

Supplemental Material

Validating Discriminative Signatures for Obstructive Sleep Apnea in Exhaled Breath

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The following inclusion and exclusion criteria were applied for participant screening as listed in Table S1.

Table S1: Inclusion and exclusion criteria for study participants.

	Inclusion Criteria			Exclusion Criteria
study cohort	Age	AHI	current condition	condition/ comorbidities
OSA untreated	≥ 18	≥ 15 ^a	OSA without CPAP treatment or other treatments for OSA	central or mixed sleep apnea pregnancy malignom lung cancer lung transplant
OSA treated	≥ 18	≥ 15 ^a	OSA with CPAP treatment	interstitial lung disease chronic obstructive lung disease (COPD, asthma) hypoventilation (e.g., adipositas hypoventilation)
healthy controls	≥ 18	≤ 5	no clinical OSA	chronic inflammatory disease relevant congenital defect renal failure drug abuse

AHI: apnea/hypopnea index (events/h), OSA: obstructive sleep apnea, CPAP: continuous positive airway pressure, COPD: chronic obstructive pulmonary disease, ^aAHI values at diagnosis.

Table S2: Most prevalent comorbidities and concomitant medications.

	OSA untreated (N=43)	OSA treated (N=43)	Control subjects (N=32)
Comorbidities			
Depression	6 (14)	4 (9)	6 (19)
Diabetes mellitus	8 (19)	6 (14)	2 (6)
Hypertension	19 (44)	23 (53)	10 (31)
Thyroid dysfunction	1 (2)	3 (7)	3 (9)
Concomitant medication			
Antidepressants	7 (16)	8 (19)	9 (28)
Antidiabetics	9 (21)	7 (16)	4 (13)
Antihypertensiva	27 (63)	29 (67)	13 (41)

Values are presented as number (%) per study group, OSA = obstructive sleep apnea.

Confirmed OSA-associated metabolites

Table S3: Confirmed m/z features in the validation study.

	m/z	metabolite	Signif.	in untreated OSA	P value	FC
1	69.0693	n.a.	no		0.854	1.02
2	71.0487	n.a.	no		0.739	1.31
3	79.0409	n.a.	no		1.000	1.19
4	81.0328	n.a.	yes	increased	0.012	1.49
5	81.0525	n.a.	no		0.818	1.23
6	83.0854	n.a.	no		0.688	1.32
7	87.0439	n.a.	no		0.083	1.22
8	91.0413	n.a.	no		0.973	1.01
9	93.0574	n.a.	no		0.484	1.53
10	95.0494	n.a.	yes	increased	0.022	1.33
11	97.0647	2-ethylfuran	no		0.079	1.44
12	101.0598	n.a.	yes	increased	0.031	1.22
13	102.0913	2-pentenal ([M+NH4]+)	no		0.845	1.03
14	105.0551	n.a.	no		0.211	1.14
15	109.0648	n.a.	no		0.558	0.81
16	111.0803	2-propylfuran	no		0.087	1.49
17	123.1165	n.a.	no		0.801	1.13
18	125.0958	2-butylfuran	yes	increased	0.029	1.44
19	128.0701	n.a.	yes	increased	0.006	1.49
20	129.0908	4-hydroxy-2-heptenal	no		0.323	1.59
21	136.0216	benzothiazole	no		0.081	1.37
22	137.0593	n.a.	no		0.081	1.77
23	138.0571	n.a.	no		0.081	0.96
24	139.1116	2-pentylfuran	no		0.491	1.15
25	143.1063	4-hydroxy-2-octenal ([M+NH4]+)	no		0.139	3.25
26	149.0971	n.a.	no		0.927	1.37
27	151.1116	n.a.	no		0.696	0.77
28	152.0699	n.a.	yes	increased	0.018	1.48
29	160.0611	n.a.	no		0.073	1.25
30	160.1329	4-hydroxy-2-octenal	no		0.054	6.13
31	167.1064	n.a.	no		0.513	0.92
32	169.0867	n.a.	yes	increased	0.020	1.36
33	175.1117	n.a.	yes	increased	0.037	1.42
34	195.1379	4-(hexyloxy)phenol	yes	increased	0.042	1.32
35	208.1776	n.a.	no		0.075	2.46
36	209.1168	n.a.	no		0.070	1.35
37	209.1536	n.a.	no		0.265	0.86
38	221.1532	n.a.	no		0.183	1.45

39	221.19	n.a.	no	0.881	1.65
40	223.1327	n.a.	no	0.155	1.34
41	237.1123	n.a.	no	0.145	0.95
42	251.1641	n.a.	no	0.318	3.61

Table S4: Cohort characteristics with stratification criterion applied for the treated OSA group.

	OSA untreated	OSA treated ^a
N	43	29
Age (y)	61 (55.0, 69)	65 (59, 70)
Sex, male, N (%)	36 (84)	25 (86)
BMI (kg/m ²)	29.5 (27.0, 34.0)	30.1 (27.8, 32.6)
AHI at diagnosis (events/h)	30.2 (24.0, 45.0)	35.0 (24.0, 13.5)
AHI at visit (events/h)	28.0 (18.8, 40.0)	1.2 (0.8, 4.4)
ESS at visit, points	5.0 (3.0, 9.0)	6.0 (2.5, 8.5)

BMI: body mass index, AHI: apnea/hypopnea index, ESS: Epworth Sleepiness Scale. Values are presented as median \pm interquartile range (IQR) unless otherwise stated. ^astratification: averaged CPAP usage \geq 5 h/night.

Testing for normality and significant differences

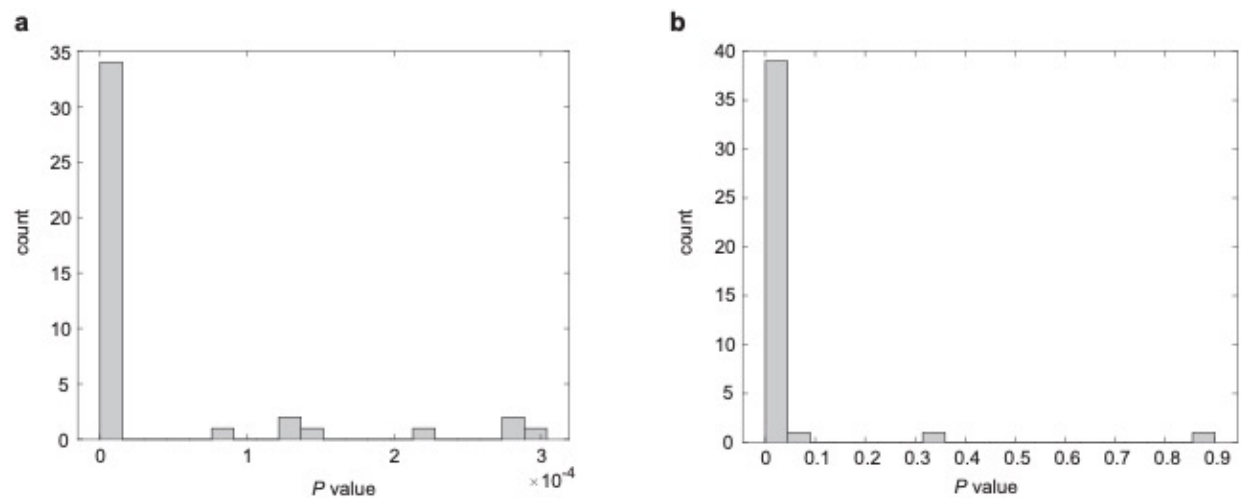


Figure S1: Shapiro-Wilk test for normality on the detected marker intensities in **a)** untreated and **b)** treated OSA. Stratification criterion: ≥ 5 h/night averaged CPAP usage.

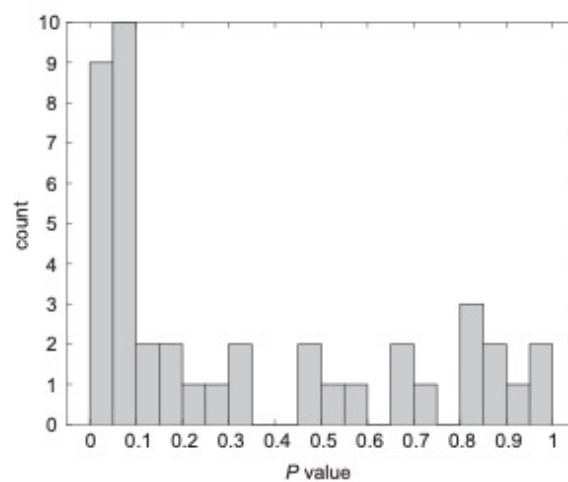


Figure S2: Mann-Whitney U test to determine significantly different OSA-associated metabolites between untreated and treated OSA patients. Stratification criterion: ≥ 5 h/night averaged CPAP usage, statistically significant markers: $P < 0.05$.

Classification performance

- 1) Prediction for patients with treated and untreated OSA, unbalanced group size

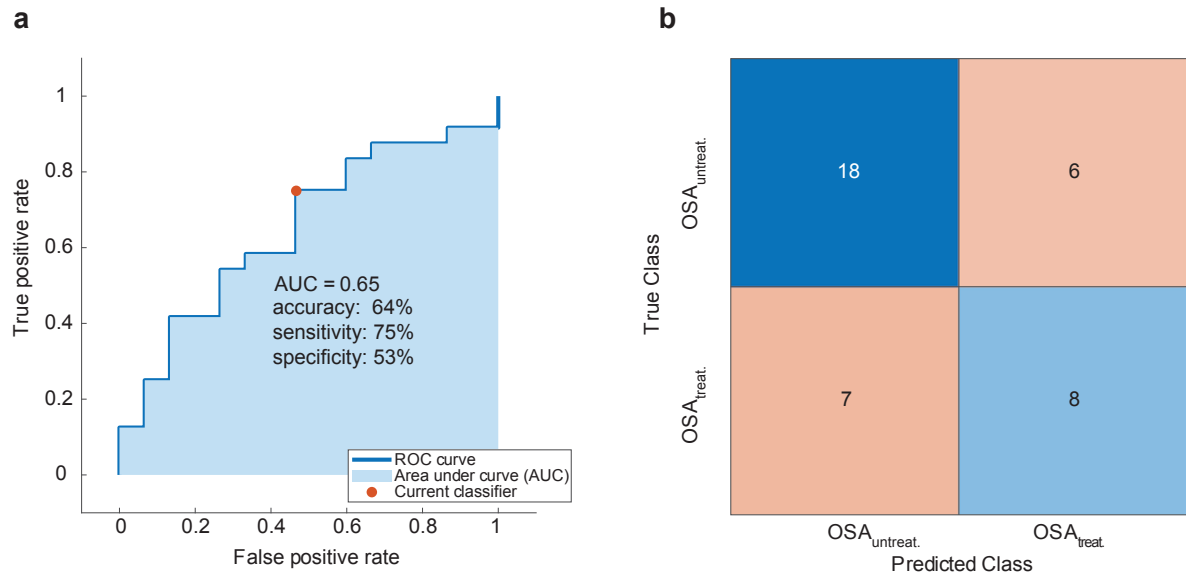


Figure S3: Classification performance of the 42 detected features for untreated OSA (OSA_{untreat.}, N=24) and treated OSA (OSA_{treat.}, N=15). Stratification criteria for the treated OSA group: ≥ 5 h/night averaged CPAP usage, and for both groups: AHI>30, or AHI>10 and ESS>10. **a)** Receiver operating characteristic (ROC) curve for OSA prediction from a 10-fold cross-validation applying the k-nearest neighbors algorithm resulted in an averaged area under the curve (AUC) of 0.65, **b)** confusion matrix for the prediction of untreated and treated OSA.

2) Prediction for patients with untreated OSA and control subjects

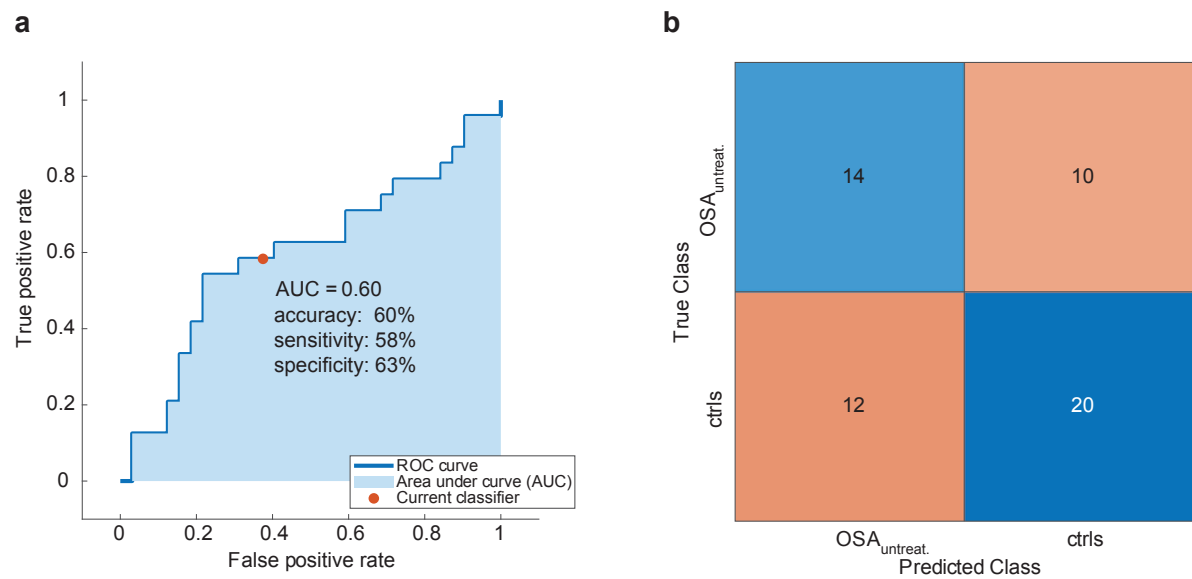


Figure S4: Classification performance of the 42 detected features for untreated OSA (N=24) and control subjects (ctrls, N=32). Stratification criteria for untreated OSA (OSA_{untreat.}): AHI>30, or AHI>10 and ESS>10. **a)** Receiver operating characteristic (ROC) curve for OSA prediction from a 10-fold cross-validation applying the ensemble methods resulted in an averaged area under the curve (AUC) of 0.60, **b)** confusion matrix for the prediction of untreated OSA and controls.

3) Predictions with different study groups

Table S5: Classification performances of 42 confirmed markers applied on the different study groups.

	N (OSA)	N (OSA _{treat.})	N (ctrls)	AUC	accuracy (%)	sensitivity (%)	specificity (%)
a) OSA, OSA _{treat.}							
a.1) unbalanced	24	15	0	0.65	64	75	53
a.2) balanced	15	15	0	0.80	77	73	80
b) OSA, ctrls							
b.1) unbalanced	24	0	32	0.60	60	58	63
b.2) balanced	24	0	24	0.61	60	63	58

Applied stratification criteria for untreated OSA (OSA) and treated OSA (OSA_{treat.}): AHI>30, or AHI>10 and ESS>10, additional for treated OSA: ≥ 5 h/night CPAP usage. AUC: area under the curve.