Obstructive Sleep Apnea and Quality of Life in Ehlers-Danlos Syndrome. A parallel-cohort study.

Supplementary material.

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Information: Informed consent for publication was obtained from all participants who are featured in the pictures.

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Supplementary Methods

Study assessments. After enrolment, socioeconomic characteristics including a structured interview and self-reported race/ethnicity and alcohol consumption were collected. The neck circumference was measured between the mid-cervical spine and the mid-anterior neck, just below the laryngeal prominence. Snoring or observed apneas were assessed using the STOP-Bang score (8 yes/no questions). Night-time sleep related quality and patterns were assessed using the Pittsburgh sleep quality index (scores each of the 9 DSM-IV criteria with a global score range 0 - 21) and day-time sleep propensity was assessed via the Epworth sleepiness scale (8 item-score; range 0–24 points), both covering a 1-month time interval. Definition of excessive daytime sleepiness required an Epworth score >10. The Short-Form 36 (SF-36) Questionnaire assessed quality of life (8 summary scores ranging 0-100) and the Patient Health Questionnaire (PHQ-9) Questionnaire Module 9 assessed common mental disorders among the study population. Levels of education were classified according to the International Standard Classification of Education (ISCED) 2011. The methods for secondary outcomes assessed in subgroups are described in the supplementary material.

Aortic diameter. Measurements of thoracic aortic diameter were be determined by a trained physician. Aortic diameter was measured in parasternal long-axis view at end-systole (T wave on electrocardiogram) in M-mode sonographically with a commercially available cardiac ultrasound system (Vivid E9 with XDclear, GE Healthcare, Little Chalfont, UK) using a 3.5-MHz transducer. Measurements included the following levels: 1. sinus of Valsalva; 2. proximal ascending aorta obtained following the method of Roman et al.[1] Measurements were conducted perpendicular to the long axis of the aorta, using the “leading edge to leading edge” technique and view showing the largest aortic diameters. Every single measurement was replicated at least three times and data were averaged by the mean.

Blood pressure. Blood pressure was measured three times and the average of the last two readings was calculated (standard digital automatic monitor, Omron Healthcare, Kyoto, Japan). Arterial hypertension was defined as either a systolic blood pressure of ≥140 mm Hg, diastolic blood pressure of ≥90 mm Hg, or current use of antihypertensive drugs. In general, studies were undertaken in a temperature-controlled room with the study subject in supine position for at least 10min before beginning with the measurements.

Craniofacial photogrammetry. Craniofacial analysis methods as well as a technique validation have been described in detail before.[2] Briefly, we followed a strictly laser-guided setup (eFigure 4) and

examples can be seen in eFigure 5. Frontal and 90-degree profile digital photographs of the head and neck were obtained with a digital single-lens reflex camera (Nikon D600 with AF Nikkor 50 mm f/1.8D prime lens and external flash unit Speedlight-600B; Nikon Corp., Japan) which was mounted on a tripod. The tripod as well as the participant were placed on predefined spots marked on the floor (eFigure 4). Standardized manual camera settings (focal length 50 mm, aperture 1.8) were used to ensure consistency of the JPEG images (resolution 6016 × 4016 pixels). A laser pointer head-clip and calibrated markers (diameter 3 cm) on a headband ensured perpendicular views and a correct scale/benchmark. Using image analysis software (Image J v1.36, NIH, Bethesda, MD), the photographs were examined for landmark digitization. Craniofacial landmarks of interest were captured as pixel coordinates (x, y) of the image which were then transferred to a custom-programmed spreadsheet for the computation of linear, angular, area, and polyhedral volume measurements.

Lung function. Lung function was assessed with a EasyOne Spirometer (ndd Medical Technologies, Andover, MA, USA) and results are expressed in percentage of predicted values according to the European reference equations.[3]

eTable 1. List of genes for inclusion criteria applied in this study.

Descriptive	Gene(s)	OMIM #
Classical	*COL5A1* / *COL5A2*	130000 / 130010
Hypermobile / Tenascin-X deficient	*TNXB*	130020 / 606408
Vascular	*COL3A1*	130050
Kyphoscoliotic	*PLOD1*	225400
Musculo-contractural	*CHST14*	601776
Arthrochalasis	*COL1A1* / *COL1A2*	130060
Dermatosparaxis	*ADAMTS2*	225410
Cardiac valvular	*COL1A2*	225320
FKBP14-related	*FKBP14*	614557
Spondylocheiro-dysplastic	*SLC39A13*	612350
Periventricular heterotopia	*FLNA*	300537

OMIM, Online Mendelian Inheritance in Man – see www.omim.org/ (effective February 2014, start of recruitment). Other very rare types (only case reports available) were not included in this study.

eTable 2. EDS subtypes included in this study

EDS subtype	N (100) = %
Classical type	46
Hypermobile type	35
Vascular type	9
Arthrochalasis type	1
FKBP14-related	1
Kyphoscoliotic type	1
Not assignable*	7

*Not clearly attributable due to overlapping features, although Villefranche inclusion criteria[4] are fulfilled. Patients fulfilling diagnostic criteria for more than one EDS subtype were labeled "not assignable" for this study.[5]

eTable 3. List of diagnostic criteria fulfilled by the EDS cohort.

Diagnostic criteria	N (100) = %
1. Clinical Villefranche criteria[4] + Gene analysis****	25
2. Clinical Villefranche criteria[4] + Electron microscopy study	32
3. Clinical Villefranche criteria[4] + Biochemical urine analysis	1
4. Clinical Villefranche criteria[4] only	17
5. Combination of **1-3***	25

Identified genes: COL5A1* (n=12); *COL3A1* (n=6); *TNXB* (n=4); *COL1A1* (n=3); *COL5A2* (n=2); *FKBP14* (n=1); *PLOD1* (n=1)

eTable 4. List of medical facilities where source data for diagnostic purposes was obtained and EDS diagnosis was established.

Medical facility	N (100) = %
University Children's Hospital Zurich, Switzerland *(university hospital)*	26
University Hospital Heidelberg, Germany *(university hospital)*	18
University Medical Center Schleswig-Holstein, Germany *(university hospital)*	12
Institute for Medical Genetics and Molecular Medicine Colonge, Germany *(university affiliated)*	9
Other university hospitals	30
Other non-university hospitals	5

eTable 5. List of countries of origin for EDS participants.

Country	**N (100) = %**
Germany	61
Switzerland	34
Austria	5

eTable 6. Detailed questionnaire results.

Components	**EDS (n=100)**	**Control (n=100)**	**p-value**
Epworth Sleepiness Scale (Daytime sleepiness)			
ESS score	11 (7-14)	7 (5-10)	p<0.001
STOP-Bang Score (OSA propensity)			
Overall score	2.24±1.53	1.24±1.26	p<0.001
Short form 36 (Quality of Life)			
Bodily pain	32 (22-62)	100 (74-100)	p<0.001
General health perceptions	36 (25-59)	82 (71-92)	p<0.001
Mental health	68 (56-84)	80 (70-88)	p<0.001
Physical functioning	55 (38-75)	100 (95-100)	p<0.001
Emotional role functioning	100 (33-100)	100 (100-100)	p<0.001
Physical role functioning	25 (0-75)	100 (100-100)	p<0.001
Social role functioning	50 (38-81)	100 (81-100)	p<0.001
Energy/Vitality	30 (20-50)	60 (50-75)	p<0.001
Pittsburgh Sleep Quality Index (Sleep Quality)			
Duration of sleep	0.98±1.01	0.57±0.73	p<0.001
Sleep disturbance	1.63±0.6	1.00±0.25	p<0.001
Sleep latency	1.70±0.96	0.94±0.84	p<0.001
Day dysfunction due to sleepiness	1.85±0.80	0.76±0.68	p<0.001
Sleep efficiency	1.22±1.18	0.32±0.68	p<0.001
Total score	9.58±4.13	4.69±2.53	p<0.001
PHQ 9 (Mental health)			
PHQ-9 total score	9.32±4.84	3.71±3.06	p<0.001

Data are reported as mean (SD) or median (25th-75th percentiles) as appropriate;
All analyses are adjusted for the matching variables

eTable 7. Spirometry data in 55 Ehlers-Danlos patients.

Parameter	Mean ± SD
FVC, liters	3.94 ± 0.94
FVC, % pred.	106.7 ± 19.7
FEV1, liters	3.20 ± 0.76
FEV1, % pred.	102.3 ± 20.26
FEV1/FVC, %	81.5 ± 6.6

FEV1, forced expiration in one second; FVC, forced vital capacity; % pred., expressed percentage of predicted values

eTable 8. Craniofacial measurements in EDS patients and controls.

Type	Facial measurements	Facial landmarks	EDS N = 59	Control N = 62	p value
Linear (cm)	Upperfacedepth	t-n	12.9±0.8	12.8±0.7	0.535
	Midfacedepth	t-sn	13.2±0.8	13.2±0.8	0.347
	Lowerfacedepth	t-gn	14.9±1.0	14.9±0.9	0.977
	Faceheight	n-gn	12.0±0.8	12.1±0.7	0.285
	Noseheight	n-sn	5.6±0.5	5.7±0.4	0.112
	Upperfaceheight	n-sto	7.7±0.6	7.8±0.5	0.707
	Lowerfaceheight	sn-gn	6.5±0.5	6.5±0.6	0.680
	Anteriormandibularheight	sto-gn	4.3±0.4	4.4±0.4	0.308
	Mandibularlength(diagonal)	gn-go	10.1±0.9	10.1±0.7	0.901
	Mandibularlength(horizontal)	me-t(TH)	9.0±1.3	9.0±1.1	0.374
	Lateralfaceheight	ex-go	10.4±0.6	10.5±0.8	0.539
	Maxillary-mandibulardepth	sn-gn(TH)	1.1±0.8	1.1±0.8	0.556
	Cricomentalspacedistance	cer-cri-me	1.3±0.6	1.5±0.5	0.156
	Facewidth	tl-tr	14.6±0.8	14.5±0.7	0.412
	Mandiblewidth	gol-gor	11.9±0.9	11.8±0.8	0.313
	Eyewidth	exl-enl	3.0±0.3	3.0±0.2	0.110
	Intercanthalwidth	enl-enr	3.4±0.4	3.2±0.3	0.007
	Biocularwidth	exl-exr	9.4±0.5	9.3±0.4	0.432
	Nosewidth	all-alr	3.8±0.4	3.9±0.3	0.253

Angle (°)	Maxillarydepthangle	t-n-sn	79.0±4.7	78.6±4.4	0.643
	Mandibulardepthangle1	t-n-sl	71.2±4.4	70.5±4.4	0.386
	Mandibulardepthangle2	t-n-gn	69.2±4.4	69.1±4.8	0.903
	Maxillary-mandibularrelationship	sn-n-sl	7.8±2.4	8.1±2.7	0.506
	Maxillary-mandibularrelationship2	sn-me-H)	75.6±6.7	74.7±6.2	0.486
	Mandibular-nasion	go-n-gn	39.1±4.1	38.8±3.4	0.666
	Mandibular-subnasion	go-sn-gn	57.0±5.8	57.1±5.2	0.896
	Headbaseinclination	t-n-TH	19.7±6.9	19.1±5.7	0.640
	Mandibularplane	go-gn-TH	19.7±7.2	20.0±6.8	0.790
	Mandibular	t-go-gn	121.2±7.9	199.8±7.0	0.317
	Facialaxis	n-tandgo-gn	39.3±5.4	39.1±6.2	0.851
	Cervicomental	nec-cer-me	136.4±17.3	131.4±12.9	0.214
	Mandibularwidth-length	gor-me-gol	75.2±5.9	74.2±4.6	0.331
	Facewidth-midfacedepth	tr-sn-tl	66.6±3.5	66.4±3.4	0.723
Volume (cm^3)	Middlecranialfossa	tl-tr-n-sn	141.6±23.1	143.1±22.4	0.726
	Maxillary	tl-tr-sn-me	181.8±28.6	181.4±32.0	0.937
	Mandibular	tl-tr-gol-gor-me	146.4±36.5	153.6±44.1	0.330
	Maxillary-mandibular	tl-tr-go-go-sn-me	328.2±55.5	335.0±68.6	0.552
Ratio (%)	Facewidth/eyewidth	tl-tr/ex-en	4.9±0.5	4.8±0.3	0.169
	Facial Index	n-gn-height/ bizygomatic-width	90.9±7.2	91.6±9.0	0.667
	Facewidth-depth	tl-tr/t-n	137.9±9.7	137.5±8.6	0.846
	Mandibularwidth-length	gol-gor/go-me	158.3±18.6	155.0±14.1	0.271

t = tragion; ex = exocanthion; sup = infraorbital rim; g = glabella; n = nasion; sn = subnasion; sto = stomion; sl = sublabiale; gn = gnathion; me = mentum; cer = cervical point; ty = thyroid; cr = cricoid; np; neck plane; ste = sternal notch; go = gonion; ra = ramus; op = opisthocranion; v = vertex; aneck = anterior neck; pneck = posterior neck; tl = tragion (L); tr = tragion (R); gol = gonion (L); gor = gonion (R); eul = euryon (L); eur = euryon (R); exl = exocanthion (L); exr = exocanthion (R); enl = endocanthion (L); enr = endocanthion (R); lal = alare (L); ral = alare (R); lneck = neck (L); rneck = neck (R); (L) = left side on the photograph, (R) = right side on the photograph

eTable 9. Characteristics of EDS patients in various cohorts.

	Current EDS Cohort N=100	USZ register N=125	EDS Netz Schweiz N=120	EDS Initiative N=230	p-value for difference (global test)
Age (years)	38±13	41±11	35±7	36±6	0.543
% female	81	61	80	73	0.121

Not data could be obtained from the EDS Selbsthilfe Verein (approx. 120 members).

eTable 10. Subgroup analysis of participant characteristics by EDS type.

	Classical type (n=46)	Hypermobility type (n=35)	Vascular type (n=9)	Unknown type (n=7)	p value for difference (global test)
AHI (/h), median (IQR)	3.6 (1.4-8.7)	1.7 (0.9-4.5)	5.9 (1.8-8.0)	3.3 (2.7-9.1)	0.241
Symptomatic OSA, %	11	14	11	14	0.779
Aortic sinus, cm mean ± SD	3.2±0.4	3.2±0.3	2.85±0.1	3.3±0.2	0.435
Aorta ascendens, cm mean ± SD	3.1±0.5	3.0±0.5	2.7±0.2	3.0±0.2	0.435

AHI = apnea-hypopnea index; EDS = Ehlers-Danlos Syndrome; IQR = interquartile range; SD = standard deviation. EDS Types with n<5 were excluded from the subgroup analysis.

eTable 11. Craniofacial measurements in the EDS sample with and without OSA.

Type	Facial measurements	Facial landmarks	EDS sample		p value
			AHI≥5 N=14	AHI<5 N=45	
Linear (cm)	Anteriormandibularheight	sto-gn	4.2±0.3	4.3±0.4	0.068
	Cricomentalspacedistance	cer-cri-me	1.0±0.6	1.5±0.6	0.011
	Mandiblewidth	gol-gor	11.8±0.9	12.4±0.9	0.061
	Eyewidth	exl-enl	2.8±0.2	3.0±0.2	0.027
	Nosewidth	all-alr	4.0±0.4	3.7±0.4	0.026
Angle (°)	Cervicomental	nec-cer-me	148.2±16.6	132.8±16	0.003

t = tragion; ex = exocanthion; sup = infraorbital rim; g = glabella; n = nasion; sn = subnasion; sto = stomion; sl = sublabiale; gn = gnathion; me = mentum; cer = cervical point; ty = thyroid; cr = cricoid; np; neck plane; ste = sternal notch; go = gonion; ra = ramus; op = opisthocranion; v = vertex; aneck = anterior neck; pneck = posterior neck; tl = tragion (L); tr = tragion (R); gol = gonion (L); gor = gonion (R); eul = euryon (L); eur = euryon (R); exl = exocanthion (L); exr = exocanthion (R); enl = endocanthion (L); enr = endocanthion (R); lal = alare (L); ral = alare (R); lneck = neck (L); rneck = neck (R); (L) = left side on the photograph, (R) = right side on the photograph

eTable 12. Subgroup analysis of primary outcomes by study subgroups.

	Study population	Echocardiography - subgroup	Blood pressure - subgroup	Craniofacial analysis - subgroup	p value for difference (global test)
n (EDS / controls)	100/100	46/40	58/59	59/62	-
AHI, h^{-1} (IQR)	1.3 (0.4-4.0)	1.7 (0.3-4.1)	1.3 (0.3-4.0)	1.3 (0.3-4.0)	0.789
OSA (AHI≥5/h), %	19.0	18.4	18.1	18.4	0.694

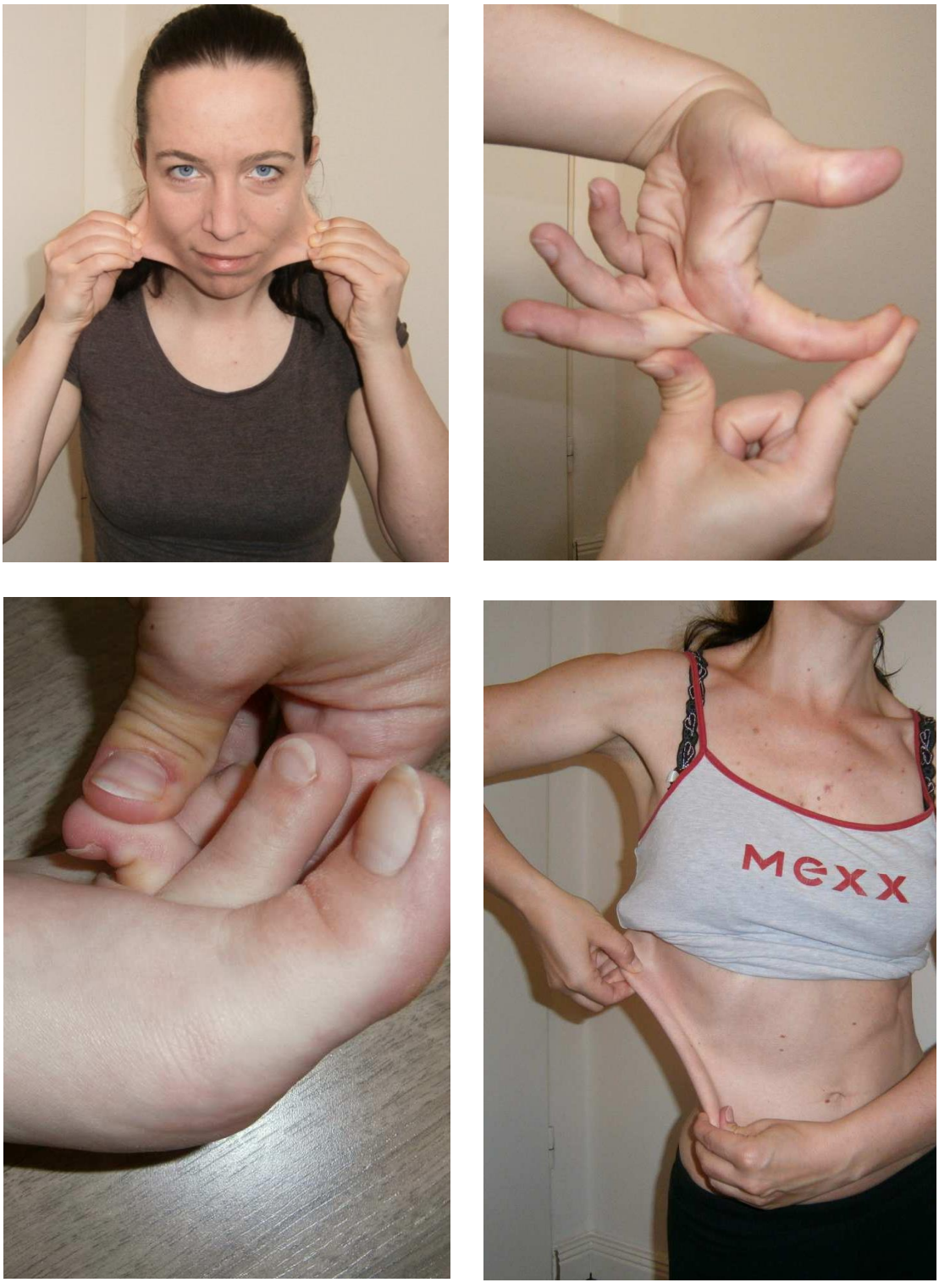
AHI = apnea-hypopnea index; EDS = Ehlers-Danlos Syndrome; IQR = interquartile range.

eTable 13. Discriminatory property of the STOP-BANG Score by EDS presence.

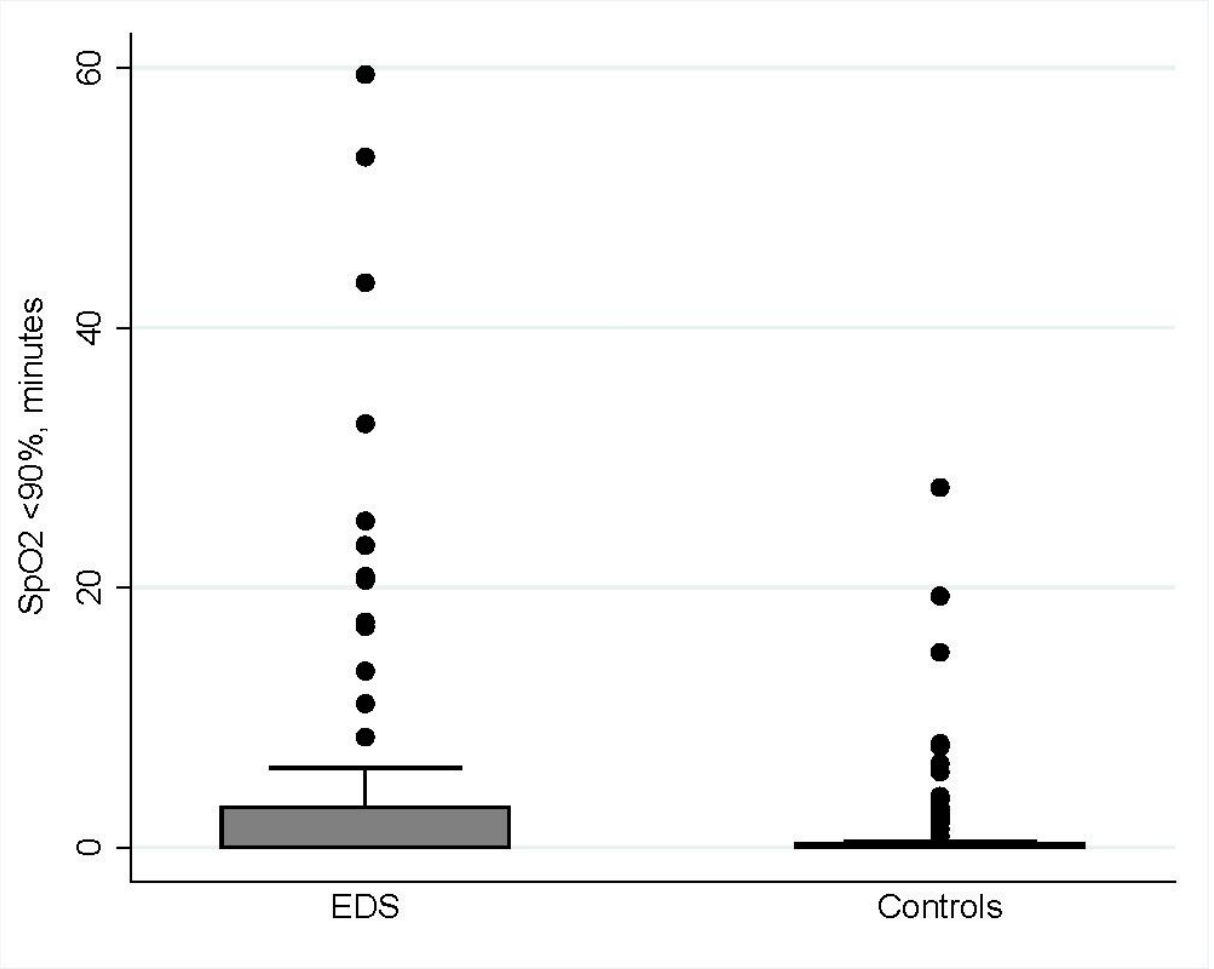
STOP BANG-Score	Sensitivity (AHI≥5/h)	Specificity (AHI≥5/h)	Correctly classified
EDS population (n=100; ROC area 0.80)			
≥1	96.88%	5.88%	35.00%
≥2	90.63%	54.41%	66.00%
≥3	65.63%	80.88%	76.00%
≥4	43.75%	92.65%	77.00%
≥5	21.88%	95.59%	72.00%
≥6	15.63%	98.53%	72.00%
Control population (n=100; ROC area 0.78)			
≥1	97.37%	21.60%	36.00%
≥2	89.47%	64.81%	69.50%
≥3	68.42%	86.42%	83.00%
≥4	44.74%	95.06%	85.50%
≥5	23.68%	96.91%	83.00%
≥6	13.16%	99.38%	83.00%

AHI = apnea-hypopnea index; EDS = Ehlers-Danlos Syndrome; *ROC = Receiver Operating Characteristic; STOP BANG Score interpretation according to www.stopbang.ca:* Low Risk 0 - 2; Intermediate Risk 3 - 4; High Risk 5 - 8

eFigure 1. Example of clinical findings in a 34-year-old female EDS patient.

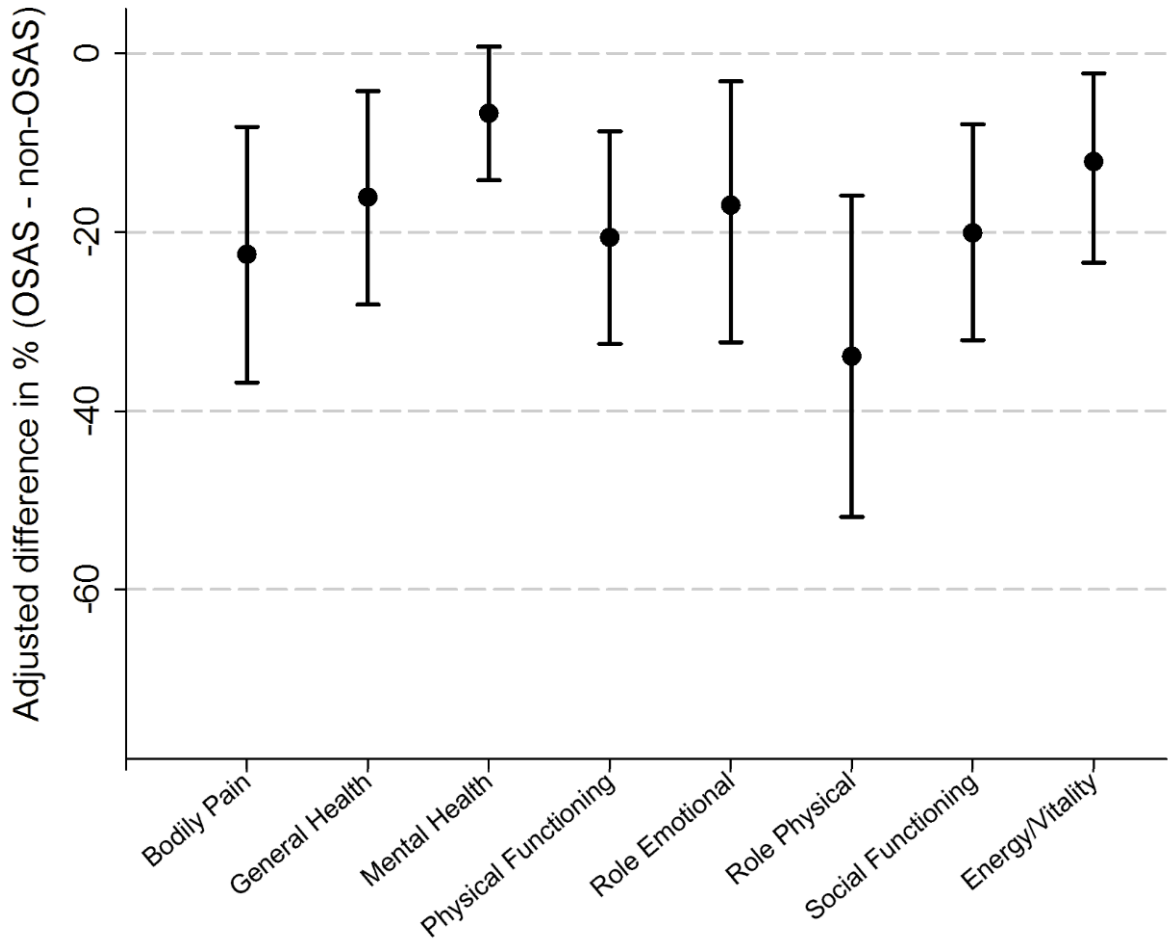


eFigure 2.Oxygen levels in the sleep studies.



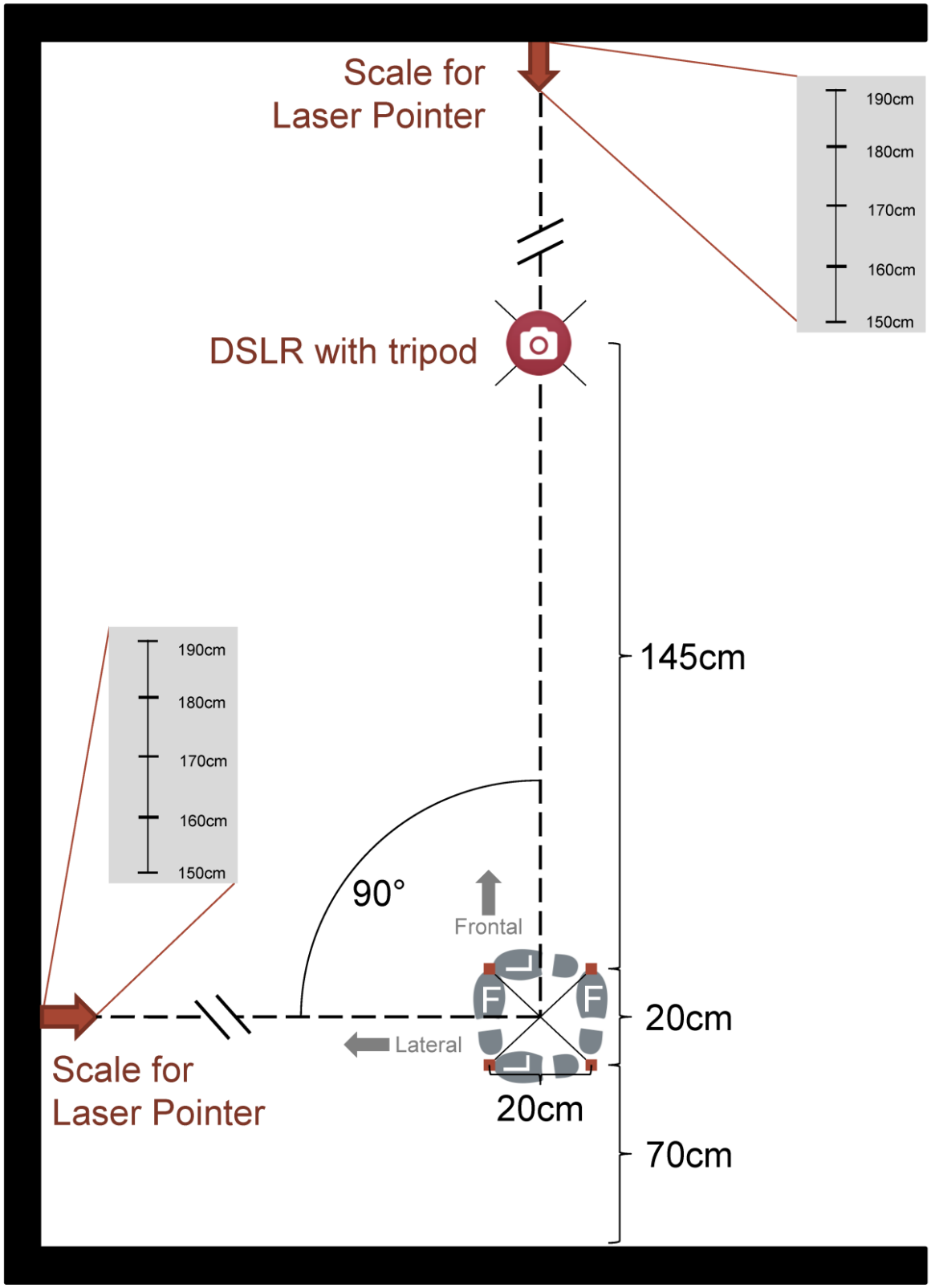
The measured median (quartiles) oxygen levels below 90% were significantly higher in the EDS group when compared to the control group, with 0 (0-22) vs. 0 (0-2); p=0.003.

eFigure 3. SF-36 adjusted difference between the OSAS population and the asymptomatic population.



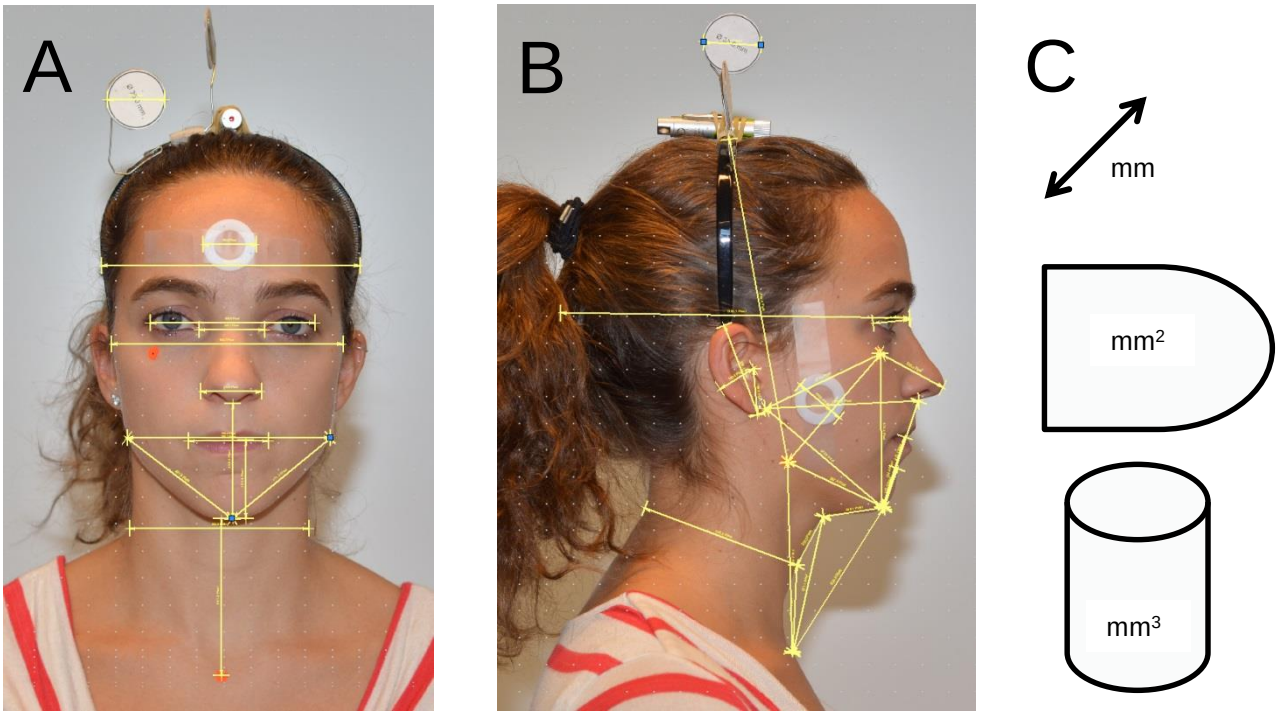
Mean and 95% confidence interval each of the Short-Form 36 (SF-36) domains displayed in % adjusted difference (OSAS – non-OSAS).

eFigure 4. Craniofacial photographic setup plan.



DSLR=digital single-lens reflex camera

eFigure 5. Example of a 28-year-old female participant undergoing craniofacial phenotyping analysis.



Photographic craniofacial phenotyping reflects underlying aspects of craniofacial skeletal abnormalities. Frontal (**A)** and profile **(B)** digital photographs from the head and neck were obtained from participants according to a standardized approach.[2] Distances, areas, and volumes of interest **(C)** can be derived from each participant.

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