The Association of Angiotensin-Converting Enzyme Gene Insertion/Deletion Polymorphisms with Obstructive Sleep Apnoea: A Meta-analysis

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Funding source: Lee Peilin was the recipient of a European Respiratory Society Fellowship (No 147)

Word count of Abstract: 187

Word count of Text: 3,258

Key words: ACE-polymorphisms; obstructive sleep apnoea; meta-analysis

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ABSTRACT

Rationale: Obstructive sleep apnoea (OSA) is an independent risk factor for hypertension. Increased angiotensin-converting enzyme (ACE) activity may be a possible promoting mechanism with different ACE insertion/deletion (I/D) genotypes influencing this activity. Studies investigating the association of ACE I/D polymorphism with OSA have shown conflicting results. We aimed to undertake a meta-analysis of existing studies exploring the association of ACE I/D polymorphisms with the risk of OSA and hypertension.

Methods: Ten studies were included in a random effects meta-analysis, comprising 1,227 OSA subjects and 1,227 controls

Results: The effect size was measured using the odds ratio (OR). The risk of having OSA for carriers of the D allele was 0.92 (95% CI 0.69-1.23). There was statistically significant heterogeneity across the studies ($I^2=42\%$, $P=0.08$ for genotype and $I^2=74\%$, $P<0.0001$ for allele frequency). The association of D- allele frequency with the risk of OSA remained non-significant after stratification based on ethnicity, source of population sample, and the presence of hypertension. Subgroup analysis failed to show any influence of genotype and allele frequency on OSA severity.

Conclusion: This meta-analysis revealed no association between the ACE I/D polymorphisms and OSA susceptibility.
INTRODUCTION

Obstructive sleep apnoea (OSA) is characterized by the recurrent collapse of the upper airway during sleep, resulting in intermittent hypoxia and sleep fragmentation which can lead to excessive daytime sleepiness (EDS). Untreated OSA is an independent risk factor for hypertension [1-3]. The exact mechanisms linking OSA and hypertension are not clear. One possibility is that OSA-related hypoxia and arousal might lead to sympathetic hyperactivity and activation of the renin-angiotensin-aldosterone system [4].

The angiotensin-converting enzyme (ACE) regulates blood pressure by hydrolyzing angiotensin I into angiotensin II and inactivating bradykinin and angiotensin 1-7 [5]. The importance of ACE in the regulation of blood pressure is demonstrated by the beneficial effect of ACE inhibitors (ACEI) on hypertension [6]. The insertion/deletion (I/D) polymorphism of ACE has been shown to account for 47% of the observed variance in serum ACE levels. The DD genotype carriers have the highest serum ACE concentrations and II genotype carriers have the lowest serum ACE concentrations [7]. The DD genotype has been associated with hypertension; the association being stronger in Asians and women [8]. However, recent meta-analyses have failed to confirm an association between ACE polymorphisms and hypertension [9-11].

In one study, increased serum ACE activity was measured in patients with OSA, compared to controls, independently of hypertension [12]. However, the distribution of the DD, II, and ID genotypes did not differ from that in controls [12]. Many studies have investigated the potential association between OSA and the ACE I/D polymorphism. The results are conflicting, as there is significant heterogeneity amongst the recruited subjects, sample sizes, ethnicities and definitions of OSA.
[2,3,6,12,20]. For example, a higher frequency of the I-allele has been reported in Chinese hypertensive patients with moderate to severe OSA, in contrast to a higher frequency of the D allele in hypertensive Caucasian patients with mild to moderate OSA [15,16, 17]. In an effort to clarify the association between ACE I/D polymorphisms and OSA, we undertook a meta-analysis on all studies published in this area to December 2010.

**METHODS**

**Data extraction**

Data extraction was carried out by one researcher (PL) (Fig.1) and the accuracy checked by an independent person (RR). First, we performed a literature search in Pub Med and EMBASE using the terms “sleep apnoea AND angiotensin converting enzyme”. No limitation on any type of study in the literature was imposed. Unpublished dissertations were accessed via the same search strategy in the UMI Dissertation Abstract database. We also conducted a web-based search through a variety of commercial internet search engines (e.g. Google™, Yahoo™) using the same technique. Additional publications were drawn from reference lists of articles obtained, including studies and reviews. Secondly, we manually reviewed all articles that included empirical data related to angiotensin-converting enzyme genotype in association with OSA. Thirdly, we excluded each potential reference for sample overlap. Reviews and other publications e.g. editorials and letters were excluded unless they contained original data. Fourthly, the remaining references were subjected to inclusion and exclusion criteria as follows: studies were considered for inclusion if the population had OSA and ACE genotyping had been performed. OSA was defined as an apnoea/hypopnoea index (AHI) ≥5 events per hr and categorized as mild to
moderate (AHI 5-30/hr) and severe (AHI>30/hr). Exclusion criteria were: (1) paediatric OSA (there is evidence that paediatric OSA may have a different aetiopathogenesis and clinical characteristics compared to adult OSA); (2) lack of baseline data; (3) lack of control subjects. See Fig. 1 for a summary of the search strategy employed.

Sample coding

Included studies were reviewed with special emphasis on extracting information about the following variables: source of the OSA sample (community vs. clinical), number of cases and controls (defined as those without OSA), ethnicity, mean age of study subjects, sex ratio, co-morbidities of study participants, body mass index (BMI in kg/m2), apnoea-hypopnoea index (AHI), proportion of study subjects with the I allele, and the geographic setting of the study. We extracted or derived a contingency table that summarized the association of the ACE I allele with the risk of having OSA. The outcome was the odds ratio (OR) of having OSA.

Statistical analysis

Analysis was performed using SPSS (v. 16, Chicago, IL, USA) and Review Manager 5.0 (The Nordic Cochrane Centre, The Cochrane Collaboration; Copenhagen, Denmark). We used a random effects model for the meta-analysis. We assessed the association of the D- or I-alleles on OSA using the dominant (DD+DI vs. II) model. For subgroup analysis, we stratified studies by ethnicity, source of OSA sample (clinic-referral vs. community), OSA severity (mild-moderate vs. severe) and hypertension. Homogeneity across the included studies was tested using the Q statistic and inconsistency quantified using the $I^2$ statistic. When only a few studies are included in a meta-analysis, the Q test has ‘low power’ for detecting heterogeneity, therefore, $p<0.1$ is taken as the threshold for statistical significance. The $I^2$ statistic is
represented by a value of 0-100%, with value directly proportional to degree of inconsistency. This latter statistic quantifies heterogeneity not explained by sampling variability in the studies included [21]. A Funnel plot was used to assess publication bias.

RESULTS

Characteristics of the Studies

In total, 14 studies (published in English, Polish, and Chinese – not all shown) were evaluated for eligibility for the analysis and 10 studies fulfilling criteria included; seven of the 10 references were in English and three were in Chinese. The characteristic of these 10 studies are shown in Table 1. Six studies [2,6,12,13,18,20] were conducted in a Caucasian population and four [14-17] were exclusively conducted in Chinese subjects. Two studies [6,18] identified OSA patients by screening a community population and eight [2, 12-17, 20] identified OSA patients through referrals to a sleep lab. Five studies [2, 6, 14-16] reported co-existent hypertension in OSA patients. Zhang’s study in 2000 [15] exclusively recruited hypertensive OSA patients. Six studies [2, 12-14, 16, 17] included subjects free of co-morbidities other than hypertension, whereas co-morbidities were not addressed in four studies [6, 15, 18, 20].

Association between OSA and ACE I/D genotype

The funnel plot showed there was no evidence for significant publication bias for the 10 studies overall (Fig. 2). The majority of the studies found no association of the ACE I/D genotype with OSA in either Caucasian [2, 6, 12, 13, 18, 20] or Chinese populations [14, 17]. The study conducted by Zhang alone, reported the presence of
the D-allele as lowering the risk of having OSA in a Chinese hypertensive population [15] (Fig. 3).

The present meta-analysis combined information on the genotype of 1,227 OSA subjects and 1,227 controls with 934 OSA subjects and 939 control subjects being carriers of the D-allele. We found a non-significant decrease in OSA risk with the D-allele (OR 0.92; 95% CI 0.69-1.23) (Fig.3). However, there was significant heterogeneity across the studies ($I^2$=42%, $P$=0.08). After performing stratified analysis for Caucasians (OR 1.09; 95% CI 0.86-1.37) and Asians (OR 0.67; 95% CI 0.35-1.29) separately, the association remained non-significant.

After further stratifying studies as community-based [6, 18] (OR 1.10; 95% CI 0.85-1.44) and clinic-referral based [2, 6, 12-15, 17, 20] (OR 0.82; 95% CI 0.55-1.24), the association of ACE I/D genotype with OSA remained non-significant. There was significant heterogeneity across the clinic-referral based studies ($I^2$=47%, $P$=0.07) which was not seen among community-based studies ($I^2$=0, $P$=0.64). Combining the information in 2 studies of 180 hypertensive OSA subjects, 115 hypertensive controls and 134 OSA subjects, 89 hypertensive controls, we found a non-significant decrease in OSA risk with the D-allele (OR 0.78; 95% CI 0.34-1.78) [15, 18].

Four studies reported genotype against OSA severity [6, 13, 15, 18]. There was no significant association in OSA severity with the D-allele (OR 0.75; 95% CI 0.49-1.16).

**Association between OSA and ACE gene D-allele frequency**

One study reported the D-allele to be associated with a decreased risk for OSA in a Caucasian population [13]. In a Chinese population three studies [14-16] (Fig. 4) showed the D-allele to decrease OSA risk and one to increase it [17]. We found a
non-significant decrease in OSA risk with D-allele frequency (OR 0.92; 95% CI 0.71 to 1.20) (Fig. 3). There was a high degree of heterogeneity across the studies ($I^2=74\%$, $P<0.0001$). After performing stratified analysis for Caucasians (OR 1.09; 95% CI 0.85-1.39) and Chinese (OR 0.67; 95% CI 0.36-1.26) separately, the association remained non-significant and heterogeneity remained significant (Caucasians: $I^2=55\%$, $P=0.05$; Chinese: $I^2=84\%$, $P=0.0003$).

Studies were further stratified as community-based [6, 18] (OR 1.16; 95% CI 0.83 to 1.63) or clinic-referral based [2, 6, 12-15, 17, 20] (OR 0.84; 95% CI 0.58-1.22), with the association remaining non-significant. There was a high degree of heterogeneity across the clinic-referral based studies ($I^2=74\%$, $P=0.0003$) and community-based studies ($I^2=71\%$, $P=0.06$). In studies of hypertensive subjects with and without OSA and controls, there was no significant association of OSA risk with D-allele frequency (OR 1.00; 95% CI 0.46-2.20) [15, 18]. The risk of having OSA, overall, was not associated with the D-allele (OR 0.87; 95% CI 0.69-1.09).

Four studies reported D-allele frequency against OSA severity [6,13,15,18]. In 238 severe OSA subjects and 624 mild-moderate OSA subjects with D-allele frequencies of 242 and 686 respectively, there was a non-significant decrease in OSA risk with the D-allele (OR 0.87; CI 95% 0.69-1.09).

**DISCUSSION**

The aim of this study was to clarify the role of the ACE I/D polymorphism and its association with OSA using meta-analysis in a series of case-control, population-based studies. Our meta-analysis combined genotype information from 10 studies representing 2,454 subjects (1,227 OSA subjects and 1,227 controls) and failed to demonstrate an association between ACE I/D polymorphism with OSA susceptibility. The analysis further explored and excluded any influence of ethnicity, source of OSA
sample, and the presence of hypertension. A subgroup analysis revealed that genotype and allele frequency did not influence OSA severity.

Our findings, showing the lack of an association of the ACE I/D genotype with OSA susceptibility, echo the negative results of the recent meta-analyses on the association of ACE I/D genotype with blood pressure [9-11]. Although the D-allele is associated with increased serum-ACE activity, the bulk of the evidence indicates that the effect of the D-allele on blood pressure is small and additional environmental or genetic factors are most likely required to determine blood pressure [5, 10, 11]. OSA subjects may have higher serum-ACE activity than control subjects, but it is unlikely that ACE I/D polymorphisms increase susceptibility to OSA per se.

Limitations of the study are those inherent to the publications available for analysis and related to the populations studied, their ethnicity and any confounding co-morbidities and other co-variates, including environmental factors that have neither been considered nor controlled for. Heterogeneity across trials is significant despite an attempt at stratification of the studies by source of referral and ethnicity. The most likely contributors to heterogeneity in this meta-analysis are related to the disparate phenotype definitions of OSAHS (see Table 1.), the small sample sizes, the low-order magnitude of the genetic effects of the ACE-gene polymorphisms in OSAHS and gene-environment interactions that can never be fully accounted for.

Notwithstanding these limitations, the present meta-analysis has failed to demonstrate an association between the ACE I/D polymorphism and OSA susceptibility irrespective of ethnicity, population sample or the presence and/or absence of co-morbid hypertension.

ACKNOWLEDGEMENTS
The authors would like to thank Dr Wen-Yi Shau for statistical guidance and Jane Thain for assistance in the preparation of this manuscript.

Peilin Lee was the recipient of European Respiratory Society Fellowship No.147.

REFERENCES


TABLE LEGEND

Table 1. Characteristics of studies included in the meta-analysis

FIGURE LEGENDS

Figure 1. Flow diagram of literature search strategy

Figure 2. The funnel plot for publication bias shown with odds ratio on the abscissa and standardized effect size on the ordinate

Figure 3. Association of ACE I/D genotype under a dominant model where the random effect model was used (n= number of OSA subjects, N= number of control subjects). Heterogeneity among overall publications (I²=42%, P=0.08) and publications for Chinese (I²=69%, P=0.02) was significant.

Figure 4. Association of ACE D-allele frequency where the random effect model was used (n= number of OSA subjects, N= number of control subjects). Heterogeneity among overall publications (I²=74%, P<0.0001), and publications for Caucasians (I²=55%, P=0.05) and Chinese (I²=84%, P=0.0003) was significant.
<table>
<thead>
<tr>
<th>Reference</th>
<th>Author</th>
<th>Year</th>
<th>Sample</th>
<th>Characteristics of Control Subjects</th>
<th>Characteristics of OSA Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No</td>
<td>Age (yrs)</td>
<td>Males (%)</td>
</tr>
<tr>
<td>12</td>
<td>Barcelo</td>
<td>2001</td>
<td>R</td>
<td>32</td>
<td>49±1</td>
</tr>
<tr>
<td>2</td>
<td>Benjamin</td>
<td>2008</td>
<td>R</td>
<td>52</td>
<td>43.59</td>
</tr>
<tr>
<td>18</td>
<td>Bostrom</td>
<td>2007</td>
<td>C</td>
<td>108</td>
<td>N/M</td>
</tr>
<tr>
<td>16</td>
<td>Li</td>
<td>2004</td>
<td>R</td>
<td>30</td>
<td>45.2</td>
</tr>
<tr>
<td>6</td>
<td>Lin</td>
<td>2004</td>
<td>C</td>
<td>626</td>
<td>N/M</td>
</tr>
<tr>
<td>13</td>
<td>Ogus</td>
<td>2010</td>
<td>R</td>
<td>79</td>
<td>60.1±10</td>
</tr>
<tr>
<td>14</td>
<td>Xiao</td>
<td>1998</td>
<td>R</td>
<td>50</td>
<td>30.9±6.6</td>
</tr>
<tr>
<td>20</td>
<td>Yildiz</td>
<td>2010</td>
<td>R</td>
<td>37</td>
<td>49.9±10.4</td>
</tr>
<tr>
<td>15</td>
<td>Zhang</td>
<td>2000</td>
<td>R</td>
<td>113</td>
<td>54.0±12.4</td>
</tr>
<tr>
<td>17</td>
<td>Zhang</td>
<td>2004</td>
<td>R</td>
<td>100</td>
<td>40.1±2.1</td>
</tr>
</tbody>
</table>

Table 1.
Abbreviation: OSA=obstructive sleep apnoea; R=obstructive sleep apnea (OSA) subjects referred from clinic; C=OSA subjects derived from a screened community sample; N/M=not mentioned; BMI=body mass index; ESS=Epworth sleepiness scale; AHI=apnea-hypopnea index; HT=percentage of the population with hypertension as defined by the authors.
Fig. 1

References identified through PubMed and EMBASE searching (n=102) → Duplicate references (n=45)

References after duplicates removed (n=61) → References excluded (n=41)

References screened (n=61) → Not relevant to ACE polymorphism (n=41)

References for detailed evaluation (n=20) → References excluded (n=6)

References assessed using inclusion and exclusion criteria (n=14) → Letter (n=1)

References excluded (n=6)

Studies included in the meta-analysis (n=10) → Review (n=4)

References after duplicates removed (n=61)

References identified through UMI Dissertation Abstract database and Google™ (n=4)

Duplicate references (n=45)

References excluded (n=4)

Pediatric OSA (n=1)

No control subjects included (n=2)

No patient number given (n=1)
Fig 2.
### Table 1: Odds Ratio (OR) - Figure 3

<table>
<thead>
<tr>
<th>Caucasian population</th>
<th>Carrier</th>
<th>Total</th>
<th>Weight</th>
<th>OR</th>
<th>95% CI</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barcelo 2001</td>
<td>37</td>
<td>23</td>
<td>44</td>
<td>32</td>
<td>5.4%</td>
<td>2.07</td>
<td>0.68</td>
</tr>
<tr>
<td>Benjamin 2008</td>
<td>20</td>
<td>42</td>
<td>26</td>
<td>52</td>
<td>5.2%</td>
<td>0.79</td>
<td>0.25</td>
</tr>
<tr>
<td>Bostrom 2007</td>
<td>189</td>
<td>85</td>
<td>230</td>
<td>108</td>
<td>13.2%</td>
<td>1.25</td>
<td>0.70</td>
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<tr>
<td>Lin 2004</td>
<td>382</td>
<td>498</td>
<td>474</td>
<td>626</td>
<td>20.9%</td>
<td>1.07</td>
<td>0.79</td>
</tr>
<tr>
<td>Ogus 2010</td>
<td>78</td>
<td>67</td>
<td>97</td>
<td>79</td>
<td>8.9%</td>
<td>0.74</td>
<td>0.33</td>
</tr>
<tr>
<td>Yakut 2010</td>
<td>54</td>
<td>30</td>
<td>64</td>
<td>37</td>
<td>5.8%</td>
<td>1.26</td>
<td>0.43</td>
</tr>
<tr>
<td>Sum (I²=0%, P=0.73)</td>
<td>760</td>
<td>745</td>
<td>935</td>
<td>934</td>
<td>59.4%</td>
<td>1.09</td>
<td>0.86</td>
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</table>

<table>
<thead>
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<th>Asian population</th>
<th>Carrier</th>
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<th>Weight</th>
<th>OR</th>
<th>95% CI</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li 2004</td>
<td>33</td>
<td>22</td>
<td>60</td>
<td>30</td>
<td>6.8%</td>
<td>0.44</td>
<td>0.17</td>
</tr>
<tr>
<td>Xiao 1998</td>
<td>28</td>
<td>34</td>
<td>50</td>
<td>50</td>
<td>8.6%</td>
<td>0.60</td>
<td>0.27</td>
</tr>
<tr>
<td>Zhang 2000</td>
<td>31</td>
<td>79</td>
<td>61</td>
<td>113</td>
<td>11.6%</td>
<td>0.44</td>
<td>0.23</td>
</tr>
<tr>
<td>Zhang 2004</td>
<td>82</td>
<td>59</td>
<td>121</td>
<td>100</td>
<td>13.6%</td>
<td>1.46</td>
<td>0.84</td>
</tr>
<tr>
<td>Sum (I²=69%, P=0.02)</td>
<td>174</td>
<td>194</td>
<td>292</td>
<td>293</td>
<td>40.6%</td>
<td>0.67</td>
<td>0.35</td>
</tr>
<tr>
<td>Overall (I²=42%, P=0.08)</td>
<td>934</td>
<td>939</td>
<td>1227</td>
<td>1227</td>
<td>100%</td>
<td>0.92</td>
<td>0.69</td>
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### Table 1: Odds Ratio (OR) - Figure 4

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<thead>
<tr>
<th>Caucasian population</th>
<th>Carrier</th>
<th>Total</th>
<th>Weight</th>
<th>OR</th>
<th>95% CI</th>
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<th>Upper</th>
</tr>
</thead>
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<tr>
<td>Barcelo 2001</td>
<td>50</td>
<td>33</td>
<td>88</td>
<td>64</td>
<td>7.9%</td>
<td>1.24</td>
<td>0.65</td>
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<tr>
<td>Benjamin 2008</td>
<td>32</td>
<td>57</td>
<td>52</td>
<td>104</td>
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<td>1.32</td>
<td>0.67</td>
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<tr>
<td>Bostrom 2007</td>
<td>268</td>
<td>107</td>
<td>460</td>
<td>216</td>
<td>12.3%</td>
<td>1.42</td>
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<tr>
<td>Lin 2004</td>
<td>514</td>
<td>678</td>
<td>948</td>
<td>1252</td>
<td>14.3%</td>
<td>1.00</td>
<td>0.85</td>
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<tr>
<td>Ogus 2010</td>
<td>105</td>
<td>104</td>
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<td>158</td>
<td>10.8%</td>
<td>0.61</td>
<td>0.40</td>
</tr>
<tr>
<td>Yakut 2010</td>
<td>81</td>
<td>41</td>
<td>128</td>
<td>74</td>
<td>8.7%</td>
<td>1.39</td>
<td>0.77</td>
</tr>
<tr>
<td>Sum (I²=55%, P=0.05)</td>
<td>1050</td>
<td>1020</td>
<td>1870</td>
<td>1868</td>
<td>61.6%</td>
<td>1.09</td>
<td>0.85</td>
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<table>
<thead>
<tr>
<th>Asian population</th>
<th>Carrier</th>
<th>Total</th>
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<th>OR</th>
<th>95% CI</th>
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<th>Upper</th>
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<tr>
<td>Li 2004</td>
<td>54</td>
<td>41</td>
<td>120</td>
<td>60</td>
<td>7.9%</td>
<td>0.38</td>
<td>0.20</td>
</tr>
<tr>
<td>Xiao 1998</td>
<td>28</td>
<td>42</td>
<td>100</td>
<td>100</td>
<td>8.6%</td>
<td>0.54</td>
<td>0.30</td>
</tr>
<tr>
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<td>42</td>
<td>107</td>
<td>122</td>
<td>228</td>
<td>10.4%</td>
<td>0.58</td>
<td>0.37</td>
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<tr>
<td>Zhang 2004</td>
<td>118</td>
<td>78</td>
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<td>200</td>
<td>11.5%</td>
<td>1.49</td>
<td>1.02</td>
</tr>
<tr>
<td>Sum (I²=84%, P=0.0003)</td>
<td>242</td>
<td>268</td>
<td>584</td>
<td>586</td>
<td>35.4%</td>
<td>0.67</td>
<td>0.36</td>
</tr>
<tr>
<td>Overall (I²=74%, P=0.0001)</td>
<td>1292</td>
<td>1288</td>
<td>2454</td>
<td>2454</td>
<td>100.0%</td>
<td>0.92</td>
<td>0.71</td>
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