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MASTER THESIS

Assess the Genetic Association Studies for the variant TNF (rs1800629) in preeclampsia using the STREGA statement

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ΜΕΤΑΠΤΥΧΙΑΚΗ ΔΙΠΛΩΜΑΤΙΚΗ ΕΡΓΑΣΙΑ

Αξιολόγηση Γενετικών Μελετών για την ποικιλία TNF (rs1800629) στην προεκλαμψία χρησιμοποιώντας το STREGA statement

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Abstract

Background/Aims

Preeclampsia is a serious disorder of human pregnancy. There is a hypothesis that Tumor Necrosis Factor and preeclampsia are associated. The aim of this systematic review and meta-analysis is to assess the genetic association studies for the variant TNF (rs1800629) in preeclampsia.

Methods

Articles related with genetic studies for TNFa -308 genotype in preeclampsia were chosen from Medline, EMBASE, Scopus, Web of Science and Google Scholar. The search was limited to the last decade, ie from 2006 onwards. The quality of the studies was evaluated using the STREGA statement. Meta-analysis was performed by using a random effect model.

Results

Finally, eleven studies were included for meta-analysis. Only one reported a statistically significant increased risk based on rs1800629. The pooled dataset was 1158 cases and 1336 controls. Meta-analysis of these data showed no significant association between TNFa -308 genotype and the risk of preeclampsia.

Conclusion

Meta-analysis of available articles documented no statistically significant association between tumor necrosis factor-a -308 polymorphism and preeclampsia.

Introduction

Pregnancy can co-exist with a variety of complications and diseases. One serious complication is preeclampsia, formerly called “toxemia of pregnancy”. It is an hypertensive disorder specific to pregnancy. Preeclampsia may develop into the more severe condition eclampsia. It is dangerous for the expectant and the unborn and responsible for maternal and infant deaths. It affects 2 to 8% of all pregnancies worldwide [12].

Principal features of preeclampsia are hypertension (a sustained diastolic blood pressure of 90 mm Hg or higher or a sustained systolic blood pressure of 140 mm Hg or higher) and proteinuria with or without edema. High blood pressure is dangerous because it may interfere with the placenta's ability to deliver oxygen and nutrition to the fetus.

Any pregnant women can get preeclampsia but it is primarily a disease of the first pregnancy. Furthermore, women who are over age 35 or under 20 have a higher risk of developing preeclampsia. This disease is more common to women with low standards of living. Other risk factors are multiple pregnancy, diabetes, high blood pressure, kidney disease, hydramnios, trophoblastic disease, gestation with down syndrome and smoking. Moreover, women with preexisting hypertension have twice the risk of getting preeclampsia [14].

The etiology of preeclampsia is still unclear. However, it is sure that the daughters of women who had preeclampsia during pregnancy have more risk of preeclampsia themselves compared with other women [1].

Many genetic studies were conducted to determine the pathophysiology of preeclampsia. The Tumor Necrosis Factor gene is a putative genetic risk factor for this disease.

The tumor necrosis factor (TNF) superfamily refers to a group of cytokines that can cause cell death. Cell death is the programmed cell death, which is a normal process by which the body controls the number of cells. TNFα or TNF alpha, is the best known member of this class. TNF alpha can be produced by many cell types such as CD4+ lymphocytes, NK cells, neutrophils, mast cells, eosinophils, neurons or by activated macrophages, and is a cell signaling protein involved in systemic inflammation and is one of the cytokines that make up the acute phase reaction.

Its role is in the regulation of immune cells. TNF, being an endogenous pyrogen, is able to induce fever, apoptotic cell death, cachexia, inflammation and to inhibit tumorigenesis and viral replication and responds to sepsis via IL1 &IL6 producing cells. Dysregulation of TNF

production has been implicated in a variety of human diseases including Alzheimer's disease, cancer, major depression, Psoriasis and inflammatory bowel disease (IBD). While still controversial, studies of depression and IBD are currently being linked to TNF levels. Recombinant TNF is used as an immunostimulant under the INN tasonermin. TNF can be produced ectopically in the setting of malignancy and parallels parathyroid hormone both in causing secondary hypercalcemia and in the cancers with which excessive production is associated [11].

As mentioned above, one more human disease that TNF is implicated is preeclampsia. One studied variant in the TNF gene is the guanine to adenine transition at position -308 nucleotides. The findings for the association of the TNF polymorphism with preeclampsia are conflicting. A systematic review and meta-analysis of data from genetic studies may give more precise estimates of effect sizes.

Methodology

Literature Search

A systematic literature search was conducted in the MEDLINE (PubMed), Embase, Scopus, Web of Science and Google Scholar databases using a combination of the following keywords: preeclampsia, TNF, TNF- a Tumor necrosis factor, rs1800629 and TNF- 308. The search was limited between January 2006 and August 2016. The language of publications was only in English. Selection of these databases allowed us to search articles from US and European journals. Moreover, the reference section of these articles was reviewed manually to find additional published studies.

Study Eligibility Criteria

Studies which assessed the association of the TNF (-308G/A) polymorphism with preeclampsia were eligible for inclusion if:

1. Preeclampsia was defined as the onset of hypertension with or without proteinuria. Hypertension was defined as systolic blood pressure ≥140 mmHg and/or ≥90 mmHg diastolic pressure blood. Proteinuria was defined as the excretion of urine protein ≥300 mg in a 24-hour.
2. The sample was women.
3. Genetic data were presented.
4. Their full texts were available.
5. These were published in the English language.

Studies were excluded if:

1. These were not related to TNF-308G/A (rs1800629) in preeclampsia.
2. These were review articles, abstracts, posters of a conference.
3. Data were unclear.

Data Extraction

From each paper information regarding was extracted the first author's name, the sample size, the study design, the study location and outcomes (Table 2).

We did not exclude any low quality papers because we wanted a complete understanding of all relative studies.

Quality Assessment

The quality of selected case-control studies assessed using the Strengthening the Reporting of Genetic Association Studies (STREGA) statement [9]. STREGA statement consists of 22 items. Specifically, the 22-item quality criteria were (Table 1): (1) indication of study design and provision of an informative and balanced summary, (2) background rationale, (3) clear statement of objectives and hypothesis (4) study design, (5) setting (location and relevant dates), (6) eligibility criteria for study participants, (7) definition of all variables, (8) sources of data/ details of methods of measurement, laboratory methods (9) efforts to address potential sources of bias, (10) study size, (11) how quantitative variables were handled in the analyses, (12) description of statistical methods, sensitivity analyses, statement of Hardy-Weinberg equilibrium, (13) report of participants, (14) sufficient descriptive data, (15) report numbers and outcomes for each genotype category, (16) report main results, (17) other analyses, (18) summarize key results with reference to study, (19) report of limitations, (20) an overall interpretation, (21) a discussion of generalizability of the study results and (22) the source of funding.

Statistical Methods

Genotype data were analyzed by merging homozygous for wild type allele versus mutant type allele-carriers (GG vs GA+AA). Review Manager 5.3.5 (Cochrane Collaboration, Oxford, United Kingdom) used for data analysis. The heterogeneity tested with Q statistic and it was used Random effect model. Also the Mantel- Haenszel method was adopted with pooled OR. The results are presented in a standard plot and 95% confidence interval.

To check for the existence of publication bias it was used a funnel plot.

Results

Characteristics of Included Studies

Ten articles were found reporting eleven case-control studies [2-8, 10, 12, 13]. A total of 1158 cases with preeclampsia and 1336 controls participated in the study. The studies provided sufficient data and had good methodological quality, according to STREGA statement [9] (Table 1). Almost all of them were performed Hardy – Weinberg equilibrium test.

Most of studies were based in Slovakia, Turkey, Austria and Hungary. The participants were of Caucasian ethnic ancestry [6-8, 10, 13]. Three studies were in Brazil and the participants were Caucasian [3] in one and Mulatto/Black in the other two [3, 4]. In a study from Mexico, participants were Maya – Mestizo ethnicity [2], whereas in studies from China [12] and Iran [5], participants were Chinese and Iranian, respectively.

Cases were women who fulfilled the criteria of preeclampsia. They had systolic blood pressure ≥140mm Hg and/or diastolic blood pressure ≥90mm Hg with proteinuria at least 300mg in a 24-hour urine specimen after 20 weeks' gestation. Controls were women with no history of preeclampsia, natural blood pressure and their gestation was without complications.

The basic characteristics of each study are presented in Table 2.

Quality Assessment

The quality of the studies was evaluated by applying the 22-item checklist of STREGA statement. All the included studies have an enlightening abstract which indicate the study design. Furthermore, their introductions declare the scientific background and the objectives of the genetic studies.

In the section of methods study design, study size, location, relevant dates and data collection (setting) are described. All included studies describe clearly the eligibility criteria for all participants, sources of data, laboratory methods, quantitative variables like age and statistical methods. Quantitative variables are used as criteria of inclusion in the studies. None of the studies did not describe potential sources of bias clearly and sensitivity analyses. Seven studies mention that the Hardy-Weinberg equilibrium test was performed [2-4, 6, 10, 12, 13]. In the part of methods the definition of potential confounders, effect modifiers and genetic exposures are unclear.

Results of all studies contain data for participants and their characteristics like age, body mass index, systolic and diastolic blood pressure or other environmental factors like smoking. They mention numbers in each genotype category. Main results and other analyses are reported. Some of them did meta-analyses [3, 6, 7], one subgroup analysis [13] and the rest examined numerous genetic variants.

In the end, in the part of discussion all articles summarize the basic results and give an overall interpretation of results. Only half of them discuss limitations for external validity and almost all give the source of funding.

Quality assessment of each study is presented in Table 1.

Genotype Association

A meta-analysis for investigating the association between preeclampsia and the TNF (wt=G and mt=A) gene polymorphism produced significant heterogeneity ($p=0.007<0.05$).

All the included studies did not find a statistical significant association between TNF -308G except for one [5].

No statistically significant association was found between TNF (rs1800629) and preeclampsia (pooled OR=1.01, 95% CI (0.69, 1.49), p=0.96) (Figure 1).

Publication Bias

An asymmetry in funnel plot indicates the existence of publication bias (Figure 2).

Conclusions

The purpose of this meta-analysis was to evaluate the association of TNF -308G/A gene polymorphism with a disease of pregnant women: preeclampsia. Eleven case-control studies were included and the search for relative articles was limited from 2006 until today. The result shows no association between the variant TNF (rs1800629) and the risk of developing preeclampsia.

Although data were not from different study designs, heterogeneity was found with I^2 = 59%. Possible reasons are the definition of phenotype, genotyping misclassification, selection of sample, confounded by ethnic origin and multiple testing.

The development of preeclampsia may be associated with genetic factors but should take into account other environmental factors. Environmental factors like fumes or environmental toxins and diet, before and during pregnancy, might play a role in the development of preeclampsia.

Because of these findings we cannot eliminate the possibility that TNF plays a role in the developing of preeclampsia. More genetic studies in larger populations, taking into consideration genetic, environmental and alimentary factors, will give an overall image and more precisely results, minimizing possible bias.

Annex

Table 1 - Quality Assessment Studies of TNF (rs1800629) in preeclampsia

Study	Title and Abstract		Introduction		Methods												
	1.(a)Study design indicated	1.(b)Informative and balanced summary	2.Background Rationale	3.Objectives and hypothesis clearly stated	4.Study design	5.Setting	6.Eligibility criteria for all participants	7.Definition of all variables	8. (a.)Data sources	8.(b)Laboratory methods	9.Bias	10.Study size	11.Quantitive variable	12.(a).Statistical methods	12.(b)Sensitivity analyses	12.(c)Hardy-Weinberg equilibrium stated
Daher et al, 2006	+	+	+	+	+	+	+	unclear	+	+	-	+	+	+	-	+
Pazarbasi et al, 2007	+	+	+	+	+	+	+	unclear	+	+	-	+	+	+	-	-
Canto-Centina et al, 2007	+	+	+	+	+	+	+	unclear	+	+	-	+	+	+	-	+
Molvarec et al, 2008	+	+	+	+	+	+	+	unclear	+	+	-	+	+	+	-	+
Stonek et al, 2008	+	+	+	+	+	+	+	unclear	+	+	-	+	+	+	-	-
Mirahmadian et al, 2008	+	+	+	+	+	+	+	unclear	+	+	-	+	+	+	-	-
De Lima et al, 2009	+	+	+	+	+	+	+	unclear	+	+	-	+	+	+	-	+
Vural et al, 2010	+	+	+	+	+	+	+	unclear	+	+	-	+	+	+	-	+
Zubor et al 2014	+	+	+	+	+	+	+	unclear	+	+	-	+	+	+	-	+
Zhou et al, 2016	+	+	+	+	+	+	+	unclear	+	+	-	+	+	+	-	+

Table 1 - Quality Assessment Studies of TNF (rs1800629) in preeclampsia

Study	Results					Discussion				Other information
	13.Participants	14.Sufficient descriptive data	15.Outcome data	16.Main results	17.Other analyses	18.Key results	19.Limitations	20.Interpretation	21.Generalizability	22.Funding
Daher et al, 2006	+	-	+	+	+	+	-	+	+	+
Pazarbasi et al, 2007	+	+	+	+	+	+	+	+	+	-
Canto-Centina et al, 2007	+	+	+	+	+	+	-	+	+	+
Molvarec et al, 2008	+	+	+	+	+	+	+	+	-	+
Stonek et al, 2008	+	-	+	+	-	+	-	+	-	-
Mirahmadian et al, 2008	+	+	+	+	-	+	-	+	-	+
De Lima et al, 2009	+	+	+	+	-	+	+	+	+	+
Vural et al, 2010	+	+	+	+	-	+	-	+	+	+
Zubor et al 2014	-	+	+	+	+	+	-	+	-	+
Zhou et al, 2016	+	+	+	+	+	+	+	+	+	+

Table 2 - Characteristics of included studies

Reference	Setting	Ethnicity	Case definition	Controls
Daher et al, 2006 (a)	Brazil	Caucasian	SBP≥140 and/or DBP≥90 on two occasions with persistent urine protein ≥ 300 mg/24 h, n = 56	Normotensive primigravidae women, n = 92)
Daher et al, 2006 (b)	Brazil	Mulatto & Black	SBP≥140 and/or DBP≥90 on two occasions with persistent urine protein ≥ 300 mg/24 h, n = 94	Normotensive primigravidae women, n = 97
Pazarbasi et al, 2007	Turkey	Caucasian	SBP≥140 and/or DBP≥90 and new onset proteinuria>300 mg/24 h, n = 152	Normotensive primigravidae women, n = 80
Canto-Centina et al, 2007	Mexico	Maya-Mestizo	SBP≥140 and/or DBP≥90 on two occasions at least 24 h apart with persistent urine protein ≥ 300 mg/24 h, n = 105	Normotensive pregnant women, n = 200
Molvarec et al, 2008	Hungary	Caucasian	SBP ≥ 140 & DBP≥90 with proteinuria ≥ 300 mg/24 h, n = 209 (69 cases had HELLP syndrome)	Normotensive pregnant women, n = 144
Stonek et al, 2008	Austria	Caucasian	SBP ≥ 160 or DBP ≥ 110 on two occasions >4 h apart with new onset proteinuria ≥ 300 mg/24 h, n = 107	Normotensive pregnant women, n = 107
Mirahmadian et al, 2008	Iran	Iranian	SBP≥140 mmHg or DBP≥90 mmHg on two occasions 6 hr with proteinuria ≥ 300 mg/24 h, n = 160	Normotensive pregnant women, n = 100
de Lima et al 2009	Brazil	Mulatto(white and black)	SBP≥140 and/or DBP≥90 and new onset proteinuria>300 mg/24 h, n = 88	Normotensive pregnant women with a history of at least one previous normal pregnancy, n = 97
Vural et al 2010	Turkey	Caucasian	SBP ≥ 140 & DBP ≥ 90 on two occasions 6 h with new onset proteinuria ≥ 300 mg/24 h, n = 101	Non pregnant healthy women, n = 95
Zubor et al 2014	Slovakia	Caucasian	SBP ≥ 140 & DBP ≥ 90 on two occasions 6 h with new onset proteinuria ≥ 300 mg/24 h, n = 38	Normotensive primigravid women, n = 38
Zhou et al, 2016	China	Chinese	SBP ≥ 140 & DBP ≥ 90 and/or proteinuria ≥ 300 mg/24 h or a positive urine dipstick test at 2+without urinary infection, n = 117	Normotensive primigravidae women, n = 286

SBP: systolic blood pressure; DBP: diastolic blood pressure; HELLP: haemolysis, elevated liver enzymes, low platelets

Figure 1

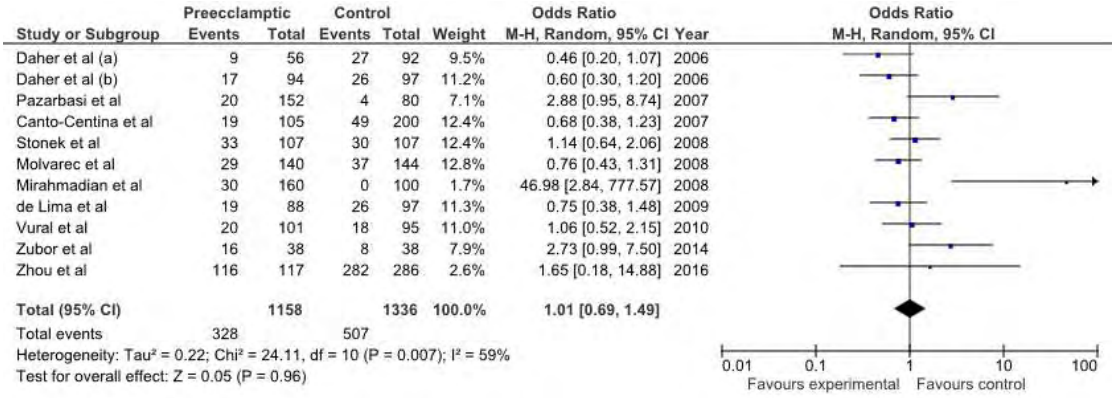
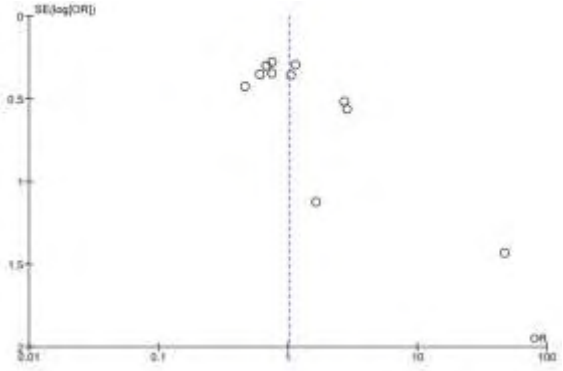


Figure 2 - Funnel plot



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