Obstructive Sleep Apnea and Circulating Potassium Channel Levels

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Background—Cardiac arrhythmias and sudden cardiac death are more frequent in patients with obstructive sleep apnea (OSA). OSA is associated with QT prolongation, and QT prolongation is an independent risk factor for sudden cardiac death. Because QT prolongation can be mediated by potassium channel loss of function, we tested whether OSA or continuous positive airway pressure therapy altered mRNA expression of circulating white blood cell potassium channels.

Methods and Results—In total, 28 patients with OSA newly diagnosed by polysomnogram and 6 participants without OSA were enrolled. Potassium channel levels in white blood cells at baseline and at a 4-week follow-up visit were compared. There was a significant inverse correlation between the severity of the OSA stratified by apnea–hypopnea index and mRNA expression of the main potassium channels assessed: KCNQ1 (r = –0.486, P = 0.007), KCNH2 (r = –0.437, P = 0.016), KCN1 (r = –0.567, P = 0.001), KCN2 (r = –0.442, P = 0.015), and KCN4 (r = –0.468, P = 0.009). In addition, KCNQ1, KCNH2, and KCNE1 inversely correlated with the oxygen desaturation index. After 4 weeks of continuous positive airway pressure therapy, circulating KCNQ1 and KCN2 were increased 1.4 ± 0.4-fold (P = 0.040) and 2.1 ± 1.4-fold (P = 0.046) in the moderate OSA group. Compared with patients with mild or moderate OSA, patients with severe OSA had a persistently higher apnea–hypopnea index (mild 2.0 ± 1.8, moderate 1.0 ± 0.9, severe 5.8 ± 5.6; P = 0.015), perhaps explaining why the potassium channel changes were not seen in the severe OSA group.

Conclusions—The mRNA expression of most potassium channels inversely correlates with the severity of OSA and hypoxemia. Continuous positive airway pressure therapy improves circulating KCNQ1 and KCN2 in patients with moderate OSA. (J Am Heart Assoc. 2016;5:e003666 doi: 10.1161/JAHA.116.003666)

Key Words: arrhythmia • gene regulation • hypoxia • ion channel • potassium-channel • sleep apnea
were representative of levels in the myocardium.\textsuperscript{16} Based on these observations, we reasoned that circulating white blood cells may experience conditions similar to those of cardiomyocytes and may reveal insights into the mechanism of QT changes with OSA and CPAP. We hypothesized that prolonged QT in OSA represented altered potassium channel regulation and that this regulation would be reflected in circulating white blood cells.

Materials and Methods

Clinical Characteristics of the OSA Population and Inclusion Criteria

This study was a multicenter prospective clinical trial entitled "Sodium Channel Splicing in Obstructive Sleep Apnea (SOCS-OSA)" and was conducted at the Lifespan Health System (Rhode Island Hospital and the Miriam Hospital) in Providence, Rhode Island, and at the University of Illinois at Chicago (UIC) in Chicago, Illinois. The study was approved by the Lifespan and UIC institutional review boards. All study participants signed written informed consent before enrollment. Participants were enrolled from July, 2014 to November, 2015. This study was preparatory for the study listed in ClinicalTrials.gov with identifier NCT02725632.

Participants were adults (aged \( \geq 18 \) years) with OSA newly diagnosed by polysomnogram who agreed to CPAP treatment (OSA group) or adults without OSA (control group). All trial participants were screened with the STOP-Bang Questionnaire\textsuperscript{19} before proceeding to the sleep study. The diagnosis of OSA was confirmed by an overnight sleep study. Based on the severity of apnea–hypopnea index (AHI), OSA patients were assigned to 3 groups: mild (AHI 5–15), moderate (AHI 15–30), or severe (AHI \( \geq 30 \)).\textsuperscript{20} The AHI was defined as the number of apneas and hypopneas per hour and calculated by adding the total number of apnea and hypopnea events and dividing by the total number of minutes of actual sleep time multiplied by 60. Control participants were those with AHI \(< 5\) by polysomnogram.

The overnight sleep study consisted of continuous polygraphic recordings from 10 PM to 7 AM, and the baseline parameters were measured. Arterial oxygen saturation was recorded by digital pulse oximeter, and the nadir arterial oxygen saturation levels (nadir \( O_2 \)) were obtained. The oxygen desaturation index is the number of times per hour of sleep that the blood’s oxygen level drops by \( \geq 3\% \) or \( \geq 4\% \) (ie, ODI4) from baseline. To quantify overall nocturnal desaturation, we used the cumulative percentages of sleep time spent at saturations \(<90\%\) or \(<85\%\). Respiratory disturbance index was defined as the average number of episodes of apnea, hypopnea, and respiratory event–related arousals per hour of sleep documented in the polysomnography.

After 4 weeks of CPAP therapy, an AHI score was measured and documented as residual AHI, which was used to assess the efficacy of CPAP therapy.

The OSA patients were evaluated with the Epworth Sleepiness Scale (ESS)\textsuperscript{21} and the Functional Outcome of Sleep Questionnaire 10 (FOSQ-10)\textsuperscript{22} at the time of initial diagnosis. CPAP compliance data were collected from the CPAP machine in the pulmonary clinic. Improvement of the symptoms were reevaluated by the ESS and FOSQ-10 after 1 month of CPAP treatment.

The exclusion criteria included chronic use of hypnotics; current drug or alcohol addiction; a rhythm other than sinus at enrollment; mandatory and biventricular pacing; a history of heart transplant or left ventricular assist device; active use of intravenous vasodilators, vasopressors, or inotropes; hemodialysis or peritoneal dialysis; active infection; acute coronary syndrome; major trauma or surgery; malignant neoplastic disease on active treatment including chemotherapy and radiation therapy; life expectancy \(< 1\) year; collagen vascular disease on active treatment including steroids and other immunomodulating drugs; systemic steroid use; or concomitant use of an investigational drug.

Sample Collection and Processing

Blood samples were collected in PAXgene blood RNA tubes (Qiagen) and were stored in a freezer at \(-80^\circ C\) before processing. Total RNA was isolated with the use of the PAXgene blood RNA isolation kit and was then converted to cDNA with the use of the SuperScript III cDNA Reverse Transcription Kit (Life Technologies). Only samples with an optical density of 260/280\( \geq 2.0\) and 260/230\( \geq 1.7\) were used. Repeated measures of the same sample varied by \(< 2\%\). Quantitative reverse transcriptase polymerase chain reaction was performed to detect the abundance of potassium channels using iQSYBR Green Supermix (Bio-Rad Laboratories) and the 7500 Fast Real-Time PCR System (Life Technologies). The primer sequences used were HKCNQ1F (5\’-TGCGAGACCCCTGACTTGT-3\’), HKCNQ1R (5\’-CTGGTGAAAGCCCTGCCTGTA-3\’), HKCNH2F (5\’-TCACCCGCTGTTACTT-3\’), HKCNH2R (5\’-CACGGGCTGATAAGTGTA-3\’), HKCNE1F (5\’-AAGCCACACCAGTGCTAAA-3\’), HKCNE1R (5\’-TATATCCA CCCCTACGCCTT-3\’), HKCNE2F (5\’-ATTTCATCTGCCACACACT-3\’), HKCNE2R (5\’-CAAGCGCTGCTAGTGAATTTGG-3\’), HKCNN5F (5\’-AGAGGATTCCACGGAAAGT3\’), HKCNN5R (5\’-ACTGTCGATTTCACGGGT-3\’), HKCN1F (5\’-TCCAGGCTTATGAAACC-3\’), HKCN1R (5\’-GAATTGGTGGAGGAGACA-3\’), HKCN2F (5\’-TGTCACGG ATGCAAATG-3\’), HKCN2R (5\’-CTGGGGCGATAGTGAAGA-3\’), HKCND3F (5\’-TGTGACCGATGATG-3\’), and HKCND3R (5\’-ATGAGTGTCTGATGAGACT-3\’). Quantitative reverse transcriptase polymerase chain reaction thermal
cycling conditions were initial incubation at 50°C for 60 minutes and then termination of the reactions at 85°C for 5 minutes. Polymerase chain reaction was performed with an initial denaturation step at 95°C for 3 seconds and annealing and nucleotide prolongation at 60°C for 30 seconds. Each sample was measured for the target genes KCNQ1, KCNH2, KCNE1, KCNE2, KCNA5, KCNJ11, KCN2, KCND3, and β-actin. Samples were normalized to the level of β-actin.

Statistical Analysis
Data were analyzed using SPSS for Windows (IBM Corp). Continuous variables were described in terms of descriptive statistics (mean and standard deviation). Categorical variables are expressed in terms of frequencies and percentages. Independent samples t tests and Pearson chi-square tests were used for bivariate analysis comparisons of continuous and categorical variables, respectively. The Pearson correlation coefficient was used to assess the correlation between the parameters measured in the sleep study, the severity of OSA, and the mRNA levels of potassium channels. Differences between gene expression at baseline and at the follow-up visit were examined by means of paired Wilcoxon tests. Differences in gene expression among the groups were examined by the Kruskal–Wallis ANOVA or the Mann–Whitney U tests. For all analyses, a P<0.05 was considered statistically significant.

Table 1. Clinical Characteristics of the SOCS-OSA Study

<table>
<thead>
<tr>
<th></th>
<th>No OSA (n=6)</th>
<th>Mild OSA (n=10)</th>
<th>Moderate OSA (n=6)</th>
<th>Severe OSA (n=12)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>43.6±16.6</td>
<td>53.1±7.4</td>
<td>51.3±11.3</td>
<td>48.1±12.2</td>
<td>0.418</td>
</tr>
<tr>
<td>BMI</td>
<td>39.5±9.7</td>
<td>35.6±6.0</td>
<td>37.5±7.6</td>
<td>36.6±10.6</td>
<td>0.776</td>
</tr>
<tr>
<td>STOP-Bang</td>
<td>5.4±0.9</td>
<td>4.1±1.4</td>
<td>5.6±1.3</td>
<td>5.6±1.1</td>
<td>0.022</td>
</tr>
<tr>
<td>AHI</td>
<td>3.5±1.6</td>
<td>9.5±2.9</td>
<td>20.2±1.6</td>
<td>65.6±40.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RDI</td>
<td>6.0±3.3</td>
<td>15.5±5.0</td>
<td>26.6±5.1</td>
<td>45.5±13.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ODI3</td>
<td>3.1±1.7</td>
<td>8.5±4.1</td>
<td>19.3±5.4</td>
<td>32.7±16.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nadir oxygen saturation</td>
<td>86.8±5.0</td>
<td>83.7±4.1</td>
<td>84.4±3.7</td>
<td>74.2±16.0</td>
<td>0.139</td>
</tr>
<tr>
<td>CT90, minute</td>
<td>0.4±0.5</td>
<td>8.8±18.2</td>
<td>7.7±10.5</td>
<td>34.0±63.9</td>
<td>0.016</td>
</tr>
<tr>
<td>CT85, minute</td>
<td>0±0.1</td>
<td>0.4±0.7</td>
<td>0.4±1.0</td>
<td>19.3±47.2</td>
<td>0.077</td>
</tr>
<tr>
<td>CPAP average use, hours/night</td>
<td>4.8±1.3</td>
<td>4.0±2.5</td>
<td>4.1±1.9</td>
<td>0.849</td>
<td></td>
</tr>
<tr>
<td>Residual AHI</td>
<td>2.0±1.8</td>
<td>1.0±0.9</td>
<td>5.8±5.6</td>
<td>0.015</td>
<td></td>
</tr>
<tr>
<td>CPAP median use, hours/night</td>
<td>5.5±0.8</td>
<td>5.1±3.7</td>
<td>4.0±1.5</td>
<td>0.171</td>
<td></td>
</tr>
</tbody>
</table>

AHI indicates apnea–hypopnea index; BMI, body mass index; CPAP, continuous positive airway pressure; CT90, the cumulative percentages of sleep time spent at saturations <85%; CT85, the cumulative percentages of sleep time spent at saturations <90%; ODI3, the number of times per hour of sleep that the blood’s oxygen level drops by ≥3% from baseline; ODI4, the number of times per hour of sleep that the blood’s oxygen level drops by ≥4% from baseline; OSA, obstructive sleep apnea; RDI, respiratory disturbance index; residual AHI, the apnea–hypopnea index after 4 weeks of continuous positive airway pressure therapy.

*P value from t tests for continuous variables and chi-square tests for categorical variables. Data are expressed as mean±SD.

Results
Demographic and Clinical Characteristics
Overall, 28 OSA patients and 6 participants without OSA met eligibility criteria and completed the baseline and follow-up visits. The mean age (±SD) was 50.7±10.1 years in OSA patients and 43.6±16.3 years in participants without OSA. The baseline body mass index was 36.4±8.1 in OSA patients and 39.5±10.7 in participants without OSA. At baseline, there was no significant difference in demographic characteristics between groups (Table 1).

The sleep characteristics and CPAP treatment among participants with OSA are shown in Table 1. As expected, CPAP therapy was associated with significant reductions in AHI. Nevertheless, compared with patients with mild or moderate OSA, patients with severe OSA had significantly higher residual AHI (mild 2.0±1.8, moderate 1.0±0.9, severe 5.8±5.6; P=0.015).

Comparison of the Questionnaires Among OSA Groups
The STOP-Bang questionnaire was used to screen for OSA. Compared with patients with mild OSA, patients with moderate OSA had markedly higher scores (mild 4.1±1.4, moderate 6.0±1.3, severe 5.4±1.1; P=0.022). There was no statistically significant difference between moderate and severe OSA, and that might be explained by use of the STOP-Bang questionnaire, which is highly sensitive at low

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scores and highly specific at high scores for OSA.\textsuperscript{23} The OSA patients were evaluated with the ESS questionnaire to measure daytime sleepiness before and after CPAP therapy. The ESS score significantly decreased after 4 weeks of CPAP in mild and moderate OSA patients (mild 12.6±5.2 versus 9.2±6.1, \(P=0.028\); moderate 11.7±4.5 versus 7.3±5.7, \(P=0.034\); severe 12.3±6.6 versus 10.7±5.3, \(P=0.497\)).

Baseline Potassium Channel mRNA Expression Correlated With the Severity of OSA

We observed a significant inverse correlation between the severity of OSA stratified by AHI and the mRNA expression of the main potassium channels assessed: KCNQ1 (\(r=-0.486\), \(P=0.007\)), KCNH2 (\(r=-0.437\), \(P=0.016\)), KCNE1 (\(r=-0.567\), \(P=0.001\)), KCNJ2 (\(r=-0.442\), \(P=0.015\)), and KCNA5 (\(r=-0.468\), \(P=0.009\)) (Figure 1 and Table 2). In addition, KCNQ1 (\(r=-0.404\), \(P=0.027\)), KCNH2 (\(r=-0.416\), \(P=0.022\)), and KCNE1 (\(r=-0.465\), \(P=0.010\)) inversely correlated with ODI4, indicating the oxygen desaturation status (Figure 2 and Table 2).

CPAP Improved Circulating KCNQ1 and KCNJ2 in Moderate OSA Patients

For patients with newly diagnosed OSA initiating CPAP for the first time, circulating KCNQ1 and KCNJ2 were increased 1.4±0.4-fold (\(P=0.040\)) and 2.1±1.4-fold (\(P=0.046\)), respectively, after 4 weeks of CPAP therapy in the moderate group (Figure 3). Although they did not reach statistical significance, CPAP improved most potassium channel gene expression in moderate OSA: KCNE1 1.5-fold, KCNE2 2.9-fold, KCNA5 1.9-fold, KCND3 1.3-fold, and KCNJ11 1.6-fold.

Discussion

The main findings of this prospective study are that the mRNA expression of at least 5 of 8 cardiac potassium channels correlated with the severity of OSA and that CPAP treatment

Table 2. Correlation of the Expression of Baseline Potassium Channels With Hypoxia

<table>
<thead>
<tr>
<th></th>
<th>KCNQ1</th>
<th>KCNH2</th>
<th>KCNE1</th>
<th>KCNE2</th>
<th>KCNJ2</th>
<th>KCNA5</th>
<th>KCNJ11</th>
<th>KCND3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log(_{10})AHI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(r)</td>
<td>-0.486</td>
<td>-0.437</td>
<td>-0.567</td>
<td>0.002</td>
<td>-0.442</td>
<td>-0.468</td>
<td>-0.329</td>
<td>-0.249</td>
</tr>
<tr>
<td>(P) value</td>
<td>0.007</td>
<td>0.016</td>
<td>0.001</td>
<td>0.993</td>
<td>0.015</td>
<td>0.009</td>
<td>0.076</td>
<td>0.184</td>
</tr>
<tr>
<td>Log(_{10})ODI4</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>(r)</td>
<td>-0.404</td>
<td>-0.416</td>
<td>-0.465</td>
<td>-0.021</td>
<td>-0.349</td>
<td>-0.326</td>
<td>-0.296</td>
<td>-0.285</td>
</tr>
<tr>
<td>(P) value</td>
<td>0.027</td>
<td>0.022</td>
<td>0.010</td>
<td>0.912</td>
<td>0.059</td>
<td>0.079</td>
<td>0.112</td>
<td>0.127</td>
</tr>
<tr>
<td>Nadir (O_2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(r)</td>
<td>0.268</td>
<td>0.269</td>
<td>0.344</td>
<td>0.180</td>
<td>0.268</td>
<td>0.146</td>
<td>0.020</td>
<td>0.370</td>
</tr>
<tr>
<td>(P) value</td>
<td>0.153</td>
<td>0.150</td>
<td>0.063</td>
<td>0.349</td>
<td>0.152</td>
<td>0.440</td>
<td>0.915</td>
<td>0.044</td>
</tr>
</tbody>
</table>

AHI indicates apnea–hypopnea index; nadir \(O_2\), nadir arterial oxygen saturation levels; ODI4, the number of times per hour of sleep that the blood’s oxygen level drops by ≥4% from baseline.
improved circulating KCNQ1 and KCNJ2 in patients with moderate OSA. There were trends toward increases in 7 of 8 potassium channels in patients with moderate OSA.

QT prolongation is an independent risk factor for SCD. In the Oregon Sudden Unexpected Death Study, idiopathic abnormal prolongation of the corrected QT interval was associated with 5-fold increased odds of SCD. Recent genetic studies have established a clear inverse relationship between QT interval and expression and function of potassium channels. Gene defects resulting in loss of function of voltage-gated potassium channels, for example, are associated with long QT syndromes: LQT1 (KCNQ1) and LQT2 (KCNH2), encoding α-subunits of the potassium channels I\(_{\text{Ks}}\) and I\(_{\text{Kr}}\); LQT5 (KCNE1) and LQT6 (KCNE2), encoding β-subunits of the potassium channels I\(_{\text{Ks}}\) and I\(_{\text{Kr}}\); and LQT7 (KCNJ2), encoding the inward rectifier potassium channel Kir2.1.12–17

OSA is associated with SCD and long QT. OSA predicted incident SCD in a longitudinal study of 10,701 adults.3 In OSA patients who had no evidence of underlying cardiac, pulmonary, or central nervous system disease, the QT interval was prolonged at the onset of apnea.7

Because OSA is associated with long QT, and long QT can be caused by potassium channel loss of function, we tested whether OSA was associated with circulating potassium channel changes. A total of 8 potassium channel genes were studied, and we found that the mRNA levels of at least 5 of 8 potassium channel genes assessed correlated inversely with hypoxemia and OSA severity. Of these channel genes, 2 were statistically significantly increased and 5 more showed trends toward an increase with CPAP therapy. These results suggest that hypoxemia may be mediating these changes.

KCNQ1 and KCNJ2 were improved with CPAP but only in the moderate OSA group. This could be explained if correction of hypoxia were the major driver for improvements in channel levels. Consistent with this idea, in a population of 10,701 adults, the magnitude of SCD risk was predicted by AHI and nocturnal oxygen desaturation.3 In our study, even with CPAP, severe OSA patients had higher residual AHI with statistical difference, and there was no change in potassium channel levels with CPAP. This supports the idea that the degree of hypoxia, rather than some other aspect of CPAP, was mediating the changes in mRNA abundance. In our patients with mild OSA, hypoxia was not severe and changed little with CPAP. This may explain why CPAP did not alter channel expression levels in this group.

The transcription factor hypoxia-inducible factor 1 (HIF-1) plays a key role in cellular response to systemic oxygen levels

Figure 2. Baseline potassium channel mRNA expression correlated with ODI4. A, Correlation between mRNA level of KCNQ1 (ordinate) and ODI4 (abscissa). \(r=-0.404\), \(P=0.027\). B, Correlation between mRNA level of KCNJ2 (ordinate) and ODI4 (abscissa). \(r=-0.349\), \(P=0.057\). ODI4 indicates the number of times per hour of sleep that the blood’s oxygen level drops by ≥4% from baseline.

Figure 3. CPAP improves KCNQ1 and KCNJ2 in patients with moderate OSA. *\(P<0.05\) comparing the post-CPAP fold change of KCNQ1 or KCNJ2 to the baseline in the moderate OSA group. CPAP indicates continuous positive airway pressure; OSA, obstructive sleep apnea.

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in humans. HIF-1α is a subunit of a heterodimeric HIF-1. There are 3 putative HIF-1α transcription factor binding sites located in the upstream sequence of the human KCNO1 gene and 4 HIF-1α binding sites located in the upstream sequence of the human KCNJ2 gene (data not shown). The systemic oxygen level affects HIF-1 through multiple mechanisms, such as HIF-1α conformational changes and stability, subcellular localization, and transcriptional activity. HIF-1 regulates the expression of >60 genes involved in angiogenesis, cell proliferation, cell survival, and glucose and iron metabolism. The presence of HIF-1α binding sites in the promoters of potassium channels may provide the mechanism by which systemic oxygen level affects the gene expression of potassium channels.

This study has a number of limitations. Although the paired design controlled for interparticipant variability, the small sample size precluded comprehensive multivariable adjustment. In addition, because of the short study duration, it is not clear if the changes in potassium channel expression with CPAP will be durable or if they correlate with improved outcomes. Furthermore, we did not correlate potassium channel mRNA levels in white blood cells with levels or currents in the myocardium. Finally, electrocardiograms were not obtained from participants in this trial.

Conclusions

The mRNA expression of most potassium channels inversely correlated with the severity of OSA and hypoxemia. CPAP therapy improved circulating KCNQ1 and KCNJ2 mRNA levels in moderate OSA patients. If downregulations in circulating potassium channel genes with OSA are mirrored in the heart, as may happen if systemic hypoxemia is a causal factor, then downregulation of potassium channel genes in the heart may explain the long QT and increased risk of SCD in OSA. Moreover, it suggests that potassium channel gene levels may represent a new circulating marker for arrhythmic risk in OSA.

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Disclosures

A provisional patent has been submitted based on this work.

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