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Short communication

Whole blood hypoxia-related gene expression reveals novel pathways to obstructive sleep apnea in humans

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ABSTRACT

In this study, our goal was to identify the key genes that are associated with obstructive sleep apnea (OSA). Thirty-five volunteers underwent full in-lab polysomnography and, according to the sleep apnea hypopnea index (AHI), were classified into control, mild-to-moderate OSA and severe OSA groups. Severe OSA patients were assigned to participate in a continuous positive airway pressure (CPAP) protocol for 6 months. Blood was collected and the expression of 84 genes analyzed using the RT^2 Profiler^{TM} PCR array. Mild-to-moderate OSA patients demonstrated down-regulation of 2 genes associated with induction of apoptosis, while a total of 13 genes were identified in severe OSA patients. After controlling for body mass index, *PRPF40A* and *PLOD3* gene expressions were strongly and independently associated with AHI scores. This research protocol highlights a number of molecular targets that might help the development of novel therapeutic strategies.

1. Introduction

Obstructive sleep apnea (OSA) is characterized by repetitive obstruction of the upper airway, resulting in pauses in breathing and subsequent oxygen desaturation. The majority of sleep disorders, including OSA, are caused by complex interactions between genes and the environment, leading studies to focus on the extent to which genes predetermine susceptibility to intermittent apneas, as well as on the effects of hypoxia and sleep fragmentation on the expression of candidate genes (Arnardottir et al., 2009). Previous studies have identified a key area of genetic influence as the cellular reaction to hypoxia (Arnardottir et al., 2009). Knowledge of the genetic factors underlying the phenotypic traits following hypoxia insults can reveal how cells confer vulnerability or resistance to hypoxia and modulate responsiveness to therapeutic interventions aimed at restoring respiratory, cardiovascular, cognitive, and metabolic functions (Cassavaugh and Lounsbury, 2011). Here, our goal was to identify the genes that are associated with hypoxia that play a role in OSA. This approach resulted in the identification of novel candidate genes of OSA, pointing the way to how genetic factors may modulate apneic susceptibility, as well as help the potential development of novel therapeutic targets.

2. Materials and methods

2.1. Study population and polysomnography

A total of 35 volunteers underwent full in-lab polysomnography (PSG; EMBLA System N7000, software RemLogic Version 2.0, CO, USA) during their habitual sleep time. Standard montage and criteria were used for scoring sleep stages, arousals, leg movements, and respiratory events according to the guidelines from the American Academy of Sleep Medicine, including the recommend rule for hypopneas – a decrease of ≥30% in the nasal pressure signal of baseline, with a duration ≥10 s, with a desaturation ≥4% associated. A total of 11 individuals were selected as controls (AHI < 5), 10 patients were diagnosed with mild-moderate (AHI 5 to <30), and 14 patients with severe OSA (AHI > 30 and clinical complaints). The research protocol was approved by the institution's ethics committee (CEP no. 985/08), and informed consent was obtained from each patient. The total number of patients recruited, inclusion and exclusion criteria, primary and secondary endpoints of the clinical trial were registered in ClinicalTrials.gov (identifier NCT01392339). Data collection, processing and analysis were completed in 10 months.

2.2. CPAP treatment

Patients with severe OSA underwent a continuous positive airway pressure (CPAP) protocol for 6 months to examine the influence of treatment on gene expression. All patients underwent a pressure titration PSG to find the ideal pressure to eliminate obstructive apnea, hypopnea, respiratory effort related arousal and

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snoring. After that they received a CPAP device (REMstar® Plus; Respironics Inc., Murrysville, PA) that allowed for pressure variance between 4 and 20 cmH_2O. A 20-min latency period was set before gradually increasing the pressure to its ideal value. The devices had a time-of-use function that measured the amount of time participants had effective delivery of treatment pressure (mask-on pressure). Patients also completed a sleep diary that consisted of questions about their use of CPAP and their amount of sleep per night. From that a mean percentage of CPAP use was calculated as a ratio of total sleep time over the number of hours using the device, as reported in the diary. A trained nurse contacted patients after 1 week, and 1, 3 and 6 months to check the adherence of treatment.

2.3. PCR array and data analysis

Blood samples were collected at 8:00 AM. Blood was collected in PAXgene® tubes (PreAnalytiX GmbH, Hombrechtikon, Switzerland), and total RNA was isolated according to the manufacturer's directions. To evaluate gene expression profile, the RT^2 Profiler^{TM} hypoxia signaling pathway PCR array, consisting of 84 genes known to be involved in the hypoxic response (Catalog #PAHS-032, SABiosciences, Frederick, MD, USA) was used according to the standard protocol. Gene expression associated with a p-value of <0.001 or absolute fold change >2 were taken as differently expressed between groups.

2.4. Functional annotation and network analysis of profile genes

Functional annotation and network analysis of the profile genes was performed using Ingenuity Pathway Analysis (IPA) software (Ingenuity Systems Inc., Redwood City, CA). Statistical significance for enrichment of functional groups was based on Fisher's exact test and corrected for multiple testing.

Table 1
List of differently expressed genes identified in mild-moderate and severe OSA patients, as well as after 6 months of CPAP treatment.

Group	Gene Name	Description	FC	FC	
Control × OSA 5 to <30					
	DAPK3	Death-associated protein kinase 3	−1.2		
	KAT5	K(lysine) acetyltransferase 5	−1.2		
Control × OSA >30				CPAP	
	ANGPTL4	Angiopoietin-like 4	−1.6	−1.9	*
	DAPK3	Death-associated protein kinase 3	−1.4	−2.2	*
	EP300	E1A binding protein p300	−2.1	−1.1	NS
	KAT5	K(lysine) acetyltransferase 5	−1.3	−1.8	*
	KHSRP	KH-type splicing regulatory protein	−1.4	−2.2	*
	M0CS3	Molybdenum cofactor synthesis 3	−1.2	−1.8	*
	MT3	Metallothionein 3	−1.8	−1.0	NS
	NAA10	ARD1 homolog A, N-acetyltransferase	−1.2	−1.4	NS
	N0TCH1	Notch homolog 1, translocation-associated	−2.4	1.3	NS
	PL0D3	Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 3	−1.4	−1.6	NS
	PRPF40A	PRP40 pre-mRNA processing factor 40 homolog A	−1.3	−1.1	NS
	RPL28	Ribosomal protein L28	−1.4	−2.3	*
	SNRNP70	Small nuclear ribonucleoprotein 70 (U1)	−1.3	1.5	NS
OSA>30 × CPAP treatment					
	ANGPTL4	Angiopoietin-like 4	−1.9		
	ARNT2	Aryl-hydrocarbon receptor nuclear translocator 2	3.5		
	BAX	BCL2-associated X protein	−2.1		
	BHLHE40	Basic helix-loop-helix family, member e40	−2.9		
	CAT	Catalase	−2.5		
	CSTB	Cystatin B (stefin B)	−2.0		
	DAPK3	Death-associated protein kinase 3	−2.2		
	ECE1	Endothelin converting enzyme 1	−6.0		
	EN01	Enolase 1, (alpha)	−2.2		
	HIF1A	Hypoxia inducible factor 1, alpha subunit	−2.4		
	HIF1AN	Hypoxia inducible factor 1, alpha subunit inhibitor	−2.3		
	HM0X1	Heme oxygenase (decycling) 1	−1.8		
	IL6ST	Interleukin 6 signal transducer (gp130, oncostatin M receptor)	−1.7		
	KAT5	K(lysine) acetyltransferase 5	−1.8		
	KHSRP	KH-type splicing regulatory protein	−2.2		
	MAN2B1	Mannosidase, alpha, class 2B, member 1	−2.4		
	M0CS3	Molybdenum cofactor synthesis 3	−1.8		
	NUDT2	Nudix (nucleoside diphosphate linked moiety X)-type motif 2	−1.4		
	PPARA	Peroxisome proliferator-activated receptor alpha	−1.9		
	PPP2CB	Protein phosphatase 2 (formerly 2A), catalytic subunit, beta isoform	−2.3		
	PRKAA1	Protein kinase, AMP-activated, alpha 1 catalytic subunit	−1.4		
	PSMB3	Proteasome (prosome, macropain) subunit, beta type, 3	−3.2		
	PTX3	Pentraxin-related gene, rapidly induced by IL-1 beta	−2.0		
	RPL28	Ribosomal protein L28	−2.3		
	RPS2	Ribosomal protein S2	−1.9		
	SAE1	SUMO1 activating enzyme subunit 1	3.6		
	SLC2A1	Solute carrier family 2 (facilitated glucose transporter), member 1	−2.8		
	SSSCA1	Sjogren syndrome/scleroderma autoantigen 1	4.2		
	SUM02	SMT3 suppressor of mif two 3 homolog 2 (S. cerevisiae)	−2.1		
	TUBA4A	Tubulin, alpha 4a	−2.3		
	UCP2	Uncoupling protein 2 (mitochondrial, proton carrier)	−2.5		
	VEGFA	Vascular endothelial growth factor A	−1.6		

Annotation of genes identified by Student's t-test ($p < 0.001$) or absolute fold change in gene expression of >2 were taken as significant. FC, fold change; NS, not significant; OSA, obstructive sleep apnea; CPAP, continuous positive airway pressure.

* <0.001