01)

(52) **U.S. Cl.**

CPC ***G06F 19/24*** (2013.01); ***G01N 30/7233*** (2013.01); ***G01N 33/6893*** (2013.01); *G01N 2800/2864* (2013.01); *G01N 2800/60* (2013.01)

(57) **ABSTRACT**

The technology concerns methods and compositions for diagnosing obstructive sleep apnea, a common condition observed in children. In certain embodiments, there are methods and compositions relating to the use of novel biomarkers to diagnose obstructive sleep apnea.



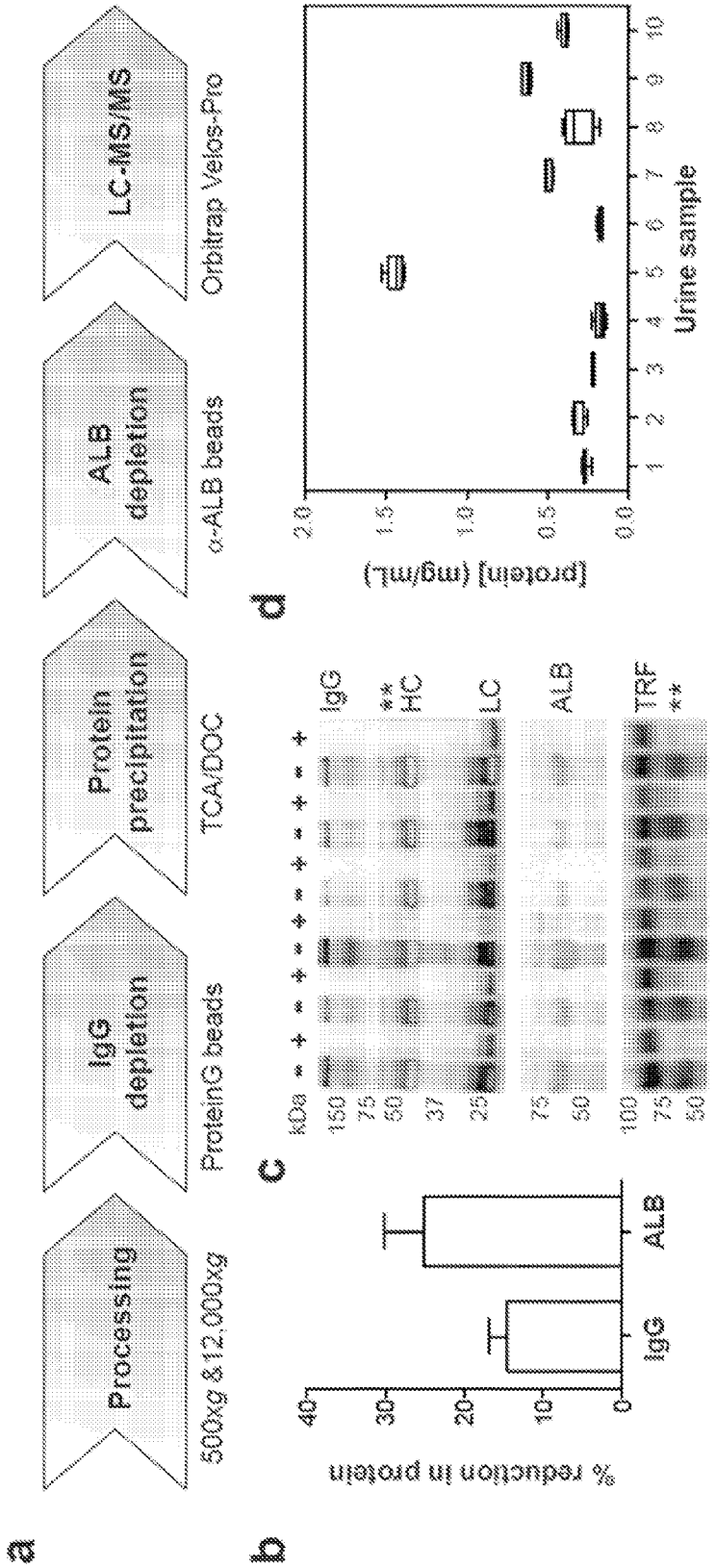
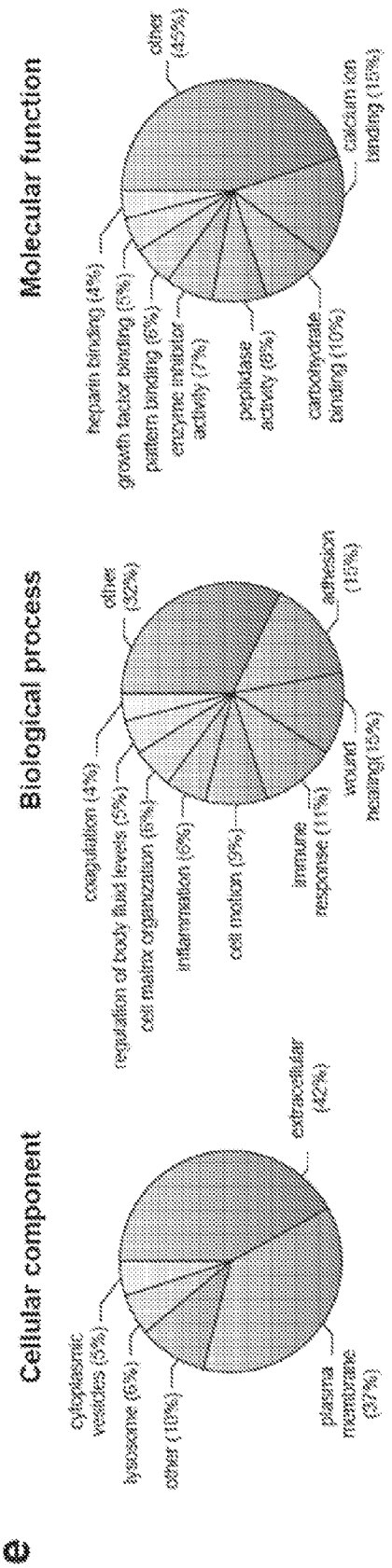

a
Processing
500xg &12,000xg
IgG depletion
ProteinG beads
Protein precipitation
TCA/DOC
ALB depletion
α-ALB beads
LC-MS/MS
Orbitrap Velos-Pro
b
% reduction in protein
IgG
ALB
c
kDa
IgG
HC
LC
ALB
TRF
d
[protein] (mg/mL)
Urine sample

FIG. 1A-1D


Molecular function
other (45%)
calcium ion binding (18%)
carbohydrate binding (10%)
peptidase (9%)
protease inhibitor (9%)
growth factor binding (5%)
heparin binding (4%)
Biological process
other (32%)
adhesion (11%)
wound healing (10%)
immune response (11%)
cell motion (9%)
inflammation (6%)
cell matrix organization (5%)
regulation of body fluid levels (5%)
coagulation (4%)
Cellular component
extracellular (42%)
plasma membrane (21%)
other (18%)
exosome (9%)
vesicle (6%)
cytoplasmic (5%)

e

FIG. 1E

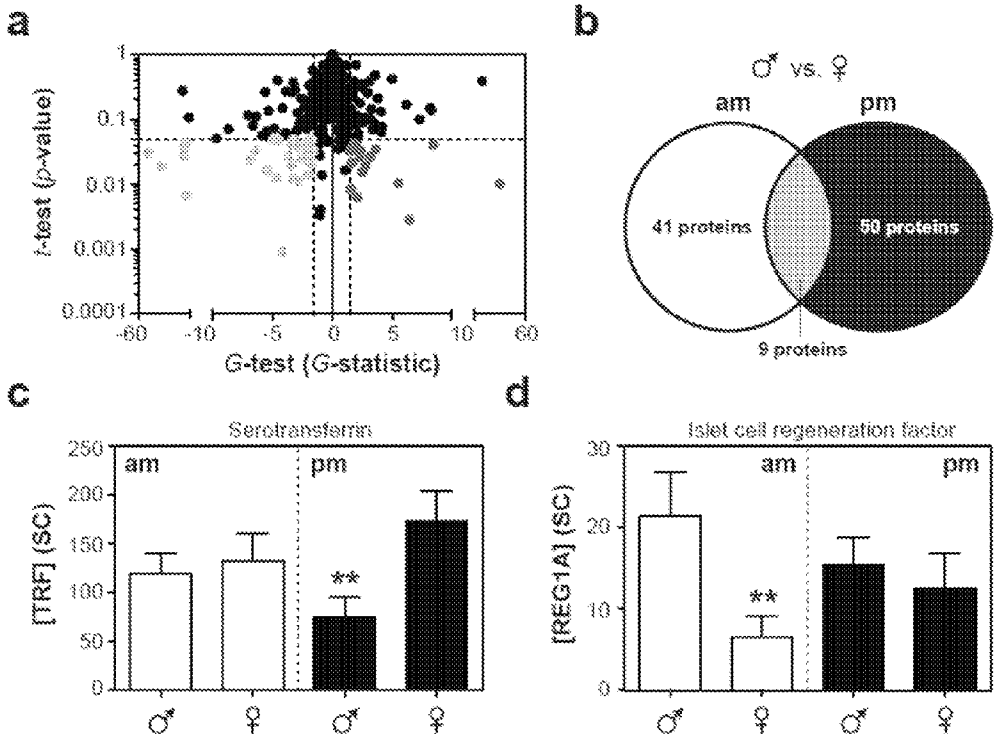

a
b
c
d
t-test (p-value)
G-test (G-statistic)
♂ vs. ♀
am
pm
41 proteins
50 proteins
9 proteins
Serotransferrin
[TRF] (SC)
Islet cell regeneration factor
[REG1A] (SC)
**

FIG. 2A-2D

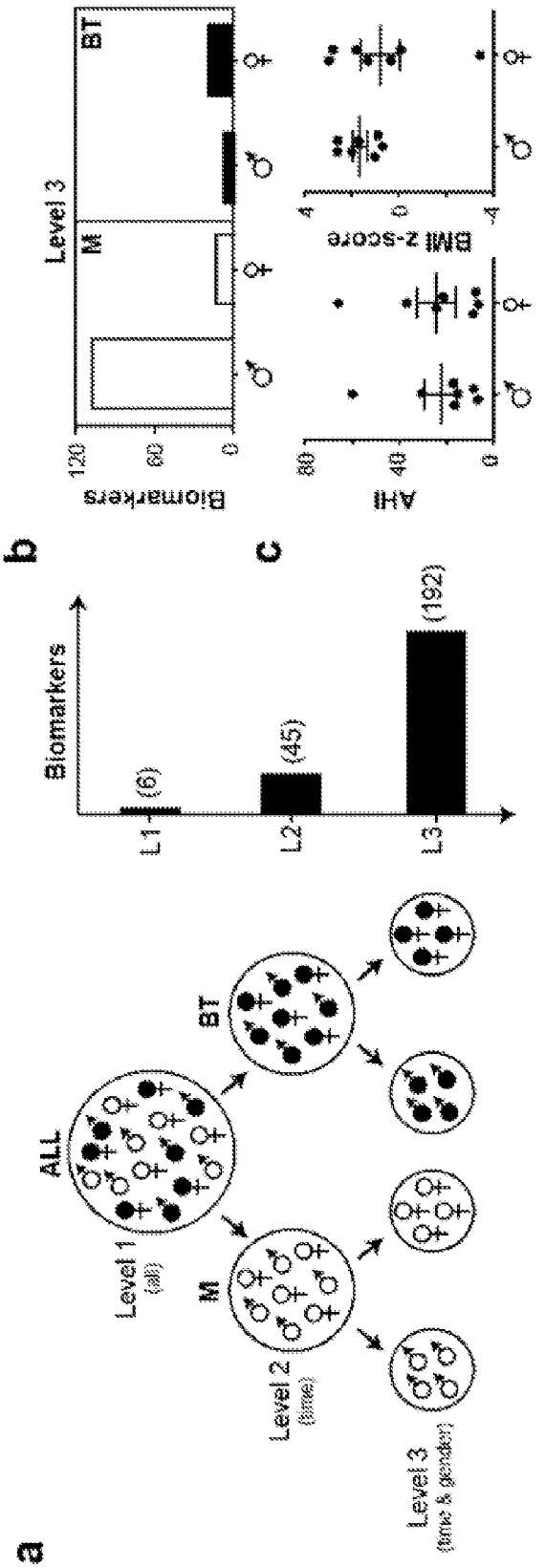

a
ALL
Level 1
(all)
M
BT
Level 2
(time)
Level 3
(time & gender)
b
Biomarkers
(6)
(45)
(192)
L1
L2
L3
c
Level 3
M
BT
Biomarkers
120
60
0
AHI
80
40
0
BMI z-score
4
0
-4

FIG. 3A-3C

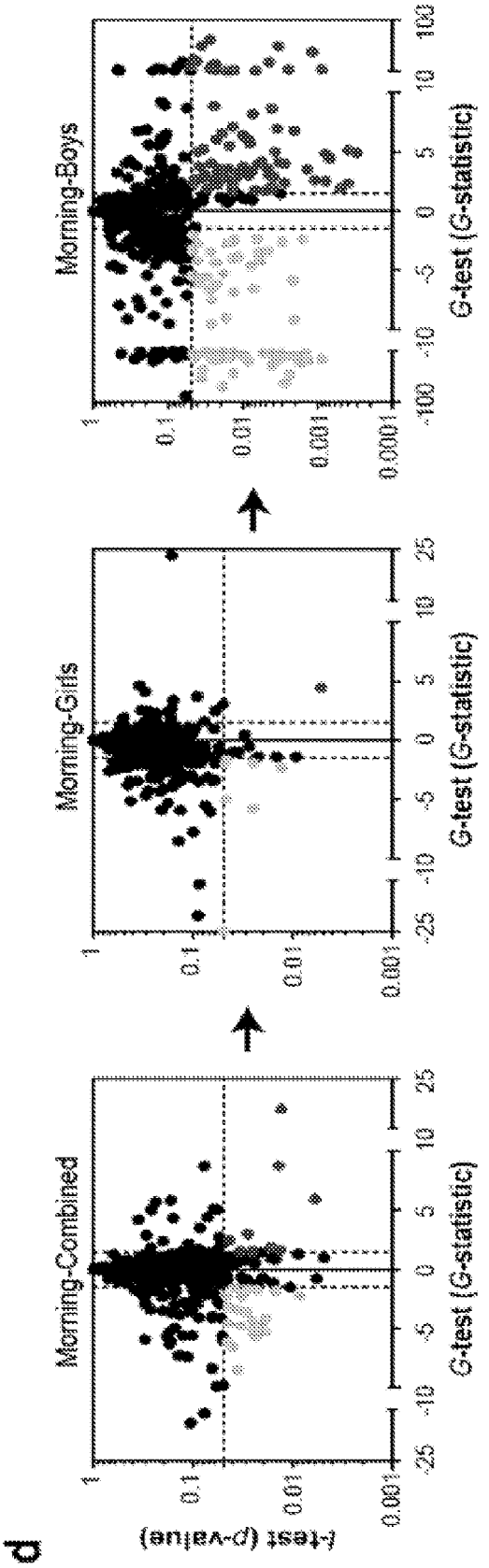

d
Morning-Combined
Morning-Girls
Morning-Boys
t-test (p-value)
G-test (G-statistic)
1
0.1
0.01
0.001
0.0001
-25
-10
-5
0
5
10
25
-100
100

FIG. 3D

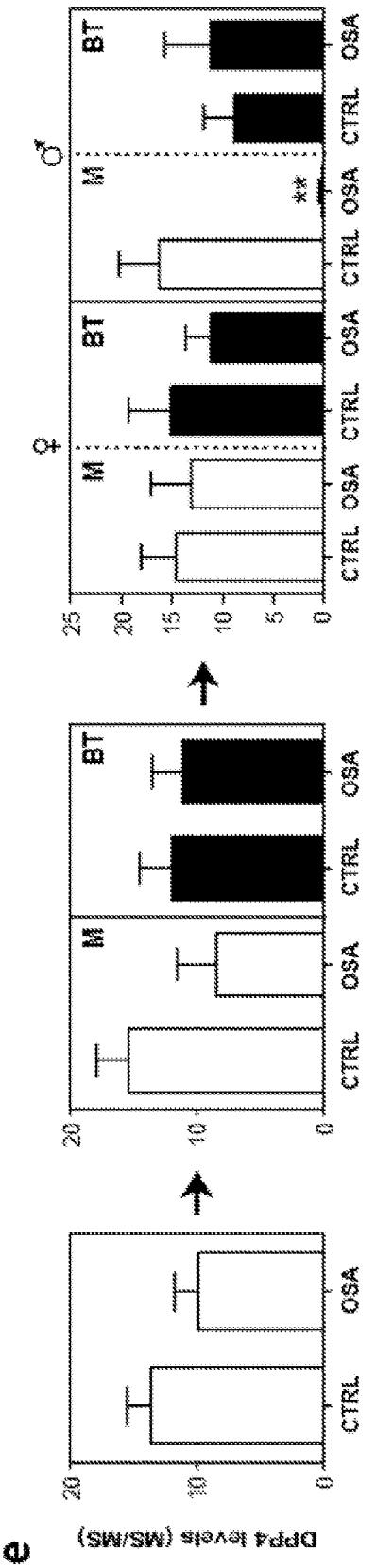

e
DPP4 levels (MS/MS)
0
10
20
CTRL
OSA
M
CTRL
OSA
BT
CTRL
OSA
♀
M
CTRL
OSA
BT
CTRL
OSA
♂
**
0
5
10
15
20
25

FIG. 3E

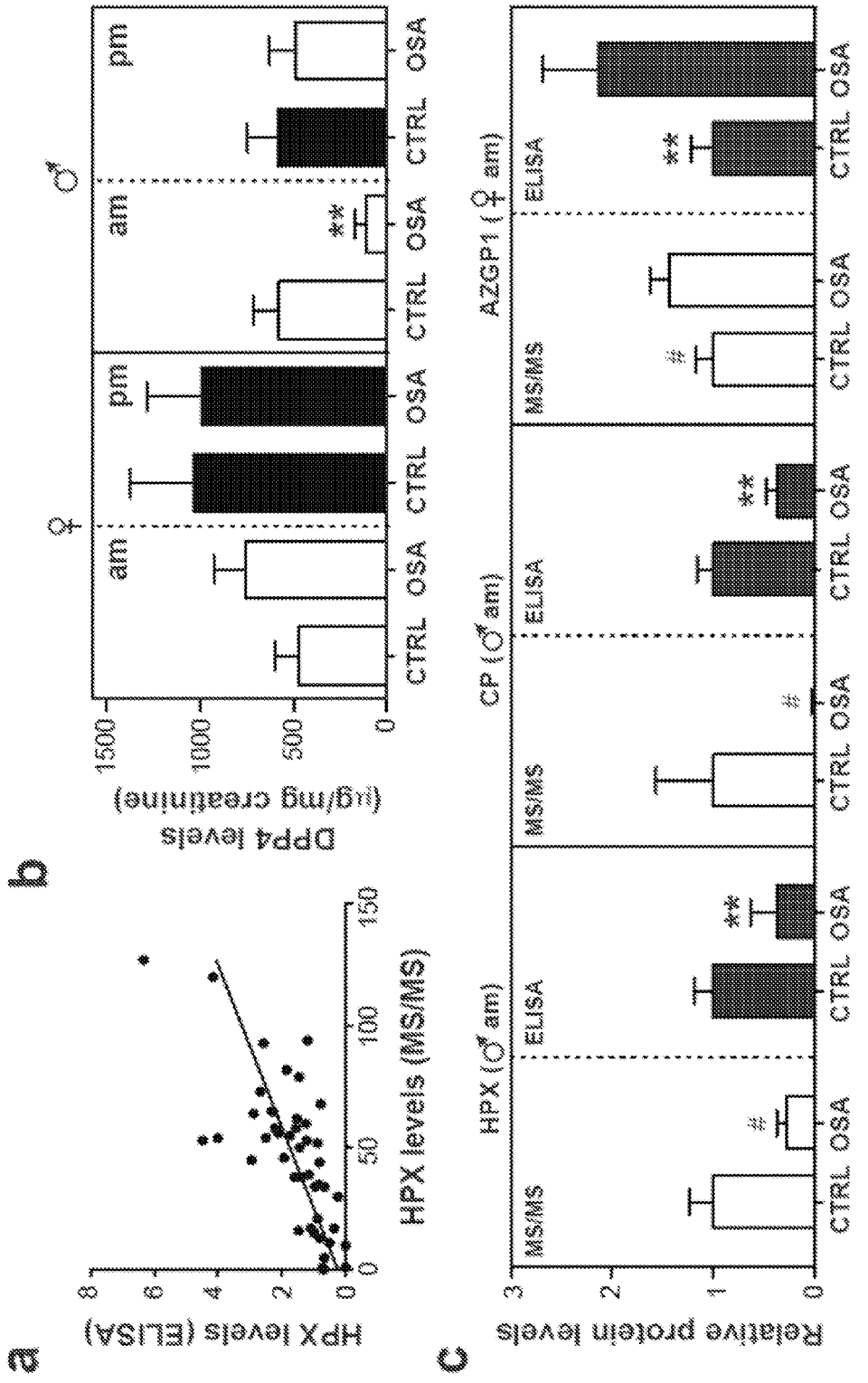

a
HPX levels (ELISA)
HPX levels (MS/MS)
b
DPP4 levels (µg/mg creatinine)
♀
♂
am
pm
CTRL OSA
c
Relative protein levels
HPX (♂ am)
CP (♂ am)
AZGP1 (♀ am)
MS/MS
ELISA
CTRL OSA

FIG. 4A-4C

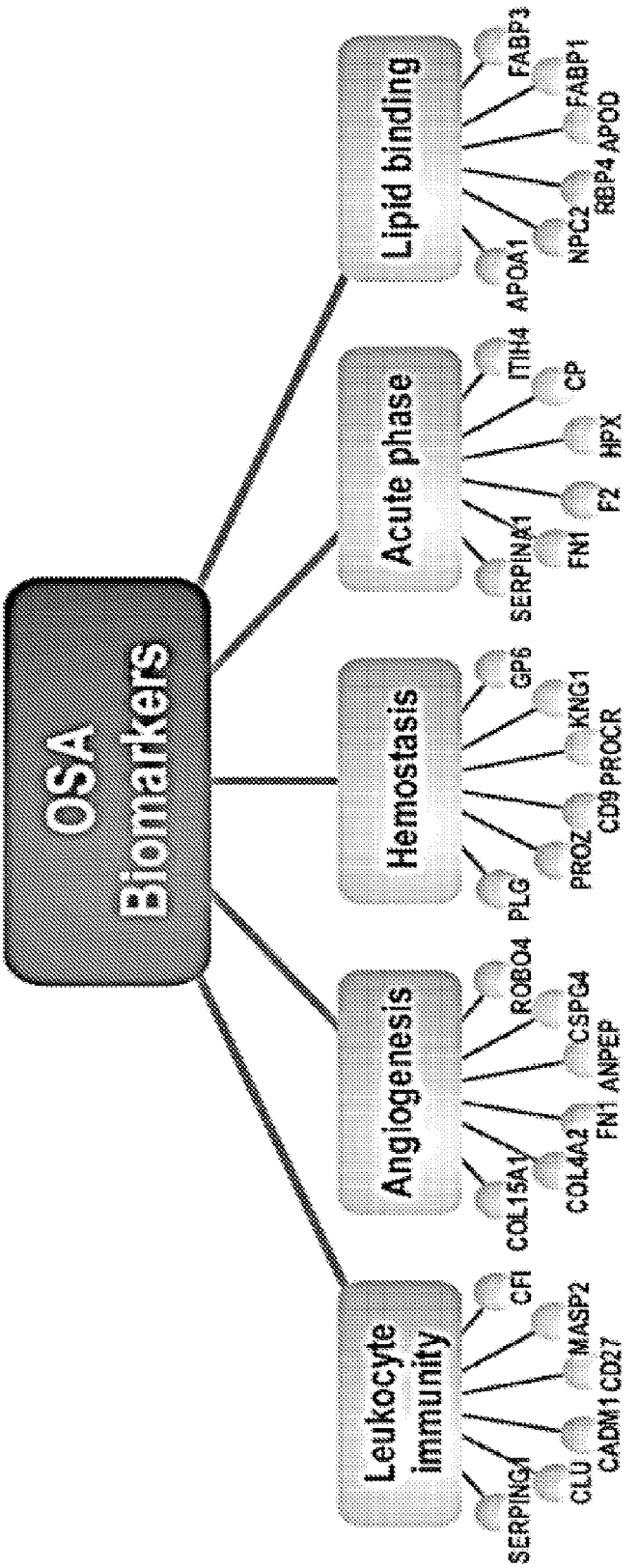

OSA Biomarkers
Lipid binding
FABP3
FABP1
APOD
RBP4
NPC2
APOA1
Acute phase
ITIH4
CP
HPX
F2
FN1
SERPINA1
Hemostasis
GP5
KNG1
PROCR
CD9
PROZ
PLG
Angiogenesis
ROBO4
CSPG4
ANPEP
FN1
COL4A2
COL15A1
Leukocyte immunity
CFI
MASP2
CD27
CADM1
CLU
SERPING1

FIG. 5

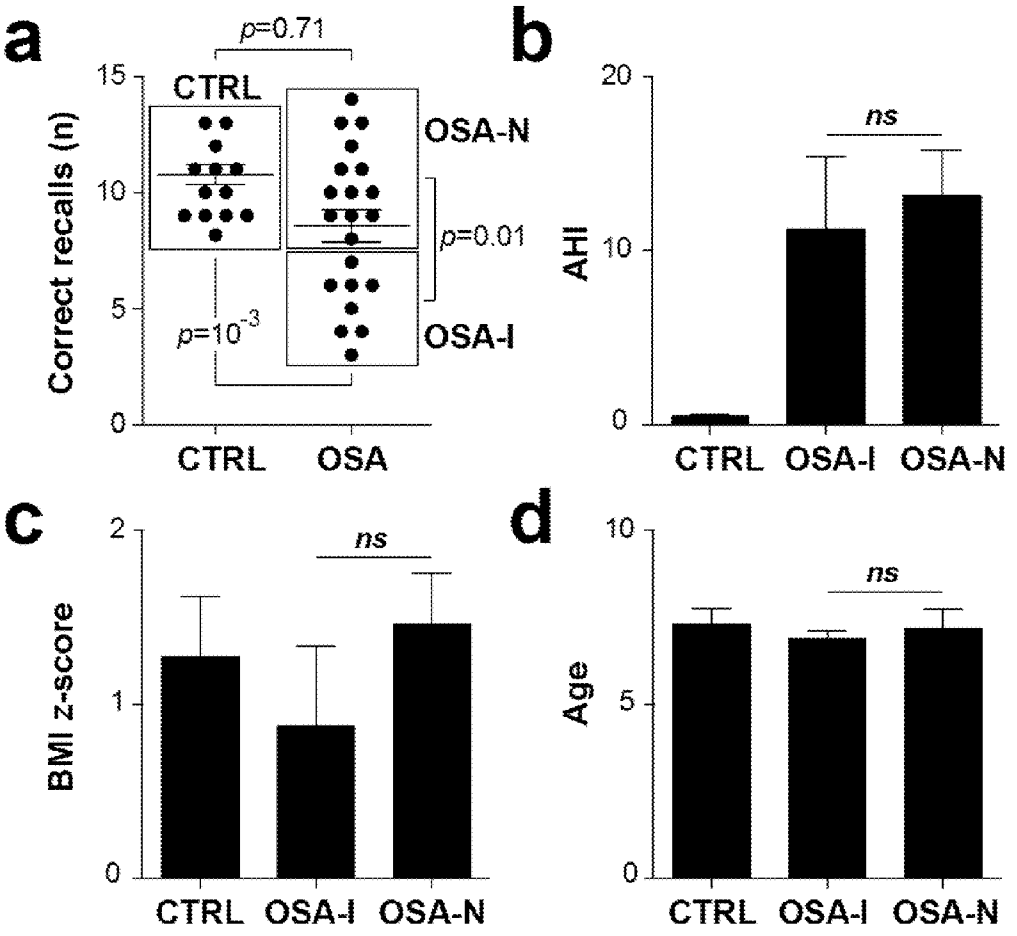

a
p=0.71
CTRL
OSA-N
OSA-I
p=0.01
p=10^-3
Correct recalls (n)
15
10
5
0
CTRL
OSA
b
ns
AHI
20
10
0
CTRL
OSA-I
OSA-N
c
ns
BMI z-score
2
1
0
CTRL
OSA-I
OSA-N
d
ns
Age
10
5
0
CTRL
OSA-I
OSA-N

FIG. 6A-6D

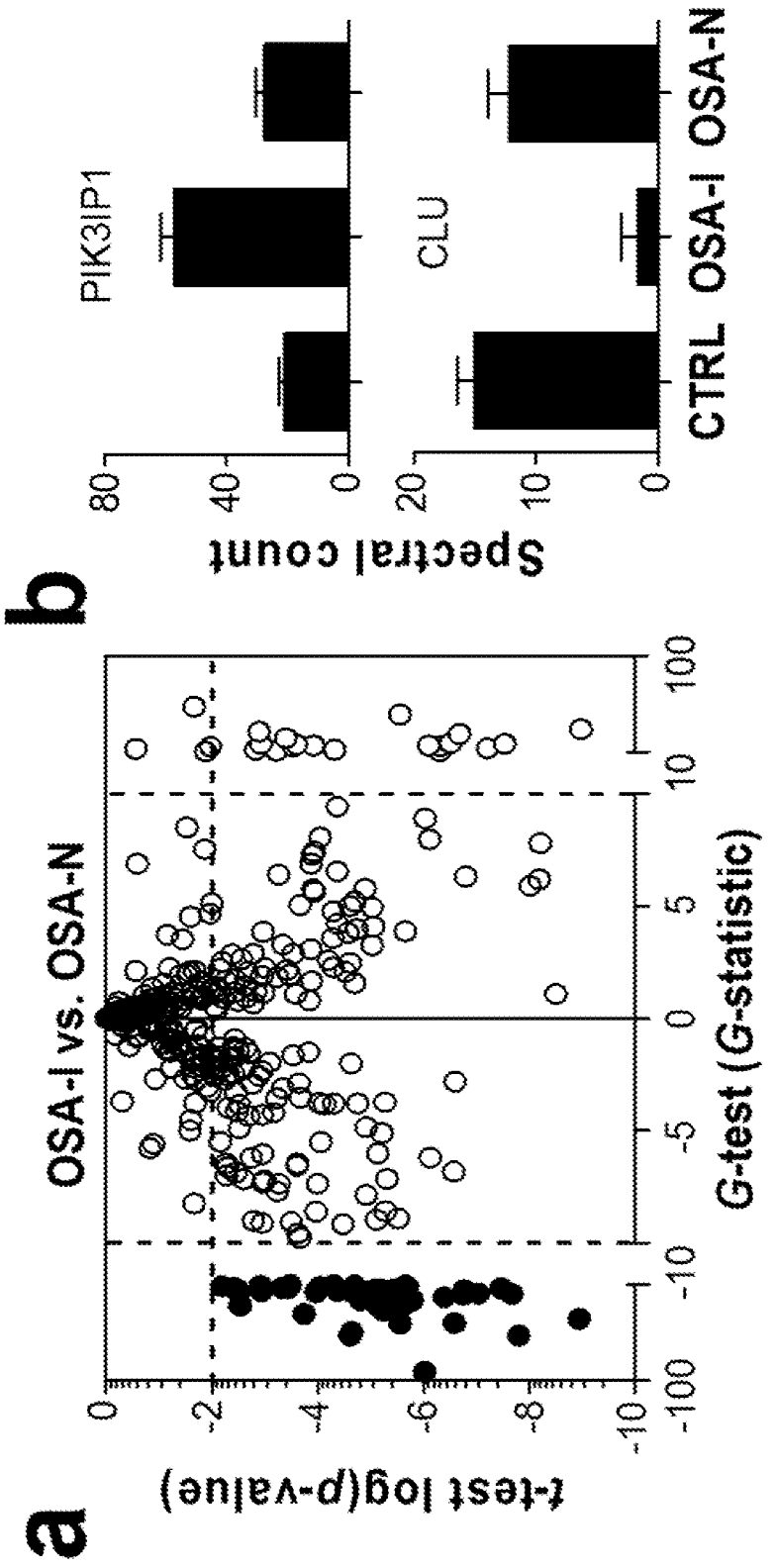

a
b
OSA-I vs. OSA-N
t-test log(p-value)
G-test (G-statistic)
Spectral count
PIK3IP1
CLU
CTRL
OSA-I
OSA-N

FIG. 7A-7B

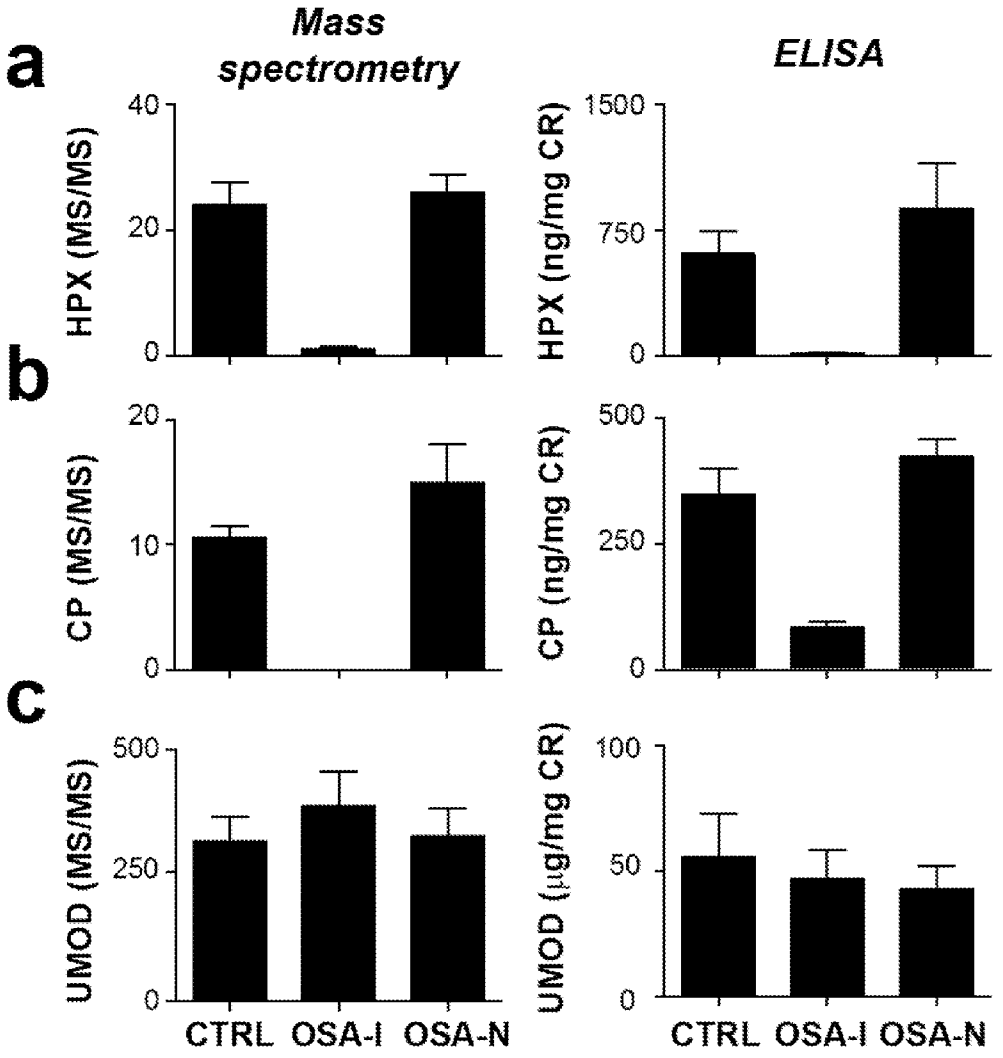

a
b
c
Mass spectrometry
ELISA
HPX (MS/MS)
HPX (ng/mg CR)
CP (MS/MS)
CP (ng/mg CR)
UMOD (MS/MS)
UMOD (μg/mg CR)
40
20
0
1500
750
0
10
500
250
100
50
CTRL
OSA-I
OSA-N

FIG. 8A-8C

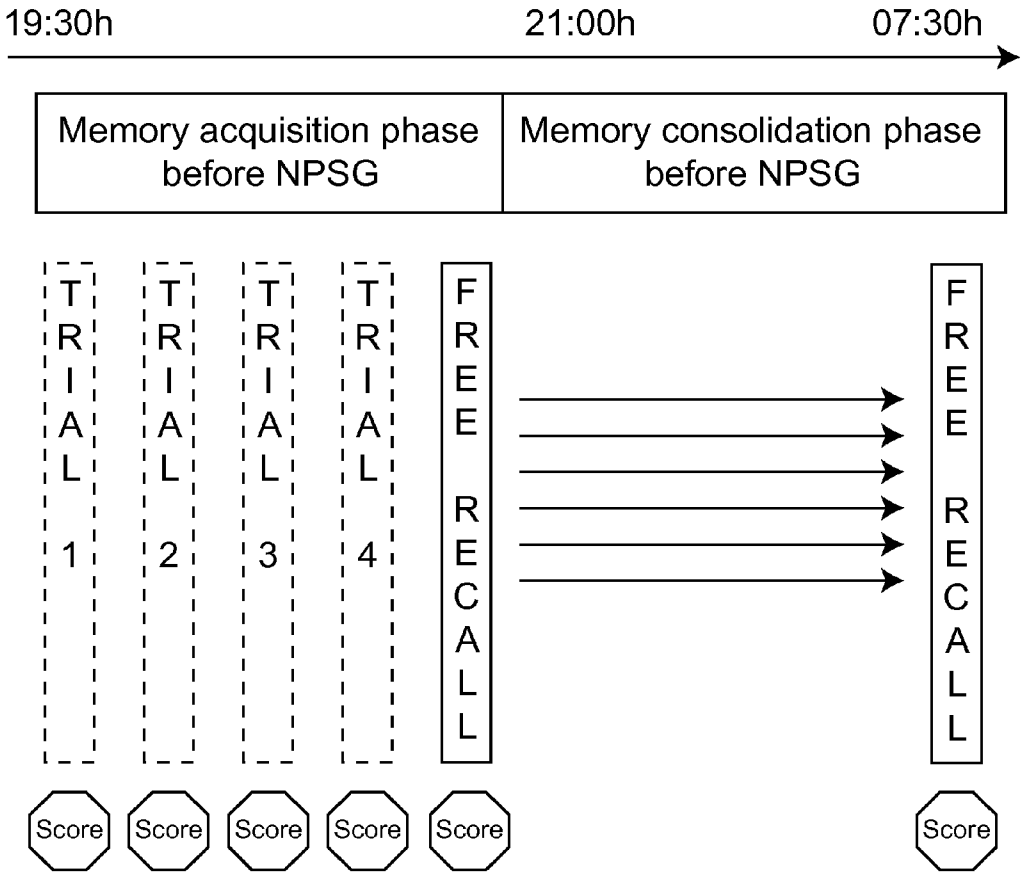

19:30h
21:00h
07:30h
Memory acquisition phase before NPSG
Memory consolidation phase before NPSG
TRIAL 1
TRIAL 2
TRIAL 3
TRIAL 4
FREE RECALL
FREE RECALL
Score
Score
Score
Score
Score
Score

FIG. 9

COMPOSITIONS AND METHODS RELATED TO OBSTRUCTIVE SLEEP APNEA

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of U.S. Provisional Application No. 61/773,936, filed on Mar. 7, 2013, which is hereby incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

[0002] I. Field of the Invention

[0003] The present invention relates generally to the field of obstructive sleep apnea. More particularly, it concerns the methods and compositions for diagnosing obstructive sleep apnea.

[0004] II. Description of the Related Art

[0005] Obstructive sleep apnea (OSA) is a prevalent disorder affecting up to 2-3% of children. It imposes substantial neurocognitive, behavioral, metabolic, and cardiovascular morbidities (Lumeng and Chervin, 2008; Capdevila et al., 2008). This condition is characterized by repeated events of partial or complete obstruction of the upper airways during sleep, leading to recurring episodes of hypercapnia, hypoxemia, and arousal throughout the night (Muzumdar and Arens, 2008). Pediatric sleep apnea is a common disorder primarily caused by enlarged tonsils and adenoids impinging upon the patency of the upper airway during sleep. Mechanisms leading to the proliferation and enlargement of the tonsils and adenoids in children who subsequently develop obstructive sleep apnea remain unknown. Adenotonsillar hypertrophy is the major pathophysiological contributor to OSA in children (Arens et al., 2003; Katz and D'Ambrosio, 2008). However, the mechanisms underlying the regulation of benign follicular lymphoid proliferation, hypertrophy, and hyperplasia are poorly understood, severely limiting the prediction of children who are at risk for developing adenotonsillar enlargement and OSA. Several epidemiological studies have demonstrated that factors such as environmental smoking, allergies, and intercurrent respiratory infections are associated with either transient or persistent hypertrophy of lymphadenoid tissue in the upper airways of snoring children (Kaditis et al., 2004; Teculescu et al., 1992; Ersu et al., 2004). Interestingly, all of these risk factors involve the generation of an inflammatory response, suggesting that the latter may promote the onset and maintenance of proliferative signals to lymphadenoid tissues.

[0006] The gold standard diagnostic approach for OSA is an overnight sleep study, or polysomnography. While this approach is reliable, it suffers from problems associated with its implementation in the clinical setting. Indeed, polysomnography is labor intensive, inconvenient, and expensive resulting in long waiting periods and unnecessary delays in diagnosis and treatment. Therefore, novel, diagnostic strategies are needed.

SUMMARY OF THE INVENTION

[0007] Embodiments concern compositions and methods that provide diagnostic applications for addressing OSA.

[0008] In some aspects, embodiments provide a method for identifying a subject as having obstructive sleep apnea (OSA) comprising measuring from a biological sample from the subject the expression levels of one or more proteins encoded by one ore more genes listed in Table 1, and identifying the subject as having OSA based on the levels of expression of the one or more proteins. In some embodiments, the method comprises comparing the level of expression of the one or more proteins to a control or reference level. In some embodiments, an elevated level of expression of the one or more proteins as compared to a control or reference level indicates that the subject is likely to have OSA. In some embodiments, a lower level of expression of the one or more proteins as compared to a control or reference level indicates that the subject is likely to have OSA. The control may be any appropriate standard. In some embodiments, the control is the level of expression of the one or more proteins in a control sample from a subject who is known not to have OSA. In some embodiments, the level of expression of the one or more proteins is standardized against the level of expression of a corresponding standard protein in the sample. In some embodiments, the standard protein is a protein encoded by one or more genes listed in Table 1.

[0009] In some embodiments, the level of expression is measured for at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 proteins. In some embodiments, the one or more proteins are encoded by a gene listed in Table 1. In some embodiments, the one or more proteins are encoded by a gene selected from the group consisting of CD14, CTSB, HPX, DPP4, TTR, DEFB1|HBD1, FABP3, CP, and AZGP1. In some embodiments, the one or more proteins are encoded by one or more genes selected from the group consisting of HPX, DPP4, CP, and AZGP1.

[0010] In some embodiments, the method further comprises obtaining the biological sample from the subject. The sample may be any appropriate sample. In some embodiments, the sample is a urine sample. In some embodiments, the corresponding standard protein is urinary creatinine. In some embodiments, the sample may be collected at a particular time of day. In some embodiments, the sample is collected in the morning, which means before 12 p.m. In certain embodiments, the sample is collected within 1 or 2 hours of waking up. In some embodiments, the sample is collected in the evening. In some embodiments, the sample is collected in the evening, which means after 4 p.m. for the subject. In other embodiments, the sample is collected after the subject has been awake for at least 8 hours or for at least 12 hours. In some embodiments, the sample is collected after the subject has been awake less than 1 hour. In some embodiments, the subject is suspected of having OSA.

[0011] In some embodiments, the subject is a male. In some embodiments, the control is the level of expression of the one or more proteins in a control male. In some embodiments, the control male is known to have OSA. In some embodiments, the control male is known to not have OSA. In some embodiments, the subject is a female. In some embodiments, the control is the level of expression of the one or more proteins in a control female. In some embodiments, the control female is known to have OSA. In some embodiments, the control female is known to not have OSA.

[0012] In some embodiments, the method further comprises using a computer algorithm to evaluate the measured levels of expression of one or more genes from Table 1. In some embodiments, the method further comprises determining a risk score for the subject for having OSA. In some embodiments, the method further comprises measuring the expression levels of RNA transcripts. In some embodiments, the expression levels of RNA transcripts are measured using

DNA complementary to the RNA transcripts. In some embodiments, expression levels of RNA transcripts are measured using an amplification or hybridization assay. In some embodiments, expression levels of proteins are measured. In some embodiments, expression levels of proteins are measured using one or more binding polypeptides. In some embodiments, one or more binding polypeptides is an antibody.

[0013] In some embodiments, the method further comprises performing a sleep study on the subject. In some embodiments, the sleep study comprises one of more of the following: using a polysomnogram (PSG), performing a multiple sleep latency test (MSLT), or performing a maintenance of wakefulness test (MWT). In some embodiments, the sleep study comprises measuring one or more physiological characteristics of the subject when sleeping. In some embodiments, the physiological characteristics include one or more of the following: brain activity, heart rate, heart rhythm, blood pressure, exhaled carbon dioxide in breath, and oxygen content in blood. In some embodiments, the sleep study comprising using an actigraph. In some embodiments, the sleep study is performed after expression levels are measured in the subject.

[0014] In some aspects, embodiments provide a method for determining whether a subject has obstructive sleep apnea (OSA) comprising assaying from a biological sample from the subject the levels of expression of one or more proteins encoded by a gene listed in Table 1, and calculating a risk score for the biological sample that identifies the likelihood of the subject having OSA. In some embodiments, calculating a risk score comprises using a computer and an algorithm. In some embodiments, calculating a risk score comprises applying model coefficients to each of the levels of expression. In some embodiments, the method further comprises identifying the patient as having a risk score indicative of 50% chance or greater of having OSA. In particular embodiments, calculating a risk score involves using or running a computer algorithm or program on a computer. In further embodiments, the risk score is reported. In further embodiments, the subject is identified as having a risk score indicative of having OSA.

[0015] In some aspects, the invention provides a method for determining whether a male subject has obstructive sleep apnea (OSA) comprising measuring from a biological sample from the subject the levels of expression of one or more proteins encoded by a gene listed in Table 1, and evaluating whether the subject has OSA based on the levels of expression of the one or more proteins. In some embodiments, the one or more proteins is encoded by a gene selected from the group consisting of DDP4, HPX, and CP. In some embodiments, the method further comprises obtaining the biological sample from the subject. The sample may be any appropriate sample. In some embodiments, the sample is a urine sample. In some embodiments, the corresponding standard protein is urinary creatinine. In some embodiments, the sample may be collected at a particular time of day. In some embodiments, the sample is collected in the morning, which means before 12 p.m. In certain embodiments, the sample is collected within 1 or 2 hours of waking up. In some embodiments, the sample is collected in the evening. In some embodiments, the sample is collected in the evening, which means after 4 p.m. for the subject. In other embodiments, the sample is collected after the subject has been awake for at least 8 hours or for at least 12 hours. In some embodiments, the sample is collected after the subject has been awake less than 1 hour. In some embodiments, the subject is suspected of having OSA. In some embodiments, a lower level of expression of the one or more proteins as compared to a control indicates that the subject is likely to have OSA. In some embodiments, the control is the level of expression of the one or more proteins in a control male. In some embodiments, the control male is known to have OSA. In some embodiments, the control male is known to not have OSA. In some embodiments, the control is the level of expression of the one or more proteins in a control female.

[0016] In some aspects, embodiments provide a method for determining whether a female subject has obstructive sleep apnea (OSA) comprising determining from a biological sample from the subject the levels of expression of one or more proteins encoded by a gene listed in Table 1, and evaluating whether the subject has OSA based on the levels of expression of the one or more proteins. In some embodiments, the one or more proteins is encoded by AZGP1. In some embodiments, the method further comprises obtaining the biological sample from the subject. The sample may be any appropriate sample. In some embodiments, the sample is a urine sample. In some embodiments, the corresponding standard protein is urinary creatinine. In some embodiments, the sample may be collected at a particular time of day. In some embodiments, the sample is collected in the morning, which means before 12 p.m. In certain embodiments, the sample is collected within 1 or 2 hours of waking up. In some embodiments, the sample is collected in the evening. In some embodiments, the sample is collected in the evening, which means after 4 p.m. for the subject. In other embodiments, the sample is collected after the subject has been awake for at least 8 hours or for at least 12 hours. In some embodiments, the sample is collected after the subject has been awake less than 1 hour. In some embodiments, the subject is suspected of having OSA. In some embodiments, an elevated level of expression of the one or more proteins as compared to a control indicates that the subject is likely to have OSA. In some embodiments, the control is the level of expression of the one or more proteins in a control female. In some embodiments, the control female is known to have OSA. In some embodiments, the control female is known to not have OSA. In some embodiments, the control is the level of expression of the one or more proteins in a control male.

[0017] In some aspects, embodiments provide a method for evaluating obstructive sleep apnea in a subject comprising subjecting the subject to a sleep study after the subject is determined to have sleep apnea based on measuring expression levels of one or more genes listed in Table 1 in a urine sample obtained from the subject. In some embodiments, the sleep study comprises one of more of the following: using a polysomnogram (PSG), performing a multiple sleep latency test (MSLT), or performing a maintenance of wakefulness test (MWT). In some embodiments, the sleep study comprises measuring one or more physiological characteristics of the subject when sleeping. In some embodiments, the physiological characteristics include one or more of the following: brain activity, heart rate, heart rhythm, blood pressure, exhaled carbon dioxide in breath, and oxygen content in blood. In some embodiments, the sleep study comprises using an actigraph.

[0018] In some aspects, provided is a method for identifying a subject as having high-risk obstructive sleep apnea (OSA) comprising a) measuring from a biological sample from the subject the expression levels of one or more products

of one or more genes listed in either Table 1 or Table 2, and b) identifying the subject as having high-risk OSA based on the levels of expression of the one or more products. In some aspects, provided is a method for identifying a subject as at risk for having high-risk obstructive sleep apnea (OSA) comprising a) measuring from a biological sample from the subject the expression levels of one or more products of one or more genes listed in either Table 1 or Table 2, and b) identifying the subject as at risk for having high-risk OSA based on the levels of expression of the one or more products. High-risk OSA is understood to be OSA which is associated with neurocognitive impairment such as memory impairment, declarative memory defects, learning delays, and issues with academic performance, mood-related disorders such as depression, behavioral issues such as ADHD, aggression, inattentiveness, impulsivity, and excessive sleepiness, cardiovascular risks including hypertension, altherosclerosis, pulmonary hypertension, and left ventricular dysfunction, a metabolic disorders such as dyslipidemia and insulin resistance. In some aspects, provided is a method for identifying a subject as having an increased risk of neurocognitive impairment such as memory impairment, declarative memory defects, learning delays, and issues with academic performance, mood-related disorders such as depression, behavioral issues such as ADHD, aggression, inattentiveness, impulsivity, and excessive sleepiness, cardiovascular risks including hypertension, altherosclerosis, pulmonary hypertension, and left ventricular dysfunction, a metabolic disorders such as dyslipidemia and insulin resistance comprising a) measuring from a biological sample from the subject the expression levels of one or more products of one or more genes listed in either Table 1 or Table 2, and b) identifying the subject as having an increased risk of neurocognitive impairment such as memory impairment, declarative memory defects, learning delays, and issues with academic performance, mood-related disorders such as depression, behavioral issues such as ADHD, aggression, inattentiveness, impulsivity, and excessive sleepiness, cardiovascular risks including hypertension, altherosclerosis, pulmonary hypertension, and left ventricular dysfunction, a metabolic disorders such as dyslipidemia and insulin resistance based on the levels of expression of the one or more products.

[0019] In some embodiments, the level of expression of the one or more products is compared to a control or reference level. The control or reference level may be any appropriate level. In some embodiments, an elevated level of expression of the one or more products as compared to a control or reference level indicates that the subject is likely to have OSA with declarative memory defects. In some embodiments, a lower level of expression of the one or more products as compared to a control or reference level indicates that the subject is likely to have OSA with declarative memory defects. In some embodiments, the control is the level of expression of the one or more products in a control sample from a subject who is known not to have OSA. In some embodiments, the control is the level of expression of the one or more products in a control sample from a subject who is known to have OSA. In some embodiments, the level of expression of the one or more products is standardized against the level of expression of a corresponding standard product in the sample.

[0020] In some embodiments, the level of expression is measured for at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 proteins. In some embodiments, the one or more proteins are encoded by a gene listed in either Table 1 or Table 2. In some embodiments, the one or more products are one or more proteins encoded by a gene selected from the group consisting of RNASE1, COL12A1, RNASE2, CD59, FN1, AMBP, FBN1, PIK3IP1, CDH1, CDH2, PLG, SLURP1, FN1 cDNA FLJ53292, TNC, C1RL, A1BG, PGLYRP2, OSCAR, AZGP1, CEL, CFI, CILP2, VASN, PLAU, SERPINA1, CD14, LRP2, CLU, FGA, NIDI, APOD, SERPING1, CADM4, CP, IGHA1, PGLYRP1, ROBO4, SERPINA5, MASP2, HPX, IGHV4-31, IGHG1, MXRA8, AMY1C, AMY1A, AMY1B, AMY2A, COL6A1, EGF, PROCR, PIGR, ITIH4, CUBN, LMAN2, TF, and KNG1. In some embodiments, the one or more products are one or more proteins encoded by one or more genes selected from the group consisting of KNG1, PIGR, PROCR, HPX, CP, RNASE1, COL12A1, CD59, APOH, and CTBS. In some embodiments, the one or more products are one or more proteins encoded by one or more genes selected from the group consisting of HPX and CP.

[0021] In some embodiments, the method further comprises obtaining the biological sample from the subject. The sample may be any appropriate sample. In some embodiments, the sample is a urine sample. In some embodiments, the corresponding standard protein is urinary creatinine. In some embodiments, the sample may be collected at a particular time of day. In some embodiments, the sample is collected in the morning, which means before 12 p.m. In certain embodiments, the sample is collected within 1 or 2 hours of waking up. In some embodiments, the sample is collected in the evening. In some embodiments, the sample is collected in the evening, which means after 4 p.m. for the subject. In other embodiments, the sample is collected after the subject has been awake for at least 8 hours or for at least 12 hours. In some embodiments, the sample is collected after the subject has been awake less than 1 hour. In some embodiments, the subject is suspected of having OSA.

[0022] In some embodiments, the subject is known to have OSA. In some embodiments, the method further comprises identifying the subject as a candidate for evaluation by the methods disclosed herein by administration of a questionnaire. In some embodiments, the method further comprises using a computer algorithm to evaluate the measured levels of expression of one or more genes from Table 1 or Table 2. In some embodiments, the method further comprises determining a risk score for the subject for having OSA with declarative memory defects. In some embodiments, the expression levels of RNA transcripts are measured. In some embodiments, the expression levels of RNA transcripts are measured using DNA complementary to the RNA transcripts. In some embodiments, expression levels of RNA transcripts are measured using an amplification or hybridization assay. In some embodiments, expression levels of proteins are measured. In some embodiments, expression levels of proteins are measured using one of more binding polypeptides. In some embodiments, one or more binding polypeptides is an antibody. In some embodiments, the method further comprises treating the subject identified as having high-risk OSA. In some embodiments, treating the subject includes pharmacological treatment with corticosteroids, leukotriene antagonists, topical nasal steroids, intranasal steroids, and/or montelukast, surgical removal of the adenoids and tonsils, applying positive airway pressure therapy (PAP), or the application of oral applicances.

[0023] In some aspects, provided is a method for determining whether a subject has obstructive sleep apnea (OSA) with declarative memory defects comprising a) assaying from a biological sample from the subject the levels of expression of one or more proteins encoded by a gene listed in Table 1 or Table 2; and b) calculating a risk score for the biological sample that identifies the likelihood of the subject having OSA with declarative memory defects. In some embodiments, calculating a risk score comprises using a computer and an algorithm. In some embodiments, calculating a risk score comprises applying model coefficients to each of the levels of expression. In some embodiments, the method further comprises identifying the patient as having a risk score indicative of 50% chance or greater of having OSA with declarative memory defects. In some aspects, provided is a method for treating high-risk obstructive sleep apnea (OSA) in a subject comprising pharmacological treatment with corticosteroids, leukotriene antagonists, topical nasal steroids, intranasal steroids, and/or montelukast, surgical removal of the adenoids and tonsils, applying positive airway pressure therapy (PAP), or the application of oral applicances after the subject is determined to have sleep apnea based on measuring expression levels of one or more genes listed in Table 1 or Table 2 in a urine sample obtained from the subject.

[0024] In some embodiments, the subject is a child or minor. In some embodiments, the child or minor is, is at least, or is at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18 years old.

[0025] Some methods also involve comparing the expression level of the at least one protein to the expression level of a control from the sample. In other embodiments, methods involve comparing the expression level of at least one protein to the expression level of that protein in a standardized sample. An increase or decrease in the level of expression will be evaluated. In some embodiments, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 comparative protein (or any range derivable therein) may be used in comparisons or compared to the expression level of a protein. In other embodiments at least or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 comparative protein are measured. In particular embodiments, at least or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 comparative protein are compared to one or more proteins.

[0026] In other embodiments, a coefficient value is applied to each protein expression level. The coefficient value reflects the weight that the expression level of that particular protein has in assessing the whether or not the subject has OSA. In certain embodiments, the coefficient values for a plurality of proteins whose expression levels are measured. The plurality may be, be at least, or be at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26 of these proteins, as well as any proteins discussed herein. Methods and computer readable medium can be implemented with coefficient values.

[0027] In some embodiments, methods will involve determining or calculating a diagnostic score based on data concerning the expression level of one or more proteins, meaning that the expression level of the one or more proteins is at least one of the factors on which the score is based. A diagnostic score will provide information about the biological sample, such as the general probability that the subject has OSA. In some embodiments, the diagnostic score represents the probability that the subject has OSA or does not have OSA. In certain embodiments, a probability value is expressed as a numerical integer or number that represents a probability of 0% likelihood to 100% likelihood that OSA. In some embodiments, the probability value is expressed as a numerical integer or number that represents a probability of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% likelihood (or any range derivable therein) that a patient has OSA. Alternatively, the probability may be expressed generally in percentiles, quartiles, or deciles.

[0028] In some embodiments, methods include evaluating one or more proteins using a scoring algorithm to generate a diagnostic score for OSA, wherein the patient is identified as having or as not having OSA based on the score. It is understood by those of skill in the art that the score is a predictive value about the classification of OSA. In some embodiments, a report is generated and/or provided that identifies the diagnostic score or the values that factor into such a score. In some embodiments, a cut-off score is employed to characterize a sample as likely having OSA. In some embodiments, the risk score for the patient is compared to a cut-off score to characterize the biological sample from the patient with respect to OSA. In certain embodiments, the diagnostic score is calculated using a weighted coefficient for each of the measured protein levels of expression. The weighted coefficients will typically reflect the significance of the expression level of a particular protein for determining risk of OSA.

[0029] Any of the methods described herein may be implemented on tangible computer-readable medium comprising computer-readable code that, when executed by a computer, causes the computer to perform one or more operations. In some embodiments, there is a tangible computer-readable medium comprising computer-readable code that, when executed by a computer, causes the computer to perform operations comprising: a) receiving information corresponding to a level of expression of at least one protein in a sample from a patient; and b) determining a protein expression level value using information corresponding to the at least one protein and information corresponding to the level of expression of a control. In some embodiments, receiving information comprises receiving from a tangible data storage device information corresponding to a level of expression of at least one protein in a sample from a patient. In additional embodiments, information is used that corresponds to the level of expression of a control. In additional embodiments the medium further comprises computer-readable code that, when executed by a computer, causes the computer to perform one or more additional operations comprising: sending information corresponding to the expression level of at least one protein to a tangible data storage device. In specific embodiments, it further comprises computer-readable code that, when executed by a computer, causes the computer to perform one or more additional operations comprising: sending information corresponding to the expression level of at least one protein to a tangible data storage device. In certain

embodiments, receiving information comprises receiving from a tangible data storage device information corresponding to a level of expression of at least one protein in a sample from a patient. In even further embodiments, the tangible computer-readable medium has computer-readable code that, when executed by a computer, causes the computer to perform operations further comprising: c) calculating a diagnostic score for the sample, wherein the diagnostic score is indicative of the probability that the subject has OSA. It is contemplated that any of the methods described above may be implemented with tangible computer readable medium that has computer readable code, that when executed by a computer, causes the computer to perform operations related to the measuring, comparing, and/or calculating a diagnostic score related to the probability of a subject having OSA.

[0030] A processor or processors can be used in performance of the operations driven by the example tangible computer-readable media disclosed herein. Alternatively, the processor or processors can perform those operations under hardware control, or under a combination of hardware and software control. For example, the processor may be a processor specifically configured to carry out one or more those operations, such as an application specific integrated circuit (ASIC) or a field programmable gate array (FPGA). The use of a processor or processors allows for the processing of information (e.g., data) that is not possible without the aid of a processor or processors, or at least not at the speed achievable with a processor or processors. Some embodiments of the performance of such operations may be achieved within a certain amount of time, such as an amount of time less than what it would take to perform the operations without the use of a computer system, processor, or processors, including no more than one hour, no more than 30 minutes, no more than 15 minutes, no more than 10 minutes, no more than one minute, no more than one second, and no more than every time interval in seconds between one second and one hour.

[0031] Some embodiments of the present tangible computer-readable media may be, for example, a CD-ROM, a DVD-ROM, a flash drive, a hard drive, or any other physical storage device. Some embodiments of the present methods may include recording a tangible computer-readable medium with computer-readable code that, when executed by a computer, causes the computer to perform any of the operations discussed herein, including those associated with the present tangible computer-readable media. Recording the tangible computer-readable medium may include, for example, burning data onto a CD-ROM or a DVD-ROM, or otherwise populating a physical storage device with the data.

[0032] The embodiments in the Example section are understood to be embodiments of the invention that are applicable to all aspects of the invention, including compositions and methods.

[0033] The use of the word "a" or "an," when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one."

[0034] The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or." It is also contemplated that anything listed using the term "or" may also be specifically excluded.

[0035] Throughout this application, the term "about" is used to indicate that a value includes the inherent variation of error for the device, the method being employed to determine the value, or the variation that exists among the study subjects.

[0036] The terms "comprise," "have" and "include" are open-ended linking verbs. Any forms or tenses of one or more of these verbs, such as "comprises," "comprising," "has," "having," "includes" and "including," are also open-ended. For example, any method that "comprises," "has" or "includes" one or more steps is not limited to possessing only those one or more steps and also covers other unlisted steps.

[0037] The term "effective," as that term is used in the specification and/or claims, means adequate to accomplish a desired, expected, or intended result.

[0038] As used herein, the term "patient" or "subject" refers to a living mammalian organism, such as a human, monkey, cow, sheep, goat, dogs, cat, mouse, rat, guinea pig, or transgenic species thereof. In certain embodiments, the patient or subject is a primate. Non-limiting examples of human subjects are adults, juveniles, infants and fetuses.

[0039] Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0040] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0041] FIGS. **1A-1E**. Pipeline for urine biomarker discovery by LC-MS/MS. Panel a: An optimized workflow for proteomic analysis of urine. Panels b-c: Immunoglobulin (IgG) and albumin (ALB) depletion. The extent of depletion was quantified by Bradford (Panel b) and visualized by SDS-PAGE (Panel c). Specificity of IgG and ALB removal was assessed by comparing serotransferrin (TRF) levels in depleted (+) and non-depleted (−) samples (Panel c). IgG, whole antibody; HC, heavy chain; LC, light chain; **, non-specific detection of ALB. Panel d: Urine samples were precipitated with TCA/DOC and protein levels were determined for 10 subjects. Results (N=6/subject) are displayed as box-and-Whisker plots (5-95% confidence intervals). Panel e: Gene ontology analysis of all urine proteins detected by mass spectrometry. All functional annotations presented are statistically significant ($p<0.05$) based on the hypergeometric test with Benjamini-Hochberg correction.

[0042] FIGS. **2A-2D**. Gender and diurnal effects on the urinary proteome of healthy children. Morning (am) and bedtime (pm) urine samples were collected from healthy boys (N=7) and girls (N=6) and subjected to LC-ESI-MS/MS analysis. Proteins were quantified by spectral counting and differentially expressed proteins were detected using the t-test and G-test. Panel a: A representative statistical analysis demonstrating proteomic differences in morning samples between boys and girls. Red, up-regulated in boys; Green,

down-regulated in boys. Confidence intervals (dashed lines; G>1.5 or G<-1.5 and α=0.05) and the FDR (<5%) were established by permutation analysis. Proteins that were down-regulated in boys were assigned negative values in the G-test. Panel b: A comparison of differentially expressed proteins in boys (relative to girls) in morning and bedtime samples. Panels c-d: Examples of proteins (TRF and REG1A) that are subjected to both gender and diurnal regulation. Results are means±SEMs, statistical significance (**) was assessed by a combination of the t-test and G-test.

[0043] FIGS. **3**A-**3**E. Identification of candidate biomarkers of pediatric OSA. Morning (am) and bedtime (pm) samples were collected from children with and without OSA and subjected to LC-MS/MS. Panel a: Analysis of proteomic data was performed as follows: Level 1 (L1), morning and bedtime measurements were averaged and boys and girls were pooled; Level 2 (L2), analyses for morning and bedtime samples were conducted independently; Level 3 (L3) analyses for morning and bedtime samples were conducted independently in both boys and girls. The number of candidate biomarkers identified at each level is shown in parentheses. Panel b: Biomarkers detected in level 3 were split according to collection time and gender. Panel c: A demonstration of the "gender effect" on global proteomic analysis (based on the t-test and G-test) of morning urine samples. Red, up-regulated in OSA; green, down-regulated in OSA; dashed lines confidence intervals (FDR <5%). Panel d: Dipeptidyl peptidase 4 (DPP4) as an example of a specific biomarker for OSA in the morning samples of boys. Protein levels (mean±SEMs) were determined by spectral counting. **, statistically significant based on the t-test and G-test.

[0044] FIGS. **4**A-**4**C. Validation of mass spectrometry data by ELISA. Differentially expressed proteins identified by proteomic analysis were validated in morning (am) and bedtime (pm) samples using commercially available ELISA assays. Panel a: Comparison of hemopexin (HPX) level quantified by mass spectrometry (MS/MS) and ELISA (ng/mg creatinine) Linear regression analysis (line) detected a strong positive correlation (R^2=0.52, p<0.0001) between both techniques. Panel b: Measurement of DPP4 levels by ELISA demonstrating specific down-regulation of dipeptidyl peptidase 4 (DPP4) in morning urine samples (compare to FIG. **3***d*). Panel c: Comparison of HPX (ng/mg creatinine), ceruloplasmin (CP; ng/mg creatinine), and zinc-α-2-glycoprotein (AZGP1; ng/mg creatinine) levels quantified by MS/MS and ELISA. Measurements were normalized relative to control samples. Where applicable results are means±SEMs. #, statistically significant based on the t-test (p<0.05) and G-test (G>1.5). **, statistically significant based on the t-test (p<0.05).

[0045] FIG. **5**. Biomarkers of pediatric OSA map to pathophysiological modules. Gene ontology analysis of the 192 candidate biomarkers identified numerous functional modules enriched in children with OSA (p<0.05, hypergeometric test with Benjamini-Hochberg correction). Six representative proteins in each functional module are presented as examples.

[0046] FIGS. **6**A-**6**D. Children with OSA exhibit heterogeneity in memory recall impairment. Healthy subjects (N=13) and children with OSA (N=20) were recruited at the University of Chicago. A: Performance on a pictoral memory recall test identified two populations of children with OSA: those with normal (OSA-N) and impaired (OSA-I) memory recall. B-D: Differences between OSA-N and OSA-I patients could not be attributed to variability in OSA severity (B), obesity (C), or age (D).

[0047] FIGS. **7**A-**7**B. Identification of candidate urine biomarkers of memory impairment in children with OSA. Proteomics analysis of morning urine samples collected from healthy subjects (CTRL) and children with OSA that had normal (OSA-N) or impaired memory (OSA-I). A: Candidate biomarkers were identified using the t-test and G-test (red lines, confidence intervals FDR=0.1%). Yellow=up, blue=down in OSA-I versus OSA-N. B: protein abundance levels (spectral count) for two candidate biomarkers.

[0048] FIGS. **8**A-**8**C. ELISA assays enable high throughput measurement of HPC and CP. Urinary levels of hemopexin (HPX; A), ceruloplasmin (CP; B), and uromodulin (UMOD; C) were quantified by mass spectrometry (MS/MS) and ELISA. For ELISA, values were standardized to urinary creatinine (CR) levels. Note the strong concordance between the two measures.

[0049] FIG. **9**. Memory recall test: Schematic of the declarative memory test for the study. NSPG: overnight polysomnography.

DETAILED DESCRIPTION

[0050] Obstructive sleep apnea (OSA) is a highly prevalent disorder in children (2-3%) characterized by repeated events of partial or complete upper airway obstruction during sleep. This frequent condition, which results in recurring episodes of hypercapnia, hypoxemia, and arousal throughout the night, and accrues substantially to the risk for the development of cardiovascular, metabolic, neurobehavioral, and cognitive problems.

[0051] Substantial evidence suggests that intermittent hypoxia and sleep fragmentation negatively influence academic achievement in children with OSA. Indeed, the inventors have previously demonstrated that children with OSA were more likely to display impairments in the acquisition, consolidation, or retrieval of declarative memories. Furthermore, work has identified declarative memory as a robust reporter on the presence or absence of global cognitive deficits in the context of OSA. Moreover, significant improvements in academic performance and cognitive deficits have been reported following treatment of OSA. Thus, the (early) detection of pediatric OSA patients who are predisposed to more severe memory impairment is of particular clinical significance. However, identifying children who have developed OSA-associated cognitive problems is complicated by the need for laborious neurocognitive tests that are unavailable in most clinical settings and therefore such assessments are not routinely pursued.

[0052] Intrinsic variance of the urine proteome limits the discriminative power of proteomic analysis and complicates biomarker detection. Using an optimized workflow for proteomic analysis of urine, the inventors demonstrate that gender and diurnal effects constitute two important sources of variability in healthy children. Indeed, by performing biomarker discovery in a gender and diurnal-dependent manner, the inventors identified ~30-fold more candidate biomarkers of pediatric obstructive sleep apnea (OSA), a highly prevalent (2-3%) condition in children characterized by repetitive episodes of intermittent hypoxia and hypercapnia, and sleep fragmentation in the context of recurrent upper airway obstructive events during sleep. Remarkably, biomarkers were highly specific for gender and sampling time since poor

overlap (~3%) was observed in the proteins identified in boys and girls across morning and bedtime samples.

[0053] Since no clinical basis to explain gender-specific effects in OSA or healthy children is apparent, the data supports the implementation of contextualized biomarker strategies to a broad range of human diseases. For example, these findings indicate that aside from providing an abundant repository of disease biomarkers, the urinary proteome also comprises a wealth of information concerning disease-related pathological processes.

A. OBSTRUCTIVE SLEEP APNEA

[0054] A person with obstructive sleep apnea (OSA) will stop breathing periodically for a short time (typically less than 60 seconds) while sleeping; it is associated with an airway that may be blocked, which prevents air from reaching the lungs. The diagnosis of this condition currently involves a physical exam and a survey about the patient's sleepiness, quality of sleep and bedtime habits. If a child is involved, questions will be posed to a parent or caregiver. A sleep study may be requested and performed to further evaluate for the presence of the condition. Other tests that may be performed include evaluation of arterial blood gases, electrocardiogram (ECG), echocardiogram, and/or thyroid function studies.

[0055] Disruption in inflammatory/immune, lipid, angiogenic, and hemostatic pathways have all been reported in patients with OSA (Adedayo, 2012; Chorostowska-Wynimko, 2005; Slupsky, 2007; von Kanel, 2007), and are proposed as the mechanistic basis for the heightened prevalence of associated co-morbidities in OSA, such as obesity, diabetes, and atherosclerosis.

[0056] OSA is a highly prevalent disease in children associated with a wide range of comorbidities. Obstructive sleep apnea (OSA) is a common disorder in children (2-3%) characterized by repeated events of partial or complete obstruction of the upper airway during sleep, resulting in recurring episodes of hypercapnia, hypoxemia, and arousal (Lumeng & Chervin, 2008). Current evidence suggests that both the sleep fragmentation, which develops as a consequence of repeated arousals, and the intermittent blood gas abnormalities (hypoxia and hypercarbia) that characterize OSA (Gozal & Kheirandish-Gozal, 2008; Kaemingk, et al., 2003; Kheirandish, et al., 2005) jointly predispose patients to a wide array of morbid consequences. The latter include reduced cognitive and academic performance and memory, behavioral deficits including attention deficit hyperactivity-like disease, aggressiveness and poor impulse control, as well as failure to thrive, enuresis and cardiovascular and metabolic dysfunction (Gozal & Kheirandish-Gozal, 2008; Gozal & Kheirandish-Gozal, 2008; Gozal, et al., 2010; Kim, et al., 2011; Spruyt, et al., 2011; Blunden, et al., 2000; Ellenbogen, et al., 2005; Gottlieb, et al., 2004; Kheirandish & Gozal, 2006; O'Brien, et al., 2003; O'Brien, et al., 2004; Rhodes, et al., 1995; Gozal, et al., 2007; Sans Capdevila, et al., 2008). Adequate treatment of OSA improves or reverses these morbidities, and is further associated with improved overall quality of life (Baldassari, et al., 2008) and reduced healthcare costs (Tarasiuk, et al., 2004).

[0057] Children with OSA exhibit reduced memory and academic performance. Preservation of both rapid eye movement (REM) sleep and non-REM sleep integrity is of great importance to the consolidation of both declarative (factual recall) and non-declarative memory (procedural skills) (Stickgold, et al., 2005). Therefore, disruption of these sleep stages may interrupt or reduce the efficacy of the processes underlying memory consolidation. In addition, sleep has been shown to strengthen memories and make them more resistant to interference in both adults (Ellenbogen, et al., 2006) and children (Hill, et al., 2007). Several studies have now shown that retention of word pairs was significantly increased after sleep, and that sleep enhanced memory performance for faces in both adults and children (Stickgold & Walker, et al., 2007; Walker & Stickgold, 2006; Backhaus, et al., 2008; Wagner, et al., 2007). Similarly, non-disrupted sleep leads to improved performance in memory recall, and enhancement of memory performance is only seen after a good night of sleep (Ellenbogen, et al., 2006; Hill, et al., 2007; Gais & Born, 2004; Ellenbogen, et al., 2006). Studies showed that children with OSA were more likely to display impairments in the acquisition, consolidation, or retrieval of memories (Kheirandish-Gozal, et al., 2010).

[0058] In addition to the diagonistic markers disclosed herein, a questionnaire may help to identify those subjects who are candidates for the methods disclosed herein. This questionnaire can request information such as the age, sex, weight, height, and race and ethnicity of the subject, in addition to more specific questions regarding the subject's sleep. Questions may include whether or not the subject stops breathing during sleep, struggles to breathe while asleep, if physical actions are ever needed to make the subject breathe again during sleep, frequency and loudness of snoring, and concerns regarding the subject's breathing while asleep. In some instances, a subject or the parent of a subject may complete such a questionnaire and, on the basis of those answers, it may be recommended that the subject be evaluated by the methods disclosed herein.

B. BIOMARKERS AND DIAGNOSTIC METHODS

[0059] In some embodiments, there are diagnostic methods related to OSA or OSA with declarative memory defects. Diagnostic methods are based on the identification of biomarkers in a sample from a subject. A "biomarker" is a molecule useful as an indicator of a biologic state in a subject.

[0060] Genetic and environmental perturbations impose dramatic variability on protein expression patterns in individuals. Epigenetic, transcriptomic, metabolomic, and proteomic studies have highlighted the dynamics of regulation of gene expression within healthy populations (Slupsky, 2007; Christensen, 2009). For example, DNA methylation patterns in healthy human tissues were highly sensitive to age and environmental factors (Christensen, 2009). Similarly, metabolites relating to mitochondrial energy metabolism were found to differentiate gender and age in healthy adults (Slupsky, 2007). Furthermore, biomarker discovery strategies based on proteomics are complicated by low protein concentrations and high levels of interfering substances (e.g., salts and nitrogenous bases) in urine. In the context of disease, complex pathophysiological perturbations magnify these proteomic differences and therefore require contextualized biomarker analysis.

[0061] In an attempt to circumvent these problems, the inventors interrogated two important likely sources of variability (gender and diurnal effects) on both the urine proteome and biomarker discovery process of pediatric OSA. To facilitate this process, the inventors optimized a proteomics workflow for biomarker discovery based on liquid chromatography tandem mass spectrometry (LC-MS/MS), an

approach that allows for deeper proteome coverage and interrogation of lower abundance proteins. Current findings demonstrate that diurnal and gender-related effects operate as powerful modulators of the urinary proteome in healthy children.

[0062] The findings demonstrate the presence of dramatic gender and diurnal effects on biomarkers of OSA, suggesting that discovery-based proteomics approaches aimed at identifying biomarkers in a contextualized manner may greatly facilitate the ability to reliably detect human disease. By incorporating these constitutive determinants of variance into the analyses, 192 putative candidate biomarkers were a priori identified in urine collected from children with OSA. Moreover, the inventors show that most if not all (~97%) of these biomarkers retained their predictive ability only if their use was implemented in the contextual setting of their collection (i.e., morning in boys, or bedtime in girls), a result that was validated by ELISA measurements. However, some biomarkers may show their predictive ability regardless of their contextualized setting or may exhibit a different contextualized setting effect as those seen for these 97%. These results highlight the complexity of the biomarker discovery process, and suggest that carefully contextualized biomarker discovery strategies will be obligatorily needed to effectively detect human disease across broad populations.

[0063] The OSA biomarkers disclosed herein can be polypeptides that exhibit a change in expression or state, which can be correlated with the presence of OSA in a subject. The OSA biomarkers are contemplated to constitute the markers identified in Table 1. In certain embodiments, specific biomarkers in Table 1 are contemplated. In certain embodiments, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 of the biomarkers in Table 1, or a range derivable therein, may be employed in embodiments described herein. In addition, the biomarkers disclosed herein can include messenger RNAs (mRNAs) encoding the biomarker polypeptides, as measurement of a change in expression of an mRNA can be correlated with changes in expression of the polypeptide encoded by the mRNA. Changes in expression may be an increase (up-regulation) in expression in OSA cells or a decrease (down-regulation) in expression in OSA cells compared to the control cells. Whether a particular biomarker is increased or decreased is shown in Table 1. As such, determining an expression level of a gene of interest in a biological sample is inclusive of determining an amount of a polypeptide biomarker and/or an amount of an mRNA encoding the polypeptide biomarker either by direct or indirect (e.g., by measure of a complementary DNA (cDNA) synthesized from the mRNA) measure of the mRNA.

TABLE 1

IPI	UniProt	Entrez	Gene name	Description	G-test	T-test
IPI00032328	P01043|P01042|B4E1C2|Q7M4P1|B2RCR2| A8K474|Q6PAU9|Q53EQ0	3827	KNG1	Kininogen-1|Kininogen 1, isoform CRA_b	72.6	0.0187
IPI00004573	P01833|Q8IZY7|Q68D81	5284	PIGR	Polymeric immunoglobulin receptor	67.3	0.0028
IPI00220143	Q75ME7|Q0VAX6|O43451|Q8TE24|Q86UM5	8972	MGAM	Maltase-glucoamylase|Maltase-glucoamylase, intestinal	65.8	0.0279
IPI00029260	Q96FR6|F1C4A7|Q9UNS3|Q96L99|B2R888| P08571|Q53XT5	929	CD14	Monocyte differentiation antigen CD14	57.4	0.0363
IPI00293088	Q16302|P10253|Q09GN4|Q8IWE7|Q14351	2548	GAA	Lysosomal alpha-glucosidase	54.4	0.0356
IPI00014048	Q1KHR2|B2R589|Q6ICS5|Q16869|Q16830| D3DS06|P07998|Q9UCB4|Q9UCB5	6035	RNASE1	Ribonuclease pancreatic	53.8	0.0034
IPI00291136	Q9BSA8|Q14040|Q14041|O00117| Q16258|O00118|Q7Z645|P12109|Q8TBN2	1291	COL6A1	Collagen alpha-1(VI) chain|Putative uncharacterized protein	50.8	0.0024
IPI00218192	Q15135|Q14624|Q9UQ54|Q9P190	3700	ITIH4	Inter-alpha-trypsin inhibitor heavy chain H4	48.7	0.0136
IPI00022620	P55000|Q6PUA6|Q53YJ6|Q92483	57152	SLURP1	Secreted Ly-6/uPAR-related protein 1	43.9	0.0012
IPI00009950	Q53HH1|Q12907|A8K7T4	10960	LMAN2	cDNA FLJ75774, highly similar to *Homo sapiens* lectin, mannose-binding 2 (LMAN2), mRNA|Vesicular integral-membrane protein VIP36	41.9	0.0351
IPI00294713	Q9H498|Q9UMV3|Q9ULC7| Q96QG4|O75754|Q9UC48|O00187| Q9H499|Q5TEQ5|Q9BZH0|Q5TER0| A8K458|A8MWJ2|Q9UBP3|Q9Y270	10747	MASP2	Mannan-binding lectin serine protease 2	34.8	0.0042
IPI00000073	E9PBF0|P01133|B4DRK7|Q52LZ6	1950	EGF	Pro-epidermal growth factor	30.3	0.0017
IPI00295741	Q6LAF9|A8K2H4|Q503A6|B3KQR5| Q96D87|P07858|B3KRR5	1508	CTSB	Cathepsin B|cDNA FLJ78235	30.3	0.0454
IPI00022488	P02790|B2R957	3263	HPX	Hemopexin	27.4	0.0086
IPI00291866	A6NMU0|Q9UC49|Q96FE0|P05155| A8KAI9|E9KL26|Q7Z455| Q16304|B2R6L5|Q59EI5|Q547W3| Q9UCF9	710	SERPING1	Plasma protease C1 inhibitor|Epididymis tissue protein Li 173	26.1	0.0036
IPI00009028	P05452|B2R582|Q6FGX6	7123	CLEC3B	Tetranectin|cDNA, FLJ92374, highly similar to *Homo sapiens* C-type lectin domain family 3, member B (CLEC3B), mRNA	26.0	0.0014
IPI00007778	F6X5H7|B2RBF5|Q5VX51|Q5VX50|Q8TC97| B3KQS3|B4DQ98|Q01459	1486	CTBS	cDNA PSEC0114 fis, clone NT2RP2006543, highly similar to DI-N-ACETYLCHITOBIASE (EC 3.2.1.—)| CTBS protein|Di-N-acetylchitobiase|cDNA FLJ55135, highly similar to Di-N-acetylchitobiase (EC 3.2.1.—)| cDNA, FLJ95483, highly similar to *Homo sapiens* chitobiase, di-N-acetyl-(CTBS), mRNA|Chitobiase, di-N-acetyl-	25.8	0.0045
IPI00006662	D3DNW6|B2R579|P05090|Q6IBG6	347	APOD	Apolipoprotein D	25.6	0.0239
IPI00299738	O14550|A4D2D2|B2R9E1|Q15113	5118	PCOLCE	Procollagen C-endopeptidase enhancer|Procollagen C-endopeptidase enhancer 1	23.9	0.0214
IPI00027843	P22891|A6NMB4|Q5JVF6|Q15213|Q5JVF5	8858	PROZ	Vitamin K-dependent protein Z	23.0	0.0009
IPI00021085	O75594|Q4VB36	8993	PGLYRP1	Peptidoglycan recognition protein 1	21.4	0.0262
IPI00009030	P13473|Q16641|D3DTF0|Q6Q3G8| Q99534|A8K4X5|Q9UD93|Q96J30	3920	LAMP2	Lysosome-associated membrane glycoprotein 2	21.2	0.0235
IPI00395488	Q6UXL4|Q6UXL5|Q96CX1|Q6EMK4	114990	VASN	Vasorin	21.2	0.0017
IPI00018953	Q53TN1|P27487	1803	DPP4	Dipeptidyl peptidase 4	20.3	0.0153
IPI00302944	Q5VYK2|Q71UR3|Q5VYK1| Q15955|Q99716|Q99715|O43853	1303	COL12A1	Collagen alpha-1(XII) chain	19.6	0.0256
IPI00293539	A8MZC8|Q9UQ94|B7WP28| Q9UQ93|A8K5D6|Q15065|P55287|Q15066	1009	CDH11	Cadherin-11	19.4	0.0246

TABLE 1-continued

IPI	UniProt	Entrez	Gene name	Description	G-test	T-test
IPI00027235	Q9UC75|Q9NTQ3|O95414|O75882| Q9UDF5|Q9NU01|A8KAE5| Q9NZ58|O60295|Q3MIT3| Q9NZ57|Q5VYW3|C9IZD4| Q5TDA4|Q5TDA2|Q9NTQ4	8455	ATRN	Uncharacterized protein|Attractin	19.3	0.0188
IPI00026314	A8MUD1|B7Z9A0|P06396|Q8WVV7| B7Z373|Q5T0I2|B7Z6N2	2934	GSN	Gelsolin (Amyloidosis, Finnish type)|cDNA FLJ56154, highly similar to Gelsolin|cDNA FLJ56212, highly similar to Gelsolin|Gelsolin	19.0	0.0436
IPI00216780	Q6NV88|Q8IUL8|Q8WV21|Q8N4A6|B2RAJ0	148113	CILP2	cDNA, FLJ94946, highly similar to *Homo sapiens* cartilage intermediate layer protein 2 (CILP2), mRNA|Cartilage intermediate layer protein 2	18.7	0.0026
IPI00021885	Q9BX62|A8K3E4|Q4QQH7|D3DP14|P02671| D3DP15|Q9UCH2	2243	FGA	cDNA FLJ78367, highly similar to *Homo sapiens* fibrinogen, A alpha polypeptide (FGA), transcriptvariant alpha, mRNA|Fibrinogen alpha chain	18.5	0.0163
IPI00012585	P07686	3074	HEXB	Beta-hexosaminidase subunit beta	18.5	0.0494
IPI00060800	Q96DA0|C3PTT6|B2R4F6|A6NIY1|Q6UW28	124220	PAUF|ZG16B	Zymogen granule protein 16 homolog B|Pancreatic adenocarcinoma upregulated factor	17.5	0.0227
IPI00176427	B2R7L5|Q9Y4A4|Q8NFZ8	199731	CADM4	Cell adhesion molecule 4	17.3	0.0021
IPI00022661	Q92692|Q96J29|Q6IBI6|O75455|Q7Z456	5819	PVRL2	Poliovirus receptor-related protein 2|Poliovirus receptor related 2	16.7	0.0454
IPI00291262	Q5HYC1|Q2TU75|B3KSE6|Q7Z5B9|B2R9Q1| P11381|P11380|P10909	1191	CLU	Clusterin	16.2	0.0096
IPI00221224	Q6GT90|Q8IVL7|B4DP01|Q59E93|Q16728| Q8IUK3|Q8IVH3|P15144|Q71E46|B4DV63| B4DPH5|B4DP96|Q9UCE0	290	ANPEP|CD13	cDNA FLJ56158, highly similar to Aminopeptidase N (EC 3.4.11.2)|Membrane alanine aminopeptidase variant|Uncharacterized protein|Aminopeptidase N|cDNA FLJ56120, highly similar to Aminopeptidase N (EC 3.4.11.2)|cDNA FLJ55496, highly similar to Aminopeptidase N (EC 3.4.11.2)	16.1	0.0111
IPI00291867	Q6LAM0|P05156|O60442	3426	CFI	Complement factor I|Light chain of factor I	15.0	0.0147
IPI00003919	Q16770|Q3KRG6|Q16769|Q53TR4	25797	tmp__locus__46|QPCT	Glutaminyl-peptide cyclotransferase|Glutaminyl-peptide cyclotransferase (Glutaminyl cyclase), isoform CRA__a	14.3	0.0121
IPI00099670	P19835|Q9UP41|Q16398|O75612|B4DSX9| Q9UCH1|Q5T7U7	1056	CEL	cDNA FLJ51297, highly similar to Bile salt-activated lipase (EC 3.1.1.3)|Bile salt-dependent lipase oncofetal isoform|Bile salt-activated lipase	13.8	0.0464
IPI00031065	Q14UV0|Q14UU9|P24855	1773	DNASE1	Deoxyribonuclease|Deoxyribonuclease-1	13.8	0.0044
IPI00043992	Q96K15|Q96NY8	81607	PVRL4	Poliovirus receptor-related protein 4	13.7	0.0332
IPI00015525	Q504V7|B4E3H8|Q6P2N2|Q9H8L6	79812	MMRN2	Multimerin-2|cDNA FLJ54082, highly similar to Multimerin-2	13.7	0.0046
IPI00009027	Q2TBE1|P05451|Q0VFX1|A8K7G6|P11379| Q4ZG28	5967	REG1A	REG1A protein|Putative uncharacterized protein REG1A|cDNA FLJ75763, highly similar to *Homo sapiens* regenerating islet-derived 1 alpha (pancreatic stone protein, pancreatic thread protein) (REG1A), mRNA|Lithostathine-1-alpha	13.6	0.0282
IPI00022432	Q9UBZ6|Q6IB96|P02766|E9KL36|Q549C7| Q9UCM9	7276	TTR	Epididymis tissue sperm binding protein Li 4a|Transthyretin	13.3	0.0042
IPI00022290	P60022|Q09753|Q86SQ8	1672	DEFB1|HBD1	Beta-defensin-1|Beta-defensin 1	13.3	0.0053
IPI00022420	D3DR38|P02753|Q9P178|Q8WWA3|Q5VY24| O43479|O43478	5950	RBP4	Retinol-binding protein 4	13.2	0.0087
IPI00102300	Q9UIF2|Q9HCN7|Q9HCN6	51206	GP6	Platelet glycoprotein VI	13.1	0.0032
IPI00240345	Q695G9|Q86T13|Q6PWT6|Q8N5V5	161198	CLEC14A	C-type lectin domain family 14 member A	12.9	0.0015
IPI00153049	Q5TA39|Q96KC3|Q9BRK3	54587	MXRA8	Matrix-remodeling-associated protein 8	12.9	0.0286
IPI00029658	A8KAJ3|Q541U7|Q12805|A8K3I4|D6W5D2| Q59G97|B2R6M6	2202	EFEMP1	EGF-containing fibulin-like extracellular matrix protein 1 isoform b variant|EGF-containing fibulin-like	12.9	0.0256

TABLE 1-continued

IPI	UniProt	Entrez	Gene name	Description	G-test	T-test
				extracellular matrix protein 1|cDNA, FLJ93024, highly similar to *Homo sapiens* EGF-containing fibulin-like extracellular matrix protein 1 (EFEMP1), transcript variant 1, mRNA|cDNA FLJ77823, highly similar to *Homo sapiens* EGF-containing fibulin-like extracellular matrix protein 1, transcript variant 3, mRNA		
IPI00219684	Q5VV93|B2RAB6|Q99957|P05413|Q6IBD7	2170	FABP3	FABP3 protein|Fatty acid-binding protein, heart	12.8	0.0009
IPI00302592	Q5HY55|Q5HY53|P21333|Q8NF52|Q60FE6|Q6NXF2|Q8TES4	2316	FLNA|FLJ00119	Filamin-A|Filamin A|FLNA protein FLJ00119 protein	12.8	0.0025
IPI00019568	P00734|B4DDT3|B2R7F7|Q53H06|Q53H04|Q9UCA1|Q69EZ8|Q4QZ40|Q7Z7P3|B4E1A7|Q69EZ7	2147	F2	Prothrombin B-chain|cDNA FLJ54622, highly similar to Prothrombin (EC 3.4.21.5)|Prothrombin	12.1	0.0383
IPI00075248	Q96HK3|P02593|P70667|Q13942|P99014|P62158|B4DJ51|Q53S29|Q61379|Q61380	801|808|805	CALM2|CALM3|CALM1	Calmodulin|Calmodulin 1 (Phosphorylase kinase, delta), isoform CRA_a	12.1	0.0234
IPI00103871	Q9NWJ8|A8K154|Q8TEG1|Q8WZ75|Q96JV6|Q9H718|Q14DU7	54538	ROBO4	Roundabout homolog 4	11.9	0.0291
IPI00009793	Q53GX9|Q9NZP8	51279	C1RL	Complement C1r subcomponent-like protein	11.7	0.0142
IPI00299086	O00173|O43391|O00560|B2R5Q7|B4DUH3|Q14CP2|B7ZLN2	6386	SDCBP	Syntenin-1|Syndecan binding protein (Syntenin)	11.7	0.0132
IPI00019157	D3DW77|Q92675|Q6UVK1	1464	CSPG4	Chondroitin sulfate proteoglycan 4	11.7	0.0185
IPI00006971	Q2M2V5|Q9HCU0|Q96KB6|Q3SX55	57124	CD248	Endosialin	11.3	0.0186
IPI00555812	Q53F31|P02774|B4DPP2|Q16309|Q16310|Q6GTG1	2638	GC	Vitamin D-binding protein	11.3	0.0073
IPI00009276	Q14218|Q9ULX1|Q96CB3|B2RC04|Q9UNN8|Q6IB56	10544	PROCR	Endothelial protein C receptor	10.9	0.0332
IPI00013955	Q9UE76|Q9UE75|Q9UQL1|Q7Z552|Q14876|Q9Y4J2|Q14128|Q16437|P13931|P17626|P15941|Q16615|P15942|Q16442|Q9BXA4	4582	MUC1	Mucin-1	10.9	0.0144
IPI00010343	Q9UPR5|B4DYQ9|B4DEZ4	6543	SLC8A2	cDNA FLJ58526, highly similar to Sodium/calcium exchanger 2|Sodium/calcium exchanger 2	10.7	0.0069
IPI00011302	P13987|Q6FHM9	966	CD59	CD59 antigen, complement regulatory protein, isoform CRA_b|CD59 glycoprotein	10.1	0.0171
IPI00017601	Q2PP18|A8K5A4|Q1L857|A5PL27|B3KTA8|Q14063|P00450|Q9UKS4	1356	CP	cDNA FLJ76826, highly similar to *Homo sapiens* ceruloplasmin (ferroxidase) (CP), mRNA|cDNA FLJ37971 fis, clone CTONG2009958, highly similar to CERULOPLASMIN (EC 1.16.3.1)|CP protein|Ceruloplasmin	9.7	0.0247
IPI00553177	E9KL23|Q0PVP5|Q53XB8|Q96BF9|B2RDQ8|Q13672|Q5U0M1|Q7M4R2|P01009|Q9P1P0|Q9UCM3|A6PX14|Q9UCE6|Q96ES1|Q86U19|Q86U18	5265	SERPINA1	Epididymis secretory sperm binding protein Li 44a|Alpha-1-antitrypsin	9.6	0.0265
IPI00032293	D3DW42|B2R5J9|P01034|E9RH26|Q6FGW9	1471	CST3	Cystatin-C|Cystatin C	9.2	0.0021
IPI00045512	Q69YJ3|Q5TYR7|Q96RW7|Q96DN8|Q96SC3|Q5TCP6|Q96DN3|Q96K89|A6NGE3	83872	DKFZp762L185|HMCN1	Hemicentin 1|cDNA FLJ14438 fis, clone HEMBB1000317, weakly similar to FIBULIN-1, ISOFORM D|Putative uncharacterized protein DKFZp762L185|Hemicentin-1	9.0	0.0171

TABLE 1-continued

IPI	UniProt	Entrez	Gene name	Description	G-test	T-test
IPI00010675	Q15854|Q03403	7032	TFF2	Trefoil factor 2	8.9	0.0247
IPI00032325	P01040|Q6IB90	1475	CSTA	CSTA protein|Cystatin-A	8.7	0.0042
IPI00298388	Q49A94|Q8NCJ9|Q96FE7|Q86YW2|O00318	113791	PIK3IP1	Phosphoinositide-3-kinase-interacting protein 1	8.2	0.0075
IPI00306322	Q14052|Q548C3|Q66K23|P08572|Q5VZA9|B4DH43	1284	COL4A2	cDNA FLJ56433, highly similar to Collagen alpha-2(IV) chain|Collagen alpha-2(IV) chain	7.5	0.0264
IPI00290085	Q14923|Q8N173|B0YIY6|P19022	1000	CDH2	Cadherin-2	7.1	0.0137
IPI00010949	Q9HAT2|B3KPB0|Q9HAU7|Q8IUT9|Q9NT71	54414	SIAE	Sialate O-acetylesterase	7.1	0.0060
IPI00295414	P39059|B3KTP7|Q5T6J4|Q9Y4W4|Q9UDC5	1306	COL15A1	Collagen alpha-1(XV) chain|cDNA FLJ38566 fis, clone HCHON2005118, highly similar to Collagen alpha-1(XV) chain	6.8	0.0135
IPI00010182	P08869|Q4VWZ6|Q53SQ7|Q9UCI8|P07108|B8ZWD8|Q6IB48	1622	DBI	Diazepam binding inhibitor, splice form 1D(1)|Acyl-CoA-binding protein	6.8	0.0021
IPI00103636	Q8WXW1|Q6IB27|A6PVD5|Q96KJ1|A2A2A5|Q14508|Q8WXV9|A2A2A6|Q8WXW0|Q8WXW2	10406	WFDC2	WAP four-disulfide core domain protein 2	6.6	0.0191
IPI00289983	Q96QM0|D3DNC6|Q96KY0|P15309|Q96QK9	55	ACPP	Prostatic acid phosphatase	6.5	0.0073
IPI00027482	B2R9F2|P08185|Q7Z2Q9|A8K456	866	SERPINA6	Corticosteroid-binding globulin|cDNA, FLJ94361, highly similar to *Homo sapiens* serine (or cysteine) proteinase inhibitor, clade A(alpha-1 antiproteinase, antitrypsin), member 6 (SERPINA6), mRNA	6.5	0.0256
IPI00175092	Q53SV6|Q8WUU3|Q8NC42|Q8NBY5|Q53S14|Q8N5I8	284996	RNF149|LOC284996	Putative uncharacterized protein LOC284996|E3 ubiquitin-protein ligase RNF149	6.4	0.0102
IPI00186826	B5A972|B5A970|Q96L35	2050	EPHB4	EPH receptor B4, isoform CRA_b|Soluble EPHB4 variant 1|Soluble EPHB4 variant 3	6.1	0.0396
IPI00019580	B2R7F8|P00747|Q9UMI2|Q15146|Q5TEH4|Q6PA00|B4DPH4	5340	PLG	PLGprotein|Plasminogen|cDNA, FLJ93426, highly similar to *Homo sapiens* plasminogen (PLG), mRNA|cDNA FLJ58778, highly similar to Plasminogen (EC 3.4.21.7)	6.1	0.0084
IPI00032258	B0QZR6|Q13160|A7E2V2|Q14033|P0C0L4|B7ZVZ6|Q6P4R1|B2RUT6|Q5JQM8|Q4LE82|P01028|Q9NPK5|P78445|Q13906|Q14835|Q9UIP5	720|721	C4A variant protein|C4A	Complement C4-A|C4A variant protein|Complement component 4A (Rodgers blood group)	6.0	0.0480
IPI00292130	A8K981|Q9UIX8|Q07507|Q8N4R2	1805	DPT	Dermatopontin	5.9	0.0022
IPI00029275	P08582|Q9BQE2	4241	MFI2	Melanotransferrin	5.8	0.0252
IPI00019906	B4DY23|P35613|Q7Z796|Q54A51|Q8IZL7	682	hEMMPRIN|BSG	Basigin|cDNA FLJ61188, highly similar to Basigin|Basigin (Ok blood group), isoform CRA_a	5.7	0.0082
IPI00218413	Q96EM9|B7Z7C9|B2R865|P43251	686	BTD	Biotinidase|cDNA FLJ50907, highly similar to Biotinidase (EC 3.5.1.12)	5.6	0.0416
IPI00026926	Q02747	2980	GUCA2A	Guanylin	5.5	0.0152
IPI00025992	B6EU04|Q9BY68|Q1HE14|P81172	57817	HAMP	Hepcidin|Hepcidin antimicrobial peptide	5.5	0.0484
IPI00179330	B2RDW1|Q9UEK8|Q8WYN8|Q91887|Q6LDU5|P62988|Q9BX98|Q9UEF2|P62979|Q5RKT7|Q9UPK7|P14798|Q9BWD6|Q6LBL4|P02248|P02249|Q91888|Q9BQ77|Q29120|P02250|Q9UEG1	6233	RPS27A	Ribosomal protein S27a|Ubiquitin-40S ribosomal protein S27a|Ribosomal protein S27a, isoform CRA_c	5.2	0.0004

TABLE 1-continued

IPI	UniProt	Entrez	Gene name	Description	G-test	T-test
IPI00099110	Q9Y4V9|B1ARE9|B1ARE8|Q5JR26|B1ARF0|Q9UGM3|Q9UGM2|Q59EX0|B1ARE7|A8E4R5|Q9UKJ4|Q9UJ57|Q96DU4A6NDG4|Q9Y211|Q6MZN4|A6NDJ5	1755	DMBT1	Deleted in malignant brain tumors 1 protein	5.0	0.0038
IPI00291488	Q8WXW1|Q6IB27|A6PVD5|Q96KJ1|A2A2A5|Q14508|Q8WXV9|A2A2A6|Q8WXW0|Q8WXW2	10406	WFDC2	WAP four-disulfide core domain protein 2	5.0	0.0413
IPI00002435	P26842|B2RDZ0	939	CD27	CD27 antigen	5.0	0.0003
IPI00021447	B3KXB7|D3DT76|P19961|Q9UBH3	280	AMY2B	Alpha-amylase 2B	4.9	0.0477
IPI00303161	Q96AP7|Q96T50	90952	ESAM	Endothelial cell-selective adhesion molecule	4.8	0.0008
IPI00000024	B4E2D8|Q8IUP2|Q08174	5097	PCDH1	cDNA FLJ59655, highly similar to Protocadherin-1|Protocadherin-1	4.6	0.0079
IPI00002280	Q9UHG2|Q4VC04	27344	PCSK1N	ProSAAS	4.5	0.0007
IPI00009650	Q5T8A1|P31025	3933	LCN1	Lipocalin-1	4.4	0.0053
IPI00021841	Q9UCS8|Q6LDN9|Q9UCT8|A8K866|P02647|Q6Q785|Q6LEJ8	335	APOA1	APOA1 protein|Apolipoprotein A-I	4.4	0.0233
IPI00977659	Q6S9E4|A8K9Q3|Q14C97|Q9ULV1|Q8TDT8	8322	GPCR|FZD4	Frizzled-4|Putative G-protein coupled receptor	4.2	0.0057
IPI00219365	Q6PJT4|P26038	4478	MSN	MSN protein|Moesin	4.1	0.0033
IPI00289334	Q9UEV9|Q13706|Q9NT26|C9JMC4|Q6MZJ1|C9JKE6|O75369|Q8WXS9|B2ZZ84|B2ZZ85|Q8WXT1|Q8WXT0|Q59EC2|Q8WXT2|Q9NRB5	2317	FLNB	Filamin-B	4.1	0.0268
IPI00216298	P10599|Q53X69|Q9UDG5|Q96KI3	7295	TXN	Thioredoxin	4.0	0.0028
IPI00013576	Q8WVV5|O00480	10385	BTN2A2	Butyrophilin subfamily 2 member A2	4.0	0.0141
IPI00376457	B4E0V9	342510		cDNA FLJ61198, highly similar to *Homo sapiens* CD300 antigen like family member E (CD300LE), mRNA	4.0	0.0064
IPI00296992	Q8N5L2|P30530|Q9UD27	558	AXL	Tyrosine-protein kinase receptor UFO	3.9	0.0454
IPI00022284	Q15216|A1YVW6|Q8TBG0|Q27H91|P04156|Q86XR1|O60489|Q5QPB4|Q6FGR8|Q15221|Q6FGN5|D4P3Q7|Q96E70|P78446|B4DDS1|Q9UP19|B2R5Q9|Q5U0K3|Q540C4|Q53YK7	5621	PRNP	Major prion protein	3.8	0.0118
IPI00289501	O15240|Q9UDW8	7425	VGF	Neurosecretory protein VGF	3.8	0.0102
IPI00001754	Q9Y624|D3DVF0|Q6FIB4	50848	F11R	F11 receptor|F11 receptor, isoform CRA_a|Junctional adhesion molecule A	3.6	0.0048
IPI00027463	P06703|Q5RHS4|D3DV39|B2R577	6277	S100A6	cDNA, FLJ92369, highly similar to *Homo sapiens* S100 calcium binding protein A6 (calcyclin) (S100A6), mRNA|Protein S100-A6	3.6	0.0207
IPI00297646	O76045|Q16050|Q9UML6|Q13902|Q14037|Q13903|Q8IVI5|Q6LAN8|P02452|Q13896|Q59F64|Q15176|D3DTX7|Q8N473|Q15201|Q14042|Q14992|Q9UMM7|Q7KZ30|P78441|Q7KZ34|Q9UMA6	1277	COL1A1	Collagen type I alpha 1|Type II procollagen gene|Collagen, type I, alpha 1, isoform CRA_a|Type I collagen alpha 1 chain|Collagen alpha-1(I) chain	3.6	0.0160
IPI00025204	A8K7M5|O43866|Q6UX63	922	CD5L	CD5 antigen-like	3.6	0.0014
IPI00470360	Q8TB15|Q5XKC6|Q9H9N1|Q7Z7N8|	55243	KIRREL	Kin of IRRE-like protein 1	3.5	0.0062

TABLE 1-continued

IPI	UniProt	Entrez	Gene name	Description	G-test	T-test
	Q5W0F8|Q96J84|Q9NVA5| Q7Z696					
IPI00002910	Q9H665|Q8N5X0	79713	IGFLR1	IGF-like family receptor 1	3.5	0.0090
IPI00641251	B2RDS5|Q53HF7|Q9NPF0|D6W668	51293	CD320	CD320 antigen	3.3	0.0078
IPI00027509	B7Z747|Q9UCJ9|B7Z8A9|P14780| Q8N725|Q9UDK2|Q3LR70| Q9UCL1|F5GY52|Q9H4Z1| B2R7V9|Q9Y354|B7Z507	4318	MMP9	cDNA FLJ51036, highly similar to Matrix metalloproteinase-9 (EC3.4.24.35)|Uncharacterized protein|Matrix metalloproteinase-9|Matrix metalloproteinase 9|cDNA FLJ51120, highly similar to Matrix metalloproteinase-9 (EC 3.4.24.35)|cDNA FLJ51166, highly similar to Matrix metalloproteinase-9 (EC 3.4.24.35)	3.3	0.0218
IPI00021968	Q9Y6Q6	8792	TNFRSF11A	Tumor necrosis factor receptor superfamily member 11A	3.2	0.0112
IPI00027436	B2R961|P08138	4804	NGFR	Tumor necrosis factor receptor superfamily member 16	3.2	0.0117
IPI00003813	Q9BY67|Q8N2F4|Q86WB8|Q6MZK6	23705	DKFZp686F1789| CADM1	Putative uncharacterized protein DKFZp686F1789|Cell adhesion molecule 1	3.1	0.0197
IPI00006705	P11684|Q9UCM4|B2R5F2|Q6FHH3| Q9UCM2	7356	SCGB1A1	Uteroglobin	3.1	0.0305
IPI00013972	Q16574|Q0Z7S6|O60399|P31997	1088	CEACAM8	Carcinoembryonic antigen-related cell adhesion molecule 8	3.1	0.0046
IPI00289831	Q16341|O75255|Q15718|Q13332| O75870|D6W633|Q2M3R7	5802	PTPRS	Receptor-type tyrosine-protein phosphatase S|Protein tyrosine phosphatase, receptor type, S, isoform CRA_a	3.0	0.0328
IPI00003101	P01589|B2R9M9|A2N4P8|Q5W007| Q53FH4	3559	IL2RA|IL2R	cDNA, FLJ94475, highly similar to *Homo sapiens* interleukin 2 receptor, alpha (IL2RA), mRNA|IL2R protein|Interleukin-2 receptor subunit alpha|Interleukin 2 receptor, alpha chain variant	3.0	0.0085
IPI00017202	Q7Z798|Q7Z7A0|Q7Z799|Q9H9P2| B2R9C0|Q9HCY3	140578	CHODL	Chondrolectin	3.0	0.0341
IPI00031121	B3KXD3|B3KR42|P16870|D3DP33| A8K4N1|Q9UIU9	1363	CPE	cDNA FLJ45230 fis, clone BRCAN2021325, highly similar to Carboxypeptidase E (EC 3.4.17.10)|Carboxypeptidase E	3.0	0.0327
IPI00010290	Q6FGL7|Q05CP7|P07148	2168	FABP1	Fatty acid-binding protein, liver|FABP1 protein	2.9	0.0039
IPI00018434	Q9BUM5|Q99816	7251	TSG101	Tumor susceptibility gene 101 protein	2.8	0.0173
IPI00219465	Q9UDM0|Q9BVI8|P20062|Q9UCI6| Q9UCI5	6948	TCN2	Transcobalamin-2	2.8	0.0339
IPI00009794	B1AME5|B1AME6|Q8NBQ3| Q96AA1|Q53HQ9|B4DSM1|B2RDF1| Q9BRK5|Q9NZP7|Q9UN53| Q53G52	51150	SDF4	45 kDa calcium-binding protein	2.8	0.0403
IPI00219860	P23468|B1ALA0	5789	PTPRD	Receptor-type tyrosine-protein phosphatase delta	2.8	0.0437
IPI00329538	Q9UCA3|Q16651	5652	PRSS8	Prostasin	2.7	0.0164
IPI00166729	O60386|Q5XKQ4|P25311|D6W5T8| Q8N4N0	563	AZGP1	Zinc-alpha-2-glycoprotein	2.6	0.0168
IPI00016786	P25763|P21181|P60953|Q9UDI2| Q7L8R5	998	CDC42	Cell division control protein 42 homolog	2.6	0.0011
IPI00215997	Q96ES4|P21926|Q5J7W6|D3DUQ9	928	CD9	CD9 antigen	2.6	0.0200
IPI00383032	Q96K94|B2RAY2|Q8WW60| Q8TDQ0	84868	HAVCR2	Hepatitis A virus cellular receptor 2	2.6	0.0202
IPI00010807	Q9H461	8325	FZD8	Frizzled-8	2.6	0.0030
IPI00034319	Q9NYQ9|O60888|Q5JXM9|Q3B784| A2BEL4|A2AB26|Q5SU05	51596	CUTA	Protein CutA	2.5	0.0245
IPI00026154	B4DJQ5|P14314|Q96BU9|Q9P0W9| E7EQZ9|Q96D06	5589	PRKCSH	Glucosidase 2 subunit beta|Uncharacterized protein|cDNA FLJ59211, highly similar to Glucosidase 2 subunit beta	2.5	0.0008

TABLE 1-continued

IPI	UniProt	Entrez	Gene name	Description	G-test	T-test
IPI00220737	Q96CJ3|Q16180|B7Z8D6|Q15829|Q05C58|P13591|P13592|P13593|Q86X47|Q59FL7|A8K8T8|Q16209	4684	NCAM1	cDNA FLJ54771, highly similar to Neural cell adhesion molecule 1, 120 kDa isoform|Neural cell adhesion molecule 1	2.4	0.0028
IPI00925540	A6NLA3|Q13350|Q14870|P26927|Q6GTN4|A8MSX3|Q53GN8|B7Z250	4485	MST1	Hepatocyte growth factor-like protein|cDNA FLJ56324, highly similar to Hepatocyte growth factor-like protein|Macrophage stimulating 1 (Hepatocyte growth factor-like) variant	2.4	0.0016
IPI00017557	Q1ZYW2|Q6PD64|Q4G124|Q6FHJ7|Q6FHM0|O14877|B4DYC1|Q05BG7	6424	SFRP4	Secreted frizzled-related protein 4	2.3	0.0460
IPI00002666	Q7M4M8|P09086|Q16648|Q9BRS4|Q9UMI6|Q9UMJ4	5452	OCT-2|POU2F2	Homeobox protein|Oct-2 factor|POU domain, class 2, transcription factor 2	2.3	0.0004
IPI00414896	Q9BZ46|Q9BZ47|B2RDA7|E1P5C3|Q8TCU2|O00584|Q5T8Q0	8635	RNASET2	Ribonuclease T2	2.3	0.0131
IPI00293836	Q8N3J6|Q658Q7|Q8IZP8|Q3KQY9	253559	CADM2	Cell adhesion molecule 2	2.3	0.0230
IPI00020557	Q59FG2|Q07954|Q6LAF4|Q2PP12|Q8IVG8|Q6LBN5	4035	LRP|LRP1	LRP protein|Alpha-2 macroglobulin receptor|Prolow-density lipoprotein receptor-related protein 1|Low density lipoprotein-related protein 1 variant	2.3	0.0465
IPI00004440	A8K604|Q16849|Q08319|Q53QD6|B4DK12	5798	PTPRN	cDNA FLJ55332, highly similar to Receptor-type tyrosine-proteinphosphatase-like N|Receptor-type tyrosine-protein phosphatase-like N|cDNA FLJ77469, highly similar to *Homo sapiens* protein tyrosine phosphatase, receptor type, N, mRNA	2.1	0.0139
IPI00016450	Q96TD2|Q6LCK3|Q6LCK5|Q6LCK4|Q6LCK6|Q93023|A5X2V1|P51170|Q93026|Q93025|Q93024|Q93027|P78437|Q6PCC2	6340	SCNN1G	Amiloride-sensitive sodium channel subunit gamma|Amiloride-sensitive epithelial sodium channel gamma subunit|Amiloride-sensitive sodium channel gamma-subunit	2.1	0.0466
IPI00221255	Q5MY99|O95797|O95796|O95799|O95798|Q15746|Q7Z4J0|Q9C0L5|Q14844|Q16794|Q5MYA0|Q9UBG5|Q9UIT9	4638	MYLK	Myosin light chain kinase, smooth muscle	2.0	0.0043
IPI00179185	O00520|Q96MX2|Q66K79	8532	CPZ	Carboxypeptidase Z	2.0	0.0485
IPI00169285	Q8NHP8	196463	PLBD2	Putative phospholipase B-like 2	1.9	0.0040
IPI00152871	B3KWI4|Q7RTN7|Q495Q6|Q8TF66	131578	LRRC15	cDNA FLJ43122 fis, clone CTONG3003737, highly similar to Leucine-rich repeat-containing protein 15|Leucine-rich repeat-containing protein 15	1.9	0.0433
IPI00015902	Q8N5L4|P09619|A8KAM8	5159	PDGFRB	cDNA FLJ76012, highly similar to *Homo sapiens* platelet-derived growth factor receptor, betapolypeptide (PDGFRB), mRNA|Platelet-derived growth factor receptor beta	1.9	0.0161

TABLE 1-continued

IPI	UniProt	Entrez	Gene name	Description	G-test	T-test
IPI00021428	P02568|Q5T8M9|P99020|P68133	58	ACTA1	Actin, alpha skeletal muscle	1.9	0.0250
IPI00005733	Q5T7S2|Q706C0|P36897|Q6IR47|Q706C1	7046	TGFBR1	TGF-beta receptor type-1|Transforming growth factor beta receptor I	1.9	0.0005
IPI00030936	Q5VST0|D3DQ14|O60745|O60635	10103	TSPAN1	Tetraspanin-1	1.9	0.0306
IPI00023974	P53801|D3DSL9|A8K274|Q9NS09|B2RDP7	754	PTTG1IP	Pituitary tumor-transforming gene 1 protein-interacting protein|cDNA FLJ78227, highly similar to *Homo sapiens* pituitary tumor-transforming 1 interacting protein (PTTG1IP), mRNA	1.8	0.0070
IPI00022830	Q5JXA5|Q5JXA4|B2RD74|Q9UI06|A2A2L1|Q9H102|Q9UNZ2|Q7Z533|Q9NVL9	55968	NSFL1C	NSFL1 cofactor p47	1.7	0.0140
IPI00000816	P42655|P29360|Q63631|Q7M4R4|D3DTH5|Q4VJB6|Q53XZ5|P62258|B3KY71	7531	YWHAE	14-3-3 protein epsilon	1.7	0.0468
IPI00163563	Q96S96|Q8WW74|Q5EVA1	157310	PEBP4	Phosphatidylethanolamine-binding protein 4	1.6	0.0470
IPI00021828	P04080|Q76LA1	1476	CSTB	Cystatin-B|CSTB protein	1.6	0.0027
IPI00029723	D3DN90|Q549Z0|A8K523|Q12841	11167	FSTL1	cDNA FLJ78447, highly similar to *Homo sapiens* follistatin-like 1 (FSTL1), mRNA|Follistatin-related protein 1	1.5	0.0075
IPI00183425	Q8WU72|Q9Y3F9|Q9ULV3|Q9Y3G0|Q9UHK4|A8K9J8|Q9H868|Q5SYW5|B4E0A3|Q9NYM8|Q5SYW3	25792	CIZ1	Cip1-interacting zinc finger protein|cDNA FLJ60074, highly similar to Cip1-interacting zinc finger protein	1.5	0.0038
IPI00007257	O94985|Q5SR52|Q5UE58|Q71MN0|A8K183|Q8N4K9	22883	CLSTN1	Calsyntenin-1	1.5	0.0118

[0064] High-risk OSA is associated with a wide variety of related disorders and vulnerabilities, and as such it has a greater need for treatment. High risk OSA is understood to be associated with neurocognitive impairment such as memory impairment, declarative memory defects, learning delays, and issues with academic performance, mood-related disorders such as depression, behavioral issues such as ADHD, aggression, inattentiveness, impulsivity, and excessive sleepiness, cardiovascular risks including hypertension, althersclerosis, pulmonary hypertension, and left ventricular dysfunction, a metabolic disorders such as dyslipidemia and insulin resistance. Review: Capdevila O S, Kheirandish-Gozal L, Dayyat E, Gozal D. Pediatric obstructive sleep apnea: complications, management, and long-term outcomes. Proc Am Thorac Soc. 2008 Feb. 15; 5(2):274-82. doi: 10.1513/pats.200708-138MG. Review. PubMed PMID: 18250221; PubMed Central PMCID: PMC2645258. Relevant treatments include pharmacological treatment with corticosteroids, leukotriene antagonists, topical nasal steroids, intranasal steroids, and/or montelukast, surgical removal of the adenoids and tonsils, applying positive airway pressure therapy (PAP), or the application of oral applicances. Kheirandish-Gozal L, Bhattacharjee R, Bandla H P, Gozal D. Anti-Inflammatory Therapy Outcomes for Mild OSA in Children. Chest. 2014 Feb. 6. doi: 10.1378/chest.13-2288. [Epub ahead of print] PubMed PMID: 24504096; Marcus C L, Brooks L J, Draper K A, Gozal D, Halbower A C, Jones J, Schechter M S, Ward S D, Sheldon S H, Shiffman R N, Lehmann C, Spruyt K; American Academy of Pediatrics. Diagnosis and management of childhood obstructive sleep apnea syndrome. Pediatrics. 2012 September; 130(3):e714-55. doi: 10.1542/peds.2012-1672. Epub 2012 August 27. Review. PubMed PMID: 22926176.

[0065] In certain embodiments, the biomarkers for high-risk OSA are contemplated to constitute the markers identified in Table 2.

TABLE 2

Candidate Biomarkers of High-Risk OSA

IPI	Gene Symbol	Description
IPI00014048	RNASE1	Ribonuclease pancreatic
IPI00302944	COL12A1	Isoform 4 of Collagen alpha-1(XII) chain
IPI00019449	RNASE2	Non-secretory ribonuclease
IPI00011302	CD59	CD59 glycoprotein
IPI00022418	FN1	Isoform 1 of Fibronectin
IPI00022426	AMBP	Protein AMBP
IPI00328113	FBN1	Fibrillin-1
IPI00829813	PIK3IP1	Isoform 2 of Phosphoinositide-3-kinase-interacting protein 1
IPI00744889	CDH1	E-cadherin
IPI00290085	CDH2	Cadherin-2
IPI00019580	PLG	Plasminogen
IPI00022620	SLURP1	Secreted Ly-6/uPAR-related protein 1
IPI00922213	FN1	cDNA FLJ53292, highly similar to *Homo sapiens* fibronectin 1 (FN1), transcript variant 5, mRNA
IPI00031008	TNC	Isoform 1 of Tenascin
IPI00872573	C1RL	Uncharacterized protein
IPI00022895	A1BG	Isoform 1 of Alpha-1B-glycoprotein
IPI00163207	PGLYRP2	Isoform 1 of N-acetylmuramoyl-L-alanine amidase
IPI00107731	OSCAR	Isoform 6 of Osteoclast-associated immunoglobulin-like receptor
IPI00166729	AZGP1	Zinc-alpha-2-glycoprotein
IPI00099670	CEL	bile salt-activated lipase precursor
IPI00291867	CFI	Complement factor I
IPI00216780	CILP2	Cartilage intermediate layer protein 2 precursor
IPI00395488	VASN	Vasorin
IPI00645018	PLAU	Isoform 2 of Urokinase-type plasminogen activator
IPI00553177	SERPINA1	Isoform 1 of Alpha-1-antitrypsin
IPI00029260	CD14	Monocyte differentiation antigen CD14
IPI00024292	LRP2	Low-density lipoprotein receptor-related protein 2
IPI00291262	CLU	Isoform 1 of Clusterin
IPI00021885	FGA	Isoform 1 of Fibrinogen alpha chain
IPI00026944	NID1	Isoform 1 of Nidogen-1
IPI00006662	APOD	Apolipoprotein D
IPI00291866	SERPING1	Plasma protease C1 inhibitor
IPI00176427	CADM4	Cell adhesion molecule 4
IPI00017601	CP	Ceruloplasmin
IPI00386879	IGHA1	cDNA FLJ14473 fis, clone MAMMA1001080, highly similar to Homo sapiens SNC73 protein (SNC73) mRNA
IPI00021085	PGLYRP1	Peptidoglycan recognition protein 1
IPI00103871	ROBO4	Isoform 1 of Roundabout homolog 4
IPI00007221	SERPINA5	Plasma serine protease inhibitor
IPI00294713	MASP2	Isoform 1 of Mannan-binding lectin serine protease 2
IPI00022488	HPX	Hemopexin
IPI00645363	IGHV4-31; IGHG1	Putative uncharacterized protein DKFZp686P15220
IPI00153049	MXRA8	Isoform 2 of Matrix-remodeling-associated protein 8
IPI00025476	AMY1C; AMY1A; AMY1B; AMY2A	Pancreatic alpha-amylase

TABLE 2-continued

Candidate Biomarkers of High-Risk OSA

IPI	Gene Symbol	Description
IPI00291136	COL6A1	Collagen alpha-1(VI) chain
IPI00000073	EGF	Isoform 1 of Pro-epidermal growth factor
IPI00009276	PROCR	Endothelial protein C receptor precursor
IPI00004573	PIGR	Polymeric immunoglobulin receptor
IPI00218192	ITIH4	Isoform 2 of Inter-alpha-trypsin inhibitor heavy chain H4
IPI00160130	CUBN	Cubilin
IPI00009950	LMAN2	Vesicular integral-membrane protein VIP36
IPI00022463	TF	Serotransferrin
IPI00215894	KNG1	Isoform LMW of Kininogen-1

[0066] 1. Nucleic Acids

[0067] Embodiments concern polynucleotides or nucleic acid molecules relating to an OSA or high-risk OSA biomarker nucleic acid sequence in diagnostic applications. Certain embodiments specifically concern a nucleic acid that can be used to diagnose OSA or high-risk OSA based on the detection of an OSA biomarker. Nucleic acids or polynucleotides may be DNA or RNA, and they may be oligonucleotides (100 residues or fewer) in certain embodiments. Moreover, they may be recombinantly produced or synthetically produced.

[0068] Other embodiments concern the use of primers or hybridizable segments that may be used to identify and/or quantify OSA biomarkers, particularly in diagnostic methods. It is contemplated that the discussion below is relevant to embodiments concerning such methods and compositions related to diagnostic applications in the context of the OSA biomarkers.

[0069] These polynucleotides or nucleic acid molecules may be isolatable and purifiable from cells or they may be synthetically produced. In some embodiments, a nucleic acid targets or identifies an OSA biomarker. In other embodiments, a nucleic acid is an inhibitor, such as a ribozyme, siRNA, or shRNA.

[0070] As used in this application, the term "polynucleotide" refers to a nucleic acid molecule, RNA or DNA, that has been isolated free of total genomic nucleic acid. Therefore, a "polynucleotide encoding an OSA or high-risk OSA biomarker" refers to a nucleic acid sequence (RNA or DNA) that contains an OSA biomarker coding sequence, yet may be isolated away from, or purified and free of, total genomic DNA and proteins. An OSA biomarker inhibitor refers to an inhibitor of an OSA biomarker.

[0071] The term "cDNA" is intended to refer to DNA prepared using RNA as a template. The advantage of using a cDNA, as opposed to genomic DNA or an RNA transcript is stability and the ability to manipulate the sequence using recombinant DNA technology (See Sambrook, 2001; Ausubel, 1996). There may be times when the full or partial genomic sequence is used. Alternatively, cDNAs may be advantageous because it represents coding regions of a polypeptide and eliminates introns and other regulatory regions. In certain embodiments, nucleic acids are complementary or identical to all or part of cDNA encoding sequences.

[0072] The term "gene" is used for simplicity to refer to a functional protein, polypeptide, or peptide-encoding nucleic acid unit. As will be understood by those in the art, this functional term includes genomic sequences, cDNA sequences, and smaller engineered gene segments that express, or may be adapted to express, proteins, polypeptides, domains, peptides, fusion proteins, and mutants. The nucleic acid molecule hybridizing to all or part of a nucleic acid sequence may comprise a contiguous nucleic acid sequence of the following lengths or at least the following lengths: 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 441, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1010, 1020, 1030, 1040, 1050, 1060, 1070, 1080, 1090, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3100, 3200, 3300, 3400, 3500, 3600, 3700, 3800, 3900, 4000, 4100, 4200, 4300, 4400, 4500, 4600, 4700, 4800, 4900, 5000, 5100, 5200, 5300, 5400, 5500, 5600, 5700, 5800, 5900, 6000, 6100, 6200, 6300, 6400, 6500, 6600, 6700, 6800, 6900, 7000, 7100, 7200, 7300, 7400, 7500, 7600, 7700, 7800, 7900, 8000, 8100, 8200, 8300, 8400, 8500, 8600, 8700, 8800, 8900, 9000, 9100, 9200, 9300, 9400, 9500, 9600, 9700, 9800, 9900, 10000, 10100, 10200, 10300, 10400, 10500, 10600, 10700, 10800, 10900, 11000, 11100, 11200, 11300, 11400, 11500, 11600, 11700, 11800, 11900, 12000 or more (or any range derivable therein) nucleotides, nucleosides, or base pairs of a sequence.

[0073] Accordingly, sequences that have or have at least or at most 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, and any range derivable therein, of nucleic acids that are identical or complementary to a nucleic acid sequence of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75,

76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 441, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1010, 1020, 1030, 1040, 1050, 1060, 1070, 1080, 1090, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3100, 3200, 3300, 3400, 3500, 3600, 3700, 3800, 3900, 4000, 4100, 4200, 4300, 4400, 4500, 4600, 4700, 4800, 4900, or 5000 contiguous bases (or any range derivable therein) of the identified biomarkers are contemplated as part of the invention.

[0074] "Isolated substantially away from other coding sequences" means that the gene of interest forms part of the coding region of the nucleic acid segment, and that the segment does not contain large portions of naturally-occurring coding nucleic acid, such as large chromosomal fragments or other functional genes or cDNA coding regions. Of course, this refers to the nucleic acid segment as originally isolated, and does not exclude genes or coding regions later added to the segment by human manipulation.

C. SAMPLES

[0075] Urine is a highly desirable biological fluid for diagnostic testing because of its ease of collection and widespread use in clinical laboratories. Biomarker discovery strategies in urine, however, have been hindered by problems associated with reproducibility and inadequate standardization of proteomic protocols. Despite these concerns, urinary proteomics analyses have been leveraged to identify numerous candidate biomarkers of a broad range of human disorders, that have included, but are not limited to renal disease, diabetes, atherosclerosis, Alzheimer's disease, and cancer (Soggiu, 2012; Zimmerli, 2008; Riaz, 2010; Zengi, 2012; Huttenhain, 2012; Zoidakis, 2012; Zurbig, 2012; Siwy, 2011). In some embodiments, the sample may be a sample of urine, saliva, tears, or serum/plasma.

D. EXAMPLES

[0076] The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1

Materials and Methods

[0077] Patient Information—

[0078] Children (ages 2-12 years) clinically referred for evaluation of OSA underwent an overnight polysomnographic evaluation at the University of Chicago Pediatric Sleep Laboratory. Healthy children were recruited from schools or well-child clinics. Exclusion criteria for all subjects included the presence of significant genetic or craniofacial syndromes, diabetes, cystic fibrosis, cancer, or treatment with oral corticosteroids, antibiotics, or anti-inflammatory medications. All parents completed a detailed intake clinical questionnaire. Height, weight and vital signs were recorded for each child, and body mass index (BMI) z-score was calculated on the basis of CDC 2000 growth standards (www.cdc.gov/growthcharts) and using online software (www.cdc.gov/epiinfo). A BMI z-score exceeding 1.65 (0.95th percentile) was considered as fulfilling criteria for obesity. The study was approved by the institutional review boards at the University of Chicago (IRB 10-708A); informed consent and, when appropriate, assents for minors were obtained.

[0079] Overnight Polysomnography—

[0080] All subjects underwent an overnight polysomnography using standard methods (Montgomery-Downs, 2006). The PSG studies were scored as per the 2007 American Association of Sleep Medicine guidelines for the scoring of sleep and associated events (Iber, 2007). The obstructive apnea-hypopnea index (AHI) was defined as the number of obstructive apneas and hypopneas per hour of total sleep time.

[0081] Urine Collection—

[0082] Mid-stream urine specimens were collected in the evening just before bedtime and as the first void in the morning after awakening. Samples (20 ml) were collected into tubes containing phenylmethylsulfonyl fluoride (PMSF, 2 mM final concentration), and immediately stored at −80° C. until analysis.

[0083] Preparation of Soluble Urine Proteins for Mass Spectrometry (MS)—

[0084] Urine (10 mL) was thawed quickly at 37° C., vortexed for 90 s, and centrifuged (500×g, 4° C.) for 5 min. Supernatants were centrifuged at 12,000×g, 4° C. for 20 min to remove urinary sediment, and incubated with 1 mL ProteinG magnetic beads (Millipore) for 30 min at 20° C. Depletion of IgG was performed according to the manufacturer's protocol. IgG-depleted urine samples were precipitated using TCA/DOC as previously described (Thongboonkerd, 2006; Becker, 2010). Briefly, urine was supplemented with 0.02% sodium deoxycholate and 20% trichloroacetic acid, and incubated overnight with rocking at 4° C. Proteins were harvested by centrifugation (18,000×g for 60 min at 4° C.). The protein pellet was washed twice with ice-cold acetone, and reconstituted in 0.1% RapiGest (Waters Corp.), 250 mM ammonium bicarbonate, pH 8.8. Protein concentration was determined by the Bradford assay with albumin as a standard. Samples (90 μg) were incubated with α-human albumin-coupled magnetic beads (90 μL, Millipore) and depletion was performed according to the manufacturer's protocol. Samples were reduced, alkylated, and digested overnight at 37° C. with sequencing-grade trypsin (1:50, w/w, trypsin/protein; Promega). Tryptic digests were mixed with acetic acid (1:1, v/v) and subjected to solid-phase extraction on a C18 column (HLB, 1 mL; Waters Corp.) according to the manufacturer's protocol. Fractions containing peptides were dried under

vacuum and resuspended in 0.3% formic acid, 5% acetonitrile (0.4 mg/mL) for LC-MS/MS analysis.

[0085] Liquid Chromatography-Electrospray Ionization-Tandem Mass Spectrometry (LC-ESI-MS/MS)—

[0086] Tryptic digests (1.5 μg) were loaded directly onto 2 cm C18 trap column (packed in-house), washed with 10 μl of solvent A (5% acetonitrile, 0.1% formic acid), and eluted on a 15 cm long, 75 μM reverse phase capillary column (ProteoPep™ II C18, 300 Å, 5 μm size, New Objective, Woburn Mass.). Peptides were separated at 300 nL/min over a 180 minute linear gradient from 5% to 35% buffer B (95% acetonitrile, 0.1% formic acid) on a Proxeon Easy n-LC II (Thermo Scientific, San Jose, Calif.). Mass spectra were acquired in the positive ion mode, using electrospray ionization and a linear ion trap mass spectrometer (LTQ Orbitrap Velos®, Thermo Scientific, San Jose, Calif.). The mass spectrometer was operated in data dependent mode, and for each MS1 precursor ion scan, the ten most intense ions were selected from fragmentation by CID (collision induced dissociation). Other parameters for mass spectrometry analysis included: resolution of MS1 was set at 60,000, normalized collision energy 35%, activation time 10 ms, isolation width 1.5, and the +1 and +4 and higher charge states were rejected.

[0087] Peptide and Protein Identification—

[0088] MS/MS spectra were searched against the International Protein Index human (v3.87, 91464 entries) primary sequence database (Kersey, 2004) using Sorcerer™-SEQUEST® (version v. 3.5) (Sage-N Research, Milpitas, Calif.). Search parameters included semi-enzyme digest with trypsin (after Arg orLys) with up to 2 missed cleavages. SEQUEST® was searched with a parent ion tolerance of 50 ppm and a fragment ion mass tolerance of 1 amu with fixed Cys alkylation, and variable Met oxidation. SEQUEST results were further validated with PeptideProphet (Keller, 2002) and ProteinProphet (Nesvizhskii, 2003), using an adjusted probability of ≥0.90 for peptides and ≥0.96 for proteins. Search results were further processed by the Computational Protemics Analysis System (CPAS) (Rauch, 2006) prior to statistical analysis (see below). Proteins considered for analysis had to be identified in at least 70% of individuals in at least one patient group (eg. healthy girls, or boys with OSA). When MS/MS spectra could not differentiate between protein isoforms, all were included in the analysis.

[0089] Protein Quantification and Statistical Analysis—

[0090] Proteins detected by LC-MS/MS were quantified by spectral counting (the total number of MS/MS spectra detected for a protein; (Liu, 2007)). Differences in relative protein abundance were assessed with the t-test and G-test (Becker, 2010; Becker, 2012; Old, 2005). Permutation analysis was used to empirically estimate the FDR (Benjamini, 1995). Significance cutoff values for the G-statistic and t-test were determined using PepC (Heinecke, 2010), a software package that maximizes the number of differentially expressed proteins identified for a given FDR.

[0091] ELISA—

[0092] Urine samples were thawed rapidly at 37° C. and clarified by centrifugation at 500×g for 10 min. Protein levels in resultant supernatants were quantified using commercially available ELISAs for DPP4 (Abnova; KA0141), AZGP1 (Abnova; KA1689), CP (Assaypro; EC4101-1), HPX (Innovative Research, Inc.; IRKTAH2562), and creatinine (Abcam; ab65340) according to the manufacturer's protocols. All protein levels were standardized to urine creatinine levels (Gardfe, 2004) and statistical significance between the groups was assessed by a two-tailed, Student's t-test.

[0093] Functional Annotation—

[0094] Functional enrichments in Gene Ontology annotations in the urine proteome or differentially expressed putative urine biomarkers (relative to the entire human genome) were identified using the Bingo 2.0 plugin in Cytoscape (V2.8.2) (Maere, 2005). Statistical significance was assessed using the hypergeometric test ($p<0.05$) with Benjamini-Hochberg correction (Benjamini, 1995) and functional categories with >5 proteins were considered.

Example 2

Proteomics Workflow for Urine Biomarker Discovery

[0095] The inventors developed a 4-step procedure involving: i) centrifugation to remove particulate material and urinary sediment, ii) depletion of IgG and albumin (ALB) to facilitate deeper proteome coverage, iii) protein precipitation to concentrate urine proteins and remove interfering substances, and iv) mass spectrometric analysis by LC-MS/MS (FIG. **1***a*).

[0096] ALB and IgG are highly abundant urine proteins (40-60% of total urinary protein) that interfere with detection of low abundance species and complicate quantification in label-free proteomic approaches (Kushnir, 2009). Magnetic beads were carefully titrated to maximize depletion of ALB and IgG (FIG. **1***b,c*) and minimize non-specific loss of unrelated proteins, as assessed by loss of serotransferrin (TRF) levels (FIG. **1***c*). Since proteins are more efficiently precipitated in concentrated solutions (due to molecular crowding), the inventors depleted ALB after protein precipitation. However, IgG depletion was incompatible with the buffer (0.1% RapiGest) used to solubilize protein pellets, and was therefore performed prior to precipitation.

[0097] The inventors incorporated a method involving trichloroaceteic acid and deoxycholate (TCA/DOC; (Thongboonkerd, 2006; Becker, 2010)) because it is well suited for precipitating proteins out of dilute solutions. The reproducibility of this method within and across samples was interrogated by precipitating 6 aliquots of the same urine sample collected from each of 10 subjects. This approach yielded highly reproducible results (6% CV, intra-sample) over a wide range of urinary protein concentrations (FIG. **1***d*).

[0098] To test the reproducibility of the proteomics workflow, urine samples from 28 children were processed and subjected to LC-MS/MS analysis. Based on a minimum of 2 unique peptide identifications per protein, the approach reliably identified 505±10 proteins per sample. Moreover, variation in sample depth, the number of high quality peptide identifications per run, was minimal (10,053±237 peptides) indicating that the method was robust and reproducible.

Example 3

Gender and Diurnal Effects Introduce Variability into the Urine Proteome of Healthy Children

[0099] The inventors collected morning and bedtime samples from healthy boys (N=7) and girls (N=6). Healthy children (ages 2-12 years) were selected by a priori excluding participants with genetic or craniofacial syndromes, diabetes,

cystic fibrosis, or cancer. Additional exclusion criteria included chronic use of medications, steroids, or immunotherapy drugs.

[0100] Samples were processed through the proteomics workflow (see FIG. **1**), and subjected to LC-MS/MS analysis. Proteins were quantified by spectral counting (Liu, 2004), and statistically significant changes in protein levels were identified using a combination of the t-test and G-test (Becker, 2010; Becker, 2012; Old, 2005) using cutoffs that minimized the false discovery rate (Benjamini, 1995; Heinecke, 2010). A representative analysis is provided in FIG. **2***a*, which demonstrates the detection of gender-regulated proteins in morning urine samples upon application of the stringent statistical criteria (G-test: G>1.5 or G≤-1.5; t-test: α=0.05; FDR<0.05).

[0101] Using this approach, the inventors detected substantial differences in the urinary proteome of healthy boys and girls, both in morning (~7%; 50 of 750 proteins) and bedtime (8%; 41 of 750) samples (FIG. **2***a,b*, Tables 2A and 2B). Tables 2A and 2B indicates data illustrating the gender and diurnal effects on the urinary proteome of healthy children. A list of the statistically significant, gender-regulated proteins detected in morning and bedtime urine samples of healthy children are represented in Tables 3A and B. Results of the t-test and G-test are also presented.

TABLE 3A

Gender effects in bedtime (pm) samples

IPI	Uniprot	Entrez	Gene	G-test	T-test
IPI00022463	P02787|Q06AH7|A0PJA6|B4DI57|O43890|Q9UHV0|Q53H26|Q1HBA5|B4E1B2|B4DEX9|Q9NQB8	7018	TF	−40.31	0.0269
IPI00553177	E9KL23|Q0PVP5|Q53XB8|Q96BF9|B2RDQ8|Q13672|Q5U0M1|Q7M4R2|P01009|Q9P1P0|Q9UCM3|A6PX14|Q9UCE6|Q96ES1|Q86U19|Q86U18	5265	SERPINA1	−17.61	0.0035
IPI00453473	Q0VAS5|B2R4R0|P02305|P02304|A2VCL0|Q6DRA9|Q6B823|P62805|Q6FGB8|Q6NWP7	8361|8360|8363|8362|8365|8364|8367|8366|8368|8359|8370|8294|554313|21504	HIST1H4C|HIST1H4B|HIST1H4A|HIST4H4|HIST1H4F|HIST1H4E|HIST1H4D|HIST1H4K|HIST1H4J|HIST1H4I|HIST1H4H|HIST1H4L|HIST2H4B|HIST2H4A	−10.47	0.0182
IPI00383164	Q8WY24			−7.65	0.0149
IPI00305457	Q9P173			−7.46	0.0155
IPI00003269	Q562X8|Q562S9|B2RPJ1|Q562R2|Q562R1	345651	ACTBL2	−4.72	0.0042
IPI00246058	Q9P2H2|Q8WUM4|Q9BX86|Q9NUN0|Q9UKL5	10015	PDCD6IP	−4.48	0.0390
IPI00219018	P04406|Q0QET7|Q2TSD0|Q53X65|P00354	2597	GAPDH	−4.10	0.0049
IPI00306322	Q14052|Q548C3|Q66K23|P08572|Q5VZA9|B4DH43	1284	COL4A2	−3.97	0.0495
IPI00012540	Q6SV49|B3KQS1|Q6SV53|Q6SV52|Q6SV51|Q6SV50|O43490|Q96EN6	8842	PROM1	−3.78	0.0361
IPI00301395	O75225|Q9NZ90|Q6UX20|Q9HB4|Q9H3G5|Q8NBL7|A4D1A4|Q96AR7|Q75MM4|B3KW79	54504	CPVL	−3.45	0.0083
IPI00034319	Q9NYQ9|O60888|Q5JXM9|Q3B784|A2BEL4|A2AB26|Q5SU05	51596	CUTA	−2.93	0.0066
IPI00029751	Q8N466|A8K0H9|Q14030|Q7M4P0|Q12860|Q12861|A8K0Y3	1272	CNTN1	−2.85	0.0042
IPI00299086	O00173|O43391|O00560|B2R5Q7|B4DUH3|Q14CP2|B7ZLN2	6386	SDCBP	−2.62	0.0151
IPI00028911	Q14118|Q969J9|A8K6M7	1605	DAG1	−2.62	0.0095
IPI00022290	P60022|Q09753|Q86SQ8	1672	DEFB1|HBD1	−2.51	0.0048
IPI00178926	P01591|	3512	IGJ	−2.49	0.0453
IPI00020687	P00995	6690	SPINK1	−2.49	0.0039
IPI00002406	P50895|A8MYF9|A9YWT5|A9YWT6|Q86VC7	4059	BCAM	−2.45	0.0195
IPI00295542	Q02818|B4DZX0|Q9BUR1|B2RD64|Q7Z4J7|B3KUR6|Q53GX6|Q15838|A8K7Q1|Q96BA4	4924	NUCB1	−2.43	0.0468
IPI00064262	Q96JQ0|O15098	8642	DCHS1	−2.29	0.0032
IPI00013446	O43653|Q6UW92|D3DWI6	8000	PSCA	−2.09	0.0393
IPI00016786	P25763|P21181|P60953|Q9UDD2|Q7L8R5	998	CDC42	−2.06	0.0453
IPI00017567	P17813|A8K2X4|B7Z6Y5|Q14926|Q5T9C0|Q96CG0|Q14248	2022	ENG	−1.87	0.0466
IPI00018279	Q59GD4|P25940|Q9NZQ6	50509	COL5A3	−1.78	0.0139
IPI00003648	O75465|Q2M3D3|Q15223|Q9HBW2|Q9HBE6	5818	PVRL1	−1.70	0.0032
IPI00329538	Q9UCA3|Q16651	5652	PRSS8	−1.66	0.0346
IPI00025846	Q63HM4|Q02487	1824	DKFZp686P18250|DSC2	1.56	0.0392
IPI00017557	Q1ZYW2|Q6PD64|Q4G124|Q6FHJ7|Q6FHM0|O14877|B4DYC1|Q05BG7	6424	SFRP4	1.61	0.0061
IPI00742682	A0PJC9|Q15655|Q99968|P12270|Q15624|Q9UE33|Q5SWY0|Q504U6|Q58F23	7175	tpr|TPR|Tpr	1.64	0.0271
IPI00022937				1.65	0.0208
IPI00552186	Q5HYH5		DKFZp313O211	1.73	0.0069
IPI00022371	B2R8I2|P04196|B9EK35|Q68DR3|D3DNU7	3273	DKFZp779H1622|HRG	2.08	0.0186
IPI00300786	B7ZMD7|Q53F26|P04745|A8K8H6|Q5T083|A6NJS5|Q13763	278|276|277	AMY1A|AMY1C|AMY1B	2.17	0.0082
IPI00021000	P10451|Q8NBK2|Q15681|A6XMV6|Q15682|Q96IZ1|Q4W597|Q15683	6696	SPP1	2.26	0.0177
IPI00024331	Q8WXR1|B9DI89|Q6IB95|Q92956|Q96J31|Q9UM65	8764	TNFRSF14	2.43	0.0028
IPI00026926	Q02747	2980	GUCA2A	2.61	0.0122
IPI00290856	Q8TC18|Q9Y5Y7|Q9UNF4|B2R672	10894	LYVE1|XLKD1	2.62	0.0469

TABLE 3A-continued

Gender effects in bedtime (pm) samples

IPI	Uniprot	Entrez	Gene	G-test	T-test
IPI00019954	Q6IBD2|Q540N7|Q15828	1474	CST6	2.62	0.0251
IPI00397820	Q9NWB4|Q8IUN3|B7WPD9|E2QRL0|Q6ZQR9|Q2KJY2|Q6ZUZ0|Q8IVR1	55083	KIF26B	2.64	0.0184
IPI00028030	O14592|Q53FR6|A8K3I0|Q16389|Q16388|P49747|Q8N4T2|Q2NL86	1311	COMP	2.67	0.0095
IPI00001662	B7ZLQ1		OPCML	2.75	0.0211
IPI00006662	D3DNW6|B2R579|P05090|Q6IBG6	347	APOD	2.77	0.0419
IPI00024046	B7Z9B1	1012		2.82	0.0060
IPI00553215	Q5NV65		IGLV2-18	3.12	0.0105
IPI00946928	B5MDQ5|C7S7U0|F5GZN4|A1L4H1|C7S7T9	284297	SSC5D	3.19	0.0295
IPI00015881	P09603|Q5VVF4|B4DTX0|Q5WF3|Q14086|A8K6J5|Q9UQR8|Q13130|Q14806	1435	CSF1	3.40	0.0165
IPI00024035	A8K5H5|Q9BWS0|P55285	1004	CDH6	3.61	0.0123
IPI00374563	O00468|Q15952|B3KMM7|Q96IC1|Q60FE1|Q5SVA2|Q8N4J5|Q7KYS8|Q9BTD4|Q5XG79	375790	AGRN	3.75	0.0278
IPI00302944	Q5VYK2|Q71UR3|Q5VYK1|Q15955|Q99716|Q99715|O43853	1303	COL12A1	3.81	0.0367
IPI00011302	P13987|Q6FHM9	966	CD59	3.85	0.0371
IPI00292130	A8K981|Q9UIX8|Q07507|Q8N4R2	1805	DPT	4.48	0.0181
IPI00299724	A6NLM2|Q8TB12|Q9Y4V0|O00241|B2R8V0|Q9H1U5|Q5TFQ9|Q5TFR0	10326	SIRPB1	5.42	0.0109
IPI00031065	Q14UV0|Q14UU9|P24855	1773	DNASE1	5.53	0.0342
IPI00293539	A8MZC8|Q9UQ94|B7WP28|Q9UQ93|A8K5D6|Q15065|P55287|Q15066	1009	CDH11	6.43	0.0488
IPI00006705	P11684|Q9UCM4|B2R5F2|Q6FHH3|Q9UCM2	7356	SCGB1A1	10.19	0.0009
IPI00018136	Q53FL7|P19320|Q6NUP8|A8K6R7	7412	VCAM1	12.52	0.0332
IPI00021447	B3KXB7|D3DT76|P19961|Q9UBH3	280	AMY2B	14.46	0.0061
IPI00166729	O60386|Q5XKQ4|P25311|D6W5T8|Q8N4N0	563	AZGP1	21.82	0.0265

TABLE 3B

Gender effect in morning (am) samples

IPI	Uniprot	Entrez	Gene	G-test	T-test
IPI00027462	Q6FGA1|Q9UCJ1|Q9NYM0|B2R4M6|P06702|D3DV36	6280	S100A9	−50.36	0.0305
IPI00007047	A8K5L3|Q9UCM6|Q9UC84|Q9UC92|Q5SY70|P05109|Q9UCJ0|D3DV37	6279	S100A8	−37.70	0.0193
IPI00019038	B2R4C5|Q13170|P00695|P61626|Q9UCF8	4069	LYZ	−15.37	0.0278
IPI00296180	Q5PY49|B2R7F2|Q969W6|Q16618|B4DPZ2|Q96SE8|Q53XS3|Q15844|P00749|Q5SWW9	5328	ATF|PLAU	−14.74	0.0386
IPI00220143	Q75ME7|Q0VAX6|O43451|Q8TE24|Q86UM5	8972	MGAM	−13.45	0.0067
IPI00384938	Q7Z351		DKFZp686N02209	−13.38	0.0447
IPI00383164	Q8WY24			−6.83	0.0237
IPI00027745	B2R6X2|Q96CL9|Q549U0|P08236	2990	GUSB	−6.36	0.0328
IPI00003807	B7Z552|Q561W5|P11117|Q9BTU7	53	ACP2	−5.41	0.0161
IPI00027827	Q6FHA2|Q16867|B2R9V7|Q5U781|P08294	6649	SOD3	−4.91	0.0485
IPI00001593	B2R7B7|P42785|B5BU34	5547	PRCP	−4.67	0.0124
IPI00025512	B2R4N8|Q9UC31|Q96EI7|Q9UC35|Q9UC34|Q9UC36|Q6FI47|P04792|Q96C20	3315	HSPB1	−4.46	0.0473
IPI00034319	Q9NYQ9|O60888|Q5JXM9|Q3B784|A2BEL4|A2AB26|Q5SU05	51596	CUTA	−4.09	0.0009
IPI00021439	Q75MN2|Q53G76|Q53G99|Q96B34|P99021|Q11211|P02570|Q96HG5|P70514|Q1KLZ0|Q8WVW5|Q64316|P60709|Q53GK6	60	PS1TP5BP1|ACTB	−3.65	0.0275
IPI00005794	B5MDX4|Q9Y646|B2RD88|Q8NBZ1|Q9Y5X6|Q9UNM8	10404	PGCP	−3.44	0.0222
IPI00018278	Q71UI9|A6NN01|Q59GV8|Q6PK98	94239	H2AFV	−3.37	0.0195
IPI00646304	Q9BVK5|Q6IBH5|A8K534|P23284	5479	PPIB	−3.36	0.0406
IPI00103871	Q9NWJ8|A8K154|Q8TEG1|Q8WZ75|Q96JV6|Q9H718|Q14DU7	54538	ROBO4	−3.25	0.0489
IPI00152871	B3KWI4|Q7RTN7|Q495Q6|Q8TF66	131578	LRRC15	−3.22	0.0117
IPI00025869	Q53HF3|Q6LER7|P06280|Q53Y83	2717	GLA|alpha-GalA	−3.19	0.0480
IPI00027166	P16035|Q9UDF7|Q16121|Q93006	7077	TIMP2	−2.83	0.0113
IPI00301395	O75225|Q9NZ90|Q6UX20|Q9HB41|Q9H3G5|Q8NBL7|A4D1A4|Q96AR7|Q75MM4|B3KW79	54504	CPVL	−2.74	0.0387
IPI00178926	P01591	3512	IGJ	−2.47	0.0251
IPI00783024	Q9UL88			−2.06	0.0140
IPI00016786	P25763|P21181|P60953|Q9UDI2|Q7L8R5	998	CDC42	−2.04	0.0377
IPI00010737	Q9UC32|P07204|Q8IV29	7056	THBD	−1.94	0.0142
IPI00021302	Q9UGT4|Q9H5Y6	56241	SUSD2	−1.93	0.0480
IPI00176427	B2R7L5|Q9Y4A4|Q8NFZ8	199731	CADM4	−1.90	0.0295
IPI00414896	Q9BZ46|Q9BZ47|B2RDA7|E1P5C3|Q8TCU2|O00584|Q5T8Q0	8635	RNASET2	−1.86	0.0223
IPI00025714	P57078|Q96KH0		RIPK4	−1.79	0.0486
IPI00178415	Q53SM0|Q9HA24|Q6UWV4|Q4ZG47|Q75T13|Q4G0R8|Q6AW92	80055	PGAP1	−1.66	0.0211
IPI00029723	D3DN90|Q549Z0|A8K523|Q12841	11167	FSTL1	−1.54	0.0331

TABLE 3B-continued

Gender effect in morning (am) samples

IPI	Uniprot	Entrez	Gene	G-test	T-test
IPI00005690	A8K491|O15232|Q4ZG02	4148	MATN3	1.52	0.0323
IPI00018019	Q86YE7|Q5VYK8|A8K5C3|Q9NW75|Q5VYK7	55105	GPATCH2	1.58	0.0084
IPI00296608	Q6P3T5|A8K2T4|P10643|B2R6W1|Q92489	730	C7	1.64	0.0213
IPI00387025	P01597			1.74	0.0374
IPI00152418	Q14UF3|Q8TD14|D3DT86|B1AP16		DAF|CD55	1.82	0.0428
IPI00644680	Q96JG9	84627	ZNF469	2.02	0.0391
IPI00045839	Q96SK8|Q9HC86|Q9HC87|Q96BR8|Q7KZR4|Q9H6K3|Q96SL5|Q96SN3|Q32P28	64175	LEPRE1	2.11	0.0063
IPI00018909	Q96NX0|E9PBB5|Q9UDA5|Q07654	7033	TFF3	2.32	0.0265
IPI00553138	P63027|Q9BUC2|P19065	6844	VAMP2	2.36	0.0331
IPI00021841	Q9UCS8|Q6LDN9|Q9UCT8|A8K866|P02647|Q6Q785|Q6LEJ8	335	APOA1	2.53	0.0156
IPI00024046	B7Z9B1	1012		2.77	0.0193
IPI00387097	P01605			3.19	0.0266
IPI00022620	P55000|Q6PUA6|Q53YJ6|Q92483	57152	SLURP1	3.64	0.0348
IPI00553215	Q5NV65		IGLV2-18	5.53	0.0104
IPI00019954	Q6IBD2|Q540N7|Q15828	1474	CST6	6.45	0.0028
IPI00009027	Q2TBE1|P05451|Q0VFX1|A8K7G6|P11379|Q4ZG28	5967	REG1A	8.45	0.0403
IPI00022426	Q9UC58|P02760|Q9UDI8|Q5TBD7|P78492|P00977|P78491|Q2TU33|P02759	259	ITIL|AMBP	34.38	0.0101

[0102] Interestingly, the inventors observed poor overlap (<10%) between differentially expressed proteins in morning and bedtime samples, suggesting that gender-related differences were also highly sensitive to diurnal effects (FIG. **2***b*). For example, TRF levels were elevated in girls at bedtime, while islet cell regeneration factor (REG1A) was specifically increased in morning urine samples collected from boys (FIG. **2***c*).

[0103] In general, urine protein composition was more substantially influenced by gender over diurnal effects. Consistent with this finding, gene ontology analysis of the gender-regulated urinary proteome in healthy children revealed significant enrichments in functional annotations that are not classically associated with gender (cell adhesion, $p=6.0\times10^{-7}$; pattern binding, $p=7.0\times10^{-3}$; complement and coagulation cascades $p=4.29\times10^{-3}$). In sharp contrast, this approach failed to identify significance in more intuitive modules such as female pregnancy (p=0.11) or embryo implantation (p=0.11).

Example 4

Urine Biomarker Discovery of Pediatric OSA is Highly Dependent Upon Gender and Diurnal Effects

[0104] Children (ages 2-12 years) with moderate to severe OSA, as assessed by the polysomnography-derived criterion of apnea hypopnea index (AHI>5 events/hour total sleep time), were recruited along with age- and sex-matched controls. Their demographic characteristics were such that no statistically significant differences in age, sex, ethnicity, or BMI distribution were present (Table 4).

TABLE 4

Demographic and polysomnographic characteristics of subjects.

	Control (N = 13)	OSA (N = 14)	t-test (p-value)
Age (years)	7.5 ± 0.8	5.9 ± 0.6	0.11
Gender (boy, girl)	7.6	7.7	N/A
BMI, z-score	0.6 ± 0.3	1.2 ± 0.5	0.27
AHI (events/hr/total sleep time)	0.4 ± 0.1	23.3 ± 5.3	0.0002

Abbreviations:
BMI = body mass index;
AHI = obstructive apnea-hypopnea index;
OSA = obstructive sleep apnea.
Where applicable, results are presented as means ± SEM.

[0105] Using stringent criteria for quality and reproducibility of protein detection, the mass spectrometric analyses of urine samples identified 742 urine proteins across all patient samples.

[0106] To investigate the impact of gender and diurnal variation on biomarker discovery, the inventors performed statistical analysis (using the t-test and G-test; (Becker, 2010; Old, 2005; Heinecke, 2010)) in three ways (FIG. **3***a*). In level 1 analysis, protein levels were averaged across morning and bedtime samples and groups were not differentiated according to gender. Level 2 analysis investigated morning and bedtime samples independently, while level 3 analysis treated samples in a collection time- and gender-dependent fashion (FIG. **3***a*).

[0107] Six candidate biomarkers of pediatric OSA were identified in level 1 analysis (Table 5A). Notably, orosomucoid 1 (ORM1), a protein that was initially identified in the previous OSA biomarker screen (Gozal, 2009), was also detected in this analysis. The statistical significance level for ORM1, however, barely cleared statistical thresholds, and subsequent ELISA measurements failed to validate this finding. A substantial increase in the number of biomarkers detected was evident when morning and bedtime samples were treated independently (level 2, 45 proteins) and a further, more dramatic, increase was visualized when gender was also accounted for in the analysis (level 3, 192 proteins) (FIG. **3***a*, Tables 5A-D). Tables 5A-D disclose the identification of urine biomarkers of pediatric OSA. Identification of differentially expressed urinary proteins in OSA relative to control samples. Results of levels 1 (all samples), 2 (corrected for diurnal effects), and 3 (corrected for both diurnal and gender effects) biomarker analysis along with corresponding t-test and G-test values are displayed.

TABLE 5A

Level 1 analysis (morning/bedtime measurements averaged, genders pooled)

IPI	UniProt	Entrez	Gene name	Description	G-test	T-test
IPI00160130	Q7LC53|B0YIZ4|O60494|Q5VTA6|Q59ED1|B3KQM7|Q96RU9	8029	CUBN	cDNA FLJ90747 fis, clone PLACE1011708, highly similar to Cubilin|Cubilin variant|Cubilin|Intrinsic factor-vitamin B12 receptor	−14.68	0.0143
IPI00291136	Q9BSA8|Q14040|Q14041|O00117|Q16258|O00118|Q7Z645|P12109|Q8TBN2	1291	COL6A1	Collagen alpha-1(VI) chain|Putative uncharacterized protein	−4.79	0.0452
IPI00022255	B4DV64|Q5VWG0|O95362|Q6UX06|Q86T22	10562	OLFM4	Olfactomedin-4|cDNA FLJ61420, highly similar to *Homo sapiens* olfactomedin 4 (OLFM4), mRNA	−2.01	0.0231
IPI00022429	B7ZKQ5|P02763|Q8TC16|Q5T539|Q5U067	5004	ORM1	Alpha-1-acid glycoprotein 1	2.02	0.0216
IPI00219684	Q5VV93|B2RAB6|Q99957|P05413|Q6IBD7	2170	FABP3	FABP3 protein|Fatty acid-binding protein, heart	2.40	0.0215
IPI00555812	Q53F31|P02774|B4DPP2|Q16309|Q16310|Q6GTG1	2638	GC	Vitamin D-binding protein	3.93	0.0079

TABLE 5B

Level 2 analysis (morning/bedtime samples treated independently, genders pooled)

IPI	UniProt	Entrez	Gene name	Description	G-test	T-test
			Morning (am) samples			
IPI00160130	Q7LC53|B0YIZ4|O60494|Q5VTA6|Q59ED1|B3KQM7|Q96RU9	8029	CUBN	cDNA FLJ90747 fis, clone PLACE1011708, highly similar to Cubilin|Cubilin variant|Cubilin|Intrinsic factor-vitamin B12 receptor	−32.91	0.0130
IPI00009276	Q14218|Q9ULX1|Q96CB3|B2RC04|Q9UNN8|Q6IB56	10544	PROCR	Endothelial protein C receptor	−8.48	0.0360
IPI00021885	Q9BX62|A8K3E4|Q4QQH7|D3DP14|P02671|D3DP15|Q9UCH2	2243	FGA	cDNA FLJ78367, highly similar to *Homo sapiens* fibrinogen, A alpha polypeptide (FGA), transcriptvariant alpha, mRNA|Fibrinogen alpha chain	−6.58	0.0405
IPI00008787	Q14769|P54802	4669	NAGLU|ufHSD2	Alpha-N-acetylglucosaminidase	−6.17	0.0457
IPI00299738	O14550|A4D2D2|B2R9E1|Q15113	5118	PCOLCE	Procollagen C-endopeptidase enhancer|Procollagen C-endopeptidase enhancer 1	−5.68	0.0242
IPI00003919	Q16770|Q3KRG6|Q16769|Q53TR4	25797	tmp__locus__46|QPCT	Glutaminyl-peptide cyclotransferase|Glutaminyl-peptide cyclotransferase (Glutaminyl cyclase), isoform CRA__a	−5.24	0.0208
IPI00029275	P08582|Q9BQE2	4241	MFI2	Melanotransferrin	−5.22	0.0190
IPI00027843	P22891|A6NMB4|Q5JVF6|Q15213|Q5JVF5	8858	PROZ	Vitamin K-dependent protein Z	−5.08	0.0257
IPI00029751	Q8N466|A8K0H9|Q14030|Q7M4P0|Q12860|Q12861|A8K0Y3	1272	CNTN1	Contactin-1	−4.78	0.0338
IPI00027827	Q6FHA2|Q16867|B2R9V7|Q5U781|P08294	6649	SOD3	Superoxide dismutase [Cu—Zn]|Extracellular superoxide dismutase [Cu—Zn]	−4.59	0.0450
IPI00176427	B2R7L5|Q9Y4A4|Q8NFZ8	199731	CADM4	Cell adhesion molecule 4	−4.54	0.0202
IPI00043992	Q96K15|Q96NY8	81607	PVRL4	Poliovirus receptor-related protein 4	−4.35	0.0257
IPI00022432	Q9UBZ6|Q6IB96|P02766|E9KL36|Q549C7|Q9UCM9	7276	TTR	Epididymis tissue sperm binding protein Li4a|Transthyretin	−4.14	0.0187
IPI00031065	Q14UV0|Q14UU9|P24855	1773	DNASE1	Deoxyribonuclease|Deoxyribonuclease-1	−3.62	0.0291
IPI00007800	Q8N2J9|B2R780|Q5JT58|Q9UKU9	23452	ANGPTL2	Angiopoietin-related protein 2|cDNA FLJ90545 fis, clone OVARC1000410, highly similar to Angiopoietin-related protein 2|cDNA, FLJ93320, highly similar to *Homo sapiens* angiopoietin-like 2 (ANGPTL2), mRNA	−3.43	0.0362
IPI00010949	Q9HAT2|B3KPB0|Q9HAU7|Q8IUT9|Q9NT71	54414	SIAE	Sialate O-acetylesterase	−3.18	0.0454
IPI00328746	B7ZLI0|Q6X813|Q17RL9|Q86UN3	349667	RTN4RL2	Reticulon 4 receptor-like 2|Reticulon-4 receptor-like 2	−2.30	0.0327
IPI00019157	D3DW77|Q92675|Q6UVK1	1464	CSPG4	Chondroitin sulfate proteoglycan 4	−2.28	0.0468
IPI00240345	Q695G9|Q86T13|Q6PWT6|Q8N5V5	161198	CLEC14A	C-type lectin domain family 14 member A	−2.23	0.0086

TABLE 5B-continued

IPI	UniProt	Entrez	Gene name	Description	G-test	T-test
Level 2 analysis (morning/bedtime samples treated independently, genders pooled)						
IPI00022255	B4DV64|Q5VWG0|O95362|Q6UX06|Q86T22	10562	OLFM4	Olfactomedin-4|cDNA FLJ61420, highly similar to *Homo sapiens* olfactomedin 4 (OLFM4), mRNA	−2.23	0.0467
IPI00102300	Q9UIF2|Q9HCN7|Q9HCN6	51206	GP6	Platelet glycoprotein VI	−2.03	0.0326
IPI00000024	B4E2D8|Q8IUP2|Q08174	5097	PCDH1	cDNA FLJ59655, highly similar to Protocadherin-1|Protocadherin-1	−1.80	0.0150
IPI00179185	O00520|Q96MX2|Q66K79	8532	CPZ	Carboxypeptidase Z	−1.75	0.0231
IPI00022039	B7Z3R8|O95660|Q9UIB8|B2R8T1|Q5H9R1|O15430|Q9UIT7|Q6FHA8|O95266|Q8WLP1|Q8WWI8|Q9UF04|Q9UIB6|Q9UIB7	8832	CD84	SLAM family member 5	−1.66	0.0281
IPI00289275	O75339|B2R8F7|Q8IYI5|Q6UW99	8483	CILP	Cartilage intermediate layer protein 1	−1.51	0.0389
IPI00298388	Q49A94|Q8NCJ9|Q96FE7|Q86YW2|O00318	113791	PIK3IP1	Phosphoinositide-3-kinase-interacting protein 1	1.53	0.0340
IPI00289501	O15240|Q9UDW8	7425	VGF	Neurosecretory protein VGF	1.57	0.0257
IPI00175092	Q53SV6|Q8WUU3|Q8NC42|Q8NBY5|Q53S14|Q8N5I8	284996	RNF149|LOC284996	Putative uncharacterized protein LOC284996|E3 ubiquitin-protein ligase RNF149	1.59	0.0138
IPI00022429	B7ZKQ5|P02763|Q8TC16|Q5T539|Q5U067	5004	ORM1	Alpha-1-acid glycoprotein 1	1.68	0.0136
IPI00922213	Q14327|Q7L553|B4DTK1|Q6PJE5|Q9H382|Q53S27|B4DTH2		FN1	Putative uncharacterized protein FN1|cDNA FLJ61165, highly similar to Fibronectin|FN1 protein|Fibronectin 1|cDNA FLJ53292, highly similar to *Homo sapiens* fibronectin 1 (FN1), transcript variant 5, mRNA	1.87	0.0165
IPI00013955	Q9UE76|Q9UE75|Q9UQL1|Q7Z552|Q14876|Q9Y4J2|Q14128|Q16437|P13931|P17626|P15941|Q16615|P15942|Q16442|Q9BXA4	4582	MUC1	Mucin-1	2.45	0.0436
IPI00010343	Q9UPR5|B4DYQ9|B4DEZ4	6543	SLC8A2	cDNA FLJ58526, highly similar to Sodium/calcium exchanger 2|Sodium/calcium exchanger 2	2.99	0.0284
IPI00219684	Q5VV93|B2RAB6|Q99957|P05413|Q6IBD7	2170	FABP3	FABP3 protein|Fatty acid-binding protein, heart	5.95	0.0060
IPI00007778	F6X5H7|B2RBF5|Q5VX51|Q5VX50|Q8TC97|B3KQS3|B4DQ98|Q01459	1486	CTBS	cDNA PSEC0114 fis, clone NT2RP2006543, highly similar to DI-N-ACETYLCHITOBIASE (EC 3.2.1.—)|CTBS protein|Di-N-acetylchitobiase|cDNA FLJ55135, highly similar to Di-N-acetylchitobiase (EC 3.2.1.—)|cDNA, FLJ95483, highly similar to *Homo sapiens* chitobiase, di-N-acetyl- (CTBS), mRNA|Chitobiase, di-N-acetyl-	8.79	0.0140
IPI00022620	P55000|Q6PUA6|Q53YJ6|Q92483	57152	SLURP1	Secreted Ly-6/uPAR-related protein 1	15.99	0.0133
Bedtime (pm) samples						
IPI00555812	Q53F31|P02774|B4DPP2|Q16309|Q16310|Q6GTG1	2638	GC	Vitamin D-binding protein	9.84	0.0034
IPI00170635	B2R7H0|Q8WVN6|Q53G27|O00466|A8K3U3|Q53G63	6398	SECTM1	Secreted and transmembrane protein 1|Secreted and transmembrane 1 precusor variant|cDNA FLJ77863, highly similar to *Homo sapiens* secreted and transmembrane 1 (SECTM1), mRNA	9.44	0.0409
IPI00022488	P02790|B2R957	3263	HPX	Hemopexin	5.80	0.0209
IPI00022432	Q9UBZ6|Q6IB96|P02766|E9KL36|Q549C7|Q9UCM9	7276	TTR	Epididymis tissue sperm binding protein Li4a|Transthyretin	5.63	0.0157
IPI00008787	Q14769|P54802	4669	NAGLU|ufHSD2	Alpha-N-acetylglucosaminidase	4.89	0.0206
IPI00022420	D3DR38|P02753|Q9P178|Q8WWA3|Q5VY24|O43479|O43478	5950	RBP4	Retinol-binding protein 4	4.32	0.0370
IPI00032258	B0QZR6|Q13160|A7E2V2|Q14033|P0C0L4|B7ZVZ6|Q6P4R1|B2RUT6|Q5JQM8|Q4LE82|P01028|Q9NPK5|P78445|Q13906|Q14835|Q9UIP5	720|721	C4A variant protein|C4A	Complement C4-A|C4A variant protein|Complement component 4A (Rodgers blood group)	3.97	0.0344

TABLE 5B-continued

Level 2 analysis (morning/bedtime samples treated independently, genders pooled)

IPI	UniProt	Entrez	Gene name	Description	G-test	T-test
IPI00021085	O75594|Q4VB36	8993	PGLYRP1	Peptidoglycan recognition protein 1	3.01	0.0373
IPI00010949	Q9HAT2|B3KPB0|Q9HAU7|Q8IUT9|Q9NT71	54414	SIAE	Sialate O-acetylesterase	2.99	0.0064
IPI00744184	Q96CJ0|P15289|B7XD04|Q63HL5|Q6ICI5|B2RCA6	410	ARSA|DKFZp686G12235	Putative uncharacterized protein DKFZp686G12235|Arylsulfatase A	2.16	0.0193

TABLE 5C

Level 3 analysis (morning/bedtime samples and genders treated independently - boys)

IPI	UniProt	Entrez	Gene name	Description	G-test	T-test
			Morning (am) samples			
IPI00032328	P01043|P01042|B4E1C2|Q7M4P1|B2RCR2|A8K474|Q6PAU9|Q53EQ0	3827	KNG1	Kininogen-1|Kininogen 1, isoform CRA_b	−72.60	0.0187
IPI00004573	P01833|Q8IZY7|Q68D81	5284	PIGR	Polymeric immunoglobulin receptor	−67.34	0.0028
IPI00029260	Q96FR6|F1C4A7|Q9UNS3|Q96L99|B2R888|P08571|Q53XT5	929	CD14	Monocyte differentiation antigen CD14	−57.39	0.0363
IPI00291136	Q9BSA8|Q14040|Q14041|O00117|Q16258|O00118|Q7Z645|P12109|Q8TBN2	1291	COL6A1	Collagen alpha-1(VI) chain|Putative uncharacterized protein	−50.80	0.0024
IPI00218192	Q15135|Q14624|Q9UQ54|Q9P190	3700	ITIH4	Inter-alpha-trypsin inhibitor heavy chain H4	−48.66	0.0136
IPI00009950	Q53HH1|Q12907|A8K7T4	10960	LMAN2	cDNA FLJ75774, highly similar to *Homo sapiens* lectin, mannose-binding 2 (LMAN2), mRNA|Vesicular integral-membrane protein VIP36	−41.87	0.0351
IPI00294713	Q9H498|Q9UMV3|Q9ULC7|Q96QG4|O75754|Q9UC48|O00187|Q9H499|Q5TEQ5|Q9BZH0|Q5TER0|A8K458|A8MWJ2|Q9UBP3|Q9Y270	10747	MASP2	Mannan-binding lectin serine protease 2	−34.82	0.0042
IPI00000073	E9PBF0|P01133|B4DRK7|Q52LZ6	1950	EGF	Pro-epidermal growth factor	−30.27	0.0017
IPI00022488	P02790|B2R957	3263	HPX	Hemopexin	−27.44	0.0086
IPI00291866	A6NMU0|Q9UC49|Q96FE0|P05155|A8KAI9|E9KL26|Q7Z455|Q16304|B2R6L5|Q59EI5|Q547W3|Q9UCF9	710	SERPING1	Plasma protease C1 inhibitor|Epididymis tissue protein Li 173	−26.09	0.0036
IPI00009028	P05452|B2R582|Q6FGX6	7123	CLEC3B	Tetranectin|cDNA, FLJ92374, highly similar to *Homo sapiens* C-type lectin domain family 3, member B (CLEC3B), mRNA	−26.01	0.0014
IPI00006662	D3DNW6|B2R579|P05090|Q6IBG6	347	APOD	Apolipoprotein D	−25.64	0.0239
IPI00299738	O14550|A4D2D2|B2R9E1|Q15113	5118	PCOLCE	Procollagen C-endopeptidase enhancer|Procollagen C-endopeptidase enhancer 1	−23.92	0.0214
IPI00027843	P22891|A6NMB4|Q5JVF6|Q15213|Q5JVF5	8858	PROZ	Vitamin K-dependent protein Z	−23.04	0.0009
IPI00021085	O75594|Q4VB36	8993	PGLYRP1	Peptidoglycan recognition protein 1	−21.41	0.0262
IPI00395488	Q6UXL4|Q6UXL5|Q96CX1|Q6EMK4	114990	VASN	Vasorin	−21.17	0.0017
IPI00018953	Q53TN1|P27487	1803	DPP4	Dipeptidyl peptidase 4	−20.33	0.0153
IPI00293539	A8MZC8|Q9UQ94|B7WP28|Q9UQ93|A8K5D6|Q15065|P55287|Q15066	1009	CDH11	Cadherin-11	−19.41	0.0246
IPI00027235	Q9UC75|Q9NTQ3|O95414|O75882|Q9UDF5|Q9NU01|A8KAE5|Q9NZ58|O60295|Q3MIT3|Q9NZ57|Q5VYW3|C9IZD4|Q5TDA4|Q5TDA2|Q9NTQ4	8455	ATRN	Uncharacterized protein|Attractin	−19.25	0.0188
IPI00026314	A8MUD1|B7Z9A0|P06396|Q8WVV7|B7Z373|Q5T0I2|B7Z6N2	2934	GSN	Gelsolin (Amyloidosis, Finnish type)|cDNA FLJ56154, highly similar to Gelsolin|cDNA FLJ56212, highly similar to Gelsolin|Gelsolin	−18.95	0.0436
IPI00216780	Q6NV88|Q8IUL8|Q8WV21|Q8N4A6|B2RAJ0	148113	CILP2	cDNA, FLJ94946, highly similar to *Homo sapiens* cartilage intermediate layer protein 2 (CILP2), mRNA|Cartilage intermediate layer protein 2	−18.69	0.0026
IPI00021885	Q9BX62|A8K3E4|Q4QQH7|D3DP14|P02671|D3DP15|Q9UCH2	2243	FGA	cDNA FLJ78367, highly similar to *Homo sapiens* fibrinogen, A alpha polypeptide (FGA), transcriptvariant alpha, mRNA|Fibrinogen alpha chain	−18.53	0.0163

TABLE 5C-continued

Level 3 analysis (morning/bedtime samples and genders treated independently - boys)

IPI	UniProt	Entrez	Gene name	Description	G-test	T-test
IPI00060800	Q96DA0|C3PTT6|B2R4F6|A6NIY1|Q6UW28	124220	PAUF|ZG16B	Zymogen granule protein 16 homolog B|Pancreatic adenocarcinoma upregulated factor	−17.51	0.0227
IPI00176427	B2R7L5|Q9Y4A4|Q8NFZ8	199731	CADM4	Cell adhesion molecule 4	−17.33	0.0021
IPI00022661	Q92692|Q96J29|Q6IBI6|O75455|Q7Z456	5819	PVRL2	Poliovirus receptor-related protein 2|Poliovirus receptor related 2	−16.66	0.0454
IPI00291262	Q5HYC1|Q2TU75|B3KSE6|Q7Z5B9|B2R9Q1|P11381|P11380|P10909	1191	CLU	Clusterin	−16.20	0.0096
IPI00221224	Q6GT90|Q8IVL7|B4DP01|Q59E93|Q16728|Q8IUK3|Q8IVH3|P15144|Q71E46|B4DV63|B4DPH5|B4DP96|Q9UCE0	290	ANPEP|CD13	cDNA FLJ56158, highly similar to Aminopeptidase N (EC 3.4.11.2)|Membrane alanine aminopeptidase variant|Uncharacterized protein|Aminopeptidase N|cDNA FLJ56120, highly similar to Aminopeptidase N (EC 3.4.11.2)|cDNA FLJ55496, highly similar to Aminopeptidase N (EC 3.4.11.2)	−16.15	0.0111
IPI00291867	Q6LAM0|P05156|O60442	3426	CFI	Complement factor I|Light chain of factor I	−15.00	0.0147
IPI00003919	Q16770|Q3KRG6|Q16769|Q53TR4	25797	tmp__locus__46|QPCT	Glutaminyl-peptide cyclotransferase|Glutaminyl-peptide cyclotransferase (Glutaminyl cyclase), isoform CRA__a	−14.35	0.0121
IPI00099670	P19835|Q9UP41|Q16398|O75612|B4DSX9|Q9UCH1|Q5T7U7	1056	CEL	cDNA FLJ51297, highly similar to Bile salt-activated lipase (EC 3.1.1.3)|Bile salt-dependent lipase oncofetal isoform|Bile salt-activated lipase	−13.80	0.0464
IPI00031065	Q14UV0|Q14UU9|P24855	1773	DNASE1	Deoxyribonuclease|Deoxyribonuclease-1	−13.80	0.0044
IPI00015525	Q504V7|B4E3H8|Q6P2N2|Q9H8L6	79812	MMRN2	Multimerin-2|cDNA FLJ54082, highly similar to Multimerin-2	−13.66	0.0046
IPI00043992	Q96K15|Q96NY8	81607	PVRL4	Poliovirus receptor-related protein 4	−13.66	0.0332
IPI00022432	Q9UBZ6|Q6IB96|P02766|E9KL36|Q549C7|Q9UCM9	7276	TTR	Epididymis tissue sperm binding protein Li 4a|Transthyretin	−13.27	0.0042
IPI00022290	P60022|Q09753|Q86SQ8	1672	DEFB1|HBD1	Beta-defensin-1|Beta-defensin 1	−13.27	0.0053
IPI00102300	Q9UIF2|Q9HCN7|Q9HCN6	51206	GP6	Platelet glycoprotein VI	−13.13	0.0032
IPI00240345	Q695G9|Q86T13|Q6PWT6|Q8N5V5	161198	CLEC14A	C-type lectin domain family 14 member A	−12.91	0.0015
IPI00153049	Q5TA39|Q96KC3|Q9BRK3	54587	MXRA8	Matrix-remodeling-associated protein 8	−12.88	0.0286
IPI00029658	A8KAJ3|Q541U7|Q12805|A8K3I4|D6W5D2|Q59G97|B2R6M6	2202	EFEMP1	EGF-containing fibulin-like extracellular matrix protein 1 isoform b variant|EGF-containing fibulin-like extracellular matrix protein 1|cDNA, FLJ93024, highly similar to *Homo sapiens* EGF-containing fibulin-like extracellular matrix protein 1 (EFEMP1), transcript variant 1, mRNA|cDNA FLJ77823, highly similar to *Homo sapiens* EGF-containing fibulin-like extracellular matrix protein 1, transcript variant 3, mRNA	−12.88	0.0256
IPI00103871	Q9NWJ8|A8K154|Q8TEG1|Q8WZ75|Q96JV6|Q9H718|Q14DU7	54538	ROBO4	Roundabout homolog 4	−11.90	0.0291
IPI00009793	Q53GX9|Q9NZP8	51279	C1RL	Complement C1r subcomponent-like protein	−11.74	0.0142
IPI00019157	D3DW77|Q92675|Q6UVK1	1464	CSPG4	Chondroitin sulfate proteoglycan 4	−11.68	0.0185
IPI00006971	Q2M2V5|Q9HCU0|Q96KB6|Q3SX55	57124	CD248	Endosialin	−11.35	0.0186
IPI00009276	Q14218|Q9ULX|Q96CB3|B2RC04|Q9UNN8|Q6IB56	10544	PROCR	Endothelial protein C receptor	−10.91	0.0332
IPI00553177	E9KL23|Q0PVP5|Q53XB8|Q96BF9|B2RDQ8|Q13672|Q5U0M1|Q7M4R2|P01009|Q9P1P0|Q9UCM3|A6PX14|Q9UCE6|Q96ES1|Q86U19|Q86U18	5265	SERPINA1	Epididymis secretory sperm binding protein Li 44a|Alpha-1-antitrypsin	−9.59	0.0265
IPI00032293	D3DW42|B2R5J9|P01034|E9RH26|Q6FGW9	1471	CST3	Cystatin-C|Cystatin C	−9.16	0.0021
IPI00045512	Q69YJ3|Q5TYR7|Q96RW7|Q96DN8|Q96SC3|Q5TCP6|Q96DN3|Q96K89|A6NGE3	83872	DKFZp762L185|HMCN1	Hemicentin 1|cDNA FLJ14438 fis, clone HEMBB1000317, weakly similar to FIBULIN-1, ISOFORM D|Putative uncharacterized protein DKFZp762L185|Hemicentin-1	−9.04	0.0171
IPI00306322	Q14052|Q548C3|Q66K23|P08572|Q5VZA9|B4DH43	1284	COL4A2	cDNA FLJ56433, highly similar to Collagen alpha-2(IV) chain|Collagen alpha-2(IV) chain	−7.50	0.0264

TABLE 5C-continued

Level 3 analysis (morning/bedtime samples and genders treated independently - boys)

IPI	UniProt	Entrez	Gene name	Description	G-test	T-test
IPI00295414	P39059|B3KTP7|Q5T6J4|Q9Y4W4| Q9UDC5	1306	COL15A1	Collagen alpha-1(XV) chain|cDNA FLJ38566 fis, clone HCHON2005118, highly similar to Collagen alpha-1(XV) chain	−6.84	0.0135
IPI00168728	Q8NF17		FLJ00385	FLJ00385 protein	−6.63	0.0282
IPI00289983	Q96QM0|D3DNC6|Q96KY0|P15309| Q96QK9	55	ACPP	Prostatic acid phosphatase	−6.55	0.0073
IPI00027482	B2R9F2|P08185|Q7Z2Q9|A8K456	866	SERPINA6	Corticosteroid-binding globulin|cDNA, FLJ94361, highly similar to *Homo sapiens* serine (or cysteine) proteinase inhibitor, clade A(alpha-1 antiproteinase, antitrypsin), member 6 (SERPINA6), mRNA	−6.54	0.0256
IPI00218851					−6.17	0.0228
IPI00186826	B5A972|B5A970|Q96L35	2050	EPHB4	EPH receptor B4, isoform CRA_b|Soluble EPHB4 variant 1|Soluble EPHB4 variant 3	−6.14	0.0396
IPI00292130	A8K981|Q9UIX8|Q07507|Q8N4R2	1805	DPT	Dermatopontin	−5.86	0.0022
IPI00218413	Q96EM9|B7Z7C9|B2R865|P43251	686	BTD	Biotinidase|cDNA FLJ50907, highly similar to Biotinidase (EC 3.5.1.12)	−5.65	0.0416
IPI00896380	P20769|P01871		IGHM	Ig mu chain C region	−5.55	0.0308
IPI00025992	B6EU04|Q9BY68|Q1HE14|P81172	57817	HAMP	Hepcidin|Hepcidin antimicrobial peptide	−5.49	0.0484
IPI00305457	Q9P173			PRO2275	−5.23	0.0184
IPI00000024	B4E2D8|Q8IUP2|Q08174	5097	PCDH1	cDNA FLJ59655, highly similar to Protocadherin-1|Protocadherin-1	−4.61	0.0079
IPI00021841	Q9UCS8|Q6LDN9|Q9UCT8| A8K866|P02647|Q6Q785| Q6LEJ8	335	APOA1	APOA1 protein|Apolipoprotein A-I	−4.35	0.0233
IPI00922041	B7Z538			cDNA FLJ60766, highly similar to Hepatocyte growth factor-like protein	−4.16	0.0058
IPI00216728	C9JJR0		NRXN3	Neurexin-3-beta, soluble form	−4.16	0.0403
IPI00013576	Q8WVV5|O00480	10385	BTN2A2	Butyrophilin subfamily 2 member A2	−4.00	0.0141
IPI00022284	Q15216|A1YVW6|Q8TBG0|Q27H91| P04156|Q86XR1|O60489|Q5QPB4| Q6FGR8|Q15221|Q6FGN5|D4P3Q7| Q96E70|P78446|B4DDS1|Q9UP19| B2R5Q9|Q5U0K3|Q540C4|Q53YK7	5621	PRNP	Major prion protein	−3.84	0.0118
IPI00470360	Q8TB15|Q5XKC6|Q9H9N1| Q7Z7N8|Q5W0F8 Q96J84|Q9NVA5| Q7Z696	55243	KIRREL	Kin of IRRE-like protein 1	−3.52	0.0062
IPI00292218	B7Z557			cDNA FLJ53076, highly similar to Hepatocyte growth factor-like protein	−3.44	0.0270
IPI01025175					−3.37	0.0047
IPI00383732	Q9Y509		VH3	VH3 protein	−3.37	0.0476
IPI00009794	B1AME5|B1AME6|Q8NBQ3| Q96AA1|Q53HQ9|B4DSM1| B2RDF1|Q9BRK5|Q9NZP7| Q9UN53|Q53G52	51150	SDF4	45 kDa calcium-binding protein	−2.77	0.0403
IPI00329538	Q9UCA3|Q16651	5652	PRSS8	Prostasin	−2.74	0.0164
IPI00010807	Q9H461	8325	FZD8	Frizzled-8	−2.57	0.0030
IPI00784865	Q6P5S8		IGK@	IGK@ protein	−2.45	0.0131
IPI00925540	A6NLA3|Q13350|Q14870|P26927| Q6GTN4|A8MSX3|Q53GN8|B7Z250	4485	MST1	Hepatocyte growth factor-like protein|cDNA FLJ56324, highly similar to Hepatocyte growth factor-like protein|Macrophage stimulating 1 (Hepatocyte growth factor-like) variant	−2.38	0.0016
IPI00556655	Q59FZ0			LAMP1 protein variant	−2.38	0.0065
IPI00016450	Q96TD2|Q6LCK3|Q6LCK5| Q6LCK4|Q6LCK6|Q93023| A5X2V1|P51170|Q93026| Q93025|Q93024|Q93027| P78437|Q6PCC2	6340	SCNN1G	Amiloride-sensitive sodium channel subunit gamma|Amiloride-sensitive epithelial sodium channel gamma subunit|Amiloride-sensitive sodium channel gamma-subunit	−2.05	0.0466
IPI00007257	O94985|Q5SR52|Q5UE58|Q71MN0| A8K183|Q8N4K9	22883	CLSTN1	Calsyntenin-1	1.50	0.0118
IPI00744007					1.70	0.0050
IPI00022830	Q5JXA5|Q5JXA4|B2RD74|Q9UI06| A2A2L1|Q9H102|Q9UNZ2|Q7Z533| Q9NVL9	55968	NSFL1C	NSFL1 cofactor p47	1.70	0.0140
IPI00023974	P53801|D3DSL9|A8K274|Q9NS09| B2RDP7	754	PTTG1IP	Pituitary tumor-transforming gene 1 protein-interacting protein|cDNA FLJ78227, highly similar to *Homo sapiens* pituitary tumor-transforming 1 interacting protein (PTTG1IP), mRNA	1.76	0.0070

TABLE 5C-continued

Level 3 analysis (morning/bedtime samples and genders treated independently - boys)

IPI	UniProt	Entrez	Gene name	Description	G-test	T-test
IPI00030936	Q5VST0|D3DQ14|O60745|O60635	10103	TSPAN1	Tetraspanin-1	1.90	0.0306
IPI00005733	Q5T7S2|Q706C0|P36897|Q6IR47|Q706C1	7046	TGFBR1	TGF-beta receptor type-1|Transforming growth factor beta receptor I	1.92	0.0005
IPI00169285	Q8NHP8	196463	PLBD2	Putative phospholipase B-like 2	1.93	0.0040
IPI00221255	Q5MY99|O95797|O95796|O95799|O95798|Q15746|Q7Z4J0|Q9C0L5|Q14844|Q16794|Q5MYA0|Q9UBG5|Q9UIT9	4638	MYLK	Myosin light chain kinase, smooth muscle	2.03	0.0043
IPI00004440	A8K604|Q16849|Q08319|Q53QD6|B4DK12	5798	PTPRN	cDNA FLJ55332, highly similar to Receptor-type tyrosine-proteinphosphatase-like N|Receptor-type tyrosine-protein phosphatase-like N|cDNA FLJ77469, highly similar to *Homo sapiens* protein tyrosine phosphatase, receptor type, N, mRNA	2.10	0.0139
IPI00216773	E7ESS9|Q8IUK7		ALB	ALB protein	2.22	0.0221
IPI00293836	Q8N3J6|Q658Q7|Q8IZP8|Q3KQY9	253559	CADM2	Cell adhesion molecule 2	2.26	0.0230
IPI00002666	Q7M4M8|P09086|Q16648|Q9BRS4|Q9UMI6|Q9UMJ4	5452	OCT-2|POU2F2	Homeobox protein|Oct-2 factor|POU domain, class 2, transcription factor 2	2.34	0.0004
IPI00017557	Q1ZYW2|Q6PD64|Q4G124|Q6FHJ7|Q6FHM0|O14877|B4DYC1|Q05BG7	6424	SFRP4	Secreted frizzled-related protein 4	2.34	0.0460
IPI00220737	Q96CJ3|Q16180|B7Z8D6|Q15829|Q05C58|P13591|P13592|P13593|Q86X47|Q59FL7|A8K8T8|Q16209	4684	NCAM1	cDNA FLJ54771, highly similar to Neural cell adhesion molecule 1, 120 kDa isoform|Neural cell adhesion molecule 1	2.41	0.0028
IPI00026154	B4DJQ5|PI4314|Q96BU9|Q9P0W9|E7EQZ9|Q96D06	5589	PRKCSH	Glucosidase 2 subunit beta|Uncharacterized protein|cDNA FLJ59211, highly similar to Glucosidase 2 subunit beta	2.53	0.0008
IPI00034319	Q9NYQ9|O60888|Q5JXM9|Q3B784|A2BEL4|A2AB26|Q5SU05	51596	CUTA	Protein CutA	2.55	0.0245
IPI00215997	Q96ES4|P21926|Q5J7W6|D3DUQ9	928	CD9	CD9 antigen	2.61	0.0200
IPI00016786	P25763|P21181|P60953|Q9UDI2|Q7L8R5	998	CDC42	Cell division control protein 42 homolog	2.61	0.0011
IPI00219860	P23468|B1ALA0	5789	PTPRD	Receptor-type tyrosine-protein phosphatase delta	2.76	0.0437
IPI00018434	Q9BUM5|Q99816	7251	TSG101	Tumor susceptibility gene 101 protein	2.79	0.0173
IPI00219465	Q9UDM0|Q9BVI8|P20062|Q9UCI6|Q9UCI5	6948	TCN2	Transcobalamin-2	2.79	0.0339
IPI00017367	A7YIJ8		RDX	Radixin	2.86	0.0122
IPI00010290	Q6FGL7|Q05CP7|P07148	2168	FABP1	Fatty acid-binding protein, liver|FABP1 protein	2.93	0.0039
IPI00017202	Q7Z798|Q7Z7A0|Q7Z799|Q9H9P2|B2R9C0|Q9HCY3	140578	CHODL	Chondrolectin	3.00	0.0341
IPI00003101	P01589|B2R9M9|A2N4P8|Q5W007|Q53FH4	3559	IL2RA|IL2R	cDNA, FLJ94475, highly similar to *Homo sapiens* interleukin 2 receptor, alpha (IL2RA), mRNA|IL2R protein|Interleukin-2 receptor subunit alpha|Interleukin 2 receptor, alpha chain variant	3.03	0.0085
IPI00289831	Q16341|O75255|Q15718|Q13332|O75870|D6W633|Q2M3R7	5802	PTPRS	Receptor-type tyrosine-protein phosphatase S|Protein tyrosine phosphatase, receptor type, S, isoform CRA_a	3.04	0.0328
IPI00013972	Q16574|Q0Z7S6|O60399|P31997	1088	CEACAM8	Carcinoembryonic antigen-related cell adhesion molecule 8	3.05	0.0046
IPI00022937					3.19	0.0000
IPI00027436	B2R961|P08138	4804	NGFR	Tumor necrosis factor receptor superfamily member 16	3.23	0.0117
IPI00021968	Q9Y6Q6	8792	TNFRSF11A	Tumor necrosis factor receptor superfamily member 11A	3.24	0.0112
IPI00027509	B7Z747|Q9UCJ9|B7Z8A9|P14780|Q8N725|Q9UDK2|Q3LR70|Q9UCL1|F5GY52|Q9H4Z1|B2R7V9|Q9Y354|B7Z507	4318	MMP9	cDNA FLJ51036, highly similar to Matrix metalloproteinase-9 (EC3.4.24.35)|Uncharacterized protein|Matrix metalloproteinase-9|Matrix metalloproteinase 9|cDNA FLJ51120, highly similar to Matrix metalloproteinase-9 (EC 3.4.24.35)|cDNA FLJ51166, highly similar to Matrix metalloproteinase-9 (EC 3.4.24.35)	3.27	0.0218
IPI00641251	B2RDS5|Q53HF7|Q9NPF0|D6W668	51293	CD320	CD320 antigen	3.34	0.0078
IPI00002910	Q9H665|Q8N5X0	79713	IGFLR1	IGF-like family receptor 1	3.49	0.0090
IPI00025204	A8K7M5|O43866|Q6UX63	922	CD5L	CD5 antigen-like	3.56	0.0014
IPI00297646	O76045|Q16050|Q9UML6|Q13902|Q14037|Q13903|Q8IVI5|Q6LAN8|P02452|Q13896|Q59F64|Q15176|D3DTX7|Q8N473|Q15201|	1277	COL1A1	Collagen type I alpha 1|Type II procollagen gene|Collagen, type I, alpha 1, isoform CRA_a|Type I collagen alpha 1 chain|Collagen alpha-1(I) chain	3.58	0.0160

TABLE 5C-continued

Level 3 analysis (morning/bedtime samples and genders treated independently - boys)

IPI	UniProt	Entrez	Gene name	Description	G-test	T-test
	Q14042|Q14992|Q9UMM7| Q7KZ30|P78441|Q7KZ34| Q9UMA6					
IPI00027463	P06703|Q5RHS4|D3DV39|B2R577	6277	S100A6	cDNA, FLJ92369, highly similar to *Homo sapiens* S100 calcium binding protein A6 (calcyclin) (S100A6), mRNA|Protein S100-A6	3.62	0.0207
IPI00001754	Q9Y624|D3DVF0|Q6FIB4	50848	F11R	F11 receptor|F11 receptor, isoform CRA_a|Junctional adhesion molecule A	3.62	0.0048
IPI00152418	Q14UF3|Q8TD14|D3DT86|B1AP16		DAF|CD55	CD55 antigen, decay accelerating factor for complement (Cromer blood group), isoform CRA_g|Decay-accelerating factor splicing variant 4|Decay-accelerating factor 1a|CD55 molecule, decay accelerating factor for complement (Cromer blood group)	3.76	0.0388
IPI00289501	O15240|Q9UDW8	7425	VGF	Neurosecretory protein VGF	3.76	0.0102
IPI00376457	B4E0V9	342510		cDNA FLJ61198, highly similar to *Homo sapiens* CD300 antigen like family member E (CD300LE), mRNA	3.97	0.0064
IPI00216298	P10599|Q53X69|Q9UDG5|Q96KI3	7295	TXN	Thioredoxin	4.02	0.0028
IPI00289334	Q9UEV9|Q13706|Q9NT26|C9JMC4| Q6MZJ1|C9JKE6|O75369|Q8WXS9| B2ZZ84|B2ZZ85|Q8WXT1| Q8WXT0|Q59EC2|Q8WXT2|Q 9NRB5	2317	FLNB	Filamin-B	4.06	0.0268
IPI00219365	Q6PJT4|P26038	4478	MSN	MSN protein|Moesin	4.15	0.0033
IPI00977659	Q6S9E4|A8K9Q3|Q14C97|Q9ULV1| Q8TDT8	8322	GPCR|FZD4	Frizzled-4|Putative G-protein coupled receptor	4.22	0.0057
IPI00002280	Q9UHG2|Q4VC04	27344	PCSK1N	ProSAAS	4.47	0.0007
IPI00303161	Q96AP7|Q96T50	90952	ESAM	Endothelial cell-selective adhesion molecule	4.85	0.0008
IPI00002435	P26842|B2RDZ0	939	CD27	CD27 antigen	4.96	0.0003
IPI00291488	Q8WXW1|Q6IB27|A6PVD5| Q96KJ1|A2A2A5|Q14508| Q8WXV9|A2A2A6|Q8WXW0| Q8WXW2	10406	WFDC2	WAP four-disulfide core domain protein 2	5.02	0.0413
IPI00099110	Q9Y4V9|B1ARE9|B1ARE8|Q5JR26| B1ARF0|Q9UGM3|Q9UGM2| Q59EX0|B1ARE7|A8E4R5| Q9UKJ4|Q9UJ57|Q96DU4| A6NDG4|Q9Y211|Q6MZN4| A6NDJ5	1755	DMBT1	Deleted in malignant brain tumors 1 protein	5.03	0.0038
IPI00179330	B2RDW1|Q9UEK8|Q8WYN8| Q91887|Q6LDU5|P62988| Q9BX98|Q9UEF2|P62979| Q5RKT7|Q9UPK7|P14798| Q9BWD6|Q6LBL4|P02248| P02249|Q91888|Q9BQ77| Q29120|P02250|Q9UEG1	6233	RPS27A	Ribosomal protein S27a|Ubiquitin-40S ribosomal protein S27a|Ribosomal protein S27a, isoform CRA_c	5.15	0.0004
IPI00008239	B7Z831		GPRC5B	G-protein-coupled receptor family C group 5 member B	5.22	0.0340
IPI00301579	E7EMS2|B4DV10		NPC2	Epididymal secretory protein E1|cDNA FLJ59142, highly similar to Epididymal secretory protein E1	5.49	0.0000
IPI00026926	Q02747	2980	GUCA2A	Guanylin	5.53	0.0152
IPI00019906	B4DY23|P35613|Q7Z796|Q54A51| Q8IZL7	682	hEMMPRIN|BSG	Basigin|cDNA FLJ61188, highly similar to Basigin|Basigin (Ok blood group), isoform CRA_a	5.71	0.0082
IPI00004901	Q9NXI0		GPRC5C	G-protein-coupled receptor family C group 5 member C	6.07	0.0228
IPI00019580	B2R7F8|P00747|Q9UMI2|Q15146| Q5TEH4|Q6PA00|B4DPH4	5340	PLG	PLG protein|Plasminogen|cDNA, FLJ93426, highly similar to *Homo sapiens* plasminogen (PLG), mRNA|cDNA FLJ58778, highly similar to Plasminogen (EC 3.4.21.7)	6.08	0.0084
IPI00175092	Q53SV6|Q8WUU3|Q8NC42| Q8NBY5|Q53S14|Q8N5I8	284996	RNF149| LOC284996	Putative uncharacterized protein LOC284996|E3 ubiquitin-protein ligase RNF149	6.39	0.0102
IPI00103636	Q8WXW1|Q6IB27|A6PVD5| Q96KJ1|A2A2A5|Q14508| Q8WXV9|A2A2A6|Q8WXW0| Q8WXW2	10406	WFDC2	WAP four-disulfide core domain protein 2	6.57	0.0191

TABLE 5C-continued

Level 3 analysis (morning/bedtime samples and genders treated independently - boys)

IPI	UniProt	Entrez	Gene name	Description	G-test	T-test
IPI00010182	P08869|Q4VWZ6|Q53SQ7|Q9UCI8|P07108|B8ZWD8|Q6IB48	1622	DBI	Diazepam binding inhibitor, splice form 1D(1)|Acyl-CoA-binding protein	6.79	0.0021
IPI00922213	Q14327|Q7L553|B4DTK1|Q6PJE5|Q9H382|Q53S27|B4DTH2		FN1	Putative uncharacterized protein FN1|cDNA FLJ61165, highly similar to Fibronectin|FN1 protein|Fibronectin 1|cDNA FLJ53292, highly similar to *Homo sapiens* fibronectin 1 (FN1), transcript variant 5, mRNA	7.00	0.0035
IPI00290085	Q14923|Q8N173|B0YIY6|PI9022	1000	CDH2	Cadherin-2	7.14	0.0137
IPI00298388	Q49A94|Q8NCJ9|Q96FE7|Q86YW2|O00318	113791	PIK3IP1	Phosphoinositide-3-kinase-interacting protein 1	8.23	0.0075
IPI00032325	P01040|Q6IB90	1475	CSTA	CSTA protein|Cystatin-A	8.67	0.0042
IPI00010675	Q15854|Q03403	7032	TFF2	Trefoil factor 2	8.89	0.0247
IPI00011302	P13987|Q6FHM9	966	CD59	CD59 antigen, complement regulatory protein, isoform CRA_b|CD59 glycoprotein	10.07	0.0171
IPI00010343	Q9UPR5|B4DYQ9|B4DEZ4	6543	SLC8A2	cDNA FLJ58526, highly similar to Sodium/calcium exchanger 2|Sodium/calcium exchanger 2	10.67	0.0069
IPI00013955	Q9UE76|Q9UE75|Q9UQL1|Q7Z552|Q14876|Q9Y4J2|Q14128|Q16437|P13931|P17626|P15941|Q16615|P15942|Q16442|Q9BXA4	4582	MUC1	Mucin-1	10.89	0.0144
IPI00299086	O00173|O43391|O00560|B2R5Q7|B4DUH3|Q14CP2|B7ZLN2	6386	SDCBP	Syntenin-1|Syndecan binding protein (Syntenin)	11.69	0.0132
IPI00075248	Q96HK3|P02593|P70667|Q13942|P99014|P62158|B4DJ51|Q53S29|Q61379|Q61380	801|808|805	CALM2|CALM3|CALM1	Calmodulin|Calmodulin 1 (Phosphorylase kinase, delta), isoform CRA_a	12.10	0.0234
IPI00302592	Q5HY55|Q5HY53|P21333|Q8NF52|Q60FE6|Q6NXF2|Q8TES4	2316	FLNA|FLJ00119	Filamin-A|Filamin A|FLNA protein|FLJ00119 protein	12.82	0.0025
IPI00219684	Q5VV93|B2RAB6|Q99957|P05413|Q6IBD7	2170	FABP3	FABP3 protein|Fatty acid-binding protein, heart	12.84	0.0009
IPI00009027	Q2TBE1|P05451|Q0VFX1|A8K7G6|P11379|Q4ZG28	5967	REG1A	REG1A protein|Putative uncharacterized protein REG1A|cDNA FLJ75763, highly similar to *Homo sapiens* regenerating islet-derived 1 alpha (pancreatic stone protein, pancreatic thread protein) (REG1A), mRNA|Lithostathine-1-alpha	13.55	0.0282
IPI00012585	P07686	3074	HEXB	Beta-hexosaminidase subunit beta	18.50	0.0494
IPI00302944	Q5VYK2|Q71UR3|Q5VYK1|Q15955|Q99716|Q99715|O43853	1303	COL12A1	Collagen alpha-1(XII) chain	19.62	0.0256
IPI00009030	P13473|Q16641|D3DTF0|Q6Q3G8|Q99534|A8K4X5|Q9UD93|Q96J30	3920	LAMP2	Lysosome-associated membrane glycoprotein 2	21.17	0.0235
IPI00007778	F6X5H7|B2RBF5|Q5VX51|Q5VX50|Q8TC97|B3KQS3|B4DQ98|Q01459	1486	CTBS	cDNA PSEC0114 fis, clone NT2RP2006543, highly similar to DI-N-ACETYLCHITOBIASE (EC 3.2.1.—)|CTBS protein|Di-N-acetylchitobiase|cDNA FLJ55135, highly similar to Di-N-acetylchitobiase (EC 3.2.1.—)|cDNA, FLJ95483, highly similar to *Homo sapiens* chitobiase, di-N-acetyl- (CTBS), mRNA|Chitobiase, di-N-acetyl-	25.84	0.0045
IPI00031008	C9J575|Q14583|Q15567|Q5T7S3|C9IYT7|C9J6D9|C9J848|Q4LE33|P24821	3371	TNC variant protein|TNC	TNC variant protein|Tenascin	27.68	0.0421
IPI00295741	Q6LAF9|A8K2H4|Q503A6|B3KQR5|Q96D87|P07858|B3KRR5	1508	CTSB	Cathepsin B|cDNA FLJ78235	30.26	0.0454
IPI00022620	P55000|Q6PUA6|Q53YJ6|Q92483	57152	SLURP1	Secreted Ly-6/uPAR-related protein 1	43.85	0.0012
IPI00014048	Q1KHR2|B2R589|Q6ICS5|Q16869|Q16830|D3DS06|P07998|Q9UCB4|Q9UCB5	6035	RNASE1	Ribonuclease pancreatic	53.77	0.0034
IPI00293088	Q16302|P10253|Q09GN4|Q8IWE7|Q14351	2548	GAA	Lysosomal alpha-glucosidase	54.43	0.0356
IPI00220143	Q75ME7|Q0VAX6|O43451|Q8TE24|Q86UM5	8972	MGAM	Maltase-glucoamylase|Maltase-glucoamylase, intestinal	65.83	0.0279
			Bedtime (pm) samples			
IPI00022420	D3DR38|P02753|Q9P178|Q8WWA3|Q5VY24|O43479|O43478	5950	RBP4	Retinol-binding protein 4	13.16	0.0087
IPI00019568	P00734|B4DDT3|B2R7F7|Q53H06|Q53H04|Q9UCA1|Q69EZ8|Q4QZ40|Q7Z7P3|B4E1A7|Q69EZ7	2147	F2	Prothrombin B-chain|cDNA FLJ54622, highly similar to Prothrombin (EC 3.4.21.5)|Prothrombin	12.12	0.0383

TABLE 5C-continued

Level 3 analysis (morning/bedtime samples and genders treated independently - boys)

IPI	UniProt	Entrez	Gene name	Description	G-test	T-test
IPI00555812	Q53F31|P02774|B4DPP2|Q16309|Q16310|Q6GTG1	2638	GC	Vitamin D-binding protein	11.29	0.0073
IPI00010949	Q9HAT2|B3KPB0|Q9HAU7|Q8IUT9|Q9NT71	54414	SIAE	Sialate O-acetylesterase	7.05	0.0060
IPI00296992	Q8N5L2|P30530|Q9UD27	558	AXL	Tyrosine-protein kinase receptor UFO	3.88	0.0454
IPI00029275	P08582|Q9BQE2	4241	MFI2	Melanotransferrin	3.43	0.0453
IPI00003813	Q9BY67|Q8N2F4|Q86WB8|Q6MZK6	23705	DKFZp686F1789|CADM1	Putative uncharacterized protein DKFZp686F1789|Cell adhesion molecule 1	3.13	0.0197
IPI00735451	A2KLM6		IGVH	Immunolgoobulin heavy chain	2.98	0.0473
IPI00334627	A6NMY6		ANXA2P2	Putative annexin A2-like protein	2.77	0.0448
IPI00023858					2.68	0.0059
IPI00383032	Q96K94|B2RAY2|Q8WW60|Q8TDQ0	84868	HAVCR2	Hepatitis A virus cellular receptor 2	2.59	0.0202
IPI00015525	Q504V7|B4E3H8|Q6P2N2|Q9H8L6	79812	MMRN2	Multimerin-2|cDNA FLJ54082, highly similar to Multimerin-2	2.14	0.0388
IPI00015902	Q8N5L4|P09619|A8KAM8	5159	PDGFRB	cDNA FLJ76012, highly similar to *Homo sapiens* platelet-derived growth factor receptor, betapolypeptide (PDGFRB), mRNA|Platelet-derived growth factor receptor beta	1.93	0.0161
IPI00021828	P04080|Q76LA1	1476	CSTB	Cystatin-B|CSTB protein	1.60	0.0027
IPI00029723	D3DN90|Q549Z0|A8K523|Q12841	11167	FSTL1	cDNA FLJ78447, highly similar to *Homo sapiens* follistatin-like 1 (FSTL1), mRNA|Follistatin-related protein 1	1.51	0.0075
IPI00183425	Q8WU72|Q9Y3F9|Q9ULV3|Q9Y3G0|Q9UHK4|A8K9J8|Q9H868|Q5SYW5|B4E0A3|Q9NYM8|Q5SYW3	25792	CIZ1	Cip1-interacting zinc finger protein|cDNA FLJ60074, highly similar to Cip1-interacting zinc finger protein	1.51	0.0038
IPI00020557	Q59FG2|Q07954|Q6LAF4|Q2PP12|Q8IVG8|Q6LBN5	4035	LRP|LRP1	LRP protein|Alpha-2 macroglobulin receptor|Prolow-density lipoprotein receptor-related protein 1|Low density lipoprotein-related protein 1 variant	−2.26	0.0465
IPI00006705	P11684|Q9UCM4|B2R5F2|Q6FHH3|Q9UCM2	7356	SCGB1A1	Uteroglobin	−3.09	0.0305

TABLE 5D

Level 3 analysis (morning/bedtime samples and genders treated independently - girls)

IPI	UniProt	Entrez	Gene name	Description	G-test	T-test
			Morning (am) samples			
IPI00029275	P08582|Q9BQE2	4241	MFI2	Melanotransferrin	−5.79	0.0252
IPI00010949	Q9HAT2|B3KPB0|Q9HAU7|Q8IUT9|Q9NT71	54414	SIAE	Sialate O-acetylesterase	−4.95	0.0473
IPI00414896	Q9BZ46|Q9BZ47|B2RDA7|E1P5C3|Q8TCU2|O00584|Q5T8Q0	8635	RNASET2	Ribonuclease T2	−2.33	0.0131
IPI00179185	O00520|Q96MX2|Q66K79	8532	CPZ	Carboxypeptidase Z	−2.02	0.0485
IPI00021428	P02568|Q5T8M9|P99020|P68133	58	ACTA1	Actin, alpha skeletal muscle	−1.93	0.0250
IPI00000816	P42655|P29360|Q63631|Q7M4R4|D3DTH5|Q4VJB6|Q53XZ5|P62258|B3KY71	7531	YWHAE	14-3-3 protein epsilon	−1.65	0.0468
IPI00166729	O60386|Q5XKQ4|P25311|D6W5T8|Q8N4N0	563	AZGP1	Zinc-alpha-2-glycoprotein	2.63	0.0168
IPI00009650	Q5T8A1|P31025	3933	LCN1	Lipocalin-1	4.43	0.0053
			Bedtime (pm) samples			
IPI00384938	Q7Z351		DKFZp686N02209	Putative uncharacterized protein DKFZp686N02209	−17.82	0.0304
IPI00009276	Q14218|Q9ULX1|Q96CB3|B2RC04|Q9UNN8|Q6IB56	10544	PROCR	Endothelial protein C receptor	−4.00	0.0368
IPI00031121	B3KXD3|B3KR42|P16870|D3DP33|A8K4N1|Q9UIU9	1363	CPE	cDNA FLJ45230 fis, clone BRCAN2021325, highly similar to Carboxypeptidase E (EC 3.4.17.10)|Carboxypeptidase E	−2.96	0.0327
IPI00152871	B3KWI4|Q7RTN7|Q495Q6|Q8TF66	131578	LRRC15	cDNA FLJ43122 fis, clone CTONG3003737, highly similar to Leucine-rich repeat-containing protein 15|Leucine-rich repeat-containing protein 15	−1.93	0.0433

TABLE 5D-continued

Level 3 analysis (morning/bedtime samples and genders treated independently - girls)

IPI	UniProt	Entrez	Gene name	Description	G-test	T-test
IPI00003111	P01594|Q6LBV5			Ig kappa chain V-I region AU|DNA rearranged by a t(2; 8) translocation leading to Burkitt's lymphoma in the cell line JI (clone JIp)	1.62	0.0240
IPI00163563	Q96S96|Q8WW74|Q5EVA1	157310	PEBP4	Phosphatidylethanolamine-binding protein 4	1.64	0.0470
IPI00009650	Q5T8A1|P31025	3933	LCN1	Lipocalin-1	2.92	0.0209
IPI00019591	Q53F89|B4E1Z4		CFB	Complement factor B	3.67	0.0386
IPI00022429	B7ZKQ5|P02763|Q8TC16|Q5T539|Q5U067	5004	ORM1	Alpha-1-acid glycoprotein 1	4.57	0.0067
IPI00021447	B3KXB7|D3DT76|P19961|Q9UBH3	280	AMY2B	Alpha-amylase 2B	4.87	0.0477
IPI00032258	B0QZR6|Q13160|A7E2V2|Q14033|P0C0L4|B7ZVZ6|Q6P4R1|B2RUT6|Q5JQM8|Q4LE82|P01028|Q9NPK5|P78445|Q13906|Q14835|Q9UIP5	720|721	C4A variant protein|C4A	Complement C4-A|C4A variant protein|Complement component 4A (Rodgers blood group)	6.02	0.0480
IPI00022488	P02790|B2R957	3263	HPX	Hemopexin	8.97	0.0165
IPI00017601	Q2PP18|A8K5A4|Q1L857|A5PL27|B3KTA8|Q14063|P00450|Q9UKS4	1356	CP	cDNA FLJ76826, highly similar to *Homo sapiens* ceruloplasmin (ferroxidase) (CP), mRNA|cDNA FLJ37971 fis, clone CTONG2009958, highly similar to CERULOPLASMIN (EC 1.16.3.1)|CP protein|Ceruloplasmin	9.65	0.0247
IPI00022417	Q68CK4|Q8N4F5|P02750|Q96QZ4	116844	LRG1|HMFT1766	Leucine-rich alpha-2-glycoprotein	11.28	0.0205

[0108] In general, morning urine samples were overrepresented in differentially expressed proteins, a result largely based on the overwhelming effect of OSA on the urinary proteome of boys (FIG. **3***b*). This observation is not surprising given that OSA is a sleep disorder characterized by repetitive respiratory events at night that should therefore be more likely to manifest in morning urine; however, the opposite results emerged among girls, in whom bedtime urine samples yielded a higher number of candidate biomarkers (FIG. **3***b*). Moreover, differentially expressed proteins were highly specific for gender and sampling time, since poor overlap (~3%) was observed in the candidate biomarkers identified in boys and girls across morning and bedtime samples (Tables 5A-D). Importantly, gender differences in the biomarkers detected could not be accounted for by differences in age, disease severity, or obesity (BMI z-score) since these parameters were not significantly different between the groups (FIG. **3***c*).

[0109] Taken together, the results suggest that failing to account for sampling time and gender substantially masks significant differences in protein expression associated with a disease state such as OSA. This concept is clearly illustrated by global proteomic analysis of morning urine samples with the t-test and G-test, which shows dramatic improvements in both number and statistical significance of biomarkers identified (FIG. **3***d*). Similar conclusions emerge at the individual protein level using dipeptidyl peptidase 4 (DPP4) as an example (FIG. **3***e*).

Example 5

Validation of Candidate Biomarkers Identified by Proteomic Analysis

[0110] To validate the findings, the inventors used commercially available ELISA assays to measure urinary levels of four candidate biomarkers. Since protein levels in urine are highly variable, and influenced by body fluid volume, all measurements were standardized against corresponding urinary creatinine levels (Garde, 2004). ELISA measurements generally correlated well with label-free quantification by MS/MS (eg. HPX, $p<0.0001$, $R^2=0.52$; FIG. **4***a*) and provided strong validation for gender and diurnal regulation of protein levels (e.g., DPP4; compare FIGS. **3***d* and **4***b*). In total, ELISA assays provided independent confirmations of changes in protein levels for four candidate biomarkers detected in the proteomic analyses: DPP4 ($p=0.02$), HPX ($p=0.02$), and CP ($p=0.01$) emerged as reliable indicators of OSA in boys, and AZGP1 ($p=0.07$) was identified in girls (FIG. **4***b,c*). Moreover, because ELISA assays involved minimal processing of urinary samples (centrifugation), while proteomic analyses required substantial processing efforts (centrifugation, IgG and ALB depletion, protein precipitation, sample digestion, etc.) the strong concordance between these two approaches further suggests that the optimized proteomic workflow approach for urine biomarker discovery is robust.

Example 6

Urinary Biomarkers of Pediatric OSA Map to Pathophysiological Functional Modules

[0111] Having identified a wide range of candidate biomarkers in urine collected from children with OSA, the inventors next sought to determine whether those proteins mapped to specific functional pathways. To this end, the inventors used gene ontology analysis to organize the 192 proteins into functional modules based on biological processes and molecular function (FIG. **5**). This strategy identified significant enrichment (relative to the entire human genome) in a number of functional annotations including acute phase proteins ($p=8.4\times10^{-5}$), angiogenesis ($p=2.7\times10^{-3}$), hemostasis ($p=4.2\times10^{-8}$), leukocyte immunity ($p=2.4\times10^{-2}$), and lipid binding ($p=2.3\times10^{-4}$). Previous studies provide evidence that all of these pathways are affected in OSA. For example, disruption in inflammatory/immune, lipid, angiogenic, and hemostatic pathways have all been reported in patients with

OSA (Adedayo, 2012; Chorostowska-Wynimko, 2005; Slupsky, 2007; von Kanel, 2007).

Example 7

Children with OSA Demonstrate Heterogeneity in Memory Impairment

[0112] It is well established that children with OSA display neurocognitive deficits and reduced academic performance (Gozal, et al., 2010; Blunden et al., 2000; Gottlieb, et al., 2004; Kheirandish & Gozal, 2006; O'Brien, et al., 2004; Rhodes, et al., 1995; Gozal & Kheirandish-Gozal, 2007; Gozal, 1998). Declarative memory function is a critical component of academic performance and studies showed that OSA children have reduced ability to acquire, consolidate, and retrieve memories (Keirandish-Gozal, et al., 2010). To follow up on this previous work, the inventors recruited children (ages 5-12) with moderate to severe OSA along with age- and gender matched controls. The inventors assessed their sleep architecture by polysomnography and quantified their memory function using a commonly used declarative memory test previously implemented to identify neurocognitive deficits in patients with OSA (Keirandish-Gozal, et al., 2010).

[0113] In total, 33 children were recruited, with 20 subjects in the OSA group and 13 subjects in the control group. The mean age was ~7.5 yrs. The two groups were matched for age, sex, ethnicity, level of maternal education, and obesity, as determined by BMI z-score (Table 6). In addition the incidence of physician-diagnosed asthma was similar between the two groups. Children with OSA had a significantly higher apnea-hypopnea index (AHI; $p<0.0001$), a measure of the severity of sleep apnea (Grigg-Damberger, et al., 2007; Redline, et al., 2007).

TABLE 6

Patient Demographics

Group	N	Gender (M/F)	Age	AHI	BMI-z
CTRL	13	(7/6)	7.8 ± 0.5	0.6 ± 0.1	1.2 ± 0.3
OSA	20	(10/10)	7.4 ± 0.6	13.1 ± 2.6	1.3 ± 0.3

[0114] The OSA group demonstrated a trend for reduced free memory recall in the morning ($p=0.1$). Upon closer inspection of the data, it was evident that OSA patients, but not control subjects, displayed substantial heterogeneity in their morning test performance scores (FIG. **6**A). Based on this heterogeneity, the inventors classified OSA children into two phenotypes—one with normal (OSA-N, >7 recalls) and one with impaired declarative memory (OSA-I, ≤7 recalls). Importantly, OSA-N and OSA-I patients did not exhibit significant differences in OSA severity (FIG. **6**B), underlying obesity (FIG. **6**C), age (FIG. **6**D), or gender (50% male for OSA-N and OSA-I). Thus, differences in morning memory recall in OSA-N and OSA-I patients could not be attributed to the severity of sleep disruption or any other potential confounder.

[0115] Urinary Proteomics Identifies Candidate Biomarkers of Impaired Memory in Children with OSA.

[0116] Our findings demonstrate that children with OSA may be separated into two phenotypes based on the severity of associated impairment of acquisition, consolidation, or retrieval of memories. On a molecular level, this observed phenotypic heterogeneity may be explained by variable systemic responses to OSA, which have been reported in children (Gozal, et al., 2007; Bhattacharjee, et al., 2010). The urinary proteome is largely derived from the systemic compartment and the inventors have previously shown that changes to urinary proteins can report pathophysiology in the context of OSA (Gozal, et al., 2009).

[0117] To define candidate biomarkers of memory impairment in children with OSA the inventors used liquid chromatography mass spectrometry (LC-MS/MS) to interrogate morning urine samples (first void) collected from healthy children (N=13), OSA-N(N=8) and OSA-I (N=12) patients. Urine was processed using a rigorous and reproducible workflow for proteomics analysis to identify 745 urinary proteins across all subjects. Protein levels were quantified by spectral counting (Liu, et al., 2004) and proteins that were differentially abundant between groups were identified using a combination of the G-test and t-test (Becker, et al., 2010; Becker, et al., 2010; Heinecke, et al., 2010; Almendros, et al., 2014). Using very stringent dual statistical criteria (G-test: G-statistic >10 and t-test: $p<0.01$) and random permutation analysis to ensure a false discovery-rate (FDR)<0.1%, the inventors identified 65 proteins that were significantly altered in OSA-I relative to OSA-N patients. (FIG. **7**A). An identical approach was implemented to identify 93 proteins that were significantly altered in OSA-I relative to control subjects (data not shown). Candidate biomarkers were defined as those proteins that showed consistent increases (or decreases) in OSA-I relative to both OSA-N and CTRL subjects. Such analyses produced a list of 52 candidate biomarkers of memory impairment in children with OSA (Table 7); clusterin (CLU) and phosphoinositide-3-kinase-interacting protein 1 (PIK3IP1) are provided as two examples of proteins that met these very stringent criteria (FIG. **7**B).

TABLE 7

Candidate Biomarkers of Memory Impairment in Children with Obstructive Sleep Apnea

	OSA-I vs CTRL		OSA-I vs OSA-N		OSA-N vs CTRL	
Protein	G-test	T-test	G-test	T-test	G-test	T-test
RNASE1	101.5	2.72E−04	68.4	2.31E−03	3.8	1.32E−01
COL12A1	45.5	2.82E−06	33.4	4.06E−04	1.0	3.24E−01
RNASE2	31.6	1.09E−09	19.2	6.25E−05	1.6	1.06E−01
CD59	29.1	1.29E−03	18.7	1.46E−02	1.2	2.35E−01
FN1	26.9	2.12E−07	20.5	2.08E−05	0.5	2.72E−01
AMBP	23.2	4.00E−04	21.6	5.58E−04	0.0	8.47E−01
FBN1	18.4	3.12E−07	13.4	7.09E−05	0.4	2.98E−01

TABLE 7-continued

Candidate Biomarkers of Memory Impairment in Children with Obstructive Sleep Apnea

	OSA-I vs CTRL		OSA-I vs OSA-N		OSA-N vs CTRL	
Protein	G-test	T-test	G-test	T-test	G-test	T-test
PIK3IP1	17.7	2.97E−08	10.8	1.08E−05	0.9	6.16E−02
CDH1	17.4	1.19E−03	11.0	9.50E−03	0.8	1.71E−01
CDH2	16.3	1.22E−04	13.5	6.27E−04	0.1	4.15E−01
PLG	16.1	8.13E−07	12.6	1.24E−04	0.2	3.78E−01
SLURP1	15.0	2.94E−04	10.5	2.30E−03	0.4	2.74E−01
FN1 cDNA FLJ53292	13.7	6.38E−08	10.7	7.56E−06	0.2	2.81E−01
TNC	11.9	4.88E−05	11.1	3.15E−04	0.0	8.32E−01
C1RL	−10.2	5.02E−05	−10.6	3.17E−04	0.0	8.72E−01
A1BG	−10.6	2.01E−05	−16.8	1.07E−03	0.8	2.27E−01
PGLYRP2	−11.2	6.58E−03	−13.5	1.55E−03	0.1	6.21E−01
OSCAR	−11.3	2.04E−06	−11.4	1.50E−05	0.0	9.81E−01
AZGP1	−12.7	4.86E−04	−11.0	3.00E−03	−0.1	7.32E−01
CEL	−12.9	4.60E−05	−12.8	1.15E−04	0.0	9.67E−01
CFI	−14.0	8.15E−06	−12.0	5.87E−05	−0.1	4.40E−01
CILP2	−14.3	3.79E−06	−15.3	2.54E−04	0.0	7.56E−01
VASN	−14.6	6.55E−06	−15.9	1.43E−04	0.0	7.36E−01
PLAU	−14.6	3.24E−03	−10.5	3.61E−03	−0.5	3.77E−01
SERPINA1	−15.2	1.72E−07	−16.6	6.25E−04	0.0	7.94E−01
CD14	−15.4	4.23E−05	−17.6	2.80E−03	0.1	6.83E−01
LRP2	−15.7	1.17E−03	−16.1	3.10E−03	0.0	9.41E−01
CLU	−15.8	4.03E−06	−11.6	4.83E−04	−0.4	2.11E−01
FGA	−16.1	3.09E−03	−24.9	1.77E−03	1.3	2.10E−01
NID1	−16.5	8.19E−06	−18.3	1.62E−04	0.1	6.78E−01
APOD	−17.0	1.17E−05	−11.5	1.81E−03	−0.6	2.30E−01
SERPING1	−17.0	1.08E−04	−14.7	1.67E−04	−0.1	5.72E−01
CADM4	−18.2	9.29E−08	−11.3	3.58E−04	−1.1	2.68E−02
CP	−18.3	2.22E−08	−26.0	7.84E−04	1.0	1.57E−01
IGHA1	−19.3	1.84E−07	−15.0	2.29E−04	−0.3	2.49E−01
PGLYRP1	−21.7	4.20E−07	−21.5	3.10E−04	0.0	9.76E−01
ROBO4	−22.5	2.07E−06	−15.0	1.20E−04	−0.9	1.06E−01
SERPINA5	−24.6	1.60E−05	−20.2	3.85E−04	−0.2	5.06E−01
MASP2	−24.7	1.67E−06	−17.6	4.18E−04	−0.8	1.39E−01
HPX	−28.9	2.40E−06	−26.3	6.67E−05	−0.1	6.71E−01
IGHV4-31	−29.3	2.94E−03	−24.5	6.38E−03	−0.2	7.21E−01
IGHG1	−29.5	3.56E−06	−20.1	7.45E−04	−1.3	9.09E−02
MXRA8	−29.7	7.39E−06	−24.6	5.00E−05	−0.3	4.86E−01
AMY1C; AMY1A; AMY1B; AMY2A	−34.3	5.75E−06	−30.5	1.03E−05	−0.1	5.77E−01
COL6A1	−37.3	1.83E−04	−23.7	1.73E−04	−1.6	2.28E−01
EGF	−42.1	1.18E−09	−27.9	7.42E−05	−1.6	6.32E−02
PROCR	−45.7	2.69E−07	−38.4	2.76E−05	−0.4	3.73E−01
PIGR	−46.5	2.86E−06	−49.4	3.64E−06	0.1	7.61E−01
ITIH4	−54.2	2.30E−05	−34.4	2.64E−04	−2.7	1.01E−01
CUBN	−57.4	1.62E−08	−48.7	1.12E−04	−0.5	3.92E−01
LMAN2	−57.4	2.50E−05	−59.2	1.59E−05	0.0	9.00E−01
TF	−91.4	9.76E−07	−45.8	2.35E−04	−8.6	1.71E−03

Proteins were quantified by spectral counting Statistical significance was assessed by t-test ($p < 0.01$) and G-test (G-statistic >10 or <−10); positive G = up-regulated in first stample relative to second, negative G = down-regulated in first sample relative to second

[0118] Interestingly, informatics analysis of the candidate biomarkers identified significant enrichment in the inflammatory response ($p=10^{-6}$; Fisher's exact test with Benjamini-Hochberg correction). These findings are consistent with previous work that demonstrated a strong correlation between plasma C-reactive protein levels (a marker of inflammation) and neurocognitive function in children with OSA (Gozal, et al., 2007). Together, these data suggest that the presence of OSA-associated inflammation may predispose children to memory deficits and neurocognitive impairments.

[0119] ELISA Assays Validate Proteomics Data and Enable High Throughput Clinical Screening.

[0120] To validate the mass spectrometric findings, the inventors used commercially available ELISA assays to measure urinary levels of hemopexin (HPX) and ceruloplasmin (CP), 2 candidate biomarkers of memory impairment in children with OSA. As a control, the inventors also quantified urinary levels of uromodulin, a protein whose levels in CTRL, OSA-I and OSA-N subjects were unchanged. Since protein levels in urine are highly variable, and influenced by body fluid volume, all measurements were standardized against corresponding urinary creatinine levels (Garde, et al., 2004). ELISA assays reproduced the regulatory patterns of HPX, CP, and UMOD predicted by mass spectrometric analyses (FIG. **8**A-C). These findings provide strong validation for the proteomic and statistical methods for identifying candidate biomarkers of memory impairment in children with OSA. Moreover, the development of ELISA assays for HPX and CP enable high throughput clinical screening.

Example 8

Develop High Throughput ELISA Assays for Candidate Urinary Biomarkers of Declarative Memory Deficit in Children with OSA

[0121] Using discovery-based proteomics, the inventors identified 52 candidate biomarkers of declarative memory impairment in children with OSA and further validated the protein abundance (measured by mass spectrometry) changes for two of these proteins (HPX and CP) by ELISA. Validated candidate biomarkers will be used to develop a multivariate classifier (a combinatorial panel) whose predictive power will be interrogated in a larger, independent patient cohort using high throughput ELISA assays.

[0122] Experimental Design.

[0123] Studies will use pre-existing urine samples (stored at −80° C.) that were analyzed by proteomics to validate candidate biomarkers that distinguish OSA-I patients from CTRL and OSA-N subjects (see FIGS. **6-8**). Based on the statistical significance and magnitude of the change in urinary protein levels (assessed by the t-test and G-test), availability of ELISA-compatible antibodies and/or kits, and biological function, the inventors have selected 10 candidates for initial testing (Table 8).

TABLE 8

Candidates for ELISA assay development

Protein	G-test*	t-test	Function
KNG1	−91	10^{-6}	Coagulation
PIGR	−46	10^{-7}	Immunity
PROCR	−42	10^{-9}	Coagulation
HPX**	−29	10^{-6}	Iron metabolism
CP**	−18	10^{-8}	Iron metabolism
RNASE1	101	10^{-6}	Nucleotide metabolism
COL12A1	46	10^{-7}	Extracellular matrix
CD59	29	10^{-9}	Complement activation
APOH	17	10^{-6}	Lipid metabolism
CTBS	15	10^{-8}	Carbohydrate metabolism

*negative G-test = reduced in OSA-I relative to OSA-N

**urinary ELISA assays already developed

[0124] Quantification of Urinary Proteins by ELISA.

[0125] Urine proteins will be quantified using commercially available ELISAs for CP, PROCR, APOH, KNG1 (Assaypro), HPX (Innovative Research, Inc.), PIGR, RNASE1, COL12A1, CTBS (USCN Life Science), CD59 (Neobiolab), and creatinine (Abcam) according to the manufacturer's protocols. To account for variable hydration states, protein levels will be standardized to urine creatinine levels (Garde, et al., 2004) and statistical significance between the groups will be assessed by a two-tailed, Student's t-test. This will corroborate that the previously identified differentiation between case and control samples (i.e., OSA-I and OSA-N) is still present when the candidate biomarkers are measured using an independent technology (i.e., ELISA). The inventors have already confirmed the proteomics findings for HPX, CP, and UMOD in previously analyzed patients (FIG. **8**).

Example 9

Determine the Predictive Power of Candidate Urinary Biomarkers in a Larger, Independent Cohort of Children with OSA

[0126] Children going through the Pediatric Sleep Laboratory at the University of Chicago will undergo polysomnography, memory testing, and provide urine samples for biochemical analysis. Initial measurements will focus on HPX and CP, which the inventors have already validated by ELISA. Additional candidate biomarkers will be tested as ELISA assays are developed in Example 8.

[0127] Experimental Design.

[0128] Children fulfilling the inclusion criteria for this study will be recruited according to the institutional human studies guidelines. All participating children will be admitted to the Pediatric Sleep Laboratory at the University of Chicago for an overnight stay. OSA severity will be assessed by polysomnography, declarative memory will be assessed by the validated pictorial memory test (Kheirandish-Gozal, et al., 2010), and morning urine samples will be collected for biochemical analysis (FIG. **9**). Initial measurements will focus on HPX and CP, as the inventors have already developed ELISA assays for these candidate biomarkers. Additional candidates will be tested as ELISA assays are developed.

[0129] Patient selection. The population targeted for this study will consist of children ages 5-12 years who are referred for clinical evaluation of snoring at the University of Chicago Sleep Medicine Center. This facility evaluates in excess of 1,250 children per year, and approximately 80% of these have snoring and suspected sleep disordered breathing as their primary reason for clinical referral. Healthy children (n=50) will be recruited from schools or well-child clinics to serve as controls. Inclusion criteria for children with OSA will include children who snore frequently >3 times/week using the extensively validated questionnaire (Spruyt-Gozal, 2012). Exclusion criteria for control and OSA children will include the presence of significant genetic or craniofacial syndromes, diabetes, cystic fibrosis, cancer, or treatment with oral corticosteroids, antibiotics, or anti-inflammatory medications. Additionally, participants will be excluded if they suffer from any chronic psychiatric condition, have a genetic syndrome known to affect cognitive abilities, or are receiving medications that are known to interfere with memory or sleep onset or sleep architecture.

[0130] Overnight Polysomnography.

[0131] All participating children will undergo an overnight polysomnography (PSG) using state of the art methods (Montgomery-Downs, 2006). The severity of OSA will be quantified by the obstructive apnea-hypopnea index (AHI), which is defined as the number of obstructive apneas and hypopneas per hour of total sleep time (Grigg-Damberger, et al., 2007; Redline, et al., 2007).

[0132] Memory Recall Test.

[0133] To assess memory recall, a blinded investigator will implement a common method (Kheirandish-Gozal, et al., 2010) to evaluate children with OSA (FIG. **9**). Children will be shown a series of 26 colorful animal pictures, all of which are highly familiar to children (e.g. dog, cat, chicken, lion, elephant, giraffe, horse, cow, camel, fish, butterfly, etc.). Subjects will be allowed to look at each animal picture for 10 s. The child will initially identify the animal and then the investigator will also name each animal (while pointing them out) as further corroboration of the adequate recognition of the

animal in each picture. After all pictures have been shown, the book will be closed and the subjects will be given 2 min to freely recall any of the animals they could remember without looking at the pictures. One point will be awarded for every correct answer, and points will not be deducted for wrong answers and subjects will be told that they are allowed to repeat animal names if they wished to do so. After the first trial, the subjects will be allowed to look at the pictures again and go over the animal names. This process will be repeated a total of four times in the evening (acquisition phase), followed by a first recall test 10 min after completion of the fourth trial. During this 10-min interval the child will be allowed to watch TV. The morning after the sleep study, within 10-15 min of awakening, the subjects will be asked to recall the pictures that they remembered from the previous evening's trials, and the morning score will be calculated.

[0134] Urine Collection and Processing.

[0135] Mid-stream urine specimens will be collected as the first void in the morning after awakening or in the evening. To minimize protein degradation, samples (20 mL) will be immediately transferred into tubes containing the serine protease inhibitor PMSF (2 mM final concentration), and stored at −80° C. until analysis (Gozal, et al., 2009).

[0136] Development of a Multivariate Classifier.

[0137] Different multivariate classifiers (groups of candidate biomarkers) will be built using ELISA measurements that sequentially incorporate corroborated proteins to evaluate their complementary contribution to classifier performance. These multivariate classifiers will be constructed using linear discriminant analysis (McLachlan, 2004), which assigns a numerical weight to each biomarker that reflects its contribution (within the aggregated classifier score) to jointly differentiate OSA-I from OSA-N subjects.

[0138] Evaluation of Candidate Biomarkers and Classifier Performance.

[0139] The sensitivity and specificity of each individual candidate biomarker or each multivariate classifier (group of biomarkers) will be calculated on the basis of tabulating the number of correctly and incorrectly classified samples (ie. OSA-I versus OSA-N). Receiver operating characteristic (ROC) plots will be obtained by plotting all sensitivity values on the y-axis against their equivalent (1-specificity) values on the x-axis for all available thresholds. The overall accuracy of each test will be evaluated by area under the curve, as it provides a single measure that is not dependent on a particular threshold (Fawcett, et al., 2006). Unadjusted p-values will be calculated on the basis of the natural logarithm-transformed intensities and the Gaussian approximation to the t distribution. Statistical adjustment for multiple testing will be performed by the method described by Reiner and colleagues (Reiner, et al., 2003).

[0140] All of the compositions and/or methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of some embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

REFERENCES

[0141] The following references and any others listed herein, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference in their entirety.

[0142] Adachi, et al., *Genome Biol.* 7(9): R80, 2006.

[0143] Adedayo, et al., "Obstructive sleep apnea and dyslipidemia: evidence and underlying mechanism. *Sleep & Breathing,* 2012.

[0144] Becker, et al., *Cell Metab.* 11(2): 125-135, 2010.

[0145] Becker, et al., *PLoS ONE.* 7(3): e33297, 2012.

[0146] Benjamini, et al., *J Roy Stat Soc B Met.* 57(1):289-300, 1995.

[0147] Chen, et al., *Proteomics: Clin Apps.* 1: 577, 2007.

[0148] Chorostowska-Wynimko, et al., *J Physiol Pharmacol.* 56(Suppl 4): 71, 2005.

[0149] Christensen, et al., *PLoS Genet.* 5(8): e1000602, 2009.

[0150] Escudero, et al., "Machine learning-based method for personalized and cost-effective detection of Alzheimer's disease." *IEEE transactions on biomedical engineering.* 2012.

[0151] Garde, et al., *Ann Occup Hyg.* 48(2): 171-9, 2004.

[0152] Gozal, et al., *Am J Respir Crit Care Med.* 177(10): 1142-1149, 2008.

[0153] Gozal, et al., *Am J Respir Crit Care Med.* 177(4): 369-75, 2008.

[0154] Gozal, et al., *Am J Respir Crit Care Med.* 180(12): 1253-1261, 2009.

[0155] Gozal, et al., *Ann N Y Acad Sci.* 1264(1): 135-141, 2012.

[0156] Gozal, et al., *Curr Op Ped.* 20(6): 654-8, 2008.

[0157] Gozal, et al., *Pediatrics.* 126(5): e1161-7, 2010.

[0158] Gozal, et al., *Sleep Med.* 11(7): 708-13, 2010.

[0159] Heinecke, et al., *Bioinformatics.* 26(12): 1574-5, 2010.

[0160] Huttenhain, et al., *Sci Trans' Med.* 4(142): 142ra94, 2012.

[0161] Iber, et al., *The AASM manual for the scoring of sleep and associated events: rules, terminology, and technical specifications.* 2007.

[0162] Keller, et al., *Anal Chem.* 74(20): 5383-92, 2002.

[0163] Kentsis, *Ped Int: Off J Japan Ped Soc.* 53(1): 1-6, 2011.

[0164] Kersey, et al., *Proteomics.* 4: 1985-8, 2004.

[0165] Kim, et al., *Respir Physiol Neurobiol.* 178(3): 465-74, 2011.

[0166] Kushnir, et al., *J Biomol Tech.* 20(2): 101-8, 2009.

[0167] La Thangue, et al., *Nat Rev Clin Oncol.* 8(10): 587-96, 2011.

[0168] Leary, et al., *Sci Trans' Med.* 2: 20ra14, 2010.

[0169] Liu, et al. *Anal Chem.* 76(14): 4193-201, 2004.

[0170] Maere, et al., *Bioinformatics.* 21(16): 3448-9, 2005.

[0171] Montgomery-Downs, et al., *Pediatrics.* 117(3): 741-53, 2006.

[0172] Nesvizhskii, et al., *Anal Chem.* 75(17): 4646-58, 2003.

[0173] Old, et al. *Mol Cell Proteomics.* 4: 1487, 2005.

[0174] Rauch, et al., *JProteome Res.*5: 112, 2006.

[0175] Riaz, et al. *Diabetes Tech Thera.* 12(12): 979-88, 2010.
[0176] Siwy, et al., *Proteomics.* 5(5-6): 367-74, 2011.
[0177] Slupsky, et al., *Anal Chem.* 79(18): 6995-7004, 2007.
[0178] Soggiu, et al., "A discovery-phase urine proteomics investigation in type 1 diabetes" *Acta Diabetol.* (In press), 2012.
[0179] Stratz, et al., *Cardiol Rev.* 20: 111, 2012.
[0180] Thongboonkerd, et al., *JProteome Res.* 5(1): 183-91, 2006.
[0181] Verrills, et al., *Am J Respir Crit Care Med.* 183(12): 1633-43, 2011.
[0182] Von Kanel, et al., *Chest.* 131(3): 733-9.
[0183] Zengi, et al., *Clin Chem Lab Med: CCLM/FESCC.* 50: 529, 2012.
[0184] Zimmerli, et al., *Mol Cell Proteomics.* 7(2): 290-8, 2007.
[0185] Zoidakis, et al., *Mol Cell Proteomics.* 11(4): M111. 009449, 2012.
[0186] Zurbig, et al., *Diabetes.* 61(12): 3304-13, 2012.

1. A method for identifying a subject as having obstructive sleep apnea (OSA) comprising:

a) using a computer and an algorithm to evaluate previously measured expression levels of one or more products of one or more genes listed in Table 1 as compared to a control or reference level in a biological sample from the subject to calculate a risk score; and

b) identifying the subject as having OSA based on the risk score.

2. (canceled)

3. The method of claim **1**, wherein a risk score calculated from an elevated level of expression of the one or more products as compared to a control or reference level indicates that the subject is likely to have OSA.

4. The method of claim **1**, wherein a risk score calculated from a lower level of expression of the one or more products as compared to a risk score calculated from a control or reference level indicates that the subject is likely to have OSA.

5. The method of claim **1**, wherein the control is the level of expression of the one or more products in a control sample from a subject who is known not to have OSA.

6. The method of claim **1**, wherein the expression level of the one or more products is standardized against the level of expression of a corresponding standard product in the sample.

7. The method of claim **1**, wherein the one or more products are one or more proteins encoded by a gene selected from the group consisting of CD14, CTSB, HPX, DPP4, TTR, DEFB1|HBD1, FABP3, CP, and AZGP1.

8. The method of claim **7**, wherein the one or more products are one or more proteins encoded by one or more genes selected from the group consisting of HPX, DPP4, CP, and AZGP1.

9. The method of claim **1**, wherein the level of expression is measured for at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 products.

10. The method of claim **1**, further comprising obtaining the biological sample from the subject.

11. The method of claim **1**, wherein the sample is a urine sample.

12. The method of claim **6**, wherein the corresponding standard product is urinary creatine.

13-23. (canceled)

24. The method of claim **1**, further comprising performing a sleep study on the subject identified as having OSA.

25-27. (canceled)

28. The method of claim **24**, wherein the sleep study comprises using an actigraph.

29-33. (canceled)

34. A method for evaluating obstructive sleep apnea in a subject comprising subjecting the subject to a sleep study after the subject is determined to have sleep apnea based on the use of a computer and an algorithm to evaluate previously measured expression levels of one or more genes listed in Table 1 in a urine sample obtained from the subject.

35. The method of claim **34**, wherein the sleep study comprises one of more of the following: using a polysomnogram (PSG), performing a multiple sleep latency test (MSLT), or performing a maintenance of wakefulness test (MWT).

36. The method of claim **34**, wherein the sleep study comprises measuring one or more physiological characteristics of the subject when sleeping.

37. (canceled)

38. The method of claim **34**, wherein the sleep study comprises using an actigraph.

39. The method of claim **1**, wherein the subject is a child.

40. The method of claim **1**, wherein calculating a risk score comprises applying model coefficients to each of the levels of expression.

41. The method of claim **1**, wherein identifying the subject as having OSA comprises identifying the patient as having a risk score indicative of 50% chance or greater of having OSA.

* * * * *