ABSTRACT: A number of studies focusing on the association between the exon 1 CAG repeat polymorphism of the androgen receptor (AR) gene and polycystic ovary syndrome (PCOS) have revealed conflicting results. The current systematic review and meta-analysis was conducted to quantify the strength of the association and to explore potential sources of heterogeneity that may have influenced the results. Studies matched to search terms from PubMed, EMBASE and HuGE Navigator published through to 31 January 2012 were retrieved. Data extraction from the included studies was carried out by two authors independently. Weighted mean differences (WMDs) of biallelic mean and odds ratios (ORs) of alleles and genotypes were pooled for meta-analysis. Sixteen articles reporting on 17 studies were included. In continuous data analysis, the summary WMD was 0.06 (95% confidence interval -0.29 to 0.16). In dichotomous data analysis, we divided the alleles into short and long alleles and calculated the summary ORs. No statistically significant results were identified by different comparison models or different cut-off point definitions. No publication bias was observed in continuous and dichotomous data analysis. In summary, the current systematic review and meta-analysis found that the AR CAG microsatellite repeat polymorphism is unlikely to be a major determining factor in the development of PCOS.

Key words: androgen receptor / CAG repeat / genetic polymorphism / meta-analysis / polycystic ovary syndrome
The NTD, in which the transactivation activity of the receptor resides, is encoded by a single large exon (exon 1). The NTD of the AR is poorly conserved among steroid nuclear receptor superfamily members and contains a polyglutamine (PolyGln) stretch coded by a highly polymorphic CAG trinucleotide repeat (rs4045402) within the first exon of the AR gene (Chamberlain et al., 1994). In vitro studies demonstrated an inverse association between CAG repeat number and AR transactivation function (Chamberlain et al., 1994; Tut et al., 1997; Irvine et al., 2000; Buchanan et al., 2004).

In addition to the microsatellite repeat polymorphism, a single-nucleotide polymorphism (SNP) in exon 1 of the AR gene, the rs6152GA polymorphism, has also been examined in a single study (Peng et al., 2010). It was concluded that the AR rs6152GA polymorphism seemed to be one of the determining factors in the development of PCOS in Han Chinese females (Peng et al., 2010).

Overall, these and other data suggest that the AR microsatellite repeat polymorphism might play a role in the pathogenesis of PCOS. On the basis of this assumption, several studies focused on the association between the CAG repeat polymorphism of the AR gene and the presence of PCOS, yielding inconsistent results (Jakuščiková et al., 1997; Mifsud et al., 2000; Hickey et al., 2002; Jääskeläinen et al., 2005; Ferk et al., 2008; Kim et al., 2008; Liu et al., 2008; Shah et al., 2008; Xie et al., 2008; Dasgupta et al., 2010; Laisk et al., 2010; Radian et al., 2010; Robeva et al., 2010; Ramos Cirilo et al., 2011; Schüring et al., 2011; Skrgetic et al., 2012). To more clearly address the question of an association between this genetic variation and PCOS, we conducted the current systematic review and meta-analysis to quantify the strength of this association and to explore potential sources of heterogeneity that may have influenced the results.

Materials and Methods

Search strategy

The searches were performed by two investigators and the final search strategies were performed with agreement. Electronic literature searches were conducted in PubMed/MEDLINE, EMBASE and HuGE Navigator (Lin et al., 2006) (http://www.hugenavigator.net/) from inception through to January, 2012, using both free words and index terms specific to each search platform (MeSH in PubMed and Emtree in Embase.com). The search strategies were based on combinations of the keywords ‘PCOS’, ‘AR’ and ‘genetic polymorphism’, as well as the abbreviations and synonyms of each term. The detailed search strategies are given in Supplementary data, ‘Materials and Methods’. The latest searches were undertaken on 31 January 2012. There were no language restrictions. Duplicate publications were considered only once. We also reviewed the reference lists of retrieved articles to identify other relevant publications. Authors of retrieved articles were contacted if the published report lacked inclusion criteria data and were asked to provide additional information.

Eligibility of relevant studies

Eligibility criteria included the following: (i) independent case–control or cohort studies; (ii) inclusion of both PCOS cases and non-PCOS controls; (iii) examination of the association between AR gene CAG repeat polymorphism and PCOS and (iv) inclusion of adequate data to calculate the effect size: (a) biallelic mean and corresponding SD or (b) allele or genotype frequencies.

When the populations of several publications overlapped, we used the article with the most extensive data. If an article presented data of different groups of research subjects, the results of those analyses were handled as separate studies. The studies published in conference proceedings or as abstracts were included if they met the aforementioned criteria. Pedigree and family based studies were excluded because such studies are generally linkage studies or family based transmission disequilibrium studies. Review articles were also excluded.

Data extraction

Data extraction from the included studies was carried out by two authors independently and disagreements were resolved by consensus. All relevant articles identified through the search were scanned on the basis of title and abstract, and were rejected in the initial screening if the article clearly did not meet the inclusion criteria. If an article could not be rejected with certainty on the basis of its title and abstract, we obtained the full text of the article for further evaluation. We attempted to contact all the correspondence authors of the included articles by e-mails and asked for detailed CAG repeat data in every PCOS and control woman.

The following information was extracted from each study: the first author’s name, year of publication, the country of origin, ethnicity and geographical location of the study population, number of cases and controls, definitions of cases and controls and method used to test the polymorphism. Biallelic mean of CAG repeat length, SD of biallelic mean and allele/genotype frequencies were extracted or calculated from published data in the included studies or data provided by the authors.

When SE was reported instead of SD, SD was calculated from SE (SD = \(SE/\sqrt{n}\)). When biallelic mean of repeats and corresponding SD for cases and controls were not reported, we reconstructed the tables with frequency distributions of biallelic mean repeats for cases and controls by measuring published frequency graphs for calculation. When no information was available to calculate the SD, it was calculated by using the \(P\) of the unpoole t-test comparison of the means between cases and controls:

\[
SD_{\text{cases}} = SD_{\text{controls}} = \frac{\text{MD}}{(Z\sqrt{(1/n_{\text{cases}} + 1/n_{\text{controls}}}))}
\]

where \(Z\) is the corresponding \(Z\) score of the \(P\) of the unpoole t-test, \(n_{\text{cases}}\) is the number of cases and \(n_{\text{controls}}\) is the number of controls (Zeegers et al., 2004). When allele frequencies were neither reported nor provided by the author, we reconstructed the tables with frequency distributions of repeats for cases and controls by measuring published frequency graphs.

Statistical analysis

We performed both continuous and dichotomous data analysis. Continuous data are expressed as the biallelic mean of the CAG length of paired alleles and SD for each group. Weighted mean differences (WMDs) with 95% confidence intervals (CIs) were calculated. Dichotomous data are presented as numbers of different genotypes or alleles for each group in the form of \(2 \times 2\) tables by using cut-off points to divide alleles into Long (L) alleles (CAG repeat length \(\geq\) cut-off point) and Short (S) alleles (CAG repeat length < cut-off point). If a \(2 \times 2\) table contained a ‘zero cell’, we added 0.5 to each cell of the \(2 \times 2\) table for the study to deal with this problem (Sterne et al., 2001). Odds ratios (ORs) and 95% CIs were reported by the article or calculated as the metrics of effect size. Hardy–Weinberg equilibrium (HWE) was tested by the \(\chi^2\) test method. Statistical analysis was carried out by using STATA (version SE 11.2; Stata Corporation, College Station, TX, USA). Meta-analysis was performed to assess pooled estimates of the effect. The between-study heterogeneity was evaluated by two methods: the \(\chi^2\)-based Cochran’s \(Q\) statistic and the \(I^2\) statistic (Higgins et al., 2003). For \(\chi^2\)-based Cochran’s
et al. (2010). When heterogeneity was significant, data were analyzed by using a random-effects model (DerSimonian and Laird method). Otherwise, a fixed-effects model (Mantel–Haenszel method) was used (Lau et al., 1997). P-values of < 0.05 or 95% CIs not containing 0 (WMD) or 1.00 (OR) were considered to be statistically significant. Meta-analysis was performed by using the ‘metaan’ command.

The following characters were investigated by subgroup analysis and meta-regression to identify whether they were sources of heterogeneity: geographic location, ethnicity, definition of PCOS and sample size of cases. In meta-regression analysis, these four factors were considered as covariates to find potential sources of heterogeneity. Covariates with \( P < 0.05 \) were considered as sources of heterogeneity between studies. Meta-regression analysis was performed by using the ‘metareg’ command.

Sensitivity analysis was performed to assess the stability of these results. A single study involved in the meta-analysis was omitted each time to reflect the influence of the individual studies on the overall effect estimate. Also, we excluded studies in which the controls deviated significantly (\( P < 0.05 \)) from HWE to examine sensitivity. The funnel plot and the Egger’s test (Egger et al., 1997) were used to explore potential publication bias. \( P < 0.05 \) was considered representative of statistically significant publication bias. Sensitivity analysis and publication bias analysis were performed by using the ‘metaninf’ and ‘metabias’ commands, respectively.

### Results

#### Characteristics of included studies

The initial search identified 60 potentially relevant articles. On the basis of \( a \) priori selection criteria (the following), screening for title or abstract identified 22 articles (18 full publications and 4 conference abstracts) for further assessment. Of these, two full publications (Mohlig et al., 2006; Van Nieuwerburgh et al., 2008) were excluded because they contained only PCOS subjects, one (Tong et al., 2010) because of data inconsistencies (no appropriate data could be extracted exactly from the allele distribution graph), one conference abstract (Diaz et al., 2010b) and one full text (Diaz et al., 2010a) because of lack of data to generate OR or WMD and one because of incomplete abstracts (Nazar enko and Biryukova, 2010) (without results or conclusions or e-mail of correspondence author). Finally, 16 articles on the AR CAG repeat polymorphism were identified (14 full publications; Jakubczka et al., 1997; Mifsud et al., 2000; Hickey et al., 2002; Jääskeläinen et al., 2005; Feret et al., 2008; Kim et al., 2008; Liu et al., 2008; Shah et al., 2008; Xita et al., 2008; Dasgupta et al., 2010; Laisk et al., 2010; Peng et al., 2010; Ramos Cirilo et al., 2011; Schüring et al., 2011; Skrgatic et al., 2012) and two conference abstracts (Radian et al., 2010; Robeva et al., 2010) were identified. Except for one German article (Jakubczka et al., 1997), the other 15 were published in English. The flow diagram of selection of studies is shown in Supplementary data, Fig. S1.

One article reported on two different study populations (Shah et al., 2008) and was considered as two separate studies. In all, 16 articles reporting 17 independent studies were included in this systematic review and meta-analysis. Considered together, the 17 studies included 4260 women: 2068 cases and 2192 controls.

The characteristics of the included 16 articles (17 studies) are shown in Table I. Of these included articles, all were case–control designs. Publication dates ranged from 1997 to 2012. They were conducted in 15 different countries and numerous geographic locations: 9 (Jakubczka et al., 1997; Jääskeläinen et al., 2005; Feret et al., 2008; Xita et al., 2008; Laisk et al., 2010; Radian et al., 2010; Robeva et al., 2010; Schüring et al., 2011; Skrgatic et al., 2012) in Europe, 4 (Mifsud et al., 2000; Kim et al., 2008; Liu et al., 2008; Dasgupta et al., 2010) in Asia, 3 (Shah et al., 2008; Ramos Cirilo et al., 2011) in the Americas and 1 (Hickey et al., 2002) in Oceania. Eleven of the studies (Jakubczka et al., 1997; Hickey et al., 2002; Jääskeläinen et al., 2005; Feret et al., 2008; Shah et al., 2008; Xita et al., 2008; Laisk et al., 2010; Radian et al., 2010; Robeva et al., 2010; Schüring et al., 2011; Skrgatic et al., 2012) were done in Caucasian populations, 4 (Mifsud et al., 2000; Kim et al., 2008; Liu et al., 2008; Dasgupta et al., 2010) were done in Asian populations, 1 (Shah et al., 2008) was done among Black women and 1 (Ramos Cirilo et al., 2011) was done in an unspecified population (Brazilian). Regarding PCOS diagnostic criteria, nine studies (Kim et al., 2008; Liu et al., 2008; Dasgupta et al., 2010; Laisk et al., 2010; Radian et al., 2010; Ramos Cirilo et al., 2011; Robeva et al., 2010; Schüring et al., 2011; Skrgatic et al., 2012) used ESHRE/ASRM criteria, three (Shah et al., 2008; Xita et al., 2008) used NIH criteria and five (Jakubczka et al., 1997; Mifsud et al., 2000; Hickey et al., 2002; Jääskeläinen et al., 2005; Feret et al., 2008) used other criteria. All the full publications reported that PCR-based methods were used for testing CAG repeat polymorphism.

#### Quantitative data synthesis

One study (Jakubczka et al., 1997) presented detailed CAG repeats of each subject in cases and controls in the full-text article. Among all the correspondence authors of the included articles we contacted via e-mails, four authors (Kim et al., 2008; Shah et al., 2008; Laisk et al., 2010; Robeva et al., 2010) provided detailed CAG repeats in every PCOS and control woman in five studies.

#### Continuous data analysis

In total, there were 11 articles (Jakubczka et al., 1997; Mifsud et al., 2000; Hickey et al., 2002; Kim et al., 2008; Liu et al., 2008; Shah et al., 2008; Laisk et al., 2010; Radian et al., 2010; Robeva et al., 2010; Schüring et al., 2011; Skrgatic et al., 2012) involving 1299 PCOS patients and 1401 controls reporting biallelic mean repeats reporting biallelic mean with 500-500000 mean and SD of CAG repeats in both groups (Supplementary data, Table S1). No significant finding was observed in the random-effect meta-analysis (Fig. 1). The comparison of biallelic mean of CAG repeats PCOS patients and controls yielded a WMD of \(-0.06\) (95% CI \(-0.29\) to 0.16). Formal testing for heterogeneity among studies yielded a Cochran’s Q test P-value of 0.06 with an \( I^2\) value of 41.9%, indicating moderate heterogeneity.

Subgroup analyses were performed to examine the potential source of heterogeneity (Table II). We classified the studies according to geographic region (Europe, Asia, America and Oceania), ethnicity (Caucasian, Asian and Black), definitions of PCOS (ESHRE/ASRM, NIH and others) and the sample size of cases (PCOS patients more than 100 and \(<100\)). Only one study was included in the Oceania subgroup, results of which indicated that PCOS patients have longer CAG biallelic mean repeats than controls (WMD = 0.66, 95% CI 0.09 – 1.24). None of the subgroup analyses results revealed a significant difference in CAG biallelic mean repeats between cases and controls. After dividing
### Table I Characteristics of the included studies of the AR CAG repeat polymorphism.

<table>
<thead>
<tr>
<th>Article</th>
<th>Geographic Country region</th>
<th>Ethnicity</th>
<th>Sample size</th>
<th>Definition</th>
<th>Method</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jakubiczka et al. (1997)</td>
<td>Germany</td>
<td>Europe</td>
<td>Caucasian</td>
<td>10 PCOS 10 Control</td>
<td>(i) PCO (≥ 10 follicles, 2–10 mm in diameter) (ii) At least score 2 for echogenicity of the ovaries (iii) Ovarian insufficiency.</td>
<td>PCR</td>
</tr>
<tr>
<td>Mifsud et al. (2000)</td>
<td>Singapore</td>
<td>Asia</td>
<td>Asian</td>
<td>91 PCOS 112 Control</td>
<td>(i) PCO (≥ 10 follicles, &lt;10 mm in diameter) (ii) Oligomenorrhea (iii) Involuntary infertility</td>
<td>PCR</td>
</tr>
<tr>
<td>Hickey et al. (2002)</td>
<td>Australia</td>
<td>Oceania</td>
<td>Caucasian</td>
<td>122 PCOS 83 Control</td>
<td>(i) Hyperandrogenism (ii) Anovulation (only infertile patients are included)</td>
<td>PCR</td>
</tr>
<tr>
<td>Jaàskelàinen et al. (2005)</td>
<td>Finland</td>
<td>Europe</td>
<td>Caucasian</td>
<td>106 PCOS 112 Control</td>
<td>(i) Anovulation (ii) PCO (iii) At least one of the following: (a) hirsutism, (b) infertility, (c) laboratory testing revealing androgen excess, (d) LH/FSH ratio &gt;2 at the luteal phase (only non-diabetic women are included)</td>
<td>PCR</td>
</tr>
<tr>
<td>Ferk et al. (2008)</td>
<td>Slovene</td>
<td>Europe</td>
<td>Caucasian</td>
<td>117 PCOS 110 Control</td>
<td>(i) Oligo-/amenorrhea (ii) PCO (iii) Hyperandrogenism</td>
<td>PCR</td>
</tr>
<tr>
<td>Kim et al. (2008)</td>
<td>South Korea</td>
<td>Asia</td>
<td>Asian</td>
<td>114 PCOS 205 ESHRE/ASRM</td>
<td></td>
<td>PCR</td>
</tr>
<tr>
<td>Liu et al. (2008)</td>
<td>China</td>
<td>Asia</td>
<td>Asian</td>
<td>148 PCOS 104 ESHRE/ASRM</td>
<td>(i) Proven fertility (ii) Normal menstrual cycles (iii) Normal ovary morphology (iv) No history of subfertility treatment</td>
<td>PCR</td>
</tr>
<tr>
<td>Article</td>
<td>Geographic</td>
<td>Sample size</td>
<td>Definition</td>
<td>Method</td>
<td>Conclusions</td>
<td></td>
</tr>
<tr>
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<td>-----------------------------------------------------------------------------</td>
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<td>-----------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Shah et al. (2008)*</td>
<td>USA America (1)</td>
<td>270 165</td>
<td>(i) Healthy women (ii) Regular menstrual cycles or a history of regular menstrual cycles before menopause (iii) Without family history of hirsutism</td>
<td>PCR</td>
<td>The risk of PCOS is associated with the AR genes with shorter CAG repeats, suggesting that genetic differences in androgen sensitivity may contribute to development of PCOS</td>
<td></td>
</tr>
<tr>
<td>Xita et al. (2008)</td>
<td>Greece Europe</td>
<td>37 84</td>
<td>(i) Healthy women (ii) Normal menstrual cycles (28–30 days) (iii) No signs of hyperandrogenism</td>
<td>PCR</td>
<td>The presence of long SHBG(TAAAA)n alleles is associated with increased risk for PCOS and in combination with short AR(CAG)n alleles may influence the hyperandrogenic phenotype of PCOS</td>
<td></td>
</tr>
<tr>
<td>Dasgupta et al. (2010)</td>
<td>India Asia</td>
<td>249 296</td>
<td>(i) No history of treatment for fertility (ii) Normal menstrual cycles (25–32 days)</td>
<td>PCR</td>
<td>CAG repeat polymorphism by itself cannot be considered as a useful marker for discriminating PCOS. In the obese PCOS women, this microsatellite variation may account for the hyperandrogenicity to a larger extent than the lean PCOS women</td>
<td></td>
</tr>
<tr>
<td>Laisk et al. (2010)</td>
<td>Estonia Europe</td>
<td>32 79</td>
<td>(i) Infertile (ii) Occluded fallopian tubes (iii) Regular menstrual cycles (26–35 days)</td>
<td>PCR</td>
<td>No direct associations between AR CAG repeats (as well as XCI) and PCOS were found</td>
<td></td>
</tr>
<tr>
<td>Radian et al. (2010)</td>
<td>Romania Europe</td>
<td>137 130</td>
<td>(i) Non-hirsute (ii) Regular menstrual cycle (iii) No history of any ovarian dysfunctions</td>
<td>NA</td>
<td>The CAG polymorphism of the AR is significantly associated with PCOS in Romanian women (Eastern Europe) and may play an important role in PCOS pathogenesis</td>
<td></td>
</tr>
<tr>
<td>Robeva et al. (2010)</td>
<td>Bulgaria Europe</td>
<td>52 41</td>
<td>(i) Regular menstrual cycles (ii) No clinical or biochemical hyperandrogenism (iii) Normal ultrasonographic images (iv) Never sought treatment for infertility (v) Without familial or personal history of ovarian or breast cancer, uterine leiomyomas or carcinomas, endometriosis and adenomyosis</td>
<td>NA</td>
<td>CAG repeat polymorphism is not crucial for the development of the PCOS. However, patients with lowest number of CAG repeats have most pronounced hirsutism; while longer allele carriers have higher LH levels and LH/testosterone ratio</td>
<td></td>
</tr>
<tr>
<td>Ramos Cinlo et al. (2011)</td>
<td>Brazil America</td>
<td>117 105</td>
<td>(i) Regular menstrual cycles (ii) No clinical or biochemical hyperandrogenism (iii) Normal ultrasonographic images (iv) Never sought treatment for infertility (v) Without familial or personal history of ovarian or breast cancer, uterine leiomyomas or carcinomas, endometriosis and adenomyosis</td>
<td>PCR</td>
<td>AR shorter alleles can be associated with higher serum levels of TT in PCOS patients</td>
<td></td>
</tr>
</tbody>
</table>
the studies into subgroups according to different characteristics, $I^2$ in subgroup analyses ranged from 0 to 66%. The results showed that the subgroups explained part of the heterogeneity, but notable between-study heterogeneity still existed.

Furthermore, we tested the effects of geographic region (Europe and others), ethnicity (Caucasian and others), definitions of PCOS (ESHRE/ASRM and others) and the sample size of cases (PCOS patients >100 and <100) on heterogeneity among the WMDs by meta-regression analysis. The $P$-values of these four confounders were 0.33, 0.57, 0.75 and 0.47, respectively. The results of meta-regression showed that none of these characteristics seemed to be significant sources of between-study heterogeneity for comparison of CAG biallelic mean repeats between cases and controls.

Sensitivity analysis was carried out by deleting every single study one at a time. The pooled WMDs ranged from $20.13 \pm 0.33$ to $20.06$, after deleting (Hickey et al., 2002) to $20.01 \pm 0.22$ to $20.21$, after deleting (Radan et al., 2010). The pooled WMDs were not materially altered, implying that the results were robust.

Funnel plot analyses (Supplementary data, Fig. S2) were employed, and symmetry of funnel plot was observed. The $P$-value of Egger’s test was 0.81. The results indicated that no publication bias was found in the included studies.

**Dichotomous data analysis**

For the CAG repeat length polymorphism in the AR gene, there has not yet been a consensus on the optimum cut-off point. Choosing the median value of CAG repeat number as the cut-off point is a routine method. Because the studies reporting 21 or 22 as the median value are more than studies reporting other median values, we chose 21 and 22 as the cut-off values for further analysis.
Table II Results of subgroup analysis of comparing AR CAG biallelic mean of cases and controls.

<table>
<thead>
<tr>
<th>Subgroups</th>
<th>Number of studies</th>
<th>WMD (95% CI)</th>
<th>Heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>12</td>
<td>-0.06 (-0.29 to 0.16)</td>
<td>0.06</td>
</tr>
<tr>
<td>Geographic location</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Europe</td>
<td>6</td>
<td>-0.20 (-0.54 to 0.13)</td>
<td>0.19</td>
</tr>
<tr>
<td>Asia</td>
<td>3</td>
<td>0.072 (-0.20 to 0.34)</td>
<td>0.70</td>
</tr>
<tr>
<td>America</td>
<td>2</td>
<td>-0.35 (-0.74 to 0.04)</td>
<td>0.60</td>
</tr>
<tr>
<td>Oceania*</td>
<td>1</td>
<td>0.66 (0.09–1.24)</td>
<td>—</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>8</td>
<td>-0.11 (-0.44 to 0.23)</td>
<td>0.02</td>
</tr>
<tr>
<td>Asian</td>
<td>3</td>
<td>0.07 (-0.20 to 0.34)</td>
<td>0.70</td>
</tr>
<tr>
<td>Black*</td>
<td>1</td>
<td>-0.13 (-1.03 to 0.77)</td>
<td>—</td>
</tr>
<tr>
<td>PCOS definition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESHRE/ASRM</td>
<td>7</td>
<td>-0.09 (-0.36 to 0.18)</td>
<td>0.11</td>
</tr>
<tr>
<td>NIH</td>
<td>2</td>
<td>-0.35 (-0.74 to 0.04)</td>
<td>0.60</td>
</tr>
<tr>
<td>Other</td>
<td>3</td>
<td>0.26 (-0.32 to 0.84)</td>
<td>0.20</td>
</tr>
<tr>
<td>Sample size of cases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 100</td>
<td>6</td>
<td>0.01 (-0.32 to 0.34)</td>
<td>0.01</td>
</tr>
<tr>
<td>&lt; 100</td>
<td>6</td>
<td>-0.22 (-0.53 to 0.08)</td>
<td>0.73</td>
</tr>
</tbody>
</table>

*Only one study was in the subgroup so that P and I² for heterogeneity could not be calculated.

1997; Jääskeläinen et al., 2005; Ferk et al., 2008; Kim et al., 2008; Shah et al., 2008; Xita et al., 2008; Dasgupta et al., 2010; Laisk et al., 2010; Robeva et al., 2010; Ramos Cirilo et al., 2011; Skrgatic et al., 2012) involving 12 studies including 1498 PCOS patients and 1584 controls reporting the frequencies of S and L alleles in cases and controls (Supplementary data, Table SII). For the meta-analysis of the allele studies, the summary results from the meta-analysis indicated no significant relationship of S allele with PCOS risk (Fig. 2a and b). When different cut-off points (21 and 22) were performed, the pooled ORs (95% CIs) were 0.93 (0.84–1.04) and 0.97 (0.85–1.04), respectively. There was no significant publication bias by performing Egger’s test (P = 0.26 and 0.79 when cut-off point = 21 and 22, respectively).

Second, we compared the genotype distribution. We included the studies reporting SS, SL and LL distributions in cases and controls. The additive model was used by comparing SS versus LL and SL versus LL, because the genetic model was not clear for the CAG repeat polymorphism. In total, we included six articles (Jakubiczka et al., 1997; Kim et al., 2008; Shah et al., 2008; Xita et al., 2008; Laisk et al., 2010; Robeva et al., 2010) involving seven studies when the cut-off value = 21; and five articles (Jakubiczka et al., 1997; Kim et al., 2008; Shah et al., 2008; Laisk et al., 2010; Robeva et al., 2010) involving six studies when the cut-off point = 22 (Supplementary data, Table SIII). One study with Hardy-Weinberg disequilibrium was observed (Xita et al., 2008).

When using 21 as the cut-off point, no significant results were found in model SS versus LL (random-effect summary OR = 0.69, 95% CI 0.31–1.55, heterogeneity: P = 0.002 and I² = 70.8%) or model SL versus LL (fixed-effect summary OR = 0.91, 95% CI 0.71–1.15, heterogeneity: P = 0.50 and I² = 0%), as shown in Fig. 3a and b. Similar results were observed in model SS versus LL (fixed-effect summary OR = 1.24, 95% CI 0.85–1.82, heterogeneity: P = 0.57 and I² = 0%) and model SL versus LL (random-effect summary OR = 1.03, 95% CI 0.67–1.59, heterogeneity: P = 0.16 and I² = 36.9%) when using 22 as the cut-off point (Fig. 3c and d).

Because of the limited number of included studies, we did not perform subgroup analysis. Sensitivity analysis was performed by deleting the Hardy-Weinberg disequilibrium study (Xita et al., 2008) (cut-off point = 21), yielding random-effect summary OR 0.56 (95% CI 0.35–0.84), heterogeneity: P = 0.004 and I² = 71.3%). When using different models, P-values of Egger’s test for publication bias were 0.25 (cut-off point = 21, SS versus LL), 0.24 (cut-off point = 21, SL versus LL), 0.28 (cut-off point = 22, SS versus LL) and 0.99 (cut-off point = 22, SL versus LL), respectively.

Thirdly, considering that the CAG repeat differs in different studies, we used the median value of CAG repeats in controls of each study as one’s cut-off value, i.e. different studies had different cut-off values (Supplementary data, Tables SII and III). We reanalyzed allele and genotype distributions in this method. When comparing S and L allele distribution, the summary OR (95% CI) by using a fixed-effect model was 0.95 (0.86–1.05), as shown in Fig. 2c. No evidence of between-study heterogeneity was observed (P = 0.51 and I² = 0%). Egger’s test indicated that no obvious publication bias existed (P = 0.54).

When comparing different genotypes, all studies were in HWE. The summary ORs (95% CIs) were 1.09 (0.76–1.55, fixed-effect model, heterogeneity: P = 0.27 and I² = 22%) and 0.97 (0.68–1.39, fixed-effect model, heterogeneity: P = 0.51 and I² = 0%).
random-effect model, heterogeneity: $P = 0.24$ and $I^2 = 26.3\%$) when models SS versus LL and SL versus LL are used, respectively (Fig. 3e and f). The $P$-values of Egger’s test for publication bias were 0.37 (SS versus LL) and 0.17 (SL versus LL).

**Discussion**

**Summary of key findings**

The present systematic review and meta-analysis on the association between AR CAG repeat polymorphism and PCOS did not provide evidence for an association between CAG repeat length and PCOS risk in both continuous data analysis and dichotomous data analysis. We recognized the difficulty in identifying an association between AR CAG repeat length and PCOS because of the presence of two alleles and the fact that the included studies had different reporting strategies.

In order to generate the overall effect, we performed both continuous data analysis and dichotomous data analysis. In continuous data analysis, biallelic means of cases and controls were compared. In dichotomous data analysis, we divided the alleles into S and L alleles according to most reported medians of controls (21 and 22) and compared allele and genotype distribution. Considering that the CAG repeat differs in different studies, we redefined S and L alleles according to the median of controls in each study and reanalyzed the data. No evidence for an association between CAG repeat length and PCOS risk was observed in any of the aforementioned methods.

The effect of heterogeneity was investigated by subgroup analysis and meta-regression according to geographic location, ethnicity, PCOS definition and sample size of cases. We found that the subgroups explained part of the heterogeneity, but between-study heterogeneity still existed in continuous data analysis, suggesting that heterogeneity might also be explained by sampling error or other confounding factors we did not analyze in the current meta-analysis. Furthermore, no evidence for an association was discovered in subgroups. In dichotomous data analysis, between-study heterogeneity varied in different genetic comparison models. Finally, no publication bias was observed in funnel plot and Egger’s test.

**Limitations of the study**

Some limitations of this systematic review and meta-analysis should be taken into consideration. Firstly, the overall ORs and WMDs were calculated on the basis of unadjusted estimates. Some confounding factors, including environmental factors and phenotypic characteristics of cases and controls, might exist in the included studies. However, such confounding factors could not be extracted from all the eligible studies because of different reporting strategies. As a result, adjusted estimates could not be used to perform a more precise evaluation and between-study heterogeneity existed. We performed subgroup analysis and meta-regression analysis, as well as random-effect modeling to solve the problem. To address this issue, individual patient data meta-analysis should be performed and confounding factors could be standardized. However, the majority of the authors of the included studies did not respond to our attempts to contact them.

Secondly, the sample sizes of cases and controls were limited. Although 2068 cases and 2192 controls were included in the analysis,
for continuous and dichotomous data analysis, <2000 subjects were analyzed in each group respectively because of different reporting strategies. Fewer subjects were included in subgroups. Of the included studies, only three (Shah et al., 2008; Dasgupta et al., 2010; Skrgatic et al., 2012) had over 200 case subjects; most of the studies had relatively small sample sizes, like majority of candidate gene studies in PCOS. We further perform power analysis via PS Power and Sample Size Calculations (Version 3.0) (Dupont and Plummer, 1990). In continuous data analysis, to achieve 80% power to detect a WMD of magnitude estimated by the meta-analysis (WMD = 0.1, SD of repeat length = 2.0), 6280 cases and 6280 controls are needed. While in dichotomous data analysis (comparing allele distribution), to achieve 80% power to detect an OR of magnitude estimated (OR = 0.9, frequency of S allele = 0.5), 2832 cases and 2832 controls are needed. It is clear that our meta-analysis is still underpowered to demonstrate the association. It is likely that many underpowered

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**Figure 3** Forrest plot of studies reporting genotype frequencies. Individual and overall ORs and 95% CIs of each study are shown. Boxes and horizontal lines represent ORs and 95% CIs of studies. The diamond represents the overall OR and 95% CI. (a) SS versus LL, cut-off point = 21, random-effect model. (b) SL versus LL, cut-off point = 21, fixed-effect model. (c) SS versus LL, cut-off point = 22, fixed-effect model. (d) SL versus LL, cut-off point = 22, random-effect model. (e) SS versus LL, cut-off point = median of CAG repeats of controls in each study, fixed-effect model. (f) SL versus LL, cut-off point = median of CAG repeats of controls in each study, random-effect model.
studies resulted in false-negative reports and that several small studies produced false-positive results (Goodarzi, 2008). In other words, owing to a limited number of individuals, many studies were underpowered to detect an association with PCOS. It is necessary to collect more samples in order to have enough statistical power to explore real association.

Thirdly, potential biases may still exist. In order to avoid selection bias and publication bias, we did not perform study quality assessment, or excluded any non-English articles or conference abstracts. Owing to lack of necessary data, we still excluded several studies, which might result in the potential selection bias and be a source of heterogeneity.

Conclusions and implications for future research

Hyperandrogenism, as with many other features of PCOS, is heterogeneous. The CAG repeat variant clearly affects hyperandrogenism and thus several studies attempted to find the association of the CAG repeat polymorphism and different clinical androgen traits as well as biochemical parameters in PCOS patients. Interestingly, these results were also inconsistent. Some studies found that neither acne (Skrgatic et al., 2012) nor hirsutism (or modified Ferriman–Gallwey score) (Mifsud et al., 2000; Shah et al., 2008; Dasgupta et al., 2010; Skrgatic et al., 2012) was significantly correlated with the CAG repeat variant in PCOS patients; while some found that short CAG repeat was associated with acne and/or hirsutism (Van Nieuwerburgh et al., 2008). Regarding biochemical parameters, some concluded that testosterone levels were not significantly correlated with the CAG repeat variant (Jääskeläinen et al., 2005; Liu et al., 2008; Dasgupta et al., 2010; Schüring et al., 2011); while some found that short (Xita et al., 2008; Ramos Cirilo et al., 2011) or long (Mifsud et al., 2000; Hickey et al., 2002; Kim et al., 2008; Ramos Cirilo et al., 2011; Skrgatic et al., 2012) CAG repeat length was associated with elevated testosterone levels. These inconsistent results cannot be simply interpreted by current theories. Further research is needed to reveal the cross-talk between the AR and coregulators that affect androgen action.

Because the AR gene is mapped to the X chromosome and the phenomenon of X chromosome inactivation (XCI) exists in females, AR activity is determined not only by AR genotype but also by the epigenetic effect of XCI. Hickey et al. (2006) demonstrated that XCI played a role in epigenetic etiology of PCOS, and both genotype and epigenotype should be considered in the etiology of PCOS. Of the included studies, six (Hickey et al., 2002; Shah et al., 2008; Dasgupta et al., 2010; Laisk et al., 2010; Radian et al., 2010; Schüring et al., 2011) had considered the potential role of XCI in the etiology of PCOS. However, these studies used different reporting strategies. Additionally, XCI analysis can be carried out only in subjects who are heterozygous at the CAG repeat, which made the analysis much more complicated. As a result, we cannot perform a further meta-analysis of the effect of XCI. All the aforementioned studies found that overall XCI was not significantly different between PCOS patients and controls. Some (Shah et al., 2008; Radian et al., 2010) found that the shorter allele was preferentially active in PCOS in the subgroup of women with non-random XCI. However, XCI can vary in different tissues and XCI of the studies were tested by using blood sample, which might not reflect the condition in androgen target tissues.

The scientific basis for most included studies was two in vitro studies (Chamberlain et al., 1994; Tut et al., 1997) suggesting an inverse association between CAG polymorphism length and AR activity. However, a recent in vitro study (Nenonen et al., 2010) demonstrated that CAG repeat number is not inversely associated with AR activity, in contrast to previous data to this effect. This might explain the inconsistent results of the included studies and the heterogeneity of the current meta-analysis. In the future, more in vitro studies are required to identify functional AR polymorphisms that affect AR transactivity and different phenotypes of PCOS.

Future genetic association studies should also be designed on the basis of new discoveries of such in vitro studies. Another strategy that should be undertaken is to perform comprehensive genotyping across the AR locus, utilizing linkage disequilibrium to capture the majority of variation across the entire gene region, as recently conducted for other logical PCOS candidate genes (Chua et al., 2012).

The first genome-wide association study (GWAS) in PCOS was published in early 2011, and three genetic susceptibility loci were mapped in Han Chinese women with PCOS (Chen et al., 2011). This GWAS did not find association of AR variants with PCOS. The CAG repeat variant is not represented on GWAS platforms. Also, none of the studies to date has attempted to assess whether any typical biallelic common SNPs are in linkage disequilibrium with the CAG repeat. Therefore, it is quite possible that GWAS cannot answer the question regarding whether the CAG repeat plays a role in PCOS. For GWAS to be able to address this, one would have to genotype the CAG repeat along with multiple SNPs in the region, to identify any common SNPs that may serve as a proxy for the CAG repeat, and such a SNP would have to be represented on the GWAS chip. What GWAS might identify is other variants in the AR that may be associated with PCOS.

In conclusion, the current systematic review and meta-analysis showed that the AR CAG microsatellite repeat polymorphism is unlikely to be a major determining factor in the development of PCOS. However, the conclusion was not a definite negative association, but simply that our study failed to show any association because of underpowered effect. Large, well-performed studies focusing on both gene–gene and gene–environment interactions are also needed to explore the mechanism of PCOS.

Supplementary data

Supplementary data are available at http://molehr.oxfordjournals.org/.

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Authors’ roles

R.W.: study conception and design, collection, analysis and interpretation of data, drafting and revising the manuscript. M.O.G.: providing additional data and revising the article for intellectual content and language. T.X.: collection, analysis and interpretation of data, drafting and revising the manuscript. D.W.: statistical analysis and interpretation of data and drafting the manuscript. R.A.: revising the article for intellectual content and assisting in generating some of the original study data. H.Z.: study conception and design, interpretation of data, drafting and revising the manuscript.

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Conflict of interest

None declared.

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