Author's response to reviews

Title: Variable number of tandem repeat polymorphisms of the interleukin-1 receptor antagonist gene IL-1RN: a novel association with the athlete status.

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Author's response to reviews: see over
The Editor in Chief

BMC Medical Genetics

Dear Editor

M. s. Variable number of tandem repeat polymorphisms of the interleukin-1 receptor antagonist gene IL-1RN: a novel association with the athlete status.

Enclosed please find a revised version signed by all the authors of the article, "Variable number of tandem repeat polymorphisms of the interleukin-1 receptor antagonist gene IL-1RN: a novel association with the athlete status"; by authors Sabina Cauci, Manuela Di Santolo, Kelli K. Ryckman, Scott M. Williams, Giuseppe Banfi.

The text has been revised by an English speaker.

We answer reviewers and accordingly send you to the revised manuscript.

1) Reviewer: Emidio Pistilli

Reviewer's report:

Article Review
Emidio E. Pistilli, PhD
University of Pennsylvania
Department of Physiology and Pennsylvania Muscle Institute

“Variable number of tandem repeat polymorphisms of the interleukin-1 receptor antagonist gene IL-1RN: a novel association with the athlete status” – Cauci et al.

Overall
The study by S. Cauci et al., examines the associations between two SNPs in the IL-1# gene and one VNTR in the IL-1RN gene with athletic status. The study utilized a population of 205 athletes (53 high-grade athletes and 152 medium grade athletes) and 458 non-athlete controls that were Caucasian and of Italian nationality. The data are interesting, especially given the observations of significant associations of the VNTR genotypes with athlete status. Please see specific comments below.

Major Compulsory Revisions

Comment 1
The authors state that haplotypes were examined in the study and report this in the results. However, it is not stated whether the SNPs were in linkage disequilibrium. The LD calculation and data should be presented, especially given the significant association of the haplotype (-511)C-(+3954)T-(VNTR)2 with athlete versus non-athlete status.

ANSWER Comment 1: As recommended we have now examined patterns of LD in both the athletes and non-athletes. In both cases, there is no evidence for strong LD. This is now mentioned in the text.

Minor Compulsory Revisions

Comment 2
The authors state in the Conclusion section that it is beyond the scope of the
paper to address the SNP associations with aerobic versus anaerobic types of sports. Why is this the case? These analyses would be extremely interesting, especially given the facts that: 1) in general anaerobic types of activities would include more forceful types of muscle contractions that would expose the muscles to more microinjuries in fibers, and 2) this study examines SNPs in immune related genes that, as the authors state, may be involved with the post-exercise inflammatory phase. In a study by David Nieman’s group in 2003, IL-1# mRNA was one of three genes that showed the biggest increase in muscle following a 2hr bout of resistance exercise in resistance trained men, and this study also states that the serum cytokine profile was different from that noted following aerobic treadmill exercise at 70% VO2max. Given the significant and interesting association observed in this study with athlete versus non-athlete, a sub-analysis of aerobic versus anaerobic athletes would be quite interesting. A sub-analysis could potentially be performed to address this fact, by separating the purely aerobic athletes in the study (triathletes, runners, swimmers) with purely anaerobic athletes.

**ANSWER Comment 2:** We agree that comparison of purely aerobic vs anaerobic athletes is extremely interesting. Unfortunately, in this respect, the majority of study athletes participated in mixed aerobic-anaerobic disciplines (like volleyball). Therefore, we had very few purely anaerobic athletes in our study. Although we observed no significant differences between purely aerobic vs anaerobic, we did not report these results as this analysis is underpowered to detect differences, even if they exist.. The Conclusion now states that studies should be performed to assess this important issue “Of major interest will be the comparison of aerobic vs. anaerobic athletes; however, in the present study this kind of evaluation was not possible due to small number of athletes participating in purely anaerobic sports”.

**Comment 3**
More clarification in the Methods section is needed on how the distinction between high grade athlete status and medium grade status was performed, especially given the fact that significant associations were found when comparing professional athletes and non-professional athletes and also when comparing athletes and non-athletes.

**ANSWER Comment 3:** To clarify the distinction between high and medium grade athletes, we added the following sentence: “Specifically, high-grade athletes group included National Italian team grade (some of these athletes participated in Olympic Games, and some were medallists in International Games) or Third Division soccer players. The “caliber” of an Italian Third Division soccer team is similar to National teams of several European countries. The non-professional athlete group included athletes who are highly involved in sports activities (training and competitions >10 h/week).”

**Comment 4**
The authors state that the specific SNPs examined in these studies were chosen because “these were the most frequently studied polymorphisms in the IL-1# and IL-1ra cytokines.” More clarification on the location of these SNPs within the gene structure would be helpful. The authors state that the -511 IL-1# SNP is in the promoter region, but do not state where the +3954 SNP or the VNTR on IL-1ra is. Also, are there additional SNPs in the IL-1RN gene that have been studied. Given the fact that the significant associations in the study were found in the VNTR of this gene, perhaps other SNPs in this gene would provide additional useful data.
ANSWER Comment 4: in the background on pg. 6 we specified.” Mostly, two single nucleotide polymorphisms (SNPs) in IL-1B have been studied for disease predisposition, one at position -511 in the promoter region [32] and another at position +3954 in exon 5 (TaqI restriction site polymorphism) [18]. In addition, a pentaallelic polymorphic site in intron 2 of the IL-1RN gene consisting in a variable number (of 86 bp identical) tandem repeats (VNTR) has been extensively investigated in relation to a variety of pathological conditions including inflammatory myopathies”.

In the Conclusion we added the following sentence: Our present results should prompt enlarged studies on additional polymorphisms in the IL-1 family in relation to physical performance and elite athletic grade, particularly, those located in the IL-1ra gene, as the SNP at position +2018.

Comment 5
The first sentence of the Discussion section states that this study “supports the notion that IL-1ra levels are critically important in physical exercise.” This statement should be revised, as IL-1ra levels were not measured in this study nor was physical exercise per se measured in this study. The study does identify SNP associations with athletes, but the study does not provide data on levels of IL-1ra following exercise. Please revise this.
ANSWER Comment 5: We changed the sentence as following:
“Our study supports the notion that the IL-1 family of genes is significantly associated with physical performance.”

Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.

3) Reviewer: Yannis Pitsiladis
Reviewer's report:
Major Compulsory Revisions:
Methods: As currently presented, the exact caliber of the studied athletes is unclear. It is of some concern that athletes may be of a fairly heterogeneous background both in terms of caliber of athlete (3rd division football) and specialism (i.e. primarily aerobic or anaerobic event as noted from Table 1 in the results section). Given the statement that some 53 of the 205 athletes were of “high-grade” status this study is clearly under powered (if one also considers gender).
ANSWER Comment 1: Please see the sentence we added in the Methods section:” “Specifically, high-grade athletes group included National Italian team grade (some of these athletes participated in Olympic Games, and some were medallists in International Games) or Third Division soccer players. The “caliber” of an Italian Third Division soccer team is similar to National teams of several European countries. The non-professional athlete group included athletes who are highly involved in sports activities (training and competitions >10 h/week).”

Third Division football players in Italy are professional athletes. The “caliber” of a Italian Third Division football team is similar to various National Teams of European countries. Thus, we do not agree with the reviewer criticism about the “caliber” of our high-grade athletes: they are top-level athletes, recruited in our National Teams, there are some athletes who participated in Olympic Games, there are medallists in International Games, there are professional athletes.
Minor Essential Revisions:
Abstract: mention of medical conditions in the abstract not appropriate as far too speculative.
**ANSWER:** We deleted the description of medical conditions in the abstract.

There is no reason for the use of the term “white” in the introduction as colour of skin has little relevance. The ethnicity of the subjects can be mentioned in the methods. It would aid the understanding of the rationale of the study if the type of athlete was mentioned in the introduction (as apposed to colour of skin). The use of the term European would suffice for the methods section.
**ANSWER:** We changed the “white” with “Caucasian” in the Introduction and Abstract. Please note that for inclusion in the study we carefully controlled for ethnicity because for example African and Asian people have a different polymorphism profile in the IL-1 family genes. Presently in Italy we have 4 million people of different ethnic groups. We anticipated in the Introduction the sentence “Athletes of National or Regional competitive standard were recruited”.

**Level of interest:** An article of insufficient interest to warrant publication in a scientific/medical journal
**Quality of written English:** Needs some language corrections before being published
**Statistical review:** Yes, and I have assessed the statistics in my report.

2) Reviewer: Agnieszka Seremak-Mrozikiewicz
**Reviewer's report:**
The analysis was performed on the large group of 205 athletes and 458 non-athlete controls. In both groups genetic polymorphisms in the IL-1beta and IL-1ra genes were determined. The Authors obtained the interesting results connected with polymorphism of IL-1ra gene and concluded that this gene predispose to athlete status among with subjects. This study refers to very interesting problem connected with inflammatory and repair reactions, the Authors apply modern molecular methods and correct statistical analysis. The purpose of the study is well defined. The methods used in the performed investigation are appropriate and well described. The research is good arranged and the results have statistical powerful. The discussion and conclusions are well balanced and adequately supported by the data. The title of manuscript is informative and clear. The abstract convey accurately the main idea and what has been found during performed investigation. The paper is clearly arranged. The writing is acceptable.
**Level of interest:** An article of limited interest
**Quality of written English:** Acceptable
**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**ANSWER:** We appreciated favourable comments of this reviewer
In consideration of the action of *BMC Medical Genetics* in reviewing and editing this submission, the authors undersigned hereby transfer, assign or otherwise convey all copyright ownership *BMC Medical Genetics* in the event that such work is published by *BMC Medical Genetics*.

We are looking forward to hearing from you.

Sincerely yours

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TO WHOM IT MAY CONCERN

I declare that I participated in the design, execution, and analysis of the paper by Sabina Cauci and colleagues entitled “Variable number of tandem repeat polymorphisms of the interleukin-1 receptor antagonist gene *IL-1RN*: a novel association with the athlete status,” and that I have seen and approved the final version. I also declare that I have no conflict of interest in connection with this paper, other than any noted in the covering letter to the editor.

Author Contributions

Conceived and designed the experiments: Sabina Cauci. Performed the experiments: Manuela Di Santolo, Sabina Cauci. Analyzed the data: Kelli K. Ryckman, Scott M. Williams. Contributed reagents/materials/analysis tools: Sabina Cauci, Giuseppe Banfi. Wrote the paper: Sabina Cauci, Scott M. Williams, Giuseppe Banfi.
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