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Journal Name:	Ophthalmology Research: An international Journal
Manuscript Number:	2014_OR_10623
Title of the Manuscript:	Association of the CASQ1 gene SNP rs3838216 with ophthalmopathy in patients with thyroid autoimmunity
Type of the Article	Original Research Article

General guideline for Peer Review process:

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound.

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PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
<u>Compulsory</u> REVISION comments	Comments to the author: The title of the article "Association of the CASQ1 gene SNP rs3838216 with ophthalmopathy in patients with thyroid autoimmunity" indicates that you are concentrating on ophthalmopathy. The aim mentioned in the abstract is directed to autoimmune thyroid diseases at large. Introduction: Page 1 line 24: Reference 8: put between brackets [8] Material and Methods: Clinical subjects: Table (1): You may include the age of the different groups (better as a range and median) in the text and remove from the table to make it less crowded. For the parameters put as mean±SD, put an * and explain in the foot note. Same for parameters mentioned as No (%), put ** and also explain in a footnote Methodology:	We are grateful to Reviewer 1 for comments. We agree that we are emphasising on ophthalmopathy, Please see our response to major comments below. Addressed in the manuscript. We thankfully followed reviewer 1 suggestion and corrected age group in manuscript accordingly. We are very grateful to reviewer 1 to



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	You mentioned that SNP rs3838216 [-ggcatt...] is an insertion or deletion. It can't be both. What is the situation in what is supposed to be the wild type? I understand from your data that it might be the absence of the sequence; if this is the case, then it's an insertion. You are using the expression of common Homozygous (both alleles are lacking the sequence) and rare homozygous (both alleles have the sequence). If the expression "wild type" will be used, then, the wild type will replace the common homozygous; the heterozygous has one -ve and one +ve allele and the homozygous will be for those cases with both alleles having the sequence (what you are calling rare homozygous). Results: You have a number of data not shown; I think they will enrich the article unless you have reason to keep these data for the moment, may be for another publication or so. Discussion: Line 176: "which unfortunately was not recorded and it is a": the sentence is lacking the verb "is" Major issue: You are orienting your data and your discussion and even the title as I mentioned in the beginning towards an association of the studied SNP with Ophthalmopathy: In fact GO is not statistically different from other thyroid subtypes; all groups are significantly different from the control. The	clarify these terminologies and we followed the suggestions. Wild type, and homozygous have replaced the term common homozygous and rare homozygous respectively. Explanations are provided for clarifying the meaning of common and rare homozygous. The data in the form of table are inserted in the results section for those results saying "data not shown" . Missing Is due to typographical error is inserted appropriately. We are grateful to Reviewer 1 for comments. We agree that we are emphasising on ophthalmopathy however, our point of emphasis is our data shows
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	polymorphism is associated with autoimmune thyroiditis at large but not specifically with ophthalmopathy associated with these cases. This last point is the most important and you have to modify your article, especially the conclusion, accordingly. The article is fine. The methodology is sound and after the major issue mentioned at the end is taken care of, it may be accepted for publication [Comments received from mail]	that this polymorphism is significantly associated with Graves' ophthalmopathy and Hashimoto's thyroiditis. It is our fault that our approach to demonstrate this association has not been adequately explained in manuscript. To prove that this polymorphism is significantly associated with thyroid disorders with and without ophthalmopathy, we measured the rs3838216 across the three genotypes of wild type, heterozygote and homozygote for GO, GH, HT and control groups which showed a significant probability as shown in table 2, $P = 0.042$. Having established the significance of this polymorphism in thyroid autoimmune disorders including ophthalmopathy, our next question was whether this polymorphism is significantly associated with ophthalmopathy. To answer this question we performed two types of statistical analysis, in one we looked for allele frequencies distribution of rs3838216 polymorphism in pair wise assessment of GO v control, GH v control, HT v Control and GH v HT, results shown in table 3. Significance of distribution of allele frequencies for SNP rs3838216 observed in GO v control with $p = 0.003$ and odds ratio
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		of 2.16 with 95% confidence interval of 1.29 - 3.64. Significant allele frequencies distribution were also observed for HT v control with $P = 0.008$, odds ratio of 1.87 with 95% confidence interval of 1.17 -2.99. No significant difference in alleles distribution frequencies were found for GH v control or GH v HT. Next to confirm this significant probability and odds ratio we observed from allele frequency data in GO and HT, we performed pair wise analysis of individual genotype and assessed the probability and odds ratio, in wild type genotype. Table 4, GO v control, showed significant probability $P = 0.004$ with odds ratio 2.42 with confidence interval of 1.30 – 4.48. Such high probability and odds ratio have been reported for HLA gene. Similarly wild type genotype showed significant probability $P = 0.011$ and odds ratio of 2.07 with confidence interval of 1.17 - 3.67 for HT v control. Above results for wild type genotype of rs3838216 was replicated for heterozygote genotype and results are shown in table 5. Based on our data, it is clear that SNP rs3838216 is associated with thyroid
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		autoimmune disorders and in particular in Graves' ophthalmopathy. Therefore, we suggested this polymorphism involvement in thyroid eye disease. It should be noted that Graves ophthalmopathy is a complex disorder and several genes including HLA gene reported to act as risk factor for this disease. Our CASQ1 gene SNP rs3838216 is one of the genes that we believe is a risk factor for thyroid eye disease as well as autoimmune thyroiditis. Several other genes are implicated to be associated with ophthalmopathy but there was not enough power to substantiate their association to ophthalmopathy. In this article with increase power and sample size we showed that this polymorphism is a risk factor for thyroid disorders and ophthalmopathy.
<u>Minor</u> REVISION comments		
<u>Optional/General</u> comments		