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Original Article

Urinary biomarkers and obstructive sleep apnea in patients with Down syndrome

Ibrahim Elsharkawi [a], David Gozal [b], Eric A. Macklin [c], Lauren Voelz [d], Gil Weintraub [e], Brian G. Skotko [a, f, *]

[a] Down Syndrome Program, Division of Genetics, Department of Pediatrics, Massachusetts General Hospital, Boston, MA, USA
[b] Department of Pediatrics, University of Chicago, Chicago, IL, USA
[c] Biostatistics Center, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA
[d] Down Syndrome Program, Division of Developmental Medicine, Department of Medicine, Boston Children's Hospital, Boston, MA, USA
[e] Beth Israel Deaconess Medical Center, Boston, MA, USA
[f] Department of Pediatrics, Harvard Medical School, Boston, MA, USA

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ABSTRACT

Study objectives: The study aimed to compare urinary biomarkers in patients with Down syndrome (DS) with and without obstructive sleep apnea (OSA) to those of age- and sex-matched neurotypically developing healthy controls (HC). We further investigated whether we could predict OSA in patients with DS using these biomarkers.

Methods: Urine samples were collected from 58 patients with DS the night before or the morning after their scheduled overnight polysomnogram or both, of whom 47 could be age- and sex-matched to a sample of 43 HC. Concentrations of 12 neurotransmitters were determined by enzyme-linked immunosorbent assay. Log-transformed creatinine-corrected assay levels were normalized. Normalized *z*-scores were compared between patients with DS vs. HC, between patients with DS with vs. without OSA, and to derive composite models to predict OSA.

Results: Most night-sampled urinary biomarkers were elevated among patients with DS relative to matched HC. No urinary biomarker levels differed between patients with DS with vs. without OSA. A combination of four urinary biomarkers predicted AHI > 1 with a positive predictive value of 90% and a negative predictive value of 68%.

Conclusions: Having DS, even in the absence of concurrent OSA, is associated with a different urinary biomarker profile when compared to that of HC. Therefore, while urinary biomarkers may be predictive of OSA in the general pediatric population, a different approach is needed in interpreting urinary biomarker assays in patients with DS. Certain biomarkers also seem promising to be predictive of OSA in patients with DS.

No clinical trial was indicated in the undertaking of this work.

* Corresponding author. Massachusetts General Hospital, 185 Cambridge Street, Room 2222, Boston, MA 02114, USA. Fax: +1 (617) 726 1566.
E-mail address: bskotko@mgh.harvard.edu (B.G. Skotko).

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Statement of significance

Urinary biomarker assays have been studied in the general pediatric population and measured as predictors of obstructive sleep apnea (OSA) compared to the current gold standard of polysomnography, which is often laborious and challenging. OSA is particularly prevalent in patients with Down syndrome, and polysomnography poses a greater challenge in these individuals. Urinary biomarkers have never been specifically studied in this population until now. We report that the urinary biomarker profile in an individual with DS, regardless of OSA status, differs from the profile in the neurotypical population and thus must be approached and interpreted with caution. Despite this variation, certain urinary biomarkers hold promise as predictors of OSA in patients with Down syndrome.

1. Introduction

OSA is a chronic condition characterized by episodes of either partial or complete obstruction of the upper airway during sleep, disrupting sleep integrity and impairing ventilation and gas exchange. If left untreated, OSA may lead to deleterious effects on cardiovascular function and neurocognitive and behavioral performance, along with impaired somatic growth [1–4].

OSA is frequently present in the general pediatric population, with prevalence estimates reported between 1% and 4% [5]. Certain pediatric populations, however, are more susceptible to develop OSA, with prevalence estimates as high as 55%–97% in children with Down syndrome (DS) [6–12]. This inordinately high OSA frequency has been attributed to the skeletal and soft tissue structural alterations that predispose to upper airway obstruction, along with potential perturbations in neural reflexes underlying the maintenance of upper airway patency. Evaluation of OSA in patients with DS is often prompted by clinical suspicion. However, parental perception of sleep disturbance is poorly correlated with abnormal polysomnography studies [9]. Overnight polysomnography remains the gold standard for diagnostic evaluation [13], and the American Academy of Pediatrics recommends that all persons with DS should have a sleep study by age four or sooner, if symptomatic [14].

Although polysomnography is a painless and noninvasive procedure, it can be challenging in children in general, and more particularly in patients with DS. As a result, alternative screening methods have been sought in recent years. In the general pediatric population, Gozal et al. demonstrated that children with polysomnographically confirmed OSA consistently expressed specific alterations in urinary concentrations of specific proteins when compared to children without OSA [15]. Using two-dimensional differential gel electrophoresis followed by mass spectrometry on morning urine samples, changes in uromodulin, urocortin 3, orosomucoid, and kallikrein emerged as reliable discriminators in children with OSA, aged 2–9 years [15]. Kheirandish-Gozal et al. also studied urinary neurotransmitters in 50 patients with OSA and 20 controls aged between 3 and 12 years. They found that urinary epinephrine and norepinephrine levels increased overnight in children with OSA, whereas taurine level decreased in this population compared to controls [16]. For both studies, the accuracy of the urinary biomarkers in predicting OSA was enhanced when the biomarkers were used in combination [15,16]. De Luca Canto et al. recently conducted a systematic review and meta-analysis, assessing the diagnostic capability of various biomarkers in predicting OSA [17]. When used as a signature panel, the set of candidate biomarkers was accurate enough to be potentially used as a diagnostic test, with a sensitivity of 100% and specificity of 97% in children [17]. Previous work has assessed urinary catecholamines in patients with DS [18], and another study assessed plasma amino acids in patients with DS [19], but these research studies did not examine their potential application for screening OSA.

We hypothesized that previously identified urinary biomarkers for pediatric OSA may also predict OSA among children with DS. This study aimed to compare an array of such candidate biomarkers in urine samples from patients with DS, both with and without OSA, to those of neurotypical controls, followed by a critical assessment of their OSA diagnostic capability.

2. Materials and methods

2.1. Patients

We enrolled 130 patients with DS in a broader study of OSA prediction [20]. Of the 130 participants, 58 provided a nighttime or morning urine sample or both. Those who were unable to provide a sample were either too young for feasible collection or unable to micturate before or after the sleep study. Of the 58, one did not complete a sleep study and was excluded from our analysis. A subset of 47 patients with DS could be age- and sex-matched to 43 healthy controls (HC) from whom samples were separately collected. Demographic data on age, sex, and racial/ethnic background were collected. Race and ethnicity were defined by the categorizations preferred by the NIH and the US Census. Additionally, BMI results were calculated for all patients with DS and were converted to percentiles using CDC 2000 growth curves. The American Academy of Pediatrics recommends using the standard National Center for Health Statistics BMI curves for patients with DS [14,21]. Mallampati and Brodsky/Friedman classification of tonsil size was assessed during physical exams for patients with DS.

The study was approved by the institutional review boards of Boston Children's Hospital (protocol 10-03-0092) and Massachusetts General Hospital (protocol 2012P002062). HC children were recruited under a separate protocol at the University of Chicago (protocol 09-115-B). These HC children resided in Chicago and were recruited through community announcements and distribution of materials in the Well Child Clinic at the University of Chicago Medical Center. All children were considered healthy, did not have any identifiable risk factors for the development of OSA, did not suffer from any specific disorder, and did not snore according to their responses to a validated questionnaire [22].

2.2. Urine collection

Each participant was scheduled for an overnight polysomnogram. On the evening before and the morning after their polysomnograms, they provided a urine sample, which was analyzed using a previously validated, multiplexed, enzyme-linked immunosorbent assay method for assessment of several neurotransmitters as previously reported (NeuroScience, Inc., Osceola, WI) [16]. Parameters Assayed included epinephrine, norepinephrine, dopamine, serotonin, glycine, taurine, γ-aminobutyric acid (GABA), glutamate, phenylethylamine (PEA), aspartic acid, histamine, 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxyindoleacetic acid (5-HIAA), tyramine, and tryptamine. We selected this panel of

biomarkers based on previous work, which demonstrated that some of these biomarkers were associated with increased risk for cognitive dysfunction [19], a significant concern in patients with DS who already have a baseline of cognitive disabilities. All samples were assayed in duplicate, and values were retained if they were within 10% of each other. Urine creatinine (Crtn) levels were measured for each sample, and individual urine neurotransmitter levels were corrected for corresponding urine creatinine concentration. The creatinine-corrected assay levels from the sample of 43 age- and gender-matched HC children were used to calculate normative values.

2.3. Statistical analyses

Demographic characteristics of the patients with DS and HC children were compared by Fisher's exact test and t-tests. Nighttime, morning, and the ratio of morning to nighttime creatinine-corrected assay levels were assessed. Z-scores for patients with DS, aged 3–12 years, were calculated after log transformation by first subtracting the predicted mean level based on a participant's age and regression coefficients from a model fit to the HC data and then dividing by the square root of residual variance from the regression model. Z-scores were compared between DS and HC samples and between patients with DS with and without at least mild OSA (apnea-hypopnea index, AHI > 1) by two-sample t-test with step-down Bonferroni adjusted two-sided p values to control for multiple comparisons across all neurotransmitters and both sampling times and their ratio.

The predictive association between age- and gender-adjusted Z-scores of each assay and sample measure and OSA status (AHI > 1 and AHI > 5) was assessed by logistic regression and summarized as area under the receiver-operator characteristic curve (ROC AUC). Assays that were significantly associated with OSA status on univariate analysis were used to construct a composite predictive model. The optimal threshold for dichotomizing the range of each significant assay was selected to maximize the sum of sensitivity and specificity. A final composite score was calculated as the number of significant assays that met the cutoff criterion for each assay-specific threshold. The performance of this composite score was summarized by its ROC AUC and by the sensitivity, specificity, and positive and negative predictive values at all possible scores.

3. Results

3.1. Demographics and clinical characteristics

Most participants (61%) were identified as white (Table 1). The mean age of patients with DS was 9.1 ± 4.0 years and that of HC was 6.5 ± 4.0 years. Moreover, BMI status, Mallampati tonsil classification, Brodsky/Friedman tonsil classification, and AHI category were identified for each patient with DS in our population. The most common BMI status was “normal,” with the BMI of 40% of the patients with DS falling between the 5th and 85th percentile on neurotypical growth curves. A Mallampati level II and Friedman level III were the most common levels observed in our patients at 53% and 59%, respectively (Table 1). Approximately 57% had no OSA, 28% had mild OSA (1 < AHI ≤ 5), 2% had moderate OSA (5 < AHI of ≤10), and 13% had severe OSA (AHI > 10).

Obese patients with DS had higher median AHI and were more likely to have at least mild OSA (AHI > 1) than nonobese patients with DS. However, BMI percentile and AHI were modestly and not

Table 1
Demographic data and clinical characteristics of individuals with Down syndrome (DS) and healthy controls (HC).

Variable	Level	Diagnosis			
		Overall % (N)	DS% (N)	HC% (N)	p Value
Overall participants		100% (90)	100.0% (47)	100.0% (43)	–
Age group	3 or 4 yrs	23.3% (21)	25.5% (12)	20.9% (9)	0.020
	5 or 6 yrs	28.8% (26)	17.0% (8)	41.9% (18)	
	7 or 8 yrs	27.7% (25)	27.7% (13)	27.9% (12)	
	9, 10, 11 yrs	20.0% (18)	29.8% (14)	9.3% (4)	
AHI category	AHI ≤ 1	77.8% (70)	57.4% (27)	100% (43)	–
	1 < AHI ≤ 5	14.4% (13)	27.7% (13)	.% (0)	
	5 < AHI ≤ 10	1.1% (1)	2.1% (1)	.% (0)	
	AHI > 10	6.7% (6)	12.8% (6)	.% (0)	
Gender	Male	56.6% (51)	61.7% (29)	51.2% (22)	0.39
	Female	43.3% (39)	38.3% (18)	48.8% (21)	
Race	White	61.1% (55)	65.9% (27)	65.1% (28)	<0.001
	Black	18.8% (17)	4.9% (2)	34.9% (15)	
	Asian	5.5% (5)	12.2% (5)	0.0% (0)	
	Other race	5.5% (5)	12.2% (5)	0.0% (0)	
	Multiracial	2.2% (2)	4.9% (2)	0.0% (0)	
	[missing]	6.6% (6)	.% (6)	.% (0)	
Ethnicity	Not Hispanic	82.2% (74)	76.7% (33)	95.3% (41)	0.026
	Hispanic/Latino	13.3% (12)	23.3% (10)	4.7% (2)	
	[missing]	4.4% (4)	.% (4)	.% (0)	
BMI category	Normal (5% ≤ BMI < 85%)	–	40.4% (19)	.% (0)	–
	Overweight (85% ≤ BMI < 95%)	–	34.0% (16)	.% (0)	
	Obese (BMI ≥ 95%)	–	25.5% (12)	.% (0)	
Mallampati classification of tonsil size	I	–	11.1% (5)	.% (0)	–
	II	–	53.3% (24)	.% (0)	
	III	–	33.3% (15)	.% (0)	
	IV	–	2.2% (1)	.% (0)	
	[missing]	–	.% (2)	.% (43)	
Brodsky and Friedman classification of tonsil size	I	–	4.3% (2)	.% (0)	–
	II	–	32.6% (15)	.% (0)	
	III	–	58.7% (27)	.% (0)	
	IV	–	4.3% (2)	.% (0)	
	[missing]	–	.% (1)	.% (43)	

significantly correlated (rank correlation r = 0.21, p = 0.16) (Supplementary Materials).

3.2. Urinary biomarkers in patients with Down syndrome

In patients with DS, regardless of OSA status, biomarker profiles differed significantly from neurotypical HC. Various biomarkers were reported to have mean Z-scores of >1.0 (Table 2). Of these, statistically significant Z-scores with an adjusted p value of <0.05 include nighttime epinephrine per Crtn (1.24), nighttime norepinephrine per Crtn (1.05), nighttime dopamine per Crtn (0.99), nighttime DOPAC per Crtn (0.80), nighttime serotonin per Crtn (1.65), nighttime 5-HIAA per Crtn (1.51), nighttime GABA per Crtn (1.24), nighttime glutamate per Crtn (1.51), nighttime PEA per Crtn (1.10), and nighttime histamine per Crtn (1.22).

3.3. Pair-wise comparisons by OSA status

When comparing patients with DS with at least mild OSA (AHI > 1) to patients with DS without OSA, no individual urinary biomarker assays differed significantly (Table 2).

3.4. Performance of urinary biomarkers as a screening tool

Levels of four biomarkers were significant univariate predictors of at least mild OSA status (AHI > 1) among patients with DS: nighttime norepinephrine with an ROC AUC of 0.68, AM/PM norepinephrine with an ROC AUC of 0.74, AM/PM dopamine with an ROC AUC of 0.69, and AM/PM taurine with an ROC AUC of 0.72. The levels of two biomarkers were significant univariate predictors of at least moderate OSA status (AHI > 5) among patients with DS: nighttime taurine with an ROC AUC of 0.76 and morning taurine with an ROC AUC of 0.94 (Supplementary Materials).

Rules for scoring abnormal urinary biomarkers levels are defined in Table 3. For example, a nighttime norepinephrine z-score <0.706 (criterion #1) would contribute one unit to an individual's composite score. An individual's score was calculated as the count of the number of biomarkers that met cutoff criteria. The operating characteristics of these composite scores are provided in Table 4. For example, a composite score of 4 yielded a positive predictive value (PPV) of 90% and a negative predictive value (NPV) of 68% in this sample. Depending on the two-biomarker rules for predicting at least moderate OSA (AHI > 5), a composite score of 2 yielded a PPV of 67% and an NPV of 100% in this sample. The ROC curves, plotting true positive rate versus false positive rate, are shown in Fig. 1.

Table 3
Component criteria of urinary biomarker prediction models.

Outcome	Assay	Direction	Threshold z-score
AHI > 1	P.M. Norepinephrine per Crtn	<	0.706
	A.M./P.M. Norepinephrine per Crtn	>	−1.092
	A.M./P.M. Dopamine per Crtn	>	−1.457
	A.M./P.M. Taurine per Crtn	>	0.072
AHI > 5	P.M. Taurine per Crtn	>	0.082
	A.M. Taurine per Crtn	>	1.020

Crtn (Creatinine).

4. Discussion

This study indicates that having DS, regardless of the presence or absence of concurrent OSA, leads to urinary biomarker findings that vary from the typical urinary biomarker patterns that can be observed in the general healthy pediatric population. As such, standard urinary biomarkers should be interpreted with caution in children with DS, given that elevated levels of particular urinary biomarkers in an individual with DS do not necessarily imply co-existing OSA, as might be the case in the general pediatric population. Nevertheless, certain urinary biomarkers can still be predictive of OSA in the DS population.

In the general pediatric population, the presence of OSA is mostly associated with an elevation in urinary biomarker levels. In patients with DS without OSA, many of these urinary biomarkers

Table 2
Urinary biomarker z-score estimates among participants with DS overall and by OSA status.

Variable	Overall for participants with DS			OSA vs. no OSA in participants with DS			
	DS	Nom P-val	Adj P-val	OSA	No OSA	Nom P-val	Adj P-val
P.M. Epinephrine per Crtn	1.24 ± 1.23 (−1.12, 4.16)	<0.001	<0.001[a]	1.07 ± 1.28 (−1.12, 4.16)	1.37 ± 1.20 (−0.80, 4.07)	0.42	>0.99
P.M. Norepinephrine per Crtn	1.05 ± 1.24 (−1.56, 5.16)	<0.001	<0.001[a]	0.67 ± 1.29 (−1.56, 4.55)	1.36 ± 1.13 (0.07, 5.16)	0.066	>0.99
P.M. Dopamine per Crtn	0.99 ± 1.20 (−1.66, 5.26)	<0.001	0.002[a]	0.79 ± 1.40 (−1.66, 5.26)	1.15 ± 1.02 (0.10, 4.48)	0.31	>0.99
P.M. DOPAC per Crtn	0.80 ± 1.06 (−1.81, 3.49)	<0.001	0.013[a]	0.47 ± 1.02 (−1.81, 2.28)	1.07 ± 1.04 (−0.83, 3.49)	0.063	>0.99
P.M. Serotonin per Crtn	1.65 ± 1.19 (−0.47, 5.19)	<0.001	<0.001[a]	1.52 ± 1.16 (−0.47, 4.36)	1.76 ± 1.22 (−0.43, 5.19)	0.49	>0.99
P.M. 5-HIAA per Crtn	1.51 ± 1.30 (−2.58, 4.14)	<0.001	<0.001[a]	1.25 ± 1.39 (−2.58, 3.59)	1.73 ± 1.20 (−0.00, 4.14)	0.22	>0.99
P.M. Glycine per Crtn	0.66 ± 1.55 (−2.36, 5.17)	0.028	0.28	0.23 ± 1.70 (−2.36, 4.73)	1.02 ± 1.35 (−1.18, 5.17)	0.09	>0.99
P.M. Taurine per Crtn	0.07 ± 1.35 (−2.43, 3.58)	0.77	>0.99	−0.01 ± 1.55 (−2.43, 3.58)	0.14 ± 1.19 (−2.10, 2.66)	0.71	>0.99
P.M. GABA per Crtn	1.24 ± 1.47 (−3.02, 5.27)	<0.001	<0.001[a]	0.87 ± 1.69 (−3.02, 5.27)	1.55 ± 1.20 (−0.31, 5.13)	0.12	>0.99
P.M. Glutamate per Crtn	1.51 ± 1.24 (−2.00, 4.85)	<0.001	<0.001[a]	1.22 ± 1.46 (−2.00, 4.85)	1.75 ± 0.98 (−0.32, 3.97)	0.16	>0.99
P.M. PEA per Crtn	1.10 ± 1.39 (−2.62, 4.64)	<0.001	0.002[a]	0.85 ± 1.65 (−2.62, 4.22)	1.31 ± 1.12 (0.03, 4.64)	0.28	>0.99
P.M. Histamine per Crtn	1.22 ± 1.38 (−1.08, 5.98)	<0.001	<0.001[a]	0.99 ± 1.36 (−1.08, 4.49)	1.41 ± 1.39 (0.00, 5.98)	0.32	>0.99
A.M. Epinephrine per Crtn	0.24 ± 1.12 (−3.73, 2.33)	0.30	>0.99	−0.04 ± 1.29 (−3.73, 1.85)	0.46 ± 0.93 (−1.48, 2.33)	0.15	>0.99
A.M. Norepinephrine per Crtn	0.02 ± 0.62 (−1.21, 1.60)	0.90	>0.99	0.11 ± 0.60 (−0.96, 1.30)	−0.05 ± 0.64 (−1.21, 1.60)	0.40	>0.99
A.M. Dopamine per Crtn	0.10 ± 0.88 (−1.30, 1.94)	0.64	>0.99	0.12 ± 0.94 (−1.29, 1.72)	0.08 ± 0.85 (−1.30, 1.94)	0.87	>0.99
A.M. DOPAC per Crtn	0.31 ± 0.66 (−1.64, 1.59)	0.10	0.94	0.24 ± 0.70 (−1.64, 1.21)	0.36 ± 0.64 (−0.66, 1.59)	0.56	>0.99
A.M. Serotonin per Crtn	0.49 ± 0.86 (−1.34, 2.01)	0.021	0.23	0.39 ± 0.82 (−1.14, 1.83)	0.56 ± 0.89 (−1.34, 2.01)	0.53	>0.99
A.M. 5-HIAA per Crtn	0.55 ± 0.76 (−1.01, 2.54)	0.007	0.10	0.31 ± 0.77 (−1.01, 1.74)	0.73 ± 0.73 (−0.50, 2.54)	0.082	>0.99
A.M. Glycine per Crtn	0.10 ± 0.87 (−2.06, 2.03)	0.62	>0.99	0.07 ± 0.96 (−2.06, 1.51)	0.13 ± 0.82 (−0.96, 2.03)	0.82	>0.99
A.M. Taurine per Crtn	−0.18 ± 1.17 (2.15, 2.18)	0.45	>0.99	0.11 ± 1.29 (−2.15, 2.18)	−0.40 ± 1.03 (−1.95, 1.53)	0.16	>0.99
A.M. GABA per Crtn	−0.12 ± 0.91 (2.28, 1.44)	0.58	>0.99	−0.21 ± 1.14 (−2.28, 1.44)	−0.04 ± 0.71 (−1.16, 1.27)	0.56	>0.99
A.M. Glutamate per Crtn	0.60 ± 0.96 (−1.40, 2.76)	0.007	0.10	0.56 ± 1.06 (−1.40, 2.45)	0.64 ± 0.90 (−0.69, 2.76)	0.79	>0.99
A.M. PEA per Crtn	−0.12 ± 1.06 (2.10, 1.78)	0.60	>0.99	−0.21 ± 1.05 (−2.10, 1.42)	−0.05 ± 1.08 (−1.72, 1.78)	0.62	>0.99
A.M. Histamine per Crtn	0.51 ± 0.83 (−0.70, 3.18)	0.015	0.18	0.53 ± 0.78 (−0.69, 2.03)	0.48 ± 0.88 (−0.70, 3.18)	0.85	>0.99

[a] Step-down Bonferroni adjusted p ≤ 0.05; Crtn (Creatinine).

Table 4
Performance characteristics of urinary biomarker prediction models for obstructive sleep apnea (OSA) outcome.

OSA outcome	Cut-off criterion	Predicted positive		Predicted negative		Sensitivity	Specificity	PPV	NPV
		True positive	False positive	True negative	False negative				
AHI > 1	≥1	17	11	9	1	94.4%	45.0%	60.7%	90.0%
	≥2	16	9	11	2	88.9%	55.0%	64.0%	84.6%
	≥3	11	4	16	7	61.1%	80.0%	73.3%	69.6%
	≥4	9	1	19	9	50.0%	95.0%	90.0%	67.9%
AHI > 5	≥1	6	13	19	0	100.0%	59.4%	31.6%	100.0%
	≥2	6	3	29	0	100.0%	90.6%	66.7%	100.0%

were elevated relative to controls even among patients without OSA. One might expect that the presence of both DS and OSA would further elevate urinary biomarkers; however, this was not the case. In fact, a polysomnographic diagnosis of OSA in patients with DS was not associated with a significant difference in biomarker profile when each biomarker was examined individually. However, using a composite score that summarized abnormal levels of several biomarkers was predictive of OSA in patients with DS. A previous study demonstrated that overnight sympathetic activation is reduced in response to obstructive events during NREM sleep in patients with DS when compared to neurotypically developing individuals. This resulted in reduced overnight urinary catecholamines in patients with DS [18]. This is consistent with some of the results in our study, where we observed a reduction in urinary biomarkers in patients with DS when compared to those in HC. Further research is needed to further explore urinary catecholamines in patients with DS and better understand the discrepant activation patterns of the autonomic nervous system in patients with DS.

A previous study found that plasma levels of taurine were higher in patients with DS aged 45 or older [19]. This was attributed to a previously demonstrated overexpression of the cystathione-β synthase gene, encoded on chromosome 21, which leads to an upregulation of homocysteine metabolism, eventually resulting in greater production of taurine from cystathione. The study also found that there is an increase in plasma homovanillic acid (HVA) levels, suggesting greater dopamine metabolism in the brain of patients with DS (based on the premise that at least 40% of circulating HVA levels originate from the brain). Of note, the ratios calculated in our study showed that urinary taurine level decreased in patients with DS when compared to that in HC, regardless of OSA status, although this was not found to be statistically significant (Table 2). There were important differences between both studies including that taurine levels were measured in the plasma in the previous study while we assessed urine levels. The study population in the previous study was also restricted to patients with DS aged 45 or older, whereas our study population consisted mostly of children. Perhaps, the biochemical abnormalities in patients with DS may become more prominent with advancing age.

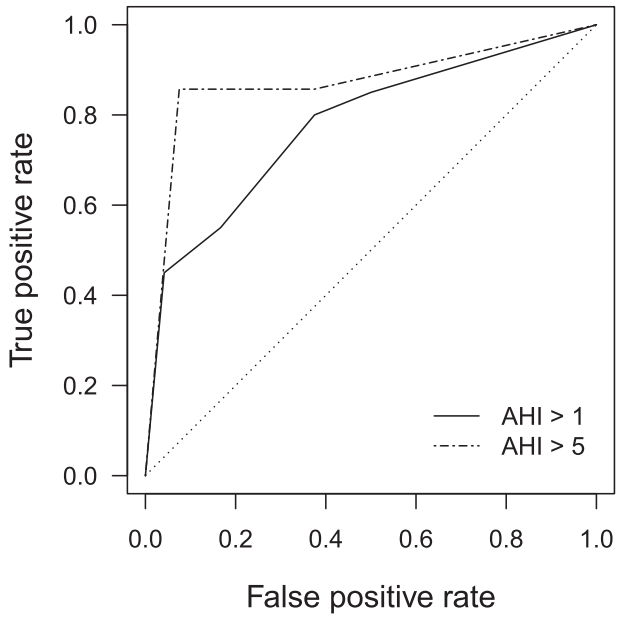

Fig. 1. Receiver operating characteristic curves for predicting OSA from urinary biomarker levels in patients with DS. Models for predicting at least mild OSA (AHI > 1, solid line) using levels of four biomarkers and for predicting moderate to severe OSA (AHI > 5, dotted line) using levels of two biomarkers are presented.

Despite the differences observed when compared to the general population, urinary biomarkers have a valuable role as a screening tool for OSA in patients with DS, similar to their application in the general population. Exploratory analyses revealed that four urinary biomarkers could be of value in predicting OSA in the DS population, and these biomarkers varied when stratified for AHI status. For an AHI > 1, these were nighttime norepinephrine/Crtn, AM/PM ratio of norepinephrine/Crtn, AM/PM ratio of dopamine/Crtn, and AM/PM ratio of taurine/Crtn, and for an AHI > 5, these were nighttime taurine/Crtn and morning taurine/Crtn particularly when specific Z-score thresholds were utilized (Table 3). Further analysis also suggested that the accuracy of biomarker-based screening was enhanced when the biomarkers were used in combination, rather than when each was assessed separately. There was no evidence to suggest that belonging to a specific racial or ethnic background or sex was associated with a specific change in urinary biomarkers in either population (patients with DS and HC.)

The discovery and implementation of diagnostic biomarkers in the context of pediatric sleep medicine has clearly evolved in the last decade but is still in the preclinical stages. Increased awareness to the elevated prevalence of pediatric sleep disorders such as OSA and the relative inaccessibility and costs associated with overnight polysomnography have motivated such approaches [23,24]. Advances in the discovery and implementation of urine-based biomarkers would be particularly attractive to unique populations such as patients with DS.

Our study was not without limitations. We found it difficult to reliably collect voluntary urine samples from children with DS under the age of seven, and this study does not include a post-hoc verification cohort. Furthermore, estimates of ROC AUC are likely optimistic, given that they were obtained from predictions derived from the same data set. Independent data are required to validate performance of the proposed models. In addition, we did not have BMI data for HC and thus were unable to adjust for BMI in our analyses. Moreover, patients with DS without OSA were demonstrated to have an alteration of urinary biomarker pattern, regardless of OSA status, where BMI is unlikely to have played a role. Furthermore, we did not explore the effect of OSA treatment on the biomarker panel. Future studies, preferably in the context a multicenter design, are needed.

5. Conclusion

Because of the practical difficulties experienced when using polysomnography as a screening tool for OSA, efforts have been aimed at developing alternative, less cumbersome methods of screening while maintaining the same accuracy in predicting disease. Our study demonstrates that having DS, even without OSA, leads to a different set of urinary biomarker assays when compared to those in the general population. The most significant urinary markers are norepinephrine, epinephrine, dopamine, and taurine. Further studies are needed in larger populations to determine the role of urinary biomarkers in patients with DS and to establish sensitivity and specificity for the use of urinary biomarkers as an OSA screening tool in this patient population.

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Research institution

Down Syndrome Program, Division of Genetics, Department of Pediatrics, Massachusetts General Hospital, Boston, Massachusetts.

Conflict of interest

Financial interests: The authors have no financial conflict of interests related to the content of this study.

Non-financial interests: B.G.S. serves in a nonpaid capacity on the Board of Directors for the Band of Angels Foundation, a nonprofit organization, and on the Medical and Science Advisory Board for the Massachusetts Down Syndrome Congress. He is a nonpaid clinical advisory to the National Center for Prenatal and Postnatal Down Syndrome Diagnoses Resources. B.G.S. occasionally gets remuneration from Down syndrome nonprofit organizations for speaking engagements about Down syndrome. He receives research support from Hoffmann-La Roche, Inc. B.G.S. receives annual royalties from Woodbine House, Inc., for the publication of his book *Fasten Your Seatbelt*: *A Crash Course on Down Syndrome for Brothers and Sisters*. B.G.S. is occasionally asked to serve as an expert witness for legal cases where Down syndrome is discussed. B.G.S. has a sister with Down syndrome. E.A.M. receives research support from Biotie Therapies, Inc. and serves on Data and Safety Monitoring Boards for Acorda Therapeutics and Shire Human Genetic Therapies. Other authors have no nonfinancial conflicts of interests related to the content of this study.

The ICMJE Uniform Disclosure Form for Potential Conflicts of Interest associated with this article can be viewed by clicking on the following link: http://dx.doi.org/10.1016/j.sleep.2017.02.005.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.sleep.2017.02.005.

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