Reviewer's report

Title: Evidence of association of a single nucleotide polymorphism in WDR77 with calcaneus bone ultrasound parameters in European men: a validation study

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Reviewer: david karasik

Reviewer's report:

In this interesting report, the authors performed an important study of replication of GWAS-derived osteoporosis-susceptibility genes. In brief, in a relative large sample of middle-aged to older men from seven European centers (EMAS consortium), they genotyped multiple SNPs, which correspond to top GWAS associations with heel QUS parameters (BUA or SOS) in the Framingham study. Some SNPs were found to be associated with the QUS parameters in the EMAS men (such as WDR77, also known as androgen receptor cofactor p44); others, with LS, FN and TH BMD. Thus SNP rs238358 on chr. 13, associated with BMD in EMAS, is in vicinity of DGKH & AKAP11 genes, which were among the genome-wide significant markers associated with lumbar spine BMD in the GEFOS meta-analysis.

This is a study by a group of experts in genetic epidemiology of osteoporosis. The manuscript's methodology in general is up-to-date and gives evidence of the authors' expertise in the field. The clinical importance of genetic determination of risk factors for fracture, such as QUS and BMD, is incontestable. However, this study has one major drawback that reduces enthusiasm:

- Indeed, the aim to validate the most significant SNPs found by GWAS utilizing an independent population, is a noble aim. However, they chose an early analysis (underpowered and based on only <100K SNPs); unfortunately, there are no other large-scale association analyses for the QUS phenotype to date. Therefore, the non-striking results of this paper are rather expected: the authors seemingly followed the false-positive hits. Despite worth to report, these results might not generate much interest in the field. Also, more recent report from the Framingham actually doesn’t find much of a QUS-specific genetics (it seems to be indistinguishable from DXA-BMD).

ESSENTIAL REVISIONS

METHODS

p. 6: - Which one collaborating site could not obtain an approval for the genetic analysis?
- self-report of the country of origin is a concern (too uncertain).

p. 7: Standardized CVs between machines were 4.8% and 9.7% for BUA and SOS, respectively, - isn’t this a lot? Please discuss these numbers.
p. 8: it is unclear, was the SNP selection (and genotyping) done in one or two stages? (“For the gene showing evidence of replication in EMAS… [additional?] tag SNPs were selected…”)

p. 9: please justify why a p < 0.05 was sufficient for replication (there were 34 SNPs and several bone traits).

RESULTS:

p. 10: Since the BUA and SOS were highly correlated (r² = 0.81), should they both still be analyzed separately, or should only one of them be representative of QUS? (also see the comment re: CV of 9.7% for SOS above). Also, standard deviations of BUA and SOS are very different, therefore variability is much higher for BUA. How this would affect the power estimates for each of these 2 traits?

DISCUSSION,

on p. 13, the authors discuss WDR77 potential functions, including a role in hypogonadism. There is thus a question, do they have an information about hypogonadism in their men?

p. 14: despite inclusion of a SNP-by-center interaction is a right thing to do, it may or may not minimize population stratification issues which obviously undermine this study design.

p. 15: this reviewer disagrees that SNP rs238358 on chr. 13 may be in LD with TNFSF11/RANKL specifically because of the recombination hotspot in-between (it’s testable though).

Also, since the association between rs238358 and aBMDs in this study nicely corroborates the genome-wide significant associations with lumbar spine BMD in the GEFOS consortium, the authors might add this info to the ABSTRACT.

Table 1: should be provided by site/center, if possible.

MINOR COMMENTS (Discretionary Revisions)

it is no obvious there’s a need in abbreviation BMD(a), since there is no other type of BMD in this paper.

p. 7: Under DXA: it is suggested to change to “scans and measurements”, to not confuse the reader with (statistical) analyses.

p. 16: 1st sentence, it should be “parameter”, not in plural.

Table 2 (and Suppl. Table): it is unclear whether the crude or adjusted analyses are shown.

Figure 1: pls. add in footnote an explanation for the vertical lines (confidence interval?)

Level of interest: An article whose findings are important to those with closely
related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

'I declare that I have no competing interests'