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REVIEWER'S REPORT

Manuscript No.: IJAR-58811

Title: The autoimmune PTPN22 variant rs2476601 is associated with rheumatoid arthritis risk but not with systemic bone loss.

Recommendation:

Accept as it is .....

Accept after minor revision.....

Accept after major revision .....

Do not accept (*Reasons below*) .....

Rating	Excel.	Good	Fair	Poor
Originality		✓		
Techn. Quality		✓		
Clarity		✓		
Significance		✓		

Reviewer Id: JPR-294

Reviewer's Comment for Publication.

The manuscript investigates the association of the autoimmune-associated **PTPN22 rs2476601 variant** with rheumatoid arthritis (RA) susceptibility and systemic bone loss in a Tunisian population. The topic is relevant and clinically significant, particularly because genetic factors may influence RA susceptibility and potentially contribute to bone complications. The study includes a relatively substantial RA cohort and healthy control group, uses an established genotyping approach, and evaluates bone mineral density at clinically relevant skeletal sites using DXA.

The results provide useful evidence supporting an association between PTPN22 rs2476601 and RA risk in the Tunisian population, while no clear association with systemic bone loss was demonstrated. The manuscript is generally well structured and the discussion provides a reasonable biological context for the findings. The study is potentially suitable for publication after addressing several minor issues of presentation, clarity, and statistical reporting.

Specific comments:

1. The title is appropriate, but the wording "autoimmune PTPN22 variant" could be changed to "autoimmune-associated PTPN22 variant" for greater scientific precision.
2. The abstract should be carefully edited for grammar and spacing. For example, "assess the association" should be written with appropriate spacing.
3. The manuscript should consistently use the standard nomenclature **PTPN22 rs2476601** throughout the text, with appropriate formatting.
4. The Methods section should clearly state the demographic characteristics of the healthy controls and confirm that they were appropriately matched to the RA patients for age and sex.
5. The number of RA patients differs between the overall cohort (363), those with clinical data (230), those with BMD measurements (216), and those evaluated for erosion (221). These differences should be clearly explained, preferably with a brief description of patient availability for each analysis.

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6. Please clarify why BMD was not available for all 363 RA patients and indicate whether missing BMD measurements could have introduced selection bias.
7. The definition of osteopenia contains a typographical error: "-1 SD < T score > -2.5 SD" should be corrected to the appropriate inequality notation, generally **−2.5 < T-score < −1.0**.
8. The manuscript should clarify whether T-scores were applied to all participants or only to postmenopausal women/appropriate patient groups, since WHO T-score criteria have specific clinical applications.
9. The statistical analysis should state whether the genetic association was evaluated under an allelic, dominant, recessive, or additive model. The analysis should also clarify whether correction for multiple comparisons was considered.
10. The very large odds ratio for RA risk (**OR = 14.27**) should be interpreted cautiously because the minor T allele was rare in the study population and the confidence interval is wide (3.44–59.13). The Discussion should acknowledge the possibility of an unstable effect estimate related to the small number of T-allele carriers.
11. There appears to be a numerical inconsistency concerning the allele frequency. The manuscript reports T allele frequencies of 6.2% in patients and 0.5% in controls, followed by the statement that the "total T allele frequency" was 4%. Please clarify how this value was calculated.
12. The reported p-value for total femur osteopenia (**p = 0.034**) is described as a "trend towards association." Since this value is below 0.05 according to the stated significance threshold, the authors should either describe it as statistically significant in the univariate analysis or explain why it is considered only a trend, particularly in view of multiple testing and the wide confidence interval.
13. The finding of no association between PTPN22 rs2476601 and systemic bone loss should be interpreted in light of the limited number of T-allele carriers. The manuscript already mentions this limitation, but it would be useful to emphasize that the study may have limited power to exclude a modest association.
14. The multivariate analysis should provide the adjusted odds ratios, 95% confidence intervals, and p-values for the variables included in the final model. This would allow readers to evaluate the robustness of the conclusion concerning systemic bone loss.
15. Table 2 should be checked carefully because it appears to be used for both the genetic association with RA and the bone-related analyses. The table should clearly identify the population and outcome corresponding to each statistical comparison.
16. The Results section contains duplicated text concerning the prevalence of osteopenia and osteoporosis at the femoral neck, total femur, and lumbar spine. The duplicated paragraph should be removed.
17. The genotyping methodology is adequately described, but the authors should indicate whether a proportion of samples were genotyped in duplicate or whether another quality-control procedure was used to confirm PCR-RFLP results.
18. The manuscript states that samples were in Hardy–Weinberg equilibrium, but the exact p-values should preferably be reported for both patients and controls.
19. Please clarify whether the 3% agarose gel and ethidium bromide staining procedure complied with the laboratory's established safety and quality-control procedures.
20. The Discussion is informative but somewhat lengthy. Some mechanistic details concerning PTPN22, PAD4, NET formation, and RANKL could be shortened to maintain focus on the principal findings of the study.
21. Statements such as "confirms the association" should preferably be changed to "supports the association" because this is a single population-based study and the confidence interval is relatively wide.

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22. The authors should avoid implying that the absence of a statistically significant association proves that PTPN22 rs2476601 has no effect on systemic bone loss. A more appropriate interpretation is that **no statistically significant association was detected in this cohort**.
23. The limitations section should mention the cross-sectional design, relatively small number of T-allele carriers, incomplete BMD data, and inability to fully account for all potential confounding factors.
24. Minor grammatical and typographical corrections are needed throughout the manuscript, including missing spaces in phrases such as "progressive disabling disease," "patients with RA," "recruited between," "osteoporosis was diagnosed," and "association between."
25. Reference formatting should be standardized, and all citations should be checked to ensure that the reference numbers correspond accurately to the statements in the manuscript.

Overall Assessment

This is a relevant and potentially valuable study addressing an important question concerning the genetic susceptibility to RA and the possible contribution of **PTPN22 rs2476601** to systemic bone loss. The main findings are interesting, and the study has sufficient merit for publication. The issues identified are primarily related to clarification of the statistical analyses, presentation of participant numbers, correction of minor inconsistencies, and more cautious interpretation of the negative findings.

Final Recommendation: Accept after Minor Revision.