Author's response to reviews

Title: Linkage Study of Fibrinogen Levels: The Strong Heart Family Study

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Author's response to reviews: see over
Dear Sirs:

We have now completed the power calculations suggested and have included them below. In addition, we believe we have addressed your concerns regarding consent for this study in revisions to the fourth sentence of the Methods, first paragraph.

We appreciate the helpful suggestions of the reviewers and will address them in order as follows:

5/29/08  Georg Endler

1) Regarding the possibility that fibrinogen is not in the causal chain of CVD; but merely a marker for existing (possibly pre-clinical) CVD.

   It is always difficult to absolutely determine that a particular biologic factor is acting independently in the causal chain of disease, especially in multifactorial conditions, such as atherosclerosis. We have strengthened our argument on this point in the second sentence of the Discussion, pointing out clearly the compelling evidence from a large meta-analysis of over 31 prospective studies involving over 150,000 participants free of CVD at baseline. This study found the predictive ability of fibrinogen was robust to adjustment for many traditional CVD risk factors; including CRP as a measure of inflammatory state. (See Fibrinogen Studies Collaboration, Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: an individual participant meta-analysis. JAMA 2005, 294:1799-809)

   Fibrinogen is correlated with other inflammatory markers, such as CRP and IL-6; but it was recently reported from the British Women’s Heart and Health Study that IL-6, which is known to stimulate fibrinogen and CRP expression in inflammatory states, was not an independent predictor of CVD in this cohort. (Fraser A, et al. Interleukin-6 and incident coronary heart disease: Results from the British Women’s Heart and Health Study. Atherosclerosis. 2008 May 15, Epub ahead of print) Although the exact role of fibrinogen is a very interesting intellectual puzzle, it seems somewhat beyond the scope of this paper, which is focused primarily on the genetic factors influencing circulating fibrinogen levels.

   Please see also comments in response to the second reviewer.

2) The suggestion to look for genes in the “hot spots” associated with cardiovascular disease or hypertension:

   This was done to some extent in the Discussion, where the angiotensin II receptor, type 1 gene (AGTR1) and angiotensin converting enzyme I (ACE1) genes were noted to be within the 1 LOD unit support interval on chromosomes 3 and 17. The products of these genes are key elements in the renin-angiotensin system (RAS), controlling blood pressure. In the 4th paragraph of the discussion we now emphasize the connection between the RAS and hypertension, but also note that there is no evidence that fibrinogen is an independent, etiologic factor in hypertension.

   In a rather exhaustive, additional search through the NCBI “Map viewer” and OMIM search functions, we found approximately 100 “hits” for search terms such as “atherosclerosis, myocardial infarction, hypertension, cardiovascular etc. Of these there were no “hits” that contributed additional, pertinent candidate genes within 20 Mb of the –1 LOD
drop range of our loci for the former search and 2 additional hits from the OMIM search on chromosome 6 that may be germane.

One of these was the ENPP1 gene at 117.5M on chromosome 6 and is related to risk of type 2 diabetes mellitus and a rare condition characterized by calcification of the aorta in infants (with pathology related to atherosclerosis). The other was the ROS1 gene at the same locus, which was found as one of 4 SNPs associated with acute myocardial infarction in a GWAS study. The authors would be willing to add additional comments in the Discussion about these last two candidates; but the connection to fibrinogen appears rather tenuous at this time. We will defer to the editors wishes on this.

5/31/08 Moniek deMaat
Major compulsory revisions:

1) Regarding the advisability of using a 500K or 1M SNP microarray in a GWAS methodology.

We are certainly aware of this methodology; but our situation is complicated by the autonomous relationship of our tribal governments and the need for community approval to conduct research among their members. Our funding agency (NIH) has refused to fund any GWAS studies unless the results will be released to qualified investigators on the internet within 6 to 12 months. The tribal governments have not been willing to agree to this condition as yet; and thus we do not have access to this methodology. We have been in extensive discussions with the tribal governments, seeking to provide information about this methodology and the implications so that they can make an informed decision.

2A) No strong linkage found, ? power calculations.

As mentioned in the discussion, each of our centers analyzes a population essentially equal in size to that reported by the Framingham Heart Study (Yang Q et al, Thromb Res 2003;110:57-64). The second largest linkage study from the GENOA Study (Ding K, et al. J Hum Hypertens 2008,22:102-10) consisted of 1300 African Americans and 700 non-Hispanic whites and ascertainment was keyed on hypertension. The only other linkage study (Soria JM et al. Arterioscler Thromb Vasc Biol 2005, 25:1287-92) reported on approximately 400 individuals in families enriched for thrombophilia.

In contrast, the present study consisted of three individual centers with about 1200 individuals in large kindreds (frequently involving 5 to 6 generations). The extended family size should significantly enhance power.

Our power calculations were completed after 2 weeks of intensive computations. A final paragraph was added to both the methods and results sections; and a sentence was included in the discussion regarding strengths of the study. These indicate that moderate power is available to detect LOD scores of ≥3, given a total heritability of 25% and a QTL variance component of between 15 and 20% for each center.

2B) Discuss in more detail why results were not consistent between centers.

I personally have been surprised by the relative lack of consistent findings between centers (in this analysis as well as in other analyses conducted in SHS). Other authors from our group, however, have not been surprised to see this apparent degree of genetic diversity between centers, which are each separated by roughly 1,000 Km and have distinct language
and cultural characteristics. We have added comments to the 8th paragraph of the discussion which address this concern.

2C) Please show comparable LOD scores from other centers, for the highlighted “hits” in Table 2.

This is done. I think the table now illustrates the clear lack of contribution from other centers to all but one locus (that of chromosome 6, 122cm) where each center contributes a rather equivalent amount to the maximum, which is for the combination of all centers.

Minor essential revisions:

1) Tell the effect of fibrinogen in the SHS population.

The fourth sentence of the background states: “The current report is derived from the Strong Heart Family Study (SHFS), an extension of the SHS, in which we have previously corroborated the ability of fibrinogen to predict CVD events [5].” (Palmieri V, Celentano A, Roman MJ, de Simone G, Best L, Lewis MR, Robbins DC, Fabsitz RR, Howard BV, Devereux RB: Relation of fibrinogen to cardiovascular events is independent of preclinical cardiovascular disease: the Strong Heart Study. Am Heart J 2003, 145:467-74.) It would not be possible to confirm the predictive value of fibrinogen in the SHFS as yet, since surveillance for CVD outcomes has not been ongoing sufficiently and this is a younger population. The SHFS participants (our source for this analysis) are all related to the original SHS members analyzed in the referenced paper. This is noted in the third sentence of the methods.

2) Was an analysis done adjusting for markers of inflammation, such as CRP?

Unfortunately, no CRP measures are available on the SHFS participants. We share the reviewer’s interest in determining the exact, specific mechanism by which fibrinogen is linked to CVD. We have since conducted post-hoc analyses using the only inflammatory marker available (WBC) and find attenuation for the chromosome 7, DK, model 2 results, and loss of signal at the chromosome 17 locus; but identical to stronger results for chromosome 7, DK, model 1 and for chromosome 3 in the OK center. This information is added to the results (page 8, lines 5-9) and the discussion (page 10, line 17-20).

In addition, there is ample evidence that fibrinogen is an independent predictor of CVD (even after adjustment for traditional risk factors and CRP, see Fibinogen Studies Collaboration, Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: an individual participant meta-analysis. JAMA 2005, 294:1799-809) and we have shown that certain genetic loci are influential in determining fibrinogen levels, independent of at least one measure of inflammatory state.

3) Were cut-off values determining smoking and drinking status too low?

We did not run the analyses with alternative cut-off values, since other analyses of the SHS data have found these to be the most optimal values. The SHS investigators have also tried to be consistent in our analyses of these covariates from manuscript to manuscript. They certainly should be definitive in categorizing the non-users (assuming some inaccuracy in reporting, as expected).
4) Write out “AZ, DK and OK” in full, when first mentioned.

    Thank you, this oversight has been corrected.

We hope that these revisions have addressed the issues adequately and appreciate the opportunity to clarify some issues and strength this manuscript.