Reviewer’s report

Title: Exome sequencing helped the fine diagnosis of two siblings afflicted with Timothy syndrome (TS2)

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Reviewer: Jill Johnsen

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Frohler et al describe a tiered approach to identifying the causative mutation in a family with arrhythmia, first by a candidate gene approach, and then utilizing whole exomes when no clear mutations were found in the candidate genes. The pedigree exhibits early childhood onset long QT syndrome (LQTS) in two siblings. Through whole exome sequencing they identified a p.G402S CACNA1C gene variant previously implicated in Timothy syndrome, a syndrome which includes cardiac arrhythmias and for which there is experimental evidence as to the function of variants at this residue. The authors conclude that a genome wide approach was effective and should be considered in the pursuit of the molecular diagnosis of suspected inherited syndromes.

Major Compulsory Revisions:

1. Manifestations of the syndrome (partial syndactyly) should be included earlier in the description of the cases (perhaps a table of characteristics?). There needs to be clarification if it has been shown that the other manifestations of the syndrome are truly absent, rather than not overtly present but not investigated?
2. Please comment if the cognitive/developmental delay in the proband could be entirely attributed to the brain injury acquired at the time of her first event and resuscitation, or could that traumatic/anoxic event be confounding clinical detection of developmental delays truly consistent with the syndrome?
3. Has the father had an EKG or other investigations?
4. The authors make the case that the father is relatively unaffected, and attribute this to mosaicism, which is entirely plausible, based upon the relative number of reads in the next gen data with the mutation, the chromatogram peak height of the variant in the Sanger sequencing, and the relative absence (?) of smaller bands using a restriction digest specific to the variant. However, there are still other explanations for these findings, such as structural variants such as CNV or artifacts. Confirmation on an independent DNA sample would be warranted if it has not been done, and consideration of sequencing DNA from another tissue might offer stronger evidence of mosaicism.
5. Page 10, the authors state, “So the less sensitive method, in this case, appeared to be more sensitive”. This needs to be revised, the methods are different, these finds are not truly a an approach to make comments on the “sensitivity” of the methods.
6. Was the informed consent that was signed under an IRB or similar?

Discretionary Revisions

1. The structure of the article is narrative, but would be improved by at least loose organization such that the methods, results, and discussion together.

2. What is the explanation for the precocious puberty in the proband? Is this suspected to be part of the syndrome?

3. What about the father’s relatives? Although seemingly unlikely, mosaicism has multiple proposed origins that could lead to risk for a relative, particularly a sibling.

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

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests