A Study of The Relationship between The Interleukin-6 Gene and Obstructive Sleep Apnea

Emma K. Larkin[1], Sanjay R. Patel[2,3], Xiaofeng Zhu[4], Russell P. Tracy[5], Nancy S. Jenny[5], Alex P. Reiner[6], Jeremy Walston[7], and Susan Redline[2,3,8]

Abstract

Because obstructive sleep apnea (OSA) is associated with increased levels of inflammatory cytokines, we examined the relationship between OSA and polymorphisms for interleukin-6 (*IL6*). Six single nucleotide polymorphisms (SNPs) within *IL6* were genotyped in 259 African Americans from the Cleveland Family Study with replication conducted in the Cardiovascular Health Study ($n = 124$). OSA was dichotomized into apnea hypopnea index (AHI) > 15, or on treatment versus absent: AHI < 5. Logistic regression was conducted, adjusting for age and sex in models with and without body mass index (BMI). SNP IL6–6021 was associated with a decreased risk of OSA after adjusting for BMI (Odds Ratio for T allele 0.24, 95%CI [0.09–0.67], $p = 0.006$, $q = 0.07$) under an additive model. This same allele was associated with increased BMI. The results from the replication sample were consistent in direction though not statistically significant ($p = 0.23$). The SNPs were studied in European- Americans, although, the minor allele frequency in *IL6*–6021 was too low (4%) for meaningful comparisons. A synonymous SNP within the *IL6* coding region was protective of OSA in African Americans; with qualitatively similar findings observed in another cohort. This suggests that variants in IL6 may influence the risk of OSA in a pathway that is not explained by obesity. Clin Trans Sci 2010; Volume 3: 337–339

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Introduction

Genetic variants that influence inflammation may contribute to obstructive sleep apnea (OSA) susceptibility through effects on airway size, muscle function, and chemoreflexes.[1] We explored whether polymorphisms within interleukin-6 (*IL6*), an important proinflammatory cytokine, are associated with OSA in members of the Cleveland Family Study (CFS). Participants from the Cardiovascular Health Study (CHS) were used for replication. Augmentation of proinflammatory pathways may affect upper airway characteristics, including pharyngeal edema and proprioceptive reflexes influential in maintaining airway patency.[1] Inflammation may feed into a cycle of worsening apnea by creating histopathologic changes in the upper airway affecting the mucosa and muscular dilator muscles.[2] Research indicates that IL-6 mRNA production in upper airway tissues is higher in patients with severe compared to mild OSA.[3] Markers for systemic inflammation including elevated IL-6 levels are also associated with OSA.[4]

Methods

The CFS is a longitudinal cohort study of large nuclear and extended pedigrees (mean pedigree size = 7.0) investigating the genetic and nongenetic epidemiology of OSA. The nested case-control sample consisted of participants older than 16 years who had attended overnight polysomnography measured from the CFS's last exam. This sample included index family members selected to provide information for genetic analyses.[5] OSA cases were identified by an elevated apnea hypopnea index (AHI ≥ 15) or current treatment with continuous positive airway pressure, while nonapneic controls were defined by an AHI < 5. The moderate level of AHI ≥ 15 was chosen as a common threshold to define physiological abnormality, compared to a clinical diagnosis. Fasting plasma IL-6 was measured using sandwich enzyme-linked immunosorbent assay (R&D systems, Minneapolis, MN, USA).

Six tag single nucleotide polymorphisms (SNPs) in *IL6* were genotyped (Taqman by Design, Applied Biosystems, Foster City, CA) based on coverage and functionality from a population of European-American descent.[6] SNPs selected include two within the 5′-untranslated flanking region (*IL6*–1111 [rs1800796], IL-6–1510 [rs1800795]), two intronic SNPs (IL6–2892 [rs2069837], *IL6*–3572 [rs1554606]), one synonymous SNP (*IL6*–6021 [rs2069849]), and one in the 3′-untranslated flanking region (*IL6*–7592 [rs1818879]).

Logistic regression (with generalized estimating equations using an exchangeable correlation structure and robust covariance estimates and adjusting for age and sex in models with and without body mass index [BMI]) was used to test the association between OSA and SNPs under an additive model (0 = no minor allele, 1 = one minor allele, 2 = 2 minor alleles), when possible as indicated in the table legend (*Table 1*). Each odds ratio (OR) represents the increased risk of OSA for each unit increase in the number of minor alleles present in cases compared to controls when coded additively.

Analyses were repeated on 272 individuals with an AHI $<$ 5 (218 European Americans) and 417 individuals with an AHI $\geq$ 15 (346 European Americans) drawn from a sample of the Cardiovascular Health Study (CHS), a community-based study of unrelated individuals (mean age 77.2 years) designed to identify cardiovascular disease risk factors.[7]

Results

The sample consisted of 259 African-American individuals (50% OSA cases) from 77 families. The mean age of the African-American

[1]Division of Allergy, Pulmonary and Critical Care, Vanderbilt University Medical Center, Nashville, Tenessee, USA; [2]Center for Clinical Investigation, Case Western Reserve University, School of Medicine, Cleveland, Ohio, USA; [3]Division of Pulmonary, Critical Care and Sleep Medicine, Case Western Reserve University, School of Medicine, Cleveland, Ohio, USA; [4]Department of Epidemiology and Biostatistics, Case Western Reserve University, School of Medicine, Cleveland, Ohio, USA; [5]Department of Pathology, University of Vermont College of Medicine, Burlington, Vermont, USA; [6]Cardiovascular Health Research Unit, University of Washington, Seattle, Washington, USA; [7]Division of Geriatric Medicine and Gerontology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA; [8]Division of Sleep Medicine, Brigham and Women's Hospital and Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, USA.

Correspondence: Emma K. Larkin (Emma.Larkin@vanderbilt.edu)

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Cleveland Family Study		No BMI adjustment			BMI adjustment		
	Minor allele (%)	OR	95%CI	*p* value	OR	95%CI	*p* value
IL6–1111†	C (7)	1.44	(0.72-2.89)	0.30	1.70	(0.77-3.74)	0.19
IL6–1510†	C (8)	1.31	(0.59-2.90)	0.50	1.43	(0.53-3.88)	0.48
IL6–2892†	G (14)	1.29	(0.67-2.49)	0.45	1.14	(0.52-2.50)	0.74
IL6–3572‡	T (40)	0.80	(0.51-1.26)	0.33	0.54	(0.25-1.17)	0.12
IL6–6021‡	T (18)	0.63	(0.31-1.28)	0.20	0.24	(0.09-0.67)	0.0061
IL6–7592‡	A (20)	1.14	(0.72-1.80)	0.58	1.24	(0.62-2.48)	0.54
IL6–1111†	C (8)	0.77	(0.30-1.96)	0.58	0.94	(0.35-2.58)	0.90
IL6–1510†	C (12)	2.37	(0.90-6.26)	0.08	2.60	(0.88-7.71)	0.09
IL6–2892†	G (9)	2.45	(0.81-7.47)	0.11	3.11	(0.90-10.75)	0.07
IL6–3572‡	T (33)	1.02	(0.57-1.83)	0.94	0.88	(0.46-1.69)	0.70
IL6–6021‡	T (13)	0.79	(0.37-1.70)	0.55	0.60	(0.26-1.40)	0.23
IL6–7592‡	A (22)	1.07	(0.59-1.93)	0.83	1.25	(0.64-2.46)	0.51

*Other covariates include age, age × age, age × sex, percent African ancestry for Cleveland Family Study and age, age × age, and sex for Cardiovascular Health Study.
†Dominant mode of inheritance.
‡Additive mode of inheritance.

Table 1. Odds Ratio (OR) for the relationship between the minor alleles of SNPs and OSA in African-Americans.*

cases was 51.1 ± 13.0 years with a mean BMI of 39.6 ± 9.2 kg/m^2 and 58% were male. The African-American controls were younger (mean age of 35.5 ± 16.2 years) with lower mean BMI of 29.4 ± 7.7 kg/m^2 and 28% were male. The 201 (47% OSA cases) European-American individuals were from 62 families. Like the African-American population, the European-American cases were older than controls (55.5 ± 13.0 vs. 39.4 ± 17.9 years, respectively), had higher BMI (37.4 ± 9.8 vs. 28.5 ± 6.3 kg/m^2, respectively), and more likely to be male (64% vs. 38%, respectively.)

In the CHS-confirmation cohort, the mean ages (75.5 ± 4.5 years) of cases and controls (75.4 ± 4.3 years) were similar. The BMI of cases was slightly higher (30.2 ± 5.1 kg/m^2) than controls (27.4 ± 4.5 kg/m^2). In the African-American cases 50% were male; in controls 26% were male. The 346 (55% male) European-American cases averaged 78.0 ± 4.4 years with a mean BMI of 28.1 ± 4.7 kg/m^2 compared to 218 controls: 77.5 years ± 4.1, 26.0 ± 4.1, and 29% were male.

In African Americans, the most significant SNP was *IL6*–6021 (OR 0.24, 95% CI: 0.09–0.67, p = 0.006) in the BMI-adjusted model (*Table 1*). In other words, each copy of the minor T allele (18% prevalence) was associated with a reduction in the risk of OSA by 76%. The minimum false discovery rate (q-value) was 0.07, allowing for multiple comparisons.[8] The *IL6*–6021 T allele also was associated with BMI and IL-6 levels in African Americans; after adjusting for age and sex, each minor allele was associated with a 2.46 kg/m^2 increase in BMI (p = 0.03) and a 1.22 pg/mL increase in IL-6 (p = 0.03), which persisted after adjusting for OSA status and BMI.

Among the 124 African-American participants from CHS, none of the SNPs were significant at the 0.05 level; however, the minor allele of *IL6*–6021 was associated with qualitatively similar findings; that is, with a decreased risk of OSA (OR: 0.60, 95%CI: 0.26–1.40) and a 0.83 kg/m^2 increase in BMI for each allele (p = 0.27).

In European Americans, the minor allele frequency for IL6–6021 was only 4%, which did not provide a stable estimate of association. Other SNPs were not associated with OSA in European Americans (p > 0.35). In European Americans from CHS, the strongest association observed was for the minor allele of IL6–1111 (rs1800796) with an OR of 1.73 (95%CI: 0.88–3.49, p = 0.11) after BMI adjustment.

Discussion

An association between an IL6 polymorphism and OSA in BMI-adjusted models provides preliminary evidence that genes in inflammation are related to OSA susceptibility. The significant variant is synonymous and may play a role in pathophysiology due to effects on splice regulation or other transcriptional mechanisms.[9] The SNP may also be in linkage disequilibrium with another causal SNP. Interestingly, the minor allele was associated with higher BMI and IL-6 plasma levels, but lower risk of OSA, and its protective association with OSA was strengthened after BMI adjustment.

The biological plausibility of this relationship is supported by evidence that OSA is associated with prominent inflammatory changes in both mucosal and muscular layers of the upper airway, changes that may affect airway collapsibility due to altered contractility or stiffness.[2] Systemic alterations in inflammatory cytokines also may influence ventilatory or sleep-wake control mechanisms. We could not address the association of *IL6* genetic variants, IL-6 tissue levels, and OSA. Although elevated plasma IL-6 levels may follow OSA-related stresses, here, plasma IL-6 levels were increased in association with the "nonrisk" allele. The possibility that genetic variants associated with higher plasma IL-6 levels and with greater obesity may have some protective effects on OSA susceptibility needs further exploration into complex relationships between inflammation, obesity, and OSA. We also note as a limitation that by selecting the extremes of the AHI distribution, our cases and controls differed substantially by age and BMI, This gap between cases and controls was lessened in the second CHS cohort.

Our findings were likely stronger in the African-American sample from the CFS because CFS included younger individuals with OSA compared to the older community-based CHS, where OSA may be associated with age-related comorbidities. Moreover, the two cohorts have different distributions of BMI, which limit the ability of CHS to serve as a true replication sample. The Spearman correlation between BMI and AHI was much higher in the younger CFS (r = 0.56) than in the older CHS (r = 0.28).

The significant allele was too rare in our sample of European Americans to provide reliable estimates of association in this group. Unfortunately, few cohorts exist with both genotyping and OSA phenotyping data, especially in AAs, limiting attempts at replication. Although the association was not statistically significant in CHS, the direction and magnitude were consistent with CFS. A recent study reported a protective association between the minor G allele in a neighboring SNP, *IL6*–1111 (rs1800796) and OSA in nonobese individuals from China.[10] Our results are similar in directionality; however, the G allele frequency is >90% in our cohorts.

These preliminary results point to the potential effects of a pleiotropic gene, which may influence OSA and obesity through different pathways. Because OSA is influenced by physiological and anatomic risk factors, evaluating models with and without BMI adjustment may help elucidate genetic underpinnings that operate distinctly from obesity.

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