Author's response to reviews

Title: Allele frequencies of hemojuvelin gene (HJV) I222N and G320V missense mutations in white and African American subjects from the general Alabama population

Authors:

James C Barton (ironmd@dnamail.com)
Charles A Rivers (rivers@uab.edu)
Sandrine Niyongere (sniyong@uab.edu)
Sean B Bohannon (sbohannon@bosco.meis.uab.edu)
Ronald T Acton (acton@uab.edu)

Version: 2 Date: 2 November 2004

Author's response to reviews:

November 2, 2004

BMC Medical Genetics

re: revision of manuscript "Frequencies of hemojuvelin gene (HJV) I222N and G320V missense mutations in Alabama white and African American subjects from the general population" by J.C. Barton, C.A. Rivers, S. Niyongere, S.B. Bohannon, and R.T. Acton

Dear Editorial Board:

Thank you very much for your prompt review and for the opportunity to respond to the criticisms and suggestions of three Reviewers. The comments of the respective Reviewers are displayed in italic font, and our suggested responses are displayed in standard font.

Reviewer Dr. Merryweather-Clarke
General
Barton et al are to be congratulated for their straightforward and coherent account of low allele frequencies of two HJV mutations in control individuals from two populations in Alabama, those with presumably European ancestry and African Americans.

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

The Reviewer did not suggest that compulsory revisions were needed.

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

The Reviewer did not suggest that minor essential revisions were needed.

Discretionary Revisions (which the author can choose to ignore)

The observation of a phenotypically normal 29-year old female double heterozygote for HFE C282Y and HJV I222N is interesting in the light of reports of double heterozygosity for mutations in HAMP and HFE causing haemochromatosis of varying severity depending on the severity of the HAMP mutation. It would be relevant to know whether this individual has had any pregnancies, is a blood donor or is a red meat eater or vegetarian if such information is accessible.

This information has been inserted in the Results section entitled Characteristics of a HJV I222N heterozygote: "She reported that she had had two normal pregnancies and one spontaneous abortion. She has always experienced heavy menstrual flow, but she reported no other significant blood loss. She reported no blood donation. She reported that she eats a variety of meats, vegetables, and other foods; there was no history of supplemental iron ingestion."
Barton et al. cite their BCMD study of JH patients (ref 10) to support their point that "it does not appear that the common HFE mutations C282Y, H63D and S65C increase the severity of iron overload in persons with JH phenotypes and two HJV mutations". It is apparent that HJV mutations cause more severe disease than those in HFE. However, only one of the six JH patients in the cited study was reported to have an HFE mutation (one was an HFE H63D heterozygote), so the authors may like to provide further observations to support their point, and for clarity should specify the relevant populations.

We deleted this sentence in the Discussion: "Similarly, it does not appear that the common HFE mutations C282Y, H63D, or S65C increase the severity of iron overload in persons with juvenile hemochromatosis phenotypes and two HJV mutations [10]." We inserted this paragraph in the Discussion in the place of the deleted sentence: "Like the present patient, some persons with JH have common HFE genotypes, including C282Y heterozygosity, H63D heterozygosity or homozygosity, and S65C heterozygosity (3, 4, 10-14) (kaltwasser 2000; rivard and mura 2000; kelly and rhodes 1998; varkonyi and kaltwasser; montes-cano 2002; roetto, totaro, and cazzola 1999). However, these HFE genotypes are infrequently associated with a severe hemochromatosis phenotype (26, 59-66) (barton and sawada-hirai 1999; feder and gnirke 1996; beutler and gelbart 1996; beutler 1997; barton and shih 1997; adams and chakrabarti 1998; mura and raguenes 1999; roetto, totaro, and cazzola 2002). Further, sequencing HFE introns and exons in English and French Canadian JH cases did not reveal novel HFE mutations that could likely explain the development of iron overload (4, 10) (rivard and mura 2000; kelly and rhodes). In 310 HFE C282Y homozygotes in France, HJV mutations were relatively common and were associated with greater severity of iron overload [8]. In 48 HFE C282Y homozygotes in the U.S. who had severe iron overload phenotypes, no HJV coding region mutation was detected [3]. The frequency of H63D is significantly greater in persons with cardiomyopathy than in normal control subjects (67) (camaschella, roetto, and cali 2000). The brother of a Spanish JH proband had evidence of iron overload associated with H63D homozygosity and heterozygosity for a putative JH-associated Ch1q haplotype (12) (montes-cano 2002). Although these reports are consistent with observations in another JH patient with cardiomyopathy who had HFE H63D (Barton and rao 2002; lee, barton, and rao 2004), other persons with JH and cardiomyopathy did not have H63D (10) (kelly and rhodes). Altogether, these reports indicate that further exploration of the potential role of variant HJV alleles in the clinical expression of iron overload with particular reference to HFE hemochromatosis is warranted (lanzara roetto 2004), and that the frequency and biological significance of HJV alleles may vary among racial/ethnic groups."

Reviewer Dr. van Duijn
I have reviewed the paper of Barton et al. The paper reports on the prevalence of HJV mutations in Alabama white and African Americans. The findings of the study are not of high scientific interest. The study is too small to address the rare mutations studied. The authors respectfully submit that the numbers of subjects in the present study are similar to (if not greater than) those in many other similar population surveys in which the frequency of hemochromatosis-associated mutations was estimated.

The methods/abstracts misses the number of persons studied and the methods misses a power calculation.

The Abstract and Results clearly state the numbers of subjects we studied. To emphasize this for the reader, we have added these numbers to additional sections of the manuscript, including the Methods section of the Abstract, the Statistical Considerations section of the main body of the paper, and the Methods section of the main body of the paper. The original section entitled Statistical Considerations clearly defines the statistical methods used in this study.

We regarded a power calculation for the present study to be inappropriate, because such predictions are based in part on previous expectations or estimates of allele frequency. At the time our work was performed, there were no population estimates of the frequency of these HJV alleles (although the work of Le Gac et al. using dHPLC technique implied that I222N and G320V did not occur in a French study population). The study of G320V detection by RFLP analysis by Pissia et al. was published after our work was completed.

In the results no information is given what the biochemical findings in the 29 year old cancer mean in terms of clinical relevance. Given these considerations. I advice to regret the paper. Reject because scientifically unsound.

The 29 year-old woman described did not have cancer; she had thrombocytopenia, as stated in the initial
version of the manuscript. Her serum iron measures were included in the original version of the manuscript in the Results section. Additional information about her case has been added to address the inquiries of Dr. Merryweather-Clarke. A revised paragraph in the Discussion addresses the possible clinical significance of the association of HJV and HFE mutations in response to a suggestion by Dr. Camaschella (please see).

Reviewer Dr. Camaschella

General

The paper by Barton et al reports the study of two mutations of juvenile hemochromatosis (HJV) gene in normal whites and African American controls from Alabama. The problem is clearly illustrated, the methodology adequate and the results well described. The results show that a single white allele out of the 480 examined was positive for HJV I222N and none for the "recurrent" G320V mutation.

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

The Reviewer did not suggest that compulsory revisions were needed.

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

It is unclear whether iron parameters were available or have been measured in the studied population.

This sentence has been added to the Methods section entitled General Criteria for Selection of Study Subjects: "Serum transferrin saturation, serum ferritin concentration, or other indicators of iron metabolism were not measured in most study subjects."

The first sentence of the Abstract is obscure. Mutations in the coding region of the HJV gene at the homozygous or compound heterozygous state are associated with juvenile hemochromatosis and not "with primary iron overload in some adults who lack typical hemochromatosis-associated HFE........". Obviously patients with juvenile hemochromatosis may present in young adulthood! The sentence should be changed according to the first Background sentence.

We used the first two sentences of the original Background section to replace the confusing sentence in the Abstract indicated above. Please see.

References are puzzling! References 1 and 2 are indicated as the papers that correlate HJV mutations and juvenile hemochromatosis, but the largest series of HJV mutations reported in the literature is in the article by Lanzara et al (ref 7), that is quoted only referring to I122N and G320V mutations! This inconsistency should be corrected.

The Lanzara et al. 2004 reference and a recent review by Robson et al (J. Med. Genet. 2004) have been inserted in the opening paragraph of the Background. These references have also been added in the revision of the Discussion (see above in Dr. Merryweather-Clarke's critique).

G320V mutation has been screened in a Greek cohort of blood donors. The paper is available in Haematologica on line (Politis et al Haematologica ,2004). This paper should be quoted in the discussion of the frequency of G320V and other HJV mutations.

We added this sentence to the Discussion, first paragraph: "In 200 Greek volunteer blood donors, none carried the HJV G320V mutation detectable by PCR-RFLP analysis, suggesting that the frequency of the G320V allele in the Greek population is lower than 0.004 (Pissia)."

Discretionary Revisions (which the author can choose to ignore)

Accept after minor essential revisions

Please see above.

We eliminated typographical errors that identified (incorrectly) the HJV mutation as I122N instead of I222N in some places in the manuscript.

Thank you in advance for your review of our revised work. The changes and the revised manuscript have been reviewed and approved by all authors. We believe that the changes that we have made are consistent with the constructive suggestions of the reviewers, and have improved the manuscript substantially.
Sincerely,

Ronald T. Acton, Ph.D.