Research Article

Rare Mutations in AHDC1 in Patients with Obstructive Sleep Apnea

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Objectives. Obstructive sleep apnea (OSA) is a common disorder influenced by genetic and environmental factors. Mutations of AT-hook DNA-binding motif containing 1 (AHDC1) gene have been implicated which could cause rare syndromes presenting OSA. This study aims to investigate some rare mutations of AHDC1 in Chinese Han individuals with OSA. Patients and Methods. Three hundred and seventy-five patients with OSA and one hundred and nine control individuals underwent polysomnography. A targeted sequencing experiment was taken in 100 patients with moderate-to-severe OSA, and genotyping was taken in 157 moderate-to-severe OSA and 100 control individuals. The effect of mutations was validated by the luciferase reporter assay. Results. One rare missense mutation (AHDC1: p.G1484D) and two mutations (c.-88C>T; c.-781C>G) in 5′-untranslated region (UTR) of AHDC1 were identified. The rare mutation (c.-781C>G) in 5′-UTR that was identified in several patients presenting more severe clinical manifestations affects expression of AHDC1. Conclusions. Our results revealed three rare mutations of AHDC1 in patients with OSA in Chinese Han individuals.

1. Introduction

Obstructive sleep apnea (OSA, OMIM 107650) is an increasingly common disorder, and it is characterized by repeated upper airway collapse during sleep that results in chronic intermittent hypoxia, hypercapnia, and sleep fragmentation [1, 2]. Affected individuals are at increased risks of cardiovascular disease, stroke, and other disorders [1]. However, the pathogenesis of OSA is unclear, so better understanding of the etiology of OSA is needed.

OSA is caused by multiple factors, most commonly obesity, muscle dysfunction, craniofacial abnormalities, and genetic predisposition [3–6]. Some Mendelian diseases also present OSA clinical features, such as auriculo-condylar syndrome [7], Costello syndrome [8], Xia–Gibbs syndrome [9], and Marfan syndrome [10]. OSA aggregates within families, having a first-degree relative with OSA increases risk of OSA by more than 1.5-fold [3]. Furthermore, approximately 35% to 40% of apnea-hypopnea index (AHI) which is the most common disease-defining metric for OSA may be explained by genetic factors [4, 11–14]. Simultaneously, from the pathological viewpoint, sympathetic nervous system activity, fat distribution, upper airway dilator muscle dysfunction, craniofacial development, heightened chemosensitivity, and a low arousal threshold may cause OSA [15, 16], whereas these pathological processes are also regulated by genes [17].
AT-hook DNA-binding motif containing 1 (AHDC1) gene is located on chromosome 1p36.11 and encodes 1603 amino acid protein AT-hook DNA-binding motif-containing protein 1. And AHDC1 is nearly AT-rich interaction domain 1A (ARID1A) gene, mutations in which cause autosomal-dominant Coffin-Siris syndrome, and its symptoms are maxillary hypoplasia, tongue cleft, poor alignment of teeth, etc. OSA could coexist with spinal-cerebellar ataxia type 1, which is a hereditary disease of the nervous system regulated by AHDC1. Abnormalities of AHDC1 probably cause pharyngeal dilator muscle incoordination, thereby leading to airway collapse [9, 18, 19]. Moreover, mutations in AHDC1 have been implicated to most likely cause Xia-Gibbs syndrome. And Xia-Gibbs syndrome presented OSA, language delay, and hypotonia. [9]. Two missense mutations in AHDC1 were found to be associated with schizophrenia and oral developmental disorders that might lead to upper airway dysfunction and perhaps OSA [20, 21]. Nevertheless, if there were any rare mutations in AHDC1 associated with OSA in Chinese Han is not clear.

The purpose of this study is to investigate rare mutations of AHDC1 in Chinese Han individuals with OSA. In this study, we used targeted sequencing which is a cost-efficient tool [22] to discover rare mutations of AHDC1 in unrelated Chinese Han individuals with OSA.

2. Patients and Methods

2.1. Patients. Subjects for sequencing came from patients at the Otolaryngological Department of Beijing An Zhen Hospital from February 2017 to December 2017. All participants were subjects of self-reported snoring symptoms; each subject completed OSA screening and was scheduled for an overnight sleep study. The sleep study was conducted using a level II portable diagnostic device (SOMNOScreen; SOMNOmedics GmbH, Randersacker, Germany) approved by the US Food and Drug Administration. In total, 484 unrelated Chinese Han adults aged ≥18 years were recruited, including 375 patients with OSA and 109 control individuals. After excluding participants with incomplete clinical data and mild OSA, a targeted sequencing experiment was taken in 100 patients with moderate-to-severe OSA. To identify additional mutation carriers, we genotyped an expanded cohort of 157 moderate-to-severe OSA and 100 control individuals. The study flow chart is shown in Figure 1. Patients with OSA and control individuals were unrelated individuals diagnosed using the American Academy of Sleep Medicine guidelines [23]. All participants underwent a complete examination, and their medical history and basic clinical and biochemical variables were collected. No participants had lung, kidney, liver, or nervous system disease.

All participants completed an informed consent before enrollment. The study was approved by the Medical Ethics Committee of Beijing An Zhen Hospital (2017005), adhered to the Declaration of Helsinki, and was registered in the Chinese Clinical Trial Register (ChiCTR-ROC-17011027).

2.2. Phenotype Definitions. The diagnostic standard of OSA is defined as AHI more than 5 times per hour, and this standard is further subdivided into mild (5 ≤ AHI ≤ 15 events/hour), moderate (15 < AHI ≤ 30 events/hour), and severe (AHI > 30 events/hour) [24]. The quantitative phenotypic outcomes were the AHI (defined by events associated with ≥3% desaturation), the lowest oxygen saturation (LSaO2) and mean oxygen saturation (MSaO2) across the sleep period. These quantitative phenotypic outcomes excluded intermittent waking episodes [25].

Covariates were obtained by questionnaires and direct measurement. Body mass index (BMI) was calculated using the standard BMI formula: body mass (in kilograms) divided by square of height (in meters). Hypertension is defined as having higher blood pressure than normal three times without antihypertensive drugs, i.e., systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg. All blood samples were collected after participants had fasted overnight. Venous blood sample was obtained before 9 am. Clinical variables included total cholesterol (TC), tri-glyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and fasting blood glucose (GLU). Serum TC, TG, HDL-C, LDL-C, GLU, and other routine serum biochemical parameters were measured using a biochemical analyzer (Hitachi-7600; Hitachi, Tokyo, Japan). Blood samples were stored in a freezer at −80°C. All measurements were obtained using blinded quality control specimens at the Department of the Biochemical Laboratory at Beijing An Zhen Hospital. All results were interpreted by a specialist.

2.3. Exome Sequencing Analysis. The genomic DNA was extracted from whole blood (details are described in the Methods section in the appendix). A custom-designed gene panel containing AHDC1 (Supplementary Table 1) was ordered from Life Technologies (Carlsbad, CA, USA) for targeted sequencing. The coverage of panel is 99.52% (Supplementary Table 2). Details regarding primers and resequencing procedures are given in Supplementary Table 3 and the Methods section in the appendix.

The read alignments were filtered by the software into mapped BAM-files using the reference genomic sequence (hg19) of the target genes. Annotation of variants was performed using Ion Reporter Software (Version 4.4; Life Technologies, Darmstadt, Germany) for the variant call format files. The annotation included genomic and complementary DNA positions, genetic reference sequences, amino acid changes, and related information available from public databases, such as the 1000 Genomes Project; National Heart, Lung, and Blood Institute Grand Opportunity Exome Sequencing Project (ESP6500) (https://esp.gs.washington.edu/drupal/); Single Nucleotide Polymorphism Database (dbSNP147) (National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/SNP/); Exome Aggregation Consortium (ExAC03) (http://exac.broadinstitute.org); ClinVar; Online Mendelian Inheritance in Man (OMIM); and Human Gene Mutation Database (HGMD). Sorting
Intolerant From Tolerant (SIFT) (http://sift.jcvi.org/), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), MutationTaster (http://www.mutationtaster.org/), and Protein Variation Effect Analyzer (PROVEAN) (http://provean.jcvi.org/index.php) were used to indicate changes in protein structure and function. PathCards (http://pathcards.genecards.org/) was used to analyze the pathway. CLUSTALW (http://www.genome.jp/tools/clustalw/) was used to analyze the consistency of the amino acid sequences. Candidate pathogenic variants were confirmed using Sanger sequencing.

2.4. Functional Analysis. Bioinformatics analysis suggests that mutation (c.-781C>G) of AHDC1 is located at 5′ UTR of AHDC1. Two 1000-bp fragments containing human AHDC1 5′ UTR-781C and -781G, respectively, were cloned into a pGL4.10[luc2] vector upstream of luciferase reporter (Promega Benelux BV, Leiden, The Netherlands), and the resultant plasmids (pGL4-C and pGL4-G) were transformed into 293T cells, respectively, to determine effects of the mutation by detecting fluorescence intensity according to the manufacturer’s instructions.

2.5. Statistical Analysis. Continuous variables are expressed as mean ± standard deviation and categorical variables as numeral (percentage). Independent Student’s t-tests for normal distributions and Wilcoxon rank sum tests for asymmetric distributions were used to analyze the differences in continuous variables. Chi-squared tests were used to analyze categorical variables between OSA and control group. Subsequently, independent Student’s t-tests and Wilcoxon rank sum tests were used to analyze the differences in continuous variables, and Fisher’s exact tests were used to analyze categorical variables between mutation and nonmutation groups. All probability values were two-sided, and \( P < 0.05 \) was considered significant. Statistical analyses were performed using SPSS 20.0 (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Baseline Characteristics of Participants. The present study included 257 patients with OSA and 100 control individuals. Basal characteristics of these individuals are presented in Table 1. No significant differences were observed in age (\( P < 0.055 \)), TC level (\( P < 0.569 \)), or LDL-C level (\( P = 0.400 \)) between OSA and the control group. Compared with the control group, the patients had a higher BMI (27.04 ± 3.36 vs. 23.71 ± 3.09 kg/m², \( < 0.001 \)), TG level (1.56 ± 1.04 vs. 1.24 ± 0.36 mmol/L, \( P = 0.002 \)), GLU level (3.46 ± 0.95 vs. 3.44 ± 0.62 mmol/L, \( P = 0.002 \)), HDL-C level (1.07 ± 0.23 vs. 1.00 ± 0.18 mmol/L, \( P = 0.018 \)), male proportion (84.82% vs. 70.00%, \( P = 0.002 \)), and prevalence of hypertension (22.96% vs. 13.00%, \( P = 0.035 \)). The OSA group exhibited severe degrees of AHI (32.17 ± 18.06 vs. 3.33 ± 1.14, \( P < 0.001 \)), significantly lower MSaO2 (93.48 ± 2.28 vs. 95.00 ± 1.37) and LSaO2 (82.14 ± 9.89 vs. 90.00 ± 2.73, \( P < 0.001 \)) in comparison with the control group.

3.2. Identification of Rare Mutations. In order to identify the mutation of AHDC1 in Chinese OSA patients, we sequenced each of 100 moderate-to-severe OSA subjects’ DNA samples using targeted sequencing technology. Among the 100 moderate-to-severe OSA patients, in total 57 variants were met the quality filtering criteria, 11 were common polymorphisms (i.e., minor allele frequency of ≥ 1%), whereas the
To find whether OSA patients with mutations had different clinical manifestations from the patients without mutations, we analyzed their clinical characteristics. No difference was found between these two groups in age (P = 0.628), sex (P = 0.578), and BMI (P = 0.503) (Supplementary Table 4). So distribution of mutations in AHDC1 is not influenced by age, sex, or BMI in the present study.

Clinical manifestations of OSA patients with AHDC1 mutations are shown in Table 3. Because HDL-C is less affected by drugs, we compared only HDL-C levels for biochemical indicators in patients with mutations. The patient with missense mutation (AHDC1: c.G451A) was a moderate OSA patient (AHI = 23.1) with lower BMI (BMI = 17.3 kg/m²) and lower HDL-C (0.83 mmol/L), while he was a diabetic and coronary heart disease (CHD) patient. The mutation (c.-88C>T) was found in a fatter male (BMI = 25.3 kg/m²) who was a moderate OSA patient (AHI = 28.2) with normal HDL-C, TG, and fasting blood sugar level. Another mutation in 5′-UTR (c.-781C>G) was found in two severe OSA patients: the first severe OSA patient (AHI = 23.6) was a height medium male (BMI = 23.6 kg/m²) who was younger (50 years) than another three patients, and he was a hypertensive and CHD patient with lower HDL-C (0.83 mmol/L). The other severe OSA patient (AHI = 68.) was a fatter female (BMI = 34 kg/m²) with lower HDL-C (0.83 mmol/L), while she was a diabetic, hypertensive, and CHD patient; this female patient presented lower oxygen saturation (LSaO2 = 69%, MSaO2 = 88%).

### Clinical Analysis in Patients with or without AHDC1 Mutations

To find whether OSA patients with AHDC1 mutations had different clinical manifestations from the patients without mutations, we analyzed their clinical characteristics. No difference was found between these two groups in age (P = 0.628), sex (P = 0.578), and BMI (P = 0.503) (Supplementary Table 4). So distribution of mutations in AHDC1 is not influenced by age, sex, or BMI in the present study.

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proteins. AHDC1 is also associated with Mesodermal Commitment Pathway (Figure 3) that may influence development of respiratory-related muscles and bones leading to OSA. These evidences suggest that AHDC1 might play an important role in OSA.

The missense mutation (AHDC1: c.G4451A) was found in a thinner moderate OSA patient. This suggests that OSA may occur in the subject with AHDC1 mutations, even if his or her BMI is small. The mutation in 5′ UTR (c.-88C>T) was found in a moderate OSA patient with normal biochemical variables, and he did not take any drugs. This suggests one interesting fact that not all OSA patients might suffer from other diagnosed diseases; every patient has his or her own characteristics, so individualized diagnosis and treatment are necessary. On the other hand, this mutation (c.-88C>T) may not cause changes in pathways related to other diseases, so we have not performed further functional analysis of this mutation. Moreover, another interesting rare mutation in 5′ UTR (c.-781C>G) was found in two patients with higher AH1, lower oxygen saturation, and lower HDL-C. Analysis of potential transcriptional binding sites in the proximate promoter region (1-kb of upstream region of the start site of AHDC1) revealed that the c.-781C>G-containing sequence is the binding consensus motif of the transcription factor SREBP-2 (Supplementary Figure 2). Mutations of SREBP-2 were found to be related to hypercholesterolemia and perhaps OSA [27]. And luciferase reporter assay suggests the mutation (c.-781C>G) in 5′UTR of AHDC1 affects expression of gene. All above suggest that AHDC1 may be related to the occurrence of OSA and 5′UTR plays a significant role in AHDC1.

Notably, a recent whole-exome sequencing analysis revealed three different de novo truncating mutations in AHDC1 in four patients with OSA, language delay, and hypotonia [9]. Two missense mutations in AHDC1 were found to be associated with schizophrenia and oral developmental disorders that may lead to upper airway dysfunction and perhaps OSA [20, 21]. Previous founded variants are not only in the coding exon [9] but also in 5′ untranslated region [21]. A de novo balanced translocation with a breakpoint in AHDC1 intron 1 that disrupted the 5′UTR of AHDC1 has been identified in a 5-year-old male patient with developmental delay and intellectual disability [21]. The disruption lead to AHDC1 expression reduced to 50% of wild-type level in lymphoblastoid cells [21]. In the present study, rare mutation (c.-781C>G) in 5′UTR of AHDC1 was found in severe OSA patients, and this rare mutation could affect expression of AHDC1. Therefore, we assumed that the mutations in AHDC1 may affect the expression of AHDC1 and lead to sleep apnea. The exact mechanism of this phenomenon needs to be investigated.

This study has some limitations. The participants may not be entirely representative of the general Han Chinese population. Potential false-positive results may still be possible. Prospective cohort studies are needed to confirm the variants in our study. We sequenced limited genes and limited sequencing regions that were most likely to harbor functional variation. Some of the patients and controls with CHD were taking drugs. The results of TC, LDL-C, and TG comparison between two groups may be different from those
of other studies. Finally, the exact mechanism of these variants is not fully understood and requires further functional studies.

5. Conclusions

In summary, we identified multiple novel variants of AHDC1 in patients with OSA. Our findings increase the mutation spectrum of associated genes for OSA, a quite complex disease. Gene analysis is helpful for individualized typing of patients with OSA and might contribute to personalized diagnosis and treatment in the future.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Ethical Approval

All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Consent

Informed consent was obtained from all individual participants included in the study. No identifying information about participants is available in this article.

Disclosure

Song Yang and Kun Li are co-first authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Supplementary Materials

Supplementary Table 1: detailed information of targeted sequencing panel. Supplementary Table 2: AmpliSeqT
amplicons and coverage details of obstructive sleep apnea-targeted sequencing assay. Supplementary Table 3: primers of panel. Supplementary Table 4: characteristics between mutation and nonmutation groups. Supplementary Figure 1: Sanger sequencing chromatograms of variants in AHDC1. Supplementary Figure 2: analysis of potential transcriptional binding sites in the proximate promoter region. Supplementary Figure 3: analysis of sequence conservation in nine species of missense mutation identified in AHDC1. (Supplementary Materials)

References

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