Urinary Neurotransmitters Are Selectively Altered in Children With Obstructive Sleep Apnea and Predict Cognitive Morbidity

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Background: Pediatric obstructive sleep apnea (OSA) is associated with cognitive dysfunction, suggesting altered neurotransmitter function. We explored overnight changes in neurotransmitters in the urine of children with and without OSA.

Methods: Urine samples were collected from children with OSA and from control subjects before and after sleep studies. A neurocognitive battery assessing general cognitive ability (GCA) was administered to a subset of children with OSA. Samples were subjected to multiple enzyme-linked immunosorbent assays for 12 neurotransmitters, and adjusted for creatinine concentrations.

Results: The study comprised 50 children with OSA and 20 control subjects. Of the children with OSA, 20 had normal GCA score (mean ± SD) (101.2 ± 14.5) and 16 had a reduced GCA score (87.3 ± 13.9; *P* < .001). Overnight increases in epinephrine, norepinephrine, and γ-aminobutyric acid (GABA) levels emerged in children with OSA; taurine levels decreased. Using combinatorial approaches and cutoff values for overnight changes of these four neurotransmitters enabled prediction of OSA (area under the curve [AUC]: 0.923; *P* < .0001). Furthermore, GABA and taurine alterations, as well as overnight reductions in phenylethylamine, were more prominent in children with OSA and low GCA than in children with OSA and normal GCA (*P* < .001), and they reliably discriminated GCA status (AUC: 0.977; *P* < .0001).

Conclusions: Pediatric OSA is associated with overnight increases in urinary concentrations of catecholamines indicative of heightened sympathetic outflow. Increases in GABA levels and decreases in taurine levels could underlie mechanisms of neuronal excitotoxicity and dysfunction. Combinatorial approaches using defined cutoffs in overnight changes in concentrations of selected neurotransmitters in urine may not only predict OSA but also the presence of cognitive deficits. Larger cohort studies appear warranted to confirm these findings.

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Abbreviations: AHI = apnea-hypopnea index; DAS = Differential Abilities Scale; GABA = γ-aminobutyric acid; GCA = General Conceptual Ability; hrTST = hours of total sleep time; OSA = obstructive sleep apnea; OSAab = obstructive sleep apnea and low general cognitive ability; OSAn = obstructive sleep apnea and normal general cognitive ability; PEA = β-phenylethylamine; ROC = receiver operating characteristic curve

Snoring is a very frequent complaint affecting 10% to 12% of all children[1] and is the primary symptom of obstructive sleep apnea (OSA). OSA is the occurrence of repeated events of partial or complete upper airway obstruction during sleep, leading to disruption of normal ventilation, and to hypoxemia and sleep fragmentation. The pathophysiology of pediatric OSA is multifactorial, but adenotonsillar hypertrophy with or without concurrent obesity constitutes the major pathophysiologic mechanism underlying OSA in children.[2] Pediatric OSA has been extensively associated with an increased risk for

behavioral and mood disturbances, as well as with cognitive deficits. Furthermore, cardiovascular and metabolic morbidities, such as pulmonary hypertension, systemic hypertension, endothelial dysfunction, insulin resistance, and serum lipid alterations, are also frequently observed.[3] However, identification of those children who have developed any such OSA-associated morbidities is difficult, and is usually complicated by the need for laborious and onerous test batteries that are not routinely available in most clinical settings. Therefore, such assessments are usually not pursued.

In children with OSA, the heightened risk for increased sympathetic tonic and reactive activity indicative of substantial changes in autonomic nervous system regulation[4] can be conveniently assessed, for example, through measurement of catecholamine levels in urine.[5-7] Considering the physiologic importance of neurotransmitters as signaling molecules in the nervous system and the potential alterations that may develop in the context of OSA, assessment of urinary neurotransmitters offers unique opportunities because of their stability, sensitivity, and particularly to the non-invasiveness of this approach.[8] The development of multiplexed techniques that enable simultaneous assessment of several neurotransmitters in biologic fluids in general, and in urine in particular, provided us with the opportunity to explore the hypothesis that pediatric OSA would be associated with a unique pattern of alterations in urinary neurotransmitters, which may reflect underlying cognitive deficits.

MATERIALS AND METHODS

The research protocol was approved by the University of Chicago (protocol 09-115-B) human research ethics committee. Informed consent was obtained from the parents of the children in the study, and age-appropriate assent was obtained from the children. Patients were recruited from the Sleep and Ear Nose and Throat (ENT) clinics of Comer Children's Hospital, as well as by advertisement. Patients who had genetic or craniofacial syndromes and chronic diseases, such as cardiac disease, diabetes, cerebral palsy, and chronic lung disease of prematurity, or were receiving any psychotropic medications were excluded.

Overnight Polysomnography

Subjects aged from 3 to 12 years were recruited and underwent standard, overnight polysomnography evaluation as previously described,[9] with assessment of eight standard EEG channels; bilateral electrooculography; electromyography; two-lead ECG; oronasal airflow measurement using thermistor, nasal pressure transducer, and end-tidal CO_2; chest and abdominal movement by respiratory inductance plethysmography; and pulse oximetry including pulse waveform. The polysomnography equipment was Polysmith (Nihon Kohden America Inc). The polysomnography studies were scored per the 2007 American Association of Sleep Medicine guidelines for the scoring of sleep and associated

events.[10-12] The proportion of time spent in each stage of sleep was calculated as a percentage of total sleep time. A respiratory event was scored as an obstructive apnea if it was associated with a > 90% fall in signal amplitude for > 90% of the entire event compared with the baseline amplitude, if the event lasted for at least two breaths, and if there was continued or increased respiratory effort throughout the period of the event. A mixed apnea was scored if there was absent inspiratory effort in the initial part of the event followed by resumption of inspiratory effort before the end of the event. A central apnea was scored if there was absent respiratory effort throughout the duration of the event, or if the event lasted for at least two missed breaths and was associated with an arousal/ awakening or with a ≥ 3% desaturation. Hypopnea was scored if the event was associated with a ≥ 50% fall in amplitude of the nasal pressure transducer, lasted at least for two breaths, and was associated with an arousal/awakening or ≥ 3% desaturation. The apnea-hypopnea index (AHI) was calculated as the number of instances of apnea or hypopnea per hour of total sleep time (hrTST). The presence of OSA was defined by the presence of an obstructive AHI ≥ 2/hrTST. Control subjects were children who did not snore and whose AHI was < 1/hrTST.

General Cognitive Abilities

A subset of the subjects included in the present study underwent neurocognitive testing by certified psychometricians experienced in working with children and who were blinded to the sleep study and urine results. The neurocognitive assessments were performed the morning after the sleep study, lasted approximately 90 min, and included the core subtests of the Differential Abilities Scales (DAS).[13] The DAS is a battery of cognitive tests designed to measure reasoning and conceptual ability in children, or general cognitive abilities. The DAS provides individual subtest scores and the General Conceptual Ability (GCA) composite score that includes the sum of verbal and nonverbal reasoning, memory, and spatial skills. The verbal tests reflect knowledge of verbal concepts and level of vocabulary development, and are also indicative of word retrieval from long-term memory. The core subtests administered included Word Definitions, which measures knowledge of word meanings as demonstrated through spoken language or the ability to formulate definition of words (verbal fluency). The subtest Similarities measures verbal reasoning and knowledge, where inductive reasoning ability or the ability to relate three words to superordinate categories is necessary to earn credit. The nonverbal ability tests measure the child's inductive and sequential reasoning abilities. The core nonverbal subtest is Matrices, measuring nonverbal reasoning, which involve perception and application of relationships among abstract figures. Sequential and quantitative reasoning tests involve detection of sequential patterns in figures or numbers. The spatial ability tests measure visuospatial construction ability, spatial memory, and spatial reasoning. The core subtest Pattern Construction measures nonverbal reasoning and spatial visualization in reproducing designs with colored blocks, incorporating response time in the individual scoring; recall of designs involves the short-term recall of visual and spatial relationships through reproduction of abstract figures.

The ability score for each subtest is converted to a T score with a mean of 50 and a SD of 10. The sum of the core subtests is then converted to yield a total standard score for the GCA, with a mean of 100 and a SD of 15. Although the raw scores were used in the modeling procedures, we expressed standardized composite scores for descriptive purposes. The DAS was normalized on a large, stratified sample of children across the United States and has good validity and reliability. The GCA scores are indicative of the "psychometric g," which is the general ability to perform complex mental processing that involves conceptualization and the transformation of information, and has been considered a structural

correlate to executive function. For study purposes, we defined abnormal general cognitive ability as ≥ 1 SD below the mean.

Urinary Neurotransmitters

Subjects urinated prior to the sleep study and a first morning urine sample was collected immediately after completing the overnight polysomnographic evaluation (<60 min after awakening in all cases). Children who urinated during the night were excluded from the study. Urine was collected in midstream after local cleaning of the genital area with a wet sterile cloth, and urine samples were placed in 5-mL tubes containing a filter disc impregnated with 250 µL of 3N hydrogen chloride as preservative. The tubes were stored in a −80°C freezer until assay. A previously validated, multiplexed, enzyme-linked immunosorbent assay method for assessment of several neurotransmitters was used, and the linearity of the response curves within the ranges pertinent to the current study was validated for each of the analytes.[14,15] The neurotransmitters included in this study were epinephrine, norepinephrine, dopamine, dihydroxyphenylacetic acid, serotonin, 5-hydroxyindoleacetic acid, glycine, taurine, γ-aminobutyric acid (GABA), glutamate, β-phenylethylamine (PEA), and histamine. All samples were assayed in duplicate and values were retained if they were within 10% of each other. Urine creatinine level was measured for each sample. Individual urine neurotransmitter levels were corrected for corresponding urine creatinine concentration.

Statistical Analysis

Data are expressed as mean ± SEM unless otherwise indicated. Significant differences within groups were analyzed using unpaired t tests or analysis of variance for continuous variables and χ^2 tests for categorical variables. Bonferroni corrections were applied for multiple comparisons. In addition, receiver operating characteristic curves (ROC) were constructed for individual overnight changes in urinary neurotransmitter levels using previously published approaches.[16] Simply, a cutoff criterion was initially identified using ROC analyses for each of the urinary neurotransmitter analytes, and then each individual subject was scrutinized as to whether they fulfilled the cutoff values for the number of analytes. Then a ROC analysis for those fulfilling one, two, or more than two cutoffs of the previously identified, significantly predictive analytes was performed to determine the predictive performance of the combinatorial approach. Statistical analyses were performed using SPSS software, version 17.0 (IBM). All P values reported are two-tailed with statistical significance set at $<.05$.

Results

A total of 50 children with OSA were included in the study. Their demographic characteristics and polysomnographic findings are shown in Table 1 along with those of 20 age-, sex-, ethnicity-, and BMI-matched control subjects. Cognitive testing could only be performed in 36 of the children with OSA; 20 of the 36 children with OSA who were tested had normal general cognitive ability (OSAn) (GCA score: 101.2 ± 14.5) and 16 had reduced general cognitive ability (OSAab) (GCA score: 87.3 ± 13.9; $P < .001$).

Significant overnight increases in creatinine-adjusted epinephrine and norepinephrine urinary levels were recorded in children with OSA (Tables 1, 2). Significant increases in GABA urinary concentrations also were apparent. Furthermore, larger decreases in urinary taurine levels were present; there were no significant differences in any of the other measured neurotransmitters (Tables 1, 2). ROC scrutiny of individual urinary

Table 1—Demographic, Polysomnographic, and Urinary Neurotransmitter Overnight Changes in 50 Children With OSA and 20 Matched Control Subjects

Characteristics	OSA Group (n = 50)	Control Group (n = 20)	P Value
Age, y	6.2 ± 1.6	6.4 ± 1.4	NS
Sex, male, %	54	55	NS
White ethnicity, %	70	70	NS
BMI Z score	1.22 ± 0.78	1.05 ± 0.84	NS
Apnea-hypopnea index, events/h	9.24 ± 0.91	0.37 ± 0.19	<.001
SaO$_2$ nadir, %	84.5 ± 3.9	93.1 ± 2.7	<.001
TAI/hrTST	16.7 ± 6.2	11.1 ± 4.9	<.04
GCAab	16/36	…	
Overnight percent change in			
Norepinephrine	114.3 ± 23.8	-9.3 ± 3.3	<.0001
Epinephrine	132.7 ± 10.5	0.8 ± 2.1	<.0001
Dopamine	13.2 ± 2.9	1.4 ± 0.2	NS
DOPAC	-30.5 ± 4.8	-23.2 ± 3.9	NS
Serotonin	51.6 ± 6.9	38.7 ± 5.6	NS
5-HIAA	-13.8 ± 2.7	-7.8 ± 2.6	NS
Glycine	-23.7 ± 3.8	-16.6 ± 3.2	NS
Taurine	-105.2 ± 11.0	9.8 ± 1.7	<.0001
GABA	45.2 ± 8.0	-7.8 ± 1.8	<.0001
Glutamate	9.2 ± 3.6	-17.8 ± 4.7	NS
PEA	-51.4 ± 10.6	-29.5 ± 9.8	NS
Histamine	-40.4 ± 9.3	-44.2 ± 9.6	NS

Data for changes in urine neurotransmitter level overnight are shown as mean ± SEM; all other values are shown as mean ± SD. 5-HIAA = 5-hydroxyindoleacetic acid; DOPAC = dihydroxyphenylacetic acid; GABA = gamma-aminobutyric acid; GCAab = low general cognitive ability; hrTST = hours of total sleep time; NS = not significant; OSA = obstructive sleep apnea; PEA = β-phenylethylamine; SaO$_2$ = hemoglobin oxygen saturation; TAI = total arousal index.

Table 2—Overnight Changes in Urinary Neurotransmitter Levels in 50 Children With OSA, 20 Control Subjects, 20 Children With OSA and Preserved GCA Scores, and 16 Children With OSA and Low GCA Scores

Neurotransmitter	OSA Group			OSAab			OSAn			Control Group			P Value	
	Overnight Percent Change	Evening	Morning	Overnight Percent Change	Evening	Morning	Overnight Percent Change	Evening	Morning	Overnight Percent Change	Evening	Morning	Control Group vs OSA Group	OSAab vs OSAn
Norepinephrine	114.3 ± 23.8	0.16 ± 0.08	0.33 ± 0.20	152.3 ± 69.6	0.13 ± 0.07	0.30 ± 0.13	90.3 ± 15.7	0.18 ± 0.08	0.35 ± 0.13	−9.3 ± 3.3	0.14 ± 0.07	0.13 ± 0.07	< .0001	.06
Epinephrine	132.7 ± 10.5	0.91 ± 0.47	2.20 ± 0.53	120.3 ± 33.8	0.89 ± 0.39	1.88 ± 0.67	143.7 ± 30.8	0.91 ± 0.53	2.24 ± 0.87	0.8 ± 2.1	0.76 ± 0.57	0.76 ± 0.64	< .0001	NS
Dopamine	13.2 ± 2.9	4.78 ± 1.88	4.90 ± 1.91	12.6 ± 4.1	4.80 ± 2.06	5.07 ± 2.22	13.4 ± 5.2	4.67 ± 2.35	4.81 ± 2.74	1.4 ± 0.2	4.26 ± 2.21	4.27 ± 2.4	NS	NS
DOPAC	−30.5 ± 4.8	19.51 ± 7.6	14.72 ± 5.8	−32.8 ± 6.6	19.77 ± 7.8	14.89 ± 6.8	−28.7 ± 7.3	18.56 ± 8.1	14.31 ± 7.6	−23.2 ± 3.9	18.7 ± 9.4	15.72 ± 9.8	NS	NS
Serotonin	51.6 ± 6.9	2.36 ± 1.23	3.13 ± 1.37	57.6 ± 10.2	2.32 ± 1.54	3.32 ± 1.76	49.2 ± 9.7	2.41 ± 1.62	3.02 ± 1.93	38.7 ± 5.6	2.22 ± 1.08	2.78 ± 1.43	NS	NS
5-HIAA	−13.8 ± 2.7	50.5 ± 22.7	45.3 ± 23.8	−15.2 ± 3.5	52.3 ± 24.9	46.7 ± 26.2	−11.4 ± 4.1	50.3 ± 23.3	44.3 ± 21.8	−7.8 ± 2.6	48.3 ± 27.4	44.7 ± 24.1	NS	NS
Glycine	−23.7 ± 3.8	18.99 ± 6.85	14.26 ± 7.21	−25.4 ± 4.9	20.32 ± 9.43	14.86 ± 10.32	−22.5 ± 8.76	17.87 ± 8.65	13.54 ± 9.32	−16.6 ± 3.2	21.55 ± 5.8	18.87 ± 9.4	NS	NS
Taurine	−105.2 ± 11.0	11.78 ± 4.31	4.83 ± 2.87	−170.3 ± 29.1	12.65 ± 5.38	2.57 ± 2.98	−45.8 ± 5.7	11.32 ± 6.43	6.65 ± 5.43	9.8 ± 1.7	13.21 ± 4.98	14.08 ± 5.76	< .0001	< .0001
GABA	45.2 ± 8.0	0.09 ± 0.03	0.14 ± 0.05	60.2 ± 5.7	0.08 ± 0.04	0.13 ± 0.05	38.6 ± 8.0	0.11 ± 0.06	0.14 ± 0.07	−7.8 ± 1.8	0.07 ± 0.03	0.06 ± 0.03	< .0001	< .0001
Glutamate	9.2 ± 3.6	0.92 ± 0.21	1.02 ± 0.26	8.7 ± 4.7	0.87 ± 0.33	0.91 ± 0.36	10.8 ± 4.2	0.94 ± 0.44	1.07 ± 0.47	−17.8 ± 4.7	0.88 ± 0.32	0.76 ± 0.43	NS	NS
PEA	−51.4 ± 10.6	1.03 ± 0.37	0.58 ± 0.23	−66.9 ± 5.3	1.13 ± 0.45	0.45 ± 0.42	−32.4 ± 5.1	0.99 ± 0.46	0.67 ± 0.37	−29.5 ± 9.8	0.89 ± 0.28	0.65 ± 0.34	NS	< .001
Histamine	−40.4 ± 9.3	0.55 ± 0.17	0.32 ± 0.23	−45.3 ± 12.3	0.57 ± 0.27	0.35 ± 0.29	−38.7 ± 17.8	0.52 ± 0.34	0.30 ± 37	−44.2 ± 9.6	0.56 ± 0.31	0.37 ± 0.28	NS	NS

Data are expressed as ratios of corresponding individual urinary creatinine levels. GCA = General Conceptual Ability score; OSAab = children with OSA and low GCA; OSAn = children with OSA and normal GCA. See Table 1 legend for expansion of other abbreviations.

neurotransmitters did not enable adequate prediction of OSA (sensitivity: 56%-76%; specificity: 38%-49%). However, use of the four urinary neurotransmitters showing statistically significant changes in children with OSA, and their expression as specific, cutoff, evening to morning changes, namely epinephrine (cutoff overnight change: > 47%), norepinephrine (cutoff overnight change: >68%), GABA (cutoff overnight change: >20%), and taurine (cutoff overnight change: < 65%), enabled relatively favorable predictions of OSA when three or more of these biomarkers fulfilled the cutoff criteria (area under the curve: 0.923; $P < .0001$) (Fig 1).

Of note, GABA and taurine alterations, as well as reduced PEA levels, were significantly more prominent in children with OSA who had OSAab than in those with OSAn ($P < .01$) (Tables 2, 3). Such differences between the two OSA subgroups were not present for urinary catecholamine levels. ROCs were constructed for each of these three urinary neurotransmitters and then used in combination for prediction of low general GCA scores in the context of OSA (Tables 2, 3) (Fig 2). Indeed, an area under the curve of 0.977, with a sensitivity of 82% and a specificity of 90%, was achieved ($P < .0001$).

DISCUSSION

This study shows that pediatric OSA is associated with significant nocturnal alterations in a selected
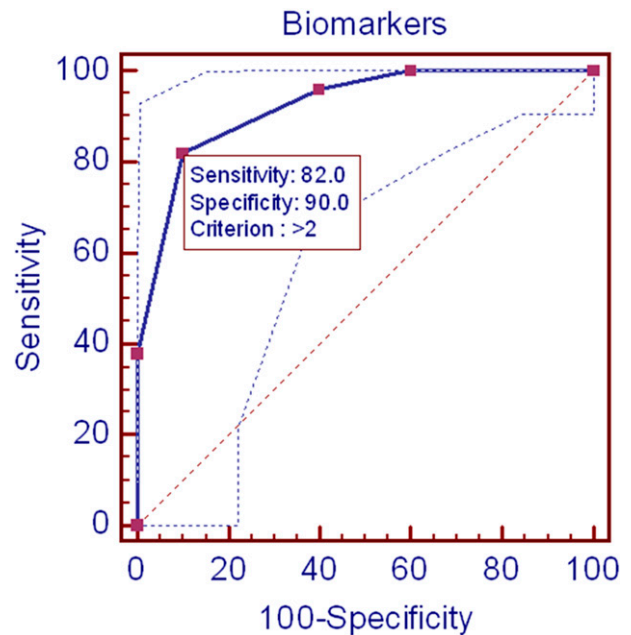

FIGURE 1. Receiver operating characteristic curve using cutoff values of overnight changes for four urinary neurotransmitters in the prediction of obstructive sleep apnea in children. Dotted lines indicate 95% CIs.

Table 3—Demographic, Polysomnographic, and Overnight Changes in Urinary Neurotransmitter Levels in 20 Children With OSA and Preserved GCA Scores and 16 Children With OSA and Low GCA Scores

Characteristics	OSAab (n = 16)	OSAn (n = 20)	P Value
Age, y	6.1 ± 1.8	6.2 ± 1.7	NS
Sex, male, %	50%	50%	NS
White ethnicity, %	68.7	65	NS
BMI Z score	1.30 ± 0.91	1.25 ± 0.87	NS
Apnea-hypopnea index (events/h)	12.24 ± 3.2	8.93 ± 4.87	NS
SaO_2 nadir, %	83.5 ± 5.1	85.6 ± 4.7	NS
TAI/hrTST	18.2 ± 8.2	17.1 ± 7.9	NS
GCA score	87.3 ± 13.9	101.2 ± 14.5	<.01
Overnight percent change in			
Norepinephrine	152.3 ± 69.6	90.3 ± 15.7	0.06
Epinephrine	120.3 ± 33.8	143.7 ± 30.8	NS
Taurine	−170.3 ± 29.1	−45.8 ± 5.7	<.0001
GABA	60.2 ± 5.7	38.6 ± 8.0	<.0001
PEA	−66.9 ± 5.3	−32.4 ± 5.1	<.0001

Data for changes in urine neurotransmitter level changes are shown as mean ± SEM; all other values are shown as mean ± SD. See Table 1 legend for expansion of abbreviations.

number of neurotransmitters when the latter are assayed as overnight changes in the urine. Furthermore, when used in combination, defined cutoffs of overnight changes among the urinary neurotransmitters with significantly altered concentrations appear to enable relatively favorable prediction of OSA. Similar approaches using a slightly different set of urinary neurotransmitters also permits relatively accurate identification of those children with OSA who manifest reduced neurocognitive function when tested using the DAS battery.

Before we discuss the potential implications of our findings, some comments regarding biomarker discovery in pediatric OSA and on the potential limitations of this study are needed. First, our study is only one of several critical steps required for the exploration of biomarker discovery in human-derived biologic samples[17]; therefore, the current findings should be viewed as preliminary. In our initial attempt to explore the presence of diagnostic biomarkers in pediatric OSA, we identified three differentially expressed proteins that were associated with OSA in plasma.[18] However, although these three proteins displayed relatively favorable predictive values, the proteomic methodologies used by Shah et al[18] did not allow for accurate identification of the proteins, and such putative biomarkers could not be confirmed. In a subsequent study using urine samples, we examined the urine proteome in children with OSA, in children with primary snoring, and in control subjects using two-dimensional gel electrophoresis.[19] In this 2009 study,[19] we identified 16 unique proteins that were differentially expressed in the OSA group, and validated four of these proteins (kallikrein-1, urocortin-3, orosomucoid-1, and uromodulin) using enzyme-linked immunosorbent assays. Using a combinatorial-biomarker, discriminative

approach similar to the one implemented in the present study, highly predictive ROC were generated. The present study undertook a different strategic approach using an a priori hypothesis positing that concentrations of some neurotransmitters would be differentially affected by the presence of OSA, and that some of such altered, overnight trajectories would reflect underlying neurocognitive outcomes.

A known limitation of urinary neurotransmitter assessment is that neurotransmitters, in any medium, are not recognized as diagnostic for any particular disease or condition with the exception of pheochromocytoma.[20] Another limitation of such assessments concerns the insufficient data available regarding the origin of urinary neurotransmitters. Neurotransmitters are synthesized in organs of the CNS and in many other organs. Therefore, multiple systems may contribute to the total urinary pool of neurotransmitters being assayed, presenting a challenge in the interpretation of urinary neurotransmitter data.[21] It also is unclear how factors other than OSA (eg, medications and supplements or even other concomitant disorders such as attention-deficit/hyperactivity disorder) that alter neurotransmitter levels in the CNS will affect neurotransmitters in the urine and vice versa.[22-24] Further limitations of the present study include a relatively small number of subjects, particularly among those who underwent neurocognitive testing; the fact that none of the control subjects were tested for cognitive function; the absence of a post hoc validation cohort for confirmatory purposes; and, finally, the need for determination of the effects of treatment on the changes reported in this study.

The overnight increases in urinary catecholamine levels reported herein were anticipated based on the several previously published studies in children with OSA, all of which indicated that morning urinary
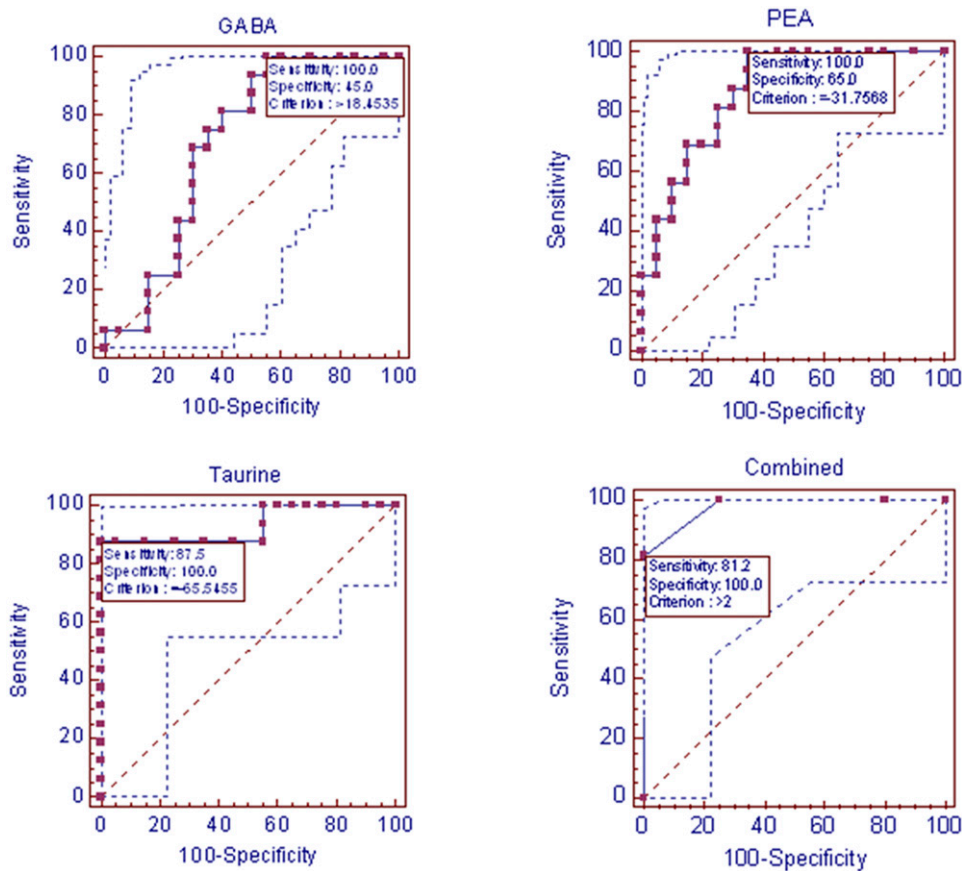
FIGURE 2. Receiver operating characteristic curves using three urinary-neurotransmitter-defined cutoff values corresponding to individual overnight changes for prediction of neurocognitive dysfunction in children with obstructive sleep apnea. Dotted lines indicate 95% CIs. GABA = γ-aminobutyric acid; PEA = β-phenylethylamine.

catecholamine levels were elevated.[5-7,25] However, the present study further indicates that the changes in urinary-catecholamine levels indeed occur during sleep, since urinary levels in the evening prior to the sleep study were similar between children with OSA and control subjects (data not shown). Thus, the recurrent hypoxemic events and the episodic arousals that characterize sleep in patients with OSA likely enhance sympathetic tonic outflow, and result in increased recovery of catecholamines in the urine.[4] Taken together, we surmise that the changes in evening-to-morning epinephrine and norepinephrine levels are probably better suited for detection of OSA and the response to specific therapeutic interventions for pediatric OSA. Notwithstanding, the individual ROCs for overnight changes in urinary catecholamine levels were not sufficiently adequate predictors of OSA to enable their use as single biomarkers. The addition of two other urinary neurotransmitters, namely GABA (with its overnight increases) and taurine (with its overnight decreases), was required to more predictably identify OSA using urinary neurotransmitter levels in evening and morning samples (Fig 1).

Although the organ sources of increases in urinary GABA levels and declines in taurine urinary levels are unclear, changes in levels of these two neurotransmitters were not unanticipated. Indeed, animal studies suggest that the presence of intermittent hypoxia is associated with significant alterations in GABAergic neurotransmission.[26-28] Similarly, taurine is known to play important roles against reactive oxygen species and hypoxia in several organs, including the those of the CNS and the vasculature.[29-33] Both intermittent hypoxia and increased oxidative stress are hallmark characteristics of OSA,[34] and the degree of oxidative stress associated with OSA has emerged as an important determinant of cognitive dysfunction in both animal models and in children.[35-37]

A unique and intriguing observation in the present study involves the differential and predictable changes in urinary neurotransmitters in children with OSA and without evidence of neurocognitive dysfunction. In this context, the patterns of overnight changes in levels of three neurotransmitters, namely GABA, taurine, and PEA, allowed accurate prediction of cognitive deficits in this small cohort (Fig 2). The potential

contributory roles of GABA and taurine have already been discussed, and their contributions to cognition will not be further expanded. Furthermore, monoaminergic systems are firmly established as playing critical roles in mood, cognition, emotion, reward, learning, and attention,[38] and PEA has been shown to affect the monoamine transporter function in brain synaptosomes, whereby aberrant levels of PEA and other monoaminergic receptor ligands have been associated with various neuropsychiatric disorders, including depression and attention-deficit/hyperactivity disorder.[39] Thus, the putative biomarkers for cognitive deficits appear as scientifically plausible. Of note, an association between a marker for systemic inflammation (ie, high-sensitivity C-reactive protein) and the presence of cognitive deficits has been previously reported in pediatric OSA,[40] lending further credence to the use of biomarkers in the detection of patients at higher risk for end-organ morbidity, particularly considering the labor-intensive nature of such seldom-performed assessments during evaluation of children with suspected OSA.

In summary, predictable overnight changes in the concentrations of specific neurotransmitters in urine of children with OSA offer the opportunity to potentially develop screening approaches for this condition in children who habitually snore, particularly considering the inability to reliably diagnose the disease when relying on history and physical examination alone.[41] Moreover, similar strategies may provide reliable indicators of children at risk for the presence of specific end-organ morbidities, such as neurocognitive dysfunction, associated with pediatric OSA.

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