Author's response to reviews

Title: SCN5A Allelic Expression Imbalance in African-Americans Heterozygous for the Common Variant p.Ser1103Tyr

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Version: 2 Date: 7 April 2010

Author's response to reviews: see over
Response to Reviewer 1:

Reviewer 1 had no concerns

Response to Reviewer 2:

1. Comments about allelic expression assays
   a. We have revised the text to remove the term “internal standards” and replaced this with “cDNA standards”.
   b. We added a statement to the methods (p. 6) about the choice of cDNA concentration for the standards:

   *The cDNA standards were diluted to a final concentration of 0.001 ng/µl after preliminary studies determined that this concentration produced cycle threshold values similar to heart tissue RNA.*

   c. We clarified that we used replicate cDNA preparations in the Methods (p. 6):

   *Assays were performed in triplicate and each RNA sample was assayed from 2-3 independent preparations of cDNA.*

2. Comments about existence of alternatively spliced transcripts.

   It is not feasible using our current assay conditions to determine allelic expression ratios separately for each splice variant. The splice variant we described involves variable inclusion of a single glutamine codon and it is not feasible to design reliable real-time RT-PCR assays that distinguish between transcripts with and without this codon at the same time evaluating presence or absence of the genetic variant. However, we added a statement to the Discussion about this as a limitation of our study (p. 9).

3. Gaussian plots Figure 1A

   The rationale for this analysis was to test the null hypothesis that allelic expression imbalance does NOT occur in which case we would have expected a single Gaussian distribution for all data points. The allelic expression ratios follow a bimodal distribution (two component mixture) rather than a normal distribution and this finding provided the first indication that allelic expression was heterogeneous.

   As this reviewer suggested, we have graphed the allelic expression ratios on the log₂ scale. The result in Fig 1 fits well with what reviewer 2 had suggested: when data are graphed on the log₂ scale, the AE ratios don't look as substantial as before, because variance of the log₂ ratios are smaller than variance of the non-log ratios. However, the graph still indicates that allelic expression ratios follow a bimodal distribution and a statistical test (likelihood ratio test) confirms the bimodal distribution fits data significantly better than a normal distribution (p<0.001).
4. Cis regulatory polymorphisms

We agree that allelic expression imbalance is best explained by the presence of cis-regulatory polymorphisms, but a systematic search for these regulatory variants is well beyond the scope of this study because there is insufficient information about SCN5A regulatory elements to determine a priori the functional significance of candidate cis-regulatory polymorphisms. A thorough systematic search would have to include extensive functional characterizations of the transcriptional control mechanisms in SCN5A and this is well beyond the scope of our present study.

We have added a statement to the Discussion about this possibility (p. 9).

5. Functional nature of the variant

We focus on this variant because of prior genetic association with cardiac arrhythmias and sudden death, as well as in vitro evidence that the variant causes dysfunction of the sodium channel. We have added a statement to the Introduction (p. 3) to emphasize that this variant is functionally significant.

Our main hypothesis tested in this study was that the variant allele (which is functional) confers increased risk of SIDS. We performed the additional analysis suggested by the reviewer and found that the proportion of cases with elevated variant allele expression was not statistically different from controls. We have added this to the Results (p. 8) and the Abstract (p. 2).