Reviewer's report

Title: Aging Syndrome Genes and Premature Coronary Artery Disease

Version: 1 Date: 9 May 2005

Reviewer: Robert Hegele

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General

The authors have evaluated an interesting hypothesis by testing the association between premature CAD and SNPs in specific candidate genes selected based upon the causative role of mutations in those genes in premature aging syndromes. The genes selected for testing were LMNA and KLOTHO. In 295 premature CAD patients and 145 controls, the authors found no associations between CAD and either individual SNP alleles or haplotype blocks of LMNA. In addition, the authors found no associations between CAD and specific KLOTHO alleles. They conclude that premature CAD is associated with common variation in genes that underlie human progeroid syndromes.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. Table 1 shows that controls and cases were not well matched for age, BMI, diabetes, hypertension and smoking. In addition, there was a significant difference in the proportion of males in the case and control groups (P=0.01), that was not commented upon by the authors. Given the imbalance in these known risk factors between cases and controls – and given the low likelihood that the authors would be able to select a more representative control groups - why was the analysis not conducted using these additional risk variables as co-variates? This may be especially relevant given that, at least in theory, there could be some rationale to hypothesize an association between the genetic markers and such risk variables as diabetes or hypertension.

2. Statistical power of the study must be addressed. The authors found no association with any of the markers tested. Thus, a pertinent question in studies with negative findings is “what was the power of the study to detect a significant between-group difference in allele frequencies?”. If one a priori selects a 1.7-fold difference in allele frequency between groups and an allele frequency of 0.2, then it would be possible to calculate, assuming alpha of 0.05, the power afforded by 295 cases and 145 controls to find a difference. Why was this not performed and shown in the text? If the power that the sample affords to find a difference is low, what then is the meaning of an essentially negative result?

3. Additional sample heterogeneity. The heterogeneity between cases and controls is already apparent given the ~3-fold differences in diabetes, smoking and hypertension between them. Additional heterogeneity may stem from differences in treatments. What were the individual treatments for dyslipidemia, hypertension and diabetes? Were these balanced between cases and controls? Also, is there some family history data to substantiate the claims of "early" CAD, since this might presumably be genetically propagated.

4. SNP selection. Please justify why all possible SNPs in LMNA were selected, but only two KLOTHO SNPs were selected for study.
5. Haplotype analysis. Given the brief description on page 15, it is unclear whether haplotypes and haplotype blocks were assigned by hand or through a computer algorithm. Please specify which was used.

6. Given the fact that most SNPs at LMNA had no functional consequence, why would not the authors consider linkage disequilibrium with other markers at this locus as a potential explanation?

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1. Page 2, line 3: spelling “vascular”
2. Page 2, line 5 and throughout: When referring to the human gene, please use capitalized italics (e.g. <italic>LMNA</italic> and <italic>KLOTHO</italic>). When referring to the mouse gene, capitalize the first italic letter only (e.g. <italic>Lmna</italic> and <italic>Klotho</italic>). Thus “lamin A/C gene” should be cited as “<italic>LMNA</italic>, which encodes lamin A/C (or lamin A and C isoforms).
3. Page 2, line 7: “<italic>KLOTHO</italic>”
4. Page 2, line 9: replace “tested” with “evaluated”
5. Page 2, line 10: refer to “<italic>LMNA</italic>" and “<italic>KLOTHO</italic>”
6. Page 2, line 15: “<italic>LMNA</italic>”
7. Page 2, line 17: space after period and “<italic>KLOTHO</italic>”
9. Page 3, line 2: spelling “support”
10. Page 3, line 3 and throughout: substitute “progeroid” for “progeric”
11. Page 4, line 21 and elsewhere: The MEF2A example (reference 8) has recently been shown to be very problematic and perhaps not correct biologically (please see Weng et al. Lack of MEF2A mutations in coronary artery disease. J Clin Invest. 2005 Apr;115(4):1016-20 and Altshuler D, Hirschhorn JN. MEF2A sequence variants and coronary artery disease: a change of heart? J Clin Invest. 2005 Apr;115(4):831-3). In light of the controversy, the authors should refrain from citing MEF2A as an example of “an alternate pathway for CAD”.
12. Page 4, line 24: replace “progeric” with “progeroid”
15. Page 5, line 18: please insert citation for Arking paper at this point in the text.
16. Page 5, line 21: replace “suggest” with “suggested”
17. Page 6, lines 7-9: use “<italic>LMNA</italic>” and “<italic>KLOTHO</italic>” instead of “<italic>LMNA</italic>” and “<italic>KLOTHO</italic>”
18. Page 6, line 17: italicize LMNA in title
19. Page 6, line 24: change “SNPs” to “SNP”
20. Page 7, line 8: show one significant digit after decimal point
21. Page 7, line 9: use “cohort” or “sample” rather than “population”
22. Page 7, line 17: insert “excess circulating LDL cholesterol which causes” between “leads to” and “focal endothelial injury”
23. Page 7, line 23: refrain from using the MEF2A example
24. Page 8, line 1: replace “lamin A/C” with “<italic>LMNA</italic>”
25. Page 8, line 7: replace “Lamin” with “LMNA”
26. Page 8, line 13: delete “progeric disorder”
27. Page 8, line 14: use “HGPS”
28. Page 8, line 16: The germline loss of the second LMNA allele is not yet accepted to be part of the mechanism underlying HGPS. If a reference exists, please cite it. Otherwise, please remove the sentence spanning lines 16-19.
29. Page 8, line 22: “The <italic>KLOTHO</italic> gene encodes a membrane protein” (a gene is not a protein)
30. Page 9, line 2 and throughout: please be more specific when referring to the KLOTHO alleles – specify the exact nucleotide and amino acids that are changed.
31. Page 9, line 15: use “<italic>LMNA</italic>” and not “lamin A/C”
32. Page 9, line 19: use “<italic>LMNA</italic>” and not “lamin A/C”
33. Page 10, line 2: space between “causation” and “[23-27]”
34. Page 10, line 13: abbreviate “CAD”
35. Page 11, lines 3-8: provide power calculations
36. Page 12, line 4: see MEF2A suggestions above
37. Page 14, line 20, 22: italicize first three letters MaeIII i.e. <italic>Mae</italic>III
38. Page 15, para 1: provide more details for haplotype estimation
39. Tables 2 and 3: please indicate in table title that it is LMNA only – if this is indeed the case.

Discretionary Revisions (which the author can choose to ignore)

1. Page 8, line 16: Note also that the discussion of link between laminopathies and atherosclerosis has been discussed in some depth in Al-Shali KZ, Hegele RA. Laminopathies and atherosclerosis. Arterioscler Thromb Vasc Biol. 2004 Sep;24(9):1591-5, and this paper could be cited for readers’ reference.

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

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

Quality of written English: Needs some language corrections before being published

Statistical review: No

Declaration of competing interests:

I declare that I have no competing interests.