### ARTICLE DETAILS

**TITLE (PROVISIONAL)**
ICAM-1 K469E polymorphism is a genetic determinant for the clinical risk factors of T2D subjects with retinopathy in Indians: a population based case control study

**AUTHORS**
Sripriya, Sarangapani; Vinita, Kumari; Prathiba, Krishnamurthy; Vaitheeswaran, Kulothungan; Sathyabaarathi, Ravichandran; Rajesh, Mahendra; Amali, John; Umashankar, Vetrivel; Kumaramanickavel, Govindasamy; Pal, Swakshyar Saumya; Ramän, Rajiv; Sharma, Tarun

### VERSION 1 - REVIEW

**REVIEWER**
Full Professor Daniel Petrovic, MD, PhD
Medical faculty, University of Ljubljana
Slovenia
There are no competing interests

**REVIEW RETURNED**
05-May-2012

**GENERAL COMMENTS**
The revision is appropriate.

**REVIEWER**
Jun Ma Professor, PhD & MD
Department of Pain Management
Xuanwu Hospital
Capital Medical University
No.45 Changchun Street, Xicheng District,
Beijing, P.R.China 100053
Competing Interests: None

**REVIEW RETURNED**
17-May-2012

**GENERAL COMMENTS**
This manuscript by Kumari Vinita, et al reports the association of K469E (rs5498) polymorphism in ICAM-1 gene and T2D patients with retinopathy in south Indian population. It shows that ICAM-1 K469E polymorphism is a genetic determinant for the clinical risk factors of T2D subjects with retinopathy, which is important for the potential clinical prognostication.

The study is a population based cohort study. It would be better if the sample size could be larger and analysed by DR subgroup, such as NPDR, PDR or CSME.

Additional comments
1. P. 12, line 4, “P<0.5” should be “P<0.05”. 
2. Reference 8 and 34 should include the volume and page.

**THE STUDY**

The manuscript has a component of the genetic factor as well as the clinical covariates. The results and discussion are based upon the logistic regression analysis. There are many points that need clarification and are as follows:

1. The title contains the key word Population based cohort study and the design in the abstract says as Case Control Study. This ambiguity needs to be clarified.

2. In the material and method section it has been specified that patients were recruited prospectively from SNDREAMS and OPD departments Sankara Nethralaya. It is quite obvious that the cases are from selected from the Hospital. Then what was the rationale behind including population based prospective cohort study in the title.

3. There is no mentioning of the time period during which these cases and controls were selected.

4. The major problem is the statistical approaches specifically the regression analysis performed. The study design has been mentioned as Case Control. But how the control were selected needs to be mentioned. Although some previous research work has been cited but then also inclusion and exclusion criteria for control selection needs to be mentioned for the completeness.

4. If the design is case control then why conditional logistic regression has not been performed with age and gender matched. The authors needs to perform the conditional logistic regression analysis and see what are the results. The conditional logistic analysis is not possible in SPSS, it can be done by STATA as it takes into account the unequal matching criteria. Even in the present study the 1:1 design is not there as number of controls are less than the number of cases, hence conditional logistic regression analysis is a must on account of unequal number of cases and controls.

5. The Hardy Weinberg Equilibrium Test should be performed for cases as well as the controls and the observed and expected frequencies for the alleles be mentioned accordingly with appropriate test of significance. Any departure from the Hardy Weinberg Equilibrium Test should be discussed.

6. Before running the conditional logistic regression analysis the authors needs to see the Bivariate correlation matrix and specifically see the presence or absence of multicollinearity and confounding. On the basis of the correlation matrix the authors should decide which variable to be included in the model and which should not be included along with the other variable on account of the
multicollinearity problem.

7. Running of the conditional logistic regression analysis holds for the table 3 and 4. Moreover the authors should cautiously see what effects what. Is the genotype effecting the phenotype (clinical factors) or vice versa.

8. At few places there are spellings mistakes. The authors should check for dependent or dependant. The authors should also see language for Odds of risk or Odds ratio for risk of DR+. The homogeneity for the interpretation and language throughout the manuscript should be maintained

9. One table depicting the results of the Genetic Model of Inheritance should be included which is the standard practice in such polymorphisms study. The table should specify the results of Dominant, Recessive and Co-dominant Genetic Models which are based upon the grouping of Wild Type, Heterozygous and Mutant type Genotypes.

10. The authors needs to mention the statistical power of the analyses performed in the table 3 and 4

11. In the conditional logistic regression analysis the P Value should be adjusted for the multiple comparison, Bonferroni or Sidak, and should be mentioned at the foot note of the respective table that which variables remained significant even after adjustment for the multiple comparison. At the foot note the exact calculation of the adjusted P value should be written.

RESULTS & CONCLUSIONS

The concurrence of the results in the discussions is based upon the cited references in Nephropathy. The concurrence should be based upon the Retinopathy too. Inclusion of reference on the Retinopathy would be a good idea for completeness.

VERSION 1 – AUTHOR RESPONSE

Reviewer: Full Professor Daniel Petrovic, MD, PhD
Medical faculty, University of Ljubljana
Slovenia

There are no competing interests.

Reviewer comments: The revision is appropriate.

Author’s reply: Thanks for reviewing our manuscript and the valuable comments to refine the same.

Reviewer: Jun Ma Professor, PhD & MD
Department of Pain Management
Xuanwu Hospital
Capital Medical University

Reviewer comments: This manuscript by Kumari Vinita, et al reports the association of K469E (rs5498) polymorphism in ICAM-1 gene and T2D patients with retinopathy in south Indian population. It shows that ICAM-1 K469E polymorphism is a genetic determinant for the clinical risk factors of T2D subjects with retinopathy, which is important for the potential clinical prognostication.
The study is a population based cohort study. It would be better if the sample size could be larger and analysed by DR subgroup, such as NPDR, PDR or CSME.

Competing Interests: None

Additional comments
Reviewer comments:
1. P. 12, line 4, “P<0.5” should be “P<0.05”.
2. Reference 8 and 34 should include the volume and page.

Author’s reply: Thanks for reviewing the article and your encouraging remarks. The changes as per the comments have now been incorporated in the manuscript.

Reviewer: ASHWANI KUMAR MISHRA
Assistant Professor (Biostatistics)
National Drug Dependence Treatment Centre (NDDTC)
All India Institute of Medical Sciences (A.I.I.M.S.)
Ansari Nagar, New Delhi-110029
INDIA

Reviewer comment: The manuscript has a component of the genetic factor as well as the clinical covariates. The results and discussion are based upon the logistic regression analysis. There are many point that needs clarification and are as follows:

Authors Reply: Thanks for reviewing and those valuable suggestions and comment. Please find below the response to your queries:

Query 1. The title contains the key word Population based cohort study and the design in the abstract says as Case Control Study. This ambiguity needs to be clarifies.

Reply 1. The study follows a case control study design. As rightly pointed sir, there is ambiguity in the title. The phrase “population based cohort study” is now being changed to “population based case-control study”

Query 2. In the material and method section it has been specified that patients were recruited prospectively from SNDREAMS and OPD departments Sankara Nethralaya. It is quite obvious that the cases are from selected from the Hospital. Then what was the rationale behind including population based prospective cohort study in the title.

Reply 2. Ours is a population based case-control study and includes patients from the SNDREAMS (an epidemiology study on the prevalence of DR in rural and urban chennai). Additionally, to increase the sample size for genetic study, we identified study subjects from the OPD of the hospital and recruited them for the SNDREAMS project. The subjects followed the same inclusion and exclusion criteria as that of the SNDREAMS project.

Query 3. There is no mentioning of the time period during which these cases and controls were selected.

Reply 3. The study subjects were enrolled between the years 2003-2010. These details are now mentioned in the methodology section of the manuscript.
Query 4. The major problem is the statistical approaches specifically the regression analysis performed. The study design has been mentioned as Case Control. But how the control were selected needs to be mentioned. Although some previous research work has been cited but then also inclusion and exclusion criteria for control selection needs to be mentioned for the completeness.

Reply 4. The inclusion and exclusion criteria for the control subjects were earlier mentioned in the sub heading “Sample collection” under the methodology section of the manuscript. It has now been further modified for clarity.

Query 5. If the design is case control then why conditional logistic regression has not been performed with age and gender matched. The authors needs to perform the conditional logistic regression analysis and see what are the results. The conditional logistic analysis is not possible in SPSS, it can be done by STATA as it takes into account the unequal matching criteria. Even in the present study the 1:1 design is not there as number of controls are less than the number of cases, hence conditional logistic regression analysis is a must on account of unequal number of cases and controls.

Reply 5. The study follows a prospective, unmatched, case control study design. Since conditional logistic regression analysis is applied for matched case control study design we have attempted only unconditional logistic regression for modeling.

Ref: Analysis of Genetic Association Studies; Gang Zheng, Yaning Yang, Xiaofeng Zhu, Robert C. Elston Single-Marker Analysis for Matched case control data (chapter 4); page 95.

Query 6. The Hardy Weinberg Equilibrium Test should be performed for cases as well as the controls and the observed and expected frequencies for the alleles be mentioned accordingly with appropriate test of significance. Any departure from the Hardy Weinberg Equilibrium Test should be discussed.

Reply 6. We did perform the HWE test and found that the results were significant. We had included this in the manuscript [page 10, line 1 – “The genotypes were in Hardy-Weinberg equilibrium (p>0.05)”]. As suggested by the reviewer, we now include them in detail in the text.

Query 7. Before running the conditional logistic regression analysis the authors needs to see the Bivariate correlation matrix and specifically see the presence or absence of multicollinearity and confounding. On the basis of the correlation matrix the authors should decide which variable to be included in the model and which should not be included along with the other variable on account of the multicollinearity problem.

Reply 7. Though unconditional logistic regression is the best fit for the unmatched case control study design followed in the current study, multicollinearity diagnostics has been done and adjustments had been made to the model accordingly.

Query 8. Running of the conditional logistic regression analysis holds for the table 3 and 4. Moreover the authors should cautiously see what effects what. Is the genotype effecting the phenotype (clinical factors) or vice versa.

Reply 8. Since we have followed an un-matched case control study design, conditional logistic regression has not been used for the analysis. To cautiously address the probable association of ICAM-1 genotype with the phenotype, we have sequentially adjusted the various clinical covariates that could probably confound the p value of significance.

Query 9. At few places there are spellings mistakes. The authors should check for dependent or dependant. The authors should also see language for Odds of risk or Odds ratio for risk of DR+. The homogeneity for the interpretation and language throughout the manuscript should be maintained
Reply 9. Thanks for the comments. We have now corrected these spelling mistakes in the manuscript.

Query 10. One table depicting the results of the Genetic Model of Inheritance should be included which is the standard practice in such polymorphisms study. The table should specify the results of Dominant, Recessive and Co-dominant Genetic Models which are based upon the grouping of Wild Type, Heterozygous and Mutant type Genotypes.

Reply 10. We have now included the results of dominant and recessive models in the Table 2 along with the co-dominant genetic models already performed in the submitted MS.

Query 11. The authors needs to mention the statistical power of the analyses performed in the table 3 and 4

Reply 11: Statistical power of the analysis was performed as suggested by the reviewer. It is observed that the overall accuracy of all models to predict subjects having DR resulted with the predictive probability (in %) range of 69.0 – 91.0 when tested against the hypothetical value of 0.5 (or 50%).

Query 12. In the conditional logistic regression analysis the P Value should be adjusted for the multiple comparison, Bonferroni or Sidak, and should be mentioned at the foot note of the respective table that which variables remained significant even after adjustment for the multiple comparison. At the foot note the exact calculation of the adjusted P value should be written.

Reply 12: Conditional logistic regression has not been used. Hence mentioned Bonferroni or Sidak adjustments have not been attempted.

Query 13. The concurrence of the results in the discussions is based upon the cited references in Nephropathy. The concurrence should be based upon the Retinopathy too. Inclusion of reference on the Retinopathy would be a good idea for completeness.

Reply 13. We do agree that the relevant references of DR need to be cited and discussed. Paragraph 2-4 of the discussion section of the original manuscript addresses the same with the citation of relevant references. Since, Diabetic nephropathy and retinopathy has common meet points, we have given additional information pertaining to nephropathy that strengthens the observations of the current study.

**VERSION 2 – REVIEW**

<table>
<thead>
<tr>
<th>REVIEWER</th>
<th>Mishra, Ashwani</th>
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<tr>
<td>REVIEW RETURNED</td>
<td>14-Jul-2012</td>
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**GENERAL COMMENTS**

The authors has replied to the query satisfactorily and hence this manuscript is acceptable.