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Polycystic Ovary Syndrome is Affected and Protected by *DD* and *DI* Genotypes of Angiotensin Converting Enzyme, Respectively: An Update of a Meta-Analysis

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Abstract

Objectives: Angiotensin converting enzyme (ACE) is an important enzyme involved in the physiopathology of renal, cardiovascular and ovarian systems. One of these ACE related diseases is polycystic ovary syndrome (PCOS).

Materials and Methods: We intend to update the only meta-analysis written by Jia et al in 2013 on the association of *ACE* gene polymorphism and risk of PCOS. The reason of our attempt to update this meta-analysis was that they found no significant relation in their meta-analysis. For this aim, we searched in databases for relevant documents.

Results: We found 8 relevant papers, 6 of which had been covered by Jia et al meta-analysis. In order to perform this meta-analysis, we used comprehensive meta-analysis software. The analysis was done through *P* value and sample size of each study based on fixed-effect model. Analyses were performed in 5 different groups of alleles and genotypes. Among these 5 analyses, 4 of them were statistically significant. Hereby, we concluded that *DD* genotype of *ACE* is a risk factor for PCOS (*P* value = 0.013; odds ratio [OR] = 1.195), while *DI* is the protecting genotype (*P* = 0.009; OR = 0.819).

Conclusion: Hence, it is suggested to use a very low dose of captopril as an ACE inhibitor in the PCOS patients having *DD* genotype in future as a clinical trial, just as a scientific model. Further investigation on ovary ACE system is needed.

Keywords: Angiotensin converting enzyme inhibitors, Meta-analysis, Polycystic ovary syndrome

Introduction

Angiotensin converting enzyme (ACE) is a renal enzyme catalyzing the conversion of angiotensin 1 to angiotensin 2. This, results in production of the aldosterone resulting in natrium retention and potassium excretion. *ACE* gene has 3 polymorphic genotypes of *II*, *DI* and *DD*. It has been observed in several studies and meta-analyses that patients with *DD* genotype are more at risk of renal and cardiovascular diseases. It seems that these effects could be due to hyperactivity of *ACE* gene in such patients (1,2). In addition to kidneys, ovaries have renin angiotensin system (RAS). The role of this system in ovulation and reproduction is not completely understood (3).

Different histopathologic and angiogenic abnormal conditions result in pregnancy complications (4). Polycystic ovary syndrome (PCOS) is one of such conditions resulting in infertility and other complications of women's reproductive system (5). Based on a meta-analysis conducted by Jalilian et al, prevalence of PCOS in Iran was 6.8%, 4.41% and 19.5% based on NIH, ultrasound and Rotterdam criteria, respectively (6). Type 2 diabetes, obesity, metabolic syndrome, hypertension and so on are considered as risk factors of PCOS (7-9). Also metformin is used for the treatment, because of insulin resistance in such patients (10). All of these evidences give us clues that hypertension related genes like *ACE* my play roles in pathogenesis of PCOS. Of course it is not clear whether this role is associated with renal RAS, ovarian RAS or both.

On the occasion of the clues and findings above, we intend to update the only meta-analysis conducted by Jia et al in 2013 on the association of *ACE* gene polymorphism and risk of PCOS (11). The reason of our attempt to update this meta-analysis was that Jia et al did not find any significant relation in their random-effect model meta-analysis.

Materials and Methods

We searched in databases for relevant keywords in the titles of the articles (Figure 1). Finally we found 8 relevant papers based on our inclusion criteria (similar protocols including similar criteria for their PCOS and control groups). In order to perform this meta-analysis, we used comprehensive meta-analysis version 2 software (Biostat, US). The analysis was done through the P value and sample size of each study using fixed-effect model. Analyses were performed in 5 different groups of allele and genotype comparisons (Table 1). This meta-analysis

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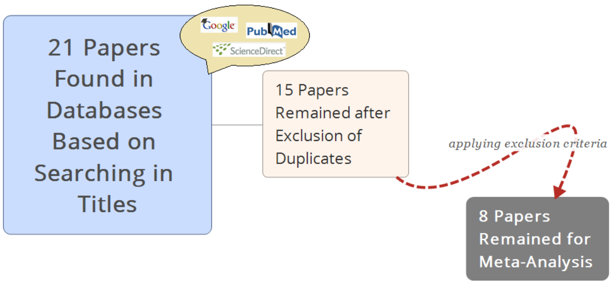


Figure 1. Searching Algorithm of Relevant Articles.

covers information of totally 3046 individuals of both PCOS and control groups. Since the *P* values had been calculated with Yate correction (or Fisher exact test if necessary), the odds ratios (ORs) achieved from these *P* values are under-estimated.

In order to recognize publication bias, we used funnel plots (12). Being in the funnel for each study was considered as homogeneity. In cases of heterogeneity, we corrected manually the weights of the heterogenic studies instead of using random-effect model. This correction (weight decreasing) was through increasing the standard error of having bias studies. In such cases we used the effect size instruction "log OR and standard error" instead of " *P* value and sample size." Of course the mentioned ORs are achieved from the P values; because conventional calculation of OR, makes the effect sizes over-estimated in comparison to using P values with Yate's correction. For multiple comparisons of the results of the mentioned 5 groups, Benjamini-Hochberg correction was used.

Results

Among the 8 studies imported in the meta-analysis, 6 of them had been covered by Jia et al meta-analysis (13-18). Analyses were performed in 5 different groups of alleles and genotypes including *D* vs *I* (Figures 2, 3), *DD* vs *II* (Figures 4, 5), *DD+DI* vs *II* (Figures 6, 7), *DD* vs *DI+II* (Figures 8, 9), and *DI* vs *DD+II* (Figures 10, 11). Among these 5 analyses, 4 of them were statistically significant (Table 1). After applying the multiple comparison correction, they still remained significant. The publication bias and their weight corrections are pointed out in the funnel plots (Figures 2A, 4A, 4B, 6A, 6B, 8A, 8B, 10A, and 10B). The meta-analysis results are shown in the forest plots (Figures 2B, 4C, 6C, 8C and 10C). Impact of ethnicities and sample sizes on the results are shown as meta-regressions (Figures 3, 5, 7, 9 and 11).

As we said, the previous meta-analysis found no significant correlation between *ACE* genotypes and PCOS. This could be due to their smaller size of total population and using random-effect model. As we checked in our funnel plots, using the random-effect model was not able to correct the heterogeneities of the studies. Therefore, we used fixed-effect model instead, and in order to correct the heterogeneities we decreased the weights of the studies having publication bias. Our statistical aim for this homogenizing was to move the co-ordination of such studies from outside to inside of the funnel in funnel plots.

For the analysis *D* vs *I*, firstly we found a significant correlation (Table 1), but after applying the needed weight correction (Figure 1A), this significance correlation did not remain (Table 1). Its justification could be the protecting

Table 1. Data Summery and *P* values of the Imported Studies

Study	Population (Ethnicity)	*D* vs *I*	*DD* vs *II*	*DD+DI* vs *II*	*DD* vs *DI+II*	*DI* vs *DD+II*
Sun et al (13)	249 (Asian)	0.4884 (-)	0.2654 (-)	0.3994 (+)	0.3078 (-)	0.8875 (+)
Sun et al (16)	582 (Asian)	0.3482 (+)	0.1167 (+)	0.2222 (+)	0.1703 (+)	0.9203 (-)
Karabulut et al (15)	63 (Caucasian)	0.4463 (-)	1 (+)	1 (-)	0.2418 (+)	0.1809 (-)
Celik et al (14)	63 (Caucasian)	0.3994 (+)	0.4054 (+)	1 (+)	0.0457 (+) *	0.0584 (-)
Bayram et al (17)	200 (Caucasian)	0.0344 (+) *	0.0239 (+) *	0.4976 (+)	0.0001 (+) *	0.0011 (-)*
Koika et al (18)	1067 (Caucasian)	0.8414 (+)	0.1502 (+)	0.0333 (+) *	0.6315 (-)	0.0488 (+) *
Deepika et al (19)	574 (Indian)	0.8230 (+)	0.8414 (-)	0.0864 (-)	0.0609 (+)	0.0010 (-) *
Ożegowska et al (20)	248 (Caucasian)	0.0002 (+) *	0.0001 (+) *	0.0001 (+) *	0.0001 (+) *	0.3348 (-)
Meta-analysis	***P* value before homogenizing**	0.046 (+) *	0.004 (+) *	0.011 (+) *	0.001 * (+)	0.077 (-)
	***P* value after homogenizing**	0.171 (+)	0.022 (+) * OR =1.169	0.013 (+) * OR =1.195	0.033 (+) * OR =1.160	0.009 (-) ** OR =0.816
Multiple comparison	**Corrected *P* value**	0.171 (+)	0.036 (+) *	0.032 (+) *	0.041 (+) *	0.045 (-) *
Homogenizing	**Corrected-weight study**	Ożegowska	Ożegowska	Ożegowska and Deepika	Ożegowska and Bayram	Koika and Bayram
	Previous/corrected standard error	0.239/0.4	0.239/0.37	0.239/0.37 0.152/0.22	0.239/0.38 0.269/0.44	0.111/0.20 0.269/0.35

Abbreviation: OR, odds ratio.
Note: (+) shows risk factor and (-) shows protecting factor.
* Significance level is at 0.05.

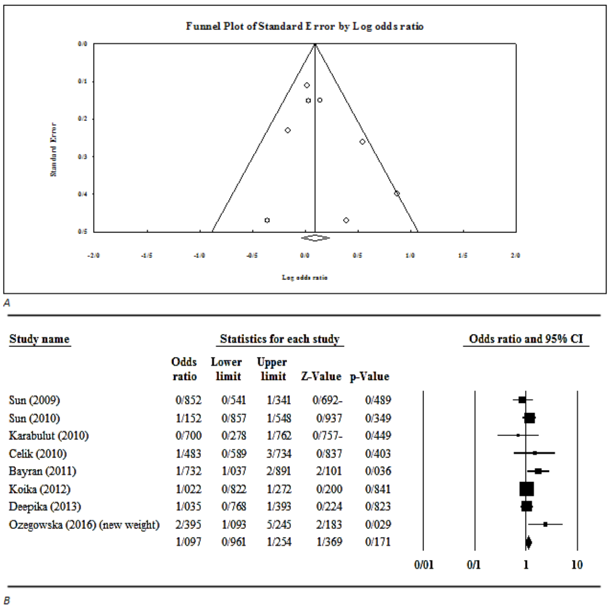


Figure 2. (A) Funnel plot for *D* vs *I* after homogenizing. (B) Forest plot for *D* vs *I*.

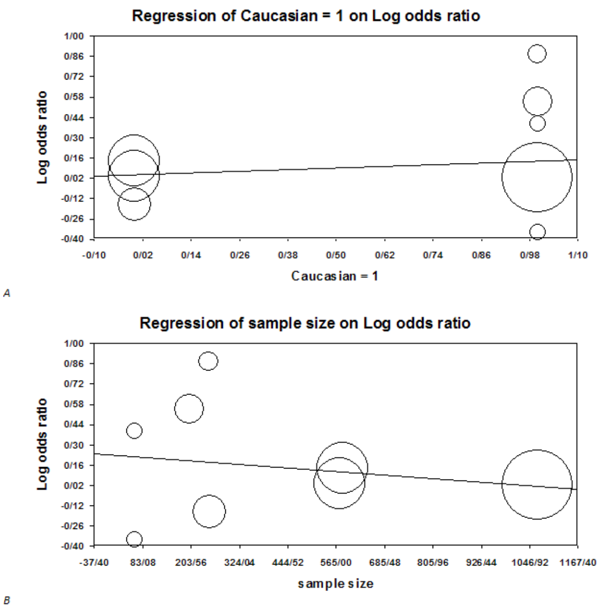


Figure 3. (A) Meta-regression of *D* vs *I* for ethnicities of the studied populations. (B) Meta-regression of *D* vs *I* for sample size of the studied populations.

effect of *DI* genotype in spite of the negative effect of *DD* genotype on PCOS. For the analysis *DD* vs *II*, after weight correction (Figure 4B), the risk factor role of *DD* genotype, remained significant (Table 1). Similarly, for the analysis *DD+DI* vs *II*, after weight correction (Figure 6B), the risk factor role of *DD* genotype remained significant (Table 1). Its justification could be the protecting effect of *II* genotype in spite of the protecting effect of *DI* genotype on PCOS. For the analysis *DD* vs *DI+II*, after weight correction (Figure 8B), the risk factor role of *DD* genotype remained

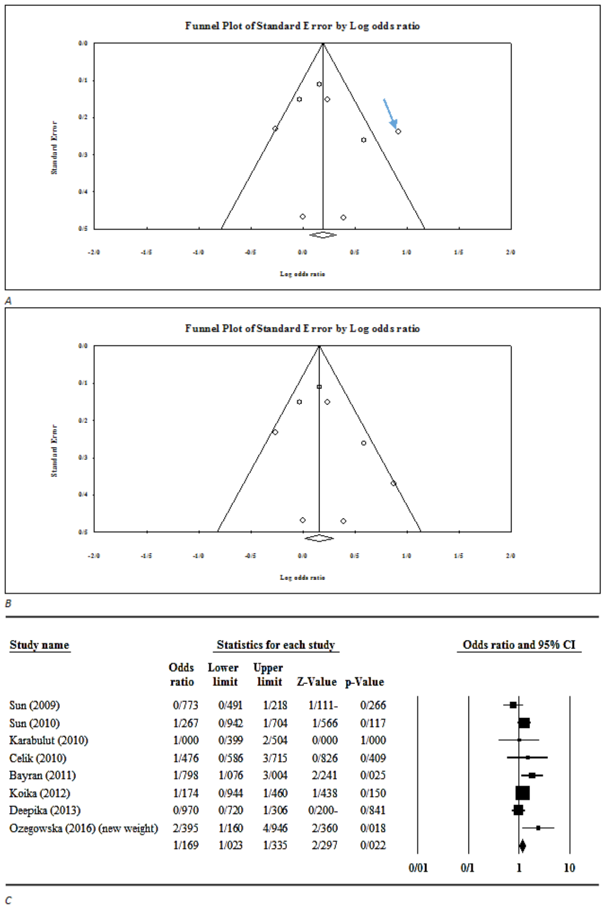


Figure 4. (A) Funnel plot for *DD* vs *II*. One publication bias has been found. B) Funnel plot for DD vs II after homogenizing. (C) Forest plot for *DD* vs *II* (*P = 0.022).

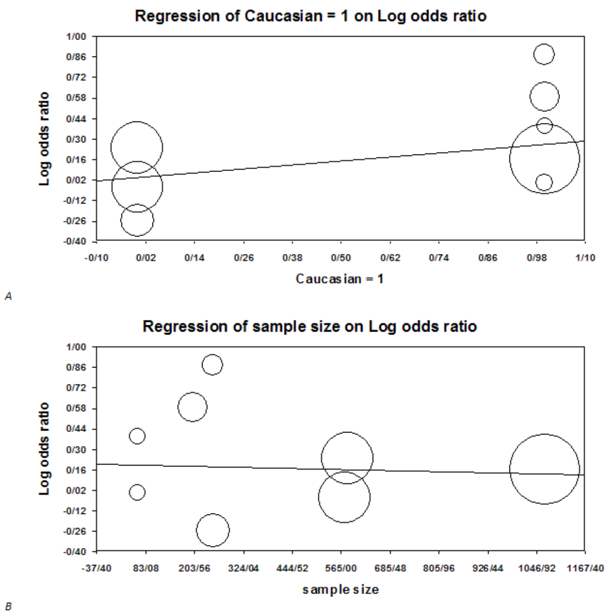


Figure 5. (A) Meta-regression of *DD* vs *II* for ethnicities of the studied populations. (B) Meta-regression of *DD* vs *II* for sample size of the studied populations.

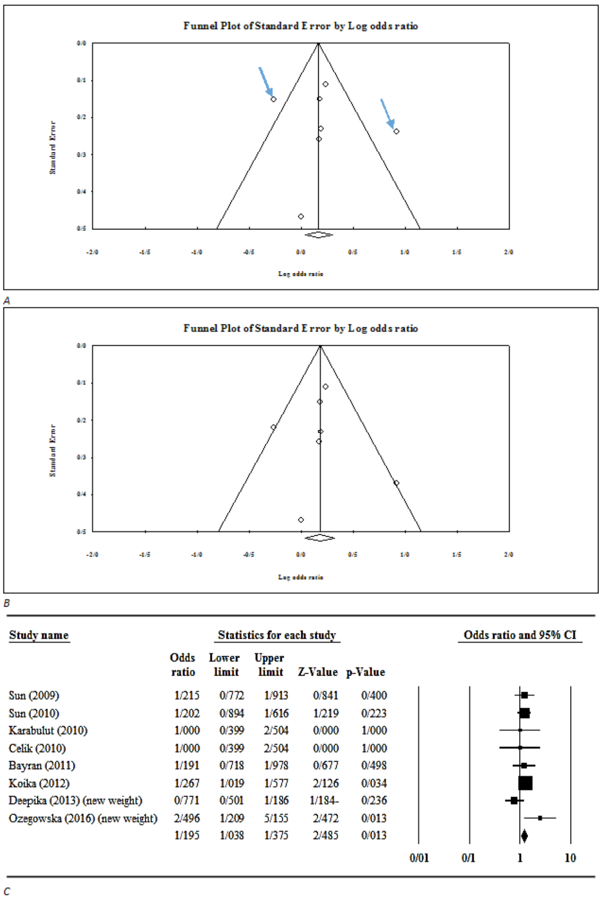


Figure 6. (A) Funnel plot for *DD+DI* vs *II*. Two publication bias analyses have been found. (B) Funnel plot for DD+DI vs II after homogenizing. (C) Forest plot for *DD+DI* vs *II*. (**P* = 0.013)

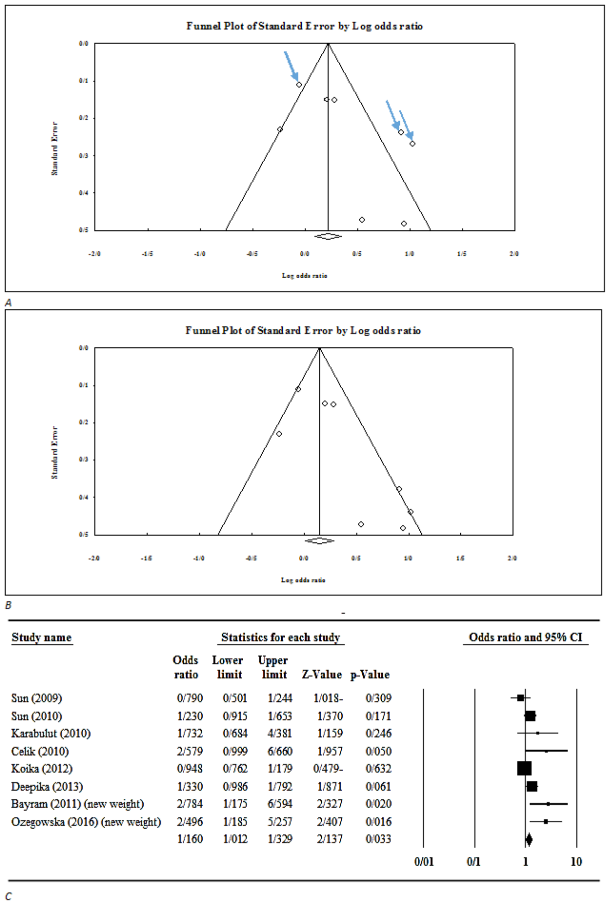


Figure 8. (A) Funnel plot for DD vs DI+II. Three publication bias have been found. Weight correction on 2 of them should be performed. (B) Funnel plot for *DD* vs *DI+II* after homogenizing. (C) Forest plot for *DD* vs *DI+II* (**P* = 0.033)

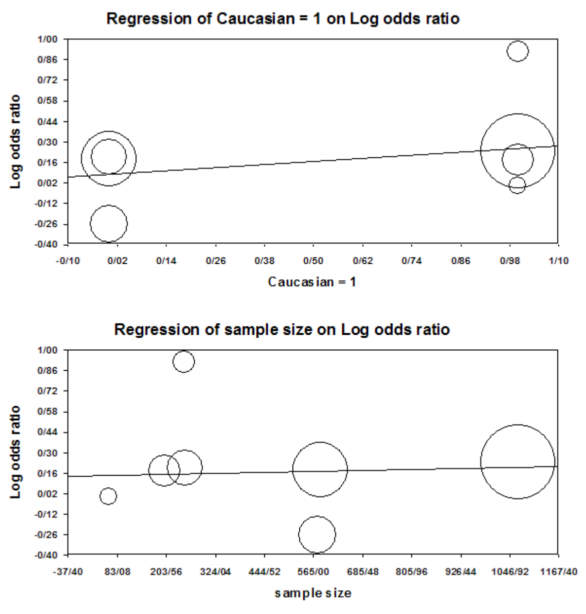


Figure 7. (A) Funnel plot for *DD+DI* vs *II*. Two publication bias analyses have been found. (B) Funnel plot for DD+DI vs II after homogenizing. (C) Forest plot for *DD+DI* vs *II*. (**P* = 0.013)

significant (Table 1). For the analysis *DI* vs *DD+II*, after weight correction (Figure 10B), the protecting role of *DI* genotype remained significant (Table 1).

The meta-regressions show that these roles of *ACE* polymorphism in PCOS are not affected by Caucasian race (Figures 3A, 5A, 7A, 9A and 11A). In meta-regressions of the study sample sizes, it is observed that the risk factor role of *DD* genotype decreases in larger populations (Figure 9B), and also the protecting role of *DI* genotype decreases in larger populations (Figure 11B). Of course these plots are based on our weight-corrected model.

Discussion

Role of genetic polymorphisms in pathogenesis of PCOS has been previously described. For instance, Panda et al have shown in a systematic review that up to now 43 different types of proteins are involved in pathogenesis of PCOS (ACE protein was not among them). Most of them were insulin related genes and proteins (21). The exact molecular pathogenesis of PCOS is still unclear. Infertility is one of the complications of PCOS (4). In such patients, controlled ovarian stimulation could be used (22), although ovarian hyper-stimulation has its own problems (23,24). For this reason, spontaneous abortion is higher

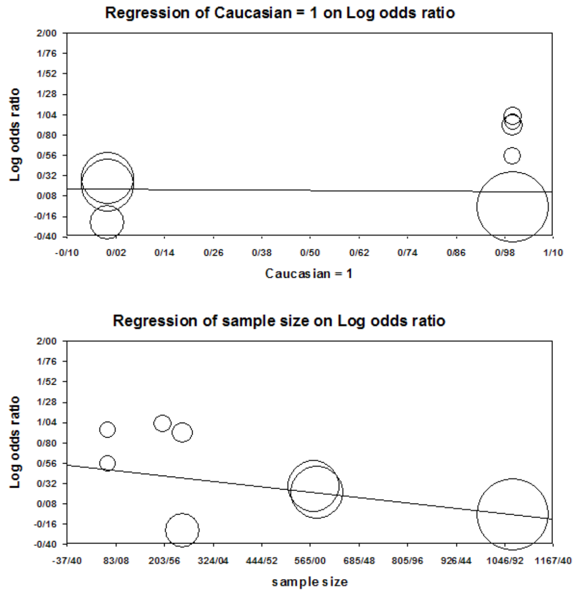


Figure 9. (A) Meta-regression of *DD* vs *DI+II* for ethnicities of the studied populations. B) Meta-regression of *DD* vs *DI+II* for sample size of the studied populations (*Slope P = 0.012)

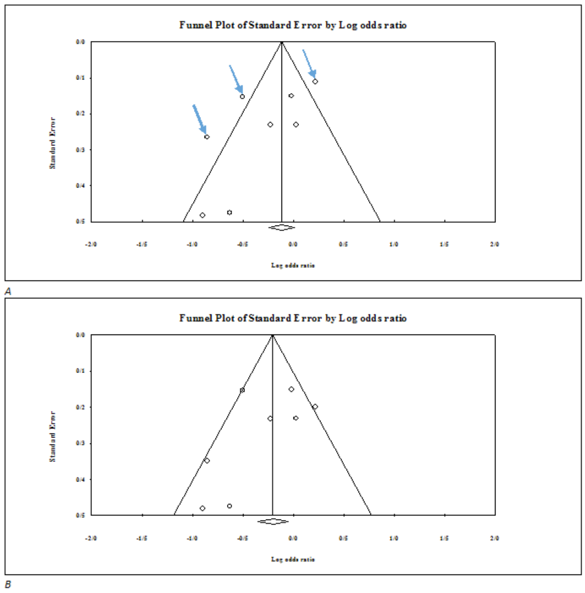
in PCOS patients even after using assisted reproductive technologies (4).

As described by Cheng et al, paternal history of diabetes mellitus and hypertension can increase the risk of PCOS (25). Another study believes that familial history of obesity, diabetes mellitus and hypertension increase the risk of PCOS (26). Since familial hypertension seems to be an angiotensin related phenotype (27), *ACE* gene polymorphism might be effective in the incidence and severity of PCOS. Of course this estimation is based on the renal RAS.

It seems and is hypothesized that both renal and ovarian RAS might be involved in physiopathology of PCOS. Ovarian RAS is involved in ovulation process whereas renal RAS is involved in blood pressure and hemodynamic changes. The role of hyper- and hypo-activity of RAS may be paradoxically different. Biochemistry wise, aldosterone as the outcome of RAS, is a part of cholesterol related cycles of metabolism. Hence the hyperlipidemia, insulin resistance and hyperandrogenism occurred in PCOS will not be unfamiliar to renal RAS. Therefor statins (28) and spironolactone (29) can be used for treatment of PCOS because they directly and indirectly related to the above-mentioned mechanisms.

Conclusion

It is concluded that the hypertension related gene *ACE* is associated with PCOS. Although this association is statistically significant, the ORs are not distant enough from one to show a highly effective role. Since the physiological activity of *DI* genotype is between *DD* and *II*, it seems that both hyper- and hypo-activity of *ACE* gene could be harmful for PCOS (but more of hyper-activity) as a scientific model. Hence it is suggested to use



Study name	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value
Sun (2009)	1/033	0/657	1/626	0/141	0/888
Sun (2010)	0/985	0/733	1/324	0/100-	0/920
Karabulut (2010)	0/533	0/210	1/354	1/323-	0/186
Celik (2010)	0/408	0/159	1/051	1/857-	0/063
Deepika (2013)	0/606	0/448	0/818	3/274-	0/001
Ozegowska (2016)	0/800	0/507	1/261	0/963-	0/336
Bayram (2011) (new weight)	0/426	0/214	0/845	2/440-	0/015
Koika (2012) (new weight)	1/245	0/841	1/843	1/096	0/273
	0/816	0/701	0/950	2/616-	0/009

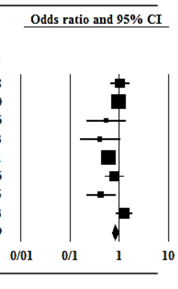


Figure 10. (A) Funnel plot for DI vs DD+II. Three publication bias analyses have been found. Weight correction on two of them should be performed. B) Funnel plot for *DI* vs *DD+II* after homogenizing. (C) Forest plot for *DI* vs *DD+II* (**P = 0.009)

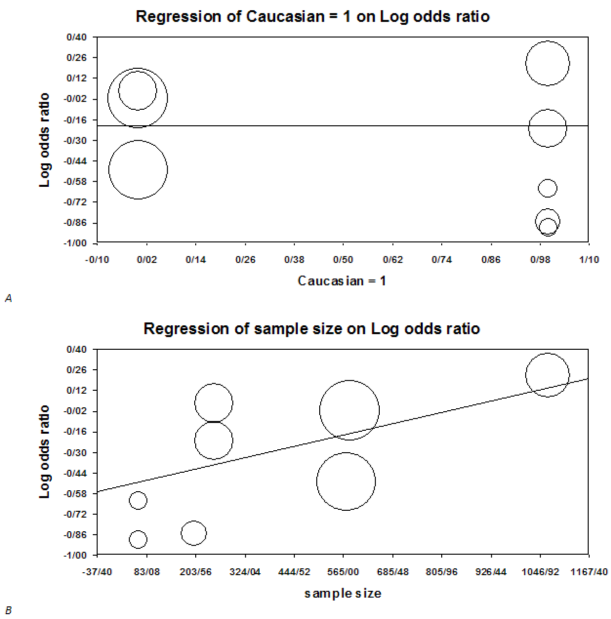


Figure 11. (A) Meta-regression of *DI* vs *DD+II* for ethnicities of the studied populations. B) Meta-regression of *DI* vs *DD+II* for sample size of the studied populations (*Slope P = 0.020)

a very low dose of captopril in the PCOS patients having *DD* genotype in future as clinical trials. This dose should be lower than usual because of the risk of hyperkalemia. Of course captopril will be just a scientific model for ACE inhibition, not a secondary usage for this medication. Further investigation on ovary ACE system is needed.

Ethical Issues
Not applicable

Conflict of Interests
Authors declare that they have no conflict of interest.

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