Genetically-reduced serum ACE activity might be a causal risk factor for obstructive sleep apnea syndrome: A meta-analysis

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We meta-analytically summarized the associations of angiotensin converting enzyme (ACE) gene insertion/deletion (I/D) polymorphism with ACE activity and obstructive sleep apnea syndrome (OSAS) to see whether ACE activity is causally associated with OSAS. Literature search and data abstraction were done in duplicate. Sixteen articles including 2060 OSAS patients and 1878 controls were summarized. Overall, no significance was observed for the association of I/D polymorphism with OSAS, whereas carriers of II genotype (weighted mean difference or WMD, 95% confidence interval or CI, $P: -11.976, -17.168$ to $-6.783, <0.001$) or I allele ($-9.842, -14.766$ to $-4.918, <0.001$) had a lower level of serum ACE activity compared with DD genotype carriers, respectively. In subgroup analyses, carriers of II genotype were 3.806 times more likely to develop OSAS (95% CI, $P: 1.865$ to $7.765, <0.001$) in OSAS patients with hypertension, without heterogeneity. Mendelian randomization analysis indicated there was 37.4% (95% CI: 1.115 to 3.142) and 32.4% (1.106 to 2.845) increased risk of OSAS by a reduction of 1 U/L in ACE activity for the II genotype and I allele carriers versus DD genotype carriers, respectively. There was no observable publication bias. Collectively, genetically-reduced serum ACE activity might be a causal risk factor for OSAS.

As the most common category of sleep apnea, obstructive sleep apnea syndrome (OSAS) is estimated to affect 4% to 5% of the general population. OSAS is characterized by recurrent episodes of upper airway obstruction for 10 seconds or more during sleep. There are several established risk factors for OSAS, such as obesity, excessive relaxation of the throat muscles and abnormal structure of the airways. The symptoms of OSAS are more common in family members affected with this disorder, and it occurs two to three times more often in males than in females. Currently, the development of OSAS is believed to, at least in part, be genetically determined with a heritability of 0.35, and the gene encoding angiotensin converting enzyme (ACE) is recognized as a promising candidate in susceptibility to OSAS.

ACE is involved in catalyzing the conversion of angiotensin I into a biologically active peptide angiotensin II, a potent vasopressor and aldosterone-stimulator that regulates blood pressure and fluid-electrolyte balance. An early study by Rohatgi et al. reported that OSAS patients had lower ACE activity than healthy controls, arguing against the finding of subsequent study by Barcelo et al., who reported that ACE activity was significantly higher in OSAS patients than in healthy controls. At present, the relation between ACE activity and OSAS susceptibility remains an open question. Converging studies suggested that serum ACE activity was controlled by an insertion/deletion (I/D) polymorphism of a 287-bp Alu element in the intron 16 of ACE gene, with the D/D, I/D and I/I genotypes respectively paralleling the high, middle and low levels of ACE activity. In view of these observations, we were
inspired to see whether there was a causal association between serum ACE activity and OSAS susceptibility with the aid of ACE gene I/D polymorphism as an instrument variable under the assumptions of Mendelian randomization by a meta-analysis in accordance with the guidelines of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Please see the Supplementary PRISMA 2009 Checklist, downloaded from http://www.prisma-statement.org/statement).

Methods

**Literature search.** Four public electronic datasets, PubMed, Web of Science, Wanfang (http://www.wanfangdata.com.cn/, Chinese) and CNKI (www.cnki.net/, Chinese) were reviewed to search potential articles that tested the associations of ACE gene I/D polymorphism or plasma/serum ACE activity with OSAS susceptibility. The key words were “angiotensin converting enzyme” or “angiotensin I converting enzyme” or “ACE,” or “ACEI” in the ABSTRACT, along with “sleep apnea” or “sleep apnoea” or “sleep disorder” or “OSAS” or “OSAHS” in the TITLE. The last search was updated on April 4, 2015. Only articles published in English or Chinese language were considered in this study. In order to avoid possible missing hits, the reference lists of major original articles and reviews were checked manually.

**Selection criteria.** Articles were qualified if they tested the associations of ACE gene I/D polymorphism or plasma/serum ACE level/activity with OSAS susceptibility; if they involved both OSAS patients and healthy controls; if they provided the genotype or allele counts of ACE gene I/D polymorphism or plasma/serum ACE level/activity with OSAS susceptibility; if they involved both OSAS patients and healthy controls.

**Data abstraction.** The following data were abstracted from each qualified article by two authors (Lan He and Bin Wang) independently based on a predetermined protocol: the first author’s last name, year of publication, race, study design, source of healthy controls, diagnostic criteria for OSAS and apnea hypopnea index (AHI) cutoff, as well as sample size, age, percentage of male gender, body mass index (BMI), smoking, drinking, AHI cutoff, Epworth sleepiness score, hypertension and diabetes mellitus between OSAS patients and healthy controls.

**Mendelian randomization.** Mendelian randomization is emerging as a novel epidemiologic study design that utilizes measured variation in genes of known biology function to examine the causal impact of a modifiable exposure on disease susceptibility in genetic association studies12. Importantly, this design can control for reverse causation and confounding which otherwise obsess observational data. To infer a causal relation between ACE activity and OSAS, we firstly suppose that the mutant II genotype increases OSAS susceptibility relative to the wild DD genotype as measured by OR II vs. DD. Secondly, we suppose that the mutant II genotype increases ACE activity with OSAS susceptibility. Finally, under the assumptions of Mendelian randomization, OR GG vs. gg ΔP is regarded as an unbiased and unconfounded estimate of the OR of OSAS for a unit change in ACE activity12,13.

**Statistical analysis.** Inconsistency index, I² quantifies the magnitude of heterogeneity in a percentile, with a higher value paralleling a higher probability of heterogeneity. In fact, I² is a derivative of the Q statistic and it measures the proportion of variability that is due to heterogeneity rather than sampling error. Generally, the cutoff of I² is set at 50% to define statistically significant heterogeneity14. Associations of ACE gene I/D polymorphism and ACE activity with OSAS susceptibility were respectively quantified as odds ratio (OR) and weighted mean difference (WMD), with 95% confidence interval (95% CI) by the DerSimonian and Laird random-effects model15.

Subgroup analyses were conducted to see whether differences in race (mainly Asian and White), language of publication (English and Chinese), study design (retrospective and prospective), source of controls (hospital-based and population-based), obesity (BMI ≥ 30 kg/m² and BMI < 30 kg/m²), hypertension status (with and without hypertension) and sample size (>300 subjects and <300 subjects) can explain heterogeneity. Moreover, meta-regression analyses were conducted on continuous covariates including age, gender, BMI, smoking, drinking to seek possible explanations for heterogeneity.

Publication bias was measured by Begg’s funnel plots and Egger’s regression tests, as well as by the trim-and-fill test, which can calculate the number of estimated missing studies to compensate for publication bias. Significant publication bias was statistically judged by the P value of Egger’s regression test at a significance level of 10%.

The above statistical analyses were managed by STATA software version 12.0 for Windows (College Station, TX: StataCorp LP).

**Results**

A comprehensive review of public electronic datasets produced a total of 179 eligible articles (62 articles in English and 117 articles in Chinese) that tested the associations of ACE gene I/D polymorphism and ACE activity with OSAS susceptibility. Only 16 articles (9 articles in English and 7 articles in Chinese)
| The first author       | Year | Race  | Study design | Source            | Criteria | BMI (kg/m²) | Smoking | Drinking | AHI Score | Hypertension | Diabetes |
|-----------------------|------|-------|--------------|-------------------|----------|-------------|---------|----------|-----------|-------------|----------|
|                       |      |       |              |                   |          |             |         |          |           |             |          |
| Chmielewska           | 2013 | White | Retrospective | Hospital          | AASM     | 33          | NA      | 0.273    | 0.800     | 0.000       | 0.000    |
|                       |      |       |              |                   |          | 32.6        | 27.2    | 0.200    | 0.8       | 10.9        | 6.5      |
|                       |      |       |              |                   |          | 32.6        | 27.2    | 0.200    | 0.8       | 10.9        | 6.5      |
|                       |      |       |              |                   |          | 30.64       | 28.51   | NA       | NA        | NA          | NA       |
|                       |      |       |              |                   |          | 30.58       | NA      | NA       | 24.24     | 11.34       | NA       |
|                       |      |       |              |                   |          | 38.4        | NA      | NA       | NA        | 14.7        | 3.1      |
|                       |      |       |              |                   |          | 28.43       | 23.22   | NA       | NA        | NA          | NA       |
|                       |      |       |              |                   |          | 28.97       | 25.01   | NA       | NA        | NA          | NA       |
|                       |      |       |              |                   |          | 28.8        | 26.2    | NA       | NA        | NA          | NA       |
|                       |      |       |              |                   |          | 29.7        | 26.9    | NA       | NA        | 65.79       | NA       |
|                       |      |       |              |                   |          | 28.9        | 26.9    | NA       | NA        | 52.75       | NA       |
|                       |      |       |              |                   |          | 31.4        | NA      | 0.130    | NA        | NA          | 0.435    |

Continued
including 2060 OSAS patients and 1878 controls were left for final analysis after applying eligibility criteria. The baseline characteristics of all qualified studies are summarized in Table 1.

When all study subjects were brought together, no significance was observed for the association of ACE gene I/D polymorphism with OSAS susceptibility under allelic, homozygous genotypic and dominant models, and heterogeneity was significant (Table 2). However, carriers of II genotype (WMD, 95% CI, P: −11.976, −17.168 to −6.783, <0.001) or I allele (−9.842, −14.766 to −4.918, <0.001) had a lower level of serum ACE activity when compared with DD genotype carriers, with significant heterogeneity (Fig. 1).

For the association of ACE gene I/D polymorphism with OSAS, further subgroup analyses were carried out to seek possible sources of heterogeneity. As shown in Table 2, grouping studies by race, language of publication, study design, source of controls and sample size failed to find any statistical significance, and no obvious improvement in heterogeneity was noticed. In subgroup analysis by obesity (BMI at a cutoff of 30 kg/m² in OSAS patients), significance was detected in OSAS patients with BMI of less than 30 kg/m² under only allelic model (OR, 95% CI, P: 1.708, 1.047–2.785, 0.032), with evident heterogeneity ($I^2 = 79.8\%$). In addition, four of 16 qualified articles that reported genotype data by hypertension status were analyzed separately. In OSAS patients complicated with hypertension, the significant associations of ACE gene I/D polymorphism with OSAS susceptibility were identified under three genetic models. For example, carriers of II genotype were 3.806 times more likely to develop OSAS (OR, 95% CI, P: 3.806, 1.865 to 7.765, <0.001), and there was no indication of significant heterogeneity ($I^2 = 48.2\%$) (Fig. 2).

Further, meta-regression analyses modeling age, gender, BMI, smoking and drinking showed no statistical significance for the association between ACE gene I/D polymorphism and OSAS susceptibility. Echoing from Beggs’s funnel plots and Egger’s regression tests, there was no suggestive publication bias (Fig. 3). As suggested by the trim-and-fill method, 3 missing studies were required for ACE gene I allele in association with OSAS susceptibility, with the trim-and-fill-adjusted OR of 1.029 (95% CI, P: 0.791 to 1.338, 0.833).

On the basis of above estimates, implementation of Mendelian randomization identified 37.4% (OR, 95% CI: 1.374, 1.115 to 3.142) and 32.4% (1.324, 1.106 to 2.845) increased risk of developing OSAS by a reduction of 1 U/L in serum ACE activity for the II genotype and I allele carriers versus the DD genotype carriers, respectively.

Discussion
Polling previous studies on the ACE-OSAS relation, our findings demonstrate that ACE gene I/D polymorphism can predict the risk for OSAS patients complicated with hypertension, and more importantly, genetically-reduced serum ACE activity might be a causal risk factor for OSAS by Mendelian randomization technique. To the best of our knowledge, this meta-analysis represents the first to test the causal contribution of ACE activity in the pathogenesis of OSAS.

Among the promising candidate genes identified so far in susceptibility to OSAS, ACE gene ranks high on the list. In spite of many candidate association studies conducted in different ethnic groups, data in medical literature are inconsistent and even contradictory, and biological implication from statistical inference remains elusive. Lately, two meta-analyses found no relationship between ACE gene I/D polymorphism and OSAS susceptibility. Moreover, there is also ongoing discordance in terms of ACE activity and OSAS. A possible explanation for these divergent findings may be due to clinical or methodological heterogeneity across studies, such as the coexistence of hypertension. Another possibility might result from confounding and reverse causation inherited in observational data. Indeed, inferring causality from genetic association studies is problematic because it is difficult to disentangle causation from an association, especially in the presence of confounding. Fortunately, Mendelian randomization provides an alternative way to deal with the problems of observational studies. This technique is alike to a randomized trial where randomization to genotypes takes place at the time of gamete formation.

Table 1. The baseline characteristics of all qualified studies. Notes: AASM, American Academy of Sleep Medicine; NA, not available; BMI, body mass index; AHI, apnea hypopnea index; (h), OSAS patients with hypertension; (n), OSAS patients without hypertension.
Table 2. Overall and subgroup analysis of ACE gene I/D polymorphism with OSAS susceptibility under three genetic models. Notes: OR, odds ratio; 95% CI, 95% confidence interval; BMI, body mass index.

With these in mind, we therefore sought to update previous meta-analyses to seek potential sources of heterogeneity and further introduce the concept of Mendelian randomization to test whether there is a causal relation between ACE activity and OSAS susceptibility.

In agreement with the previous two meta-analyses 31,32, our overall analyses repeated the overall neutral association between ACE gene I/D polymorphism and OSAS susceptibility. To produce more information, we subsequently conducted a wide range of subgroup analyses to seek possible sources of heterogeneity resulting from clinical and methodological aspects, and interestingly a positive signal emerged after restricting data to OSAS patients complicated with hypertension and normotensive healthy controls. It is well recognized that OSAS is a risk factor for the development of hypertension and approximately half of OSAS patients have hypertension 36,37. Elucidating the pathophysiological mechanisms of ACE gene underlying OSAS susceptibility in connection with hypertension is beyond the capability of this meta-analysis, it is rational to speculate that the predictive role of ACE genetic alterations is manifested in the coexistence of hypertension.
The most noteworthy finding of this study is that our Mendelian randomization analysis demonstrated that genetically-reduced serum ACE activity might be a causal risk factor for OSAS with the aid of ACE gene I/D polymorphism as an instrument variable. Factually, the selection of I/D polymorphism as a surrogate to infer causality between ACE activity and OSAS is biologically robust as this polymorphism was reported to interpret half the variance of serum ACE activity. As expected from our results, the II genotype was associated with a significant lower level of ACE activity as compared with the DD genotype, in agreement with the findings of most studies. In this meta-analysis, we for the first time put forward that a unit reduction of serum ACE activity can lead to 30% increased risk of predisposing OSAS. However, given the complex pathogenesis of OSAS and its underlying relation with hypertension, more and more large studies are warranted to validate this significant finding.

There are several research limitations for this meta-analysis. The conclusion of this study was based on 16 independent articles and 3938 subjects, which might not be sufficient enough to quantify the effect estimate reliably. Only one polymorphism in ACE gene was selected as a surrogate marker, and
it is encouraged to bring other functional polymorphisms within or flanking ACE gene together to examine their joint impact on OSAS susceptibility and ACE activity. Moreover, as with a majority of meta-analyses, heterogeneity is a disturbing issue for both overall and subgroup analyses, and seeking other sources of between-study heterogeneity still remains a challengeable task. Further, all retrieved articles were published in English or Chinese language, leading to a possibility of selection bias. However, as viewed from Begg's funnel plots and Egger's tests, there was no indicative of publication bias, substantiating the robustness of our findings.

In conclusion, through a well-designed Mendelian randomization meta-analysis, our findings demonstrate that ACE gene I/D polymorphism can predict the risk for OSAS complicated with hypertension, and more importantly, genetically-reduced serum ACE activity might be a causal risk factor for OSAS. Nevertheless, the results of this meta-analysis need further replication in other ethnic groups, and in-vivo and in-vitro functional studies are also required to unravel potential molecule mechanisms underlying the associations of ACE genetic defects and its serum activity with OSAS susceptibility.

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Author Contributions
H.M.P. and L.H.Z. conceived and designed the experiments; L.H. and B.W. performed the experiments; H.M.P., W.Y.L. and J.X. analyzed the data; J.X., D.L.Z. and G.F.L. contributed materials/analysis tools; H.M.P. and L.H.Z. wrote the manuscript. All authors reviewed and approved the manuscript prior to submission.

Additional Information
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