ON-LINE DATA SUPPLEMENT

FAMILY AGGREGATION OF UPPER AIRWAY SOFT TRUCTURES IN NORMALS AND PATIENTS WITH SLEEP APNEA

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METHODS

Subjects

Probands were primarily recruited from the Penn Sleep Center outpatient practice and were newly diagnosed with sleep apnea. Probands already using CPAP therapy were excluded from the study since CPAP has the potential to alter upper airway tissue properties (E1). Controls were recruited through local advertisements and had to live in the neighborhood (same school district) of the matched proband. Controls were of the same ethnic background and gender as the proband and were within 10 years of age of the matched proband. Controls found to have symptoms of sleep apnea and an apnea hypopnea index greater than 15 events/hour were re-categorized as probands. Sixteen of our 55 probands came from this source. Controls with apnea hypopnea indices between 5 and 15 events/hour were considered indeterminate and were not studied further. Subjects were paid $100.00 for the polysomnography and $100.00 for the MRI. The patients received the MRI and sleep study report at the same time so weight loss secondary to knowing that they had OSA could not have occurred. The MRI was performed within one week of the sleep study. Thus, there was no time for them to lose weight. We did not know the duration of the apnea in the probands. The probands were newly diagnosed and we do not know how many years they may have had apnea. The University of Pennsylvania Institutional Review Board approved the study, and written informed consent was obtained from all subjects. Exclusionary criteria included: 1) age under 18; 2) subjects chronically taking medications that affected upper airway caliber (i.e., sedatives or benzodiazepines); and 3) MRI exclusions: specifically: a) body weight > 136 kg (table limit of the magnetic resonance scanner); b) presence of metallic implants (pacemaker), ferromagnetic clips, etc.; or c) severe claustrophobia.

Polysomnography

Standard polysomnography operating procedures and scoring were performed, as previously described from our laboratory (E2, E3), in the Penn Center for Sleep Disorders using a computerized polysomnography system (Sandman, Mellville Diagnostics, Ottawa, Ontario). Controls,

siblings of controls and siblings of probands underwent full night polysomnography. Probands (defined by an AHI > 15 events/hour) initially had a clinical sleep study. In the event that this was a split night protocol (diagnostic study in the first half of the night and CPAP in the second half of the night) the probands had a repeat sleep study before starting CPAP, i.e., a full night study without CPAP in order to determine the AHI over the entire night analogous to the rest of the subjects in our protocol. Polysomnograms were scored by registered sleep technologists and interpreted by certified sleep physicians using the standard criteria of Rechtschaffen and Kales (E4) and the more recently proposed criteria of the American Academy of Sleep Medicine (E5). Obstructive apneas were defined as airflow cessation for > 10 seconds; hypopneas were defined as a 50% reduction in airflow for > 10 seconds and associated with > than 3% decrement in oxyhemoglobin saturation and/or an arousal. Nasal pressure monitors were used in all subjects to measure airflow. In addition to AHI, sleep efficiency, total sleep minutes, arousal index, minutes in NREM (stages 1-4) and REM sleep and latency to REM sleep were assessed. We did not record the duration of the apneic events themselves on the sleep studies. Snoring was noted but not quantified.

MR Analysis

The technicians who performed the MR analysis were not blinded to the name of the subject but they did not know the results of the sleep study. Thus they did not know if the subject had sleep apnea or was normal. See figures E1A,B for anatomy and segmentation of the structures including the tongue, soft palate, lateral pharyngeal walls, parapharyngeal fat pads and mandible. Although the soft palate and tongue are adjacent there is usually a thin white line that separates the structures.

Statistical Analysis

Family aggregation of the airway and soft tissue risk factors was assessed with three analysis strategies (see Figure 1). The first analytic approach compared mean values across subject groups using mixed model analyses of variance. We hypothesized that the size of the surrounding soft

tissues for the proband siblings would be, on average, intermediate between proband values and control values. Therefore we compared the adjusted mean values among proband sibs to adjusted means among probands and controls to determine if this hypothesis was true. In addition, we examined the data to determine if the size of these upper airway anatomic risk factors was more similar within family groupings (proband vs. proband sib and control vs. control sib) compared to between family groupings (proband vs. control and proband sibling vs. control sibling), i.e., suggesting but not proving family aggregation.

The second analysis approach employed an analogous mixed model ANOVA but focused on the variance components in order to quantify the degree of heritability for each measurement. As part of this analysis we also estimated intraclass correlations (adjusted for gender, age, craniofacial size, ethnicity and visceral neck fat) for the upper airway soft tissues volumes independently for probands/proband siblings and controls/ control siblings in order to determine if the heritability of the upper airway soft tissue structures is different in normals than apneics.

The third analysis approach employed a reconstituted cohort design comparing proband siblings to control siblings. We hypothesized that familial aggregation, whether arising from genetic or environmental influences, would cause proband siblings to differ from control siblings in terms of soft tissue structures associated with increased risk of sleep apnea. These differences are expected to arise assuming sleep apnea has a genetic basis because siblings of probands share at least some of their genetic identity with the proband. Multiple logistic regression models were used to obtain adjusted odds ratios (OR) for having a sibling with sleep apnea in order to quantify the relative magnitudes among soft tissue structures, proband sibling versus control sibling differences. Odds ratios with a 95% CI lower bound (LB) greater than one indicate that an increase in the size of that structure is associated with an increased risk of having a sibling with apnea. In contrast, odds ratios with a 95% CI upper bound (UB) below one indicate that a reduction in the size of that structure is associated with an increased risk of having a sibling with obstructive sleep apnea.

RESULTS

Polysomnography

Sleep efficiency and the amount of time spent in stage 1, stage 2 were not significantly different (see Table E1 in web-based repository) between the subject groups. However, the amount of REM sleep and the total amount of NREM sleep were significantly different across the groups with the least amount of REM sleep occurring in the probands. The arousal frequency was also significantly different across groups with the probands manifesting the greatest number of arousals. The proband siblings had the second largest number of arousals.

Airway Measurements

Descriptive comparisons of mean values for the airway measurements between the 4 groups are displayed in Table 7. There were significant group differences in the retropalatal region for airway volume, airway area per slice, minimum airway area, and the lateral and anterior-posterior dimensions of the retropalatal airway after adjusting for age, gender, height, ethnicity and BMI. There were no significant differences in the volume of the upper airway, or measurements obtained in the retroglossal region between the groups (Table 7). In the RP region, airway area per slice (h^2 = 35.0%), minimum airway area (h^2 = 46.0%) and lateral dimension (h^2 = 17.0%) demonstrated significant heritability after adjusting for gender, ethnicity, age, craniofacial size and visceral neck fat (Table 8). No airway measure demonstrated an increased risk for having a sibling with sleep apnea.

Two Dimensional Soft Tissue Measurements

We found significant group differences for the thickness of the retropalatal lateral pharyngeal wall after controlling for gender, ethnicity, age, craniofacial size and visceral neck fat (Table E2). However, there were no significant group differences in retroglossal lateral pharyngeal wall width, parapharyngeal fat pad width, thickness of the ptergyoid muscles or width of soft tissue between the mandibular rami. The retropalatal lateral pharyngeal wall width was largest in the probands with the proband sibling being intermediate between probands and controls (Table E2). No two

dimensional soft tissue measurements demonstrated significant heritability after adjusting for gender, ethnicity, age, craniofacial size and visceral neck fat (Table E3). Similarly, no two dimensional soft tissue measurement demonstrated an increased risk for having a sibling with sleep apnea.

On Line References

E1. Schwab RJ, Pack AI, Gupta KB, Metzger LJ, Oh E, Getsy JE, Hoffman EA, Gefter WB. Upper airway and soft tissue structural changes induced by CPAP in normal subjects. Am J Respir Crit Care Med 1996;154:1106-1116.

E2. Welch KC, Foster GD, Ritter CT, Schellenberg JB, Wadden TA, Arens R, Maislin G, Schwab RJ. A novel volumetric magnetic resonance imaging paradigm to study upper airway anatomy. Sleep 2002;25:532-542.

E3. Schwab RJ, Gupta KB, Gefter WB, Hoffman EA, Pack AI. Upper airway soft tissue anatomy in normals and patients with sleep disordered breathing. Significance of the lateral pharyngeal walls. Am J Respir Crit Care Med 1995;152:1673-1689.

E4. Rechtschaffen, A., and A. Kales. A manual of standardized terminology: techniques and scoring system for sleep stages of human subjects. Brain Info Service/Brain Research Institute, UCLA, Los Angeles. 1968. NIH Publication No. 204.

E5. American Academy of Sleep Medicine Task Force. Sleep-disorders breathing disorders in adults: recommendations for syndrome definition and measurement techniques in clinical research. Sleep 1999;22:667-689.

Figure Legends

Figure E1. A. T1-weighted axial MR image in the retropalatal region in a normal subject B. Segmentation of the tongue (blue), mandible (brown), soft palate (red), parapharyngeal fat pads (yellow) and lateral walls (green) on the same image (Figure 1A).

Table E1: Summary of Polysomnography Results for Probands, Proband Sibs, Controls and Control Sibs

Sleep Study Variable	Probands		Proband Sibs		Controls		Control Sibs		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	p value*
Sleep efficiency	77.2	15.6	79.7	12.7	77.5	11.8	80.3	12.1	0.523
Arousal index	46.6	31.5	24.3	16.7	17.5	7.2	19.6	8.3	0.0001
Minutes in stage 1	36.2	22.6	39.2	23.0	31.2	18.7	31.1	21.4	0.087
Minutes in stage 2	227.4	68.5	229.5	57.9	233.2	46.2	240.8	49.2	0.597
Minutes in stage 3/4	6.8	19.6	10.4	19.4	13.7	18.3	12.1	21.7	--[&]
REM stage minutes	58.9	31.3	70.7	29.7	73.3	28.3	72.4	28.0	0.033
Latency to stage REM	126.4	79.2	105.4	69.1	122.4	79.7	112.5	68.5	0.427
NREM stage minutes	249.3	79.3	279.1	57.8	278.1	44.5	284.1	48.9	0.009
Total test time	430.8	81.5	440.5	53.2	453.6	28.5	443.8	37.6	0.171

* ANOVA between all 4 subject groups

[&] In the mixed model the p value did not converge for this parameter

Table E2: Comparisons of Two-Dimensional Soft Tissue Measurements in Probands, Proband Sibs, Controls and Control Sibs

2D - Soft Tissue Measurements	Probands		Proband Sibs		Controls		Control Sibs		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	p value*
Width of soft tissue between mandibular rami (mm)	94.3	5.2	93.9	6.2	91.8	6.8	93.5	7.4	0.053[&]
RP lateral pharyngeal wall width (mm)	**29.3**	**8.1**	**27.2**	**8.1**	**26.5**	**7.5**	**25.8**	**6.9**	**0.029**
RG lateral pharyngeal wall width (mm)	23.6	8.8	24.4	8.7	22.9	8.3	22.3	6.3	0.168
RP fat pad width (mm)	20.1	9.2	19.8	9.7	17.0	5.8	17.0	7.7	0.732
Thickness of ptergyoid muscles (mm)	34.7	8.6	32.8	7.8	33.5	7.7	34.59	9.3	0.341

*ANOVA between all 4 subject groups adjusting for age, gender, craniofacial size, ethnicity and visceral neck fat

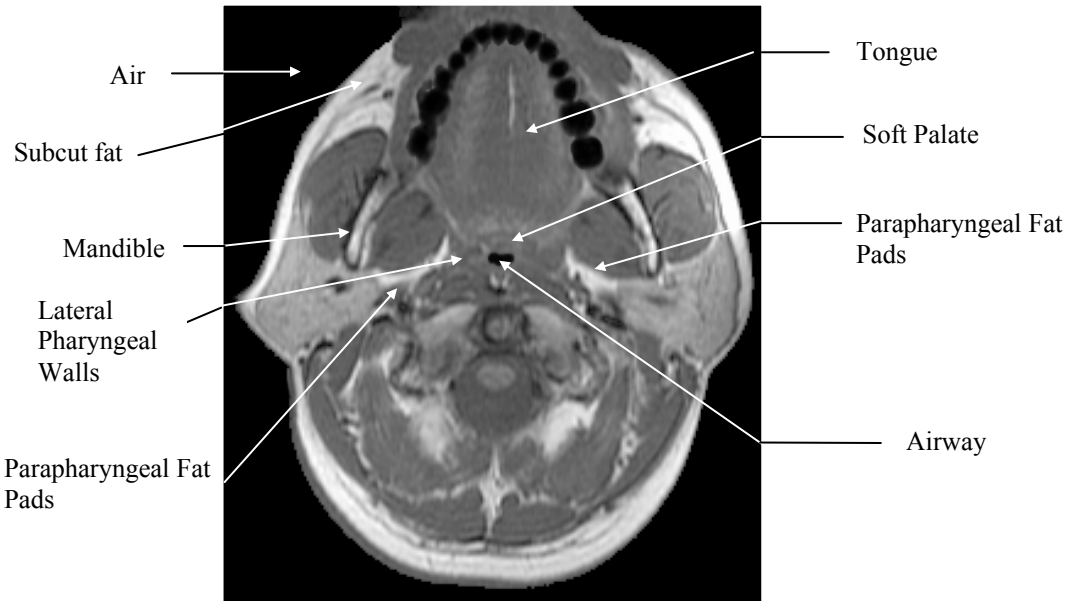
[&]p value adjusting for age, gender, visceral neck fat and ethnicity

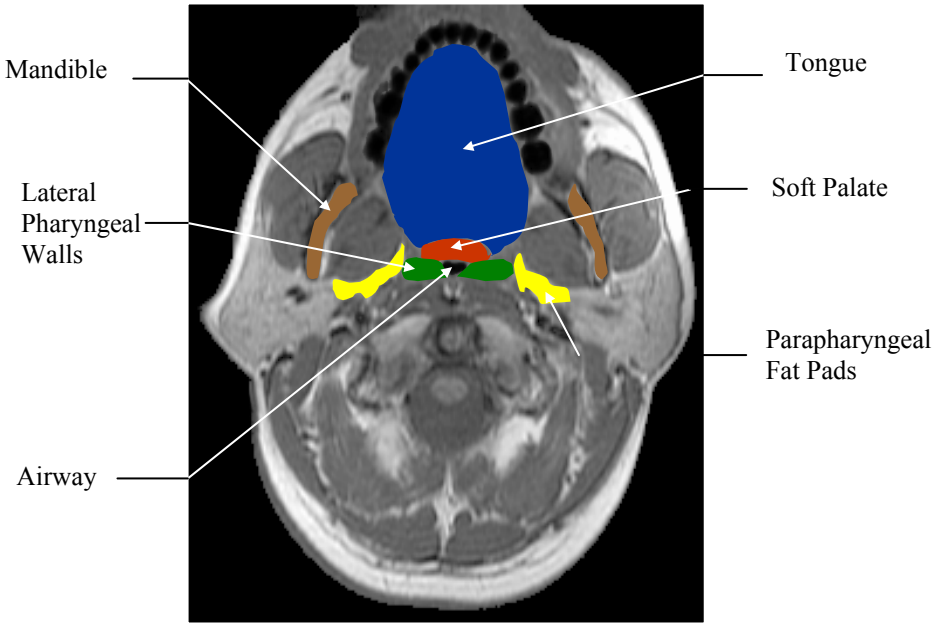
Table E3: Heritability Indices for Two-Dimensional Soft Tissue Measurements

Two Dimensional Soft Tissue Measurements	Unadjusted	Adjusted for Age, Gender, Craniofacial Size and Race	Adjusted for Age, Gender, Craniofacial Size, Race and Visceral Neck Fat	
	h^2	h^2	h^2	p value
Width of soft tissue between mandibular rami (mm)	17.3%	10.9%	10.9%	0.152[&]
RP lateral pharyngeal wall width (mm)	10.2%	1.8%	1.9%	0.437
RG lateral pharyngeal wall width (mm)	14.0%	17.5%	17.3%	0.098
Fat pad width (mm)	11.6%	3.6%	10.6%	0.199
Thickness of ptergyoid muscles (mm)	4.2%	15.8%	14.6%	0.086

[&]p value adjusting for age, gender, visceral neck fat and ethnicity

Fig. E1


Air
Subcut fat
Mandible
Lateral Pharyngeal Walls
Parapharyngeal Fat Pads
Tongue
Soft Palate
Parapharyngeal Fat Pads
Airway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