Reviewer's report

Title: Comparative integratomics approach for cryptorchidism evidences joint genomic relations with muscle-contraction pathway and Noonan syndrome

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Reviewer: julia barthold

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The authors use their previously published "integratomics" methodology, which integrates genomic, transcriptisoic and protein interaction data to identify potential pathways and gene candidates associated with, in this case, cryptorchidism. Although this specific methodology is not validated at this time, in the sense that genes so identified have yet to be shown reproducibly as functional candidates for disease, it is generally a reasonable way to generate hypotheses that can be tested in animal and/or human populations. However, a key step is inclusion of all available high quality data and avoidance of less reliable data that could potentially skew the analyses. In that regard, I believe that the genes included in the analysis require more careful screening to avoid false positives.

Major Compulsory Revisions

1. Genes associated with syndromes that include cryptorchidism as a phenotypic manifestation likely provide the most reliable data for inclusion in the authors' analysis. However, many syndromes, including Noonan's, are genotypically heterogeneous and genotype-phenotype correlations for cryptorchidism are lacking. Similarly, there is variation in the prevalence of cryptorchidism in the various syndromes and if rare, may be coincidental since the overall prevalence of this phenotype is relatively high in the population. I feel it is important to validate inclusion of known genetic syndromes in this analysis based on a careful, primary review of the literature; only those in which cryptorchidism is a regular feature should be included. Conversely, the authors failed to include some genes recognized as causative for syndromes whose phenotype includes cryptorchidism based on documentation in the literature, OMIM and/or the London Dysmorphology Database.

2. Similarly, as the authors note, gene candidates were not consistently associated with cryptorchidism in association studies; one may question whether the level of validation is sufficient for some to warrant inclusion in the present analysis. A recent GWAS reported for cryptorchidism (Dalgaard et al, J Med Genet. 2012 Jan;49(1):58-65. Epub 2011), although not independently validated, was not included. Unfortunately, many existing case-control association analyses of cryptorchidism may be underpowered and/or have other methodological shortcomings that limit their reliability.

3. As noted for human candidates, MGI data should be validated to assure that
specific transgenic models are associated with cryptorchidism. Also, surgical and antiandrogen models of cryptorchidism referenced by the authors are probably not relevant to the present analysis.

4. A major concern is the use of a single rat gene expression study as the source of >50% of gene candidates for this analysis. Thousands of genes were differentially expressed in fetal gubernaculum in that study between the wild type and mutant rat strains, but only 112 were included in the present analysis. This approach clearly provides a bias toward the same conclusions drawn by the previous authors. The present authors should address the concern that these data may not provide a reliable source of primary gene candidates, and likely less reliable than transgenic or well-validated syndromic gene candidates.

5. The significance of genetic variants within putative gene candidates is potentially interesting, but does not indicate functional significance per se and should be excluded (Table 4).

6. The enrichment analysis (DAVID 6.7) is biased in that it uses the candidates themselves and their "first neighbours" as background for the analysis. The appropriate background for human orthologs should be the human genome.

7. The meaning of the term "joint genomic relations" in the title is unclear, and based on the above methodological concerns, the link made by the authors between cryptorchidism and cardiomyopathy may not be generalizable, and is not supported by epidemiologic data linking the two phenotypes.

Minor Essential Revisions
1. The phrase "genetic cause for CO" on page 9 is not supported by the present data and should be modified.

2. It is unclear why "biomarker development" was used in the title of section #4, as this topic doesn't seem relevant nor was it specifically discussed in that section.

3. In the Conclusions, I question how unreliable data (as discussed above) could "help in solving the problem of fragmented and often contradictory data extracted from methodologically focused studies." There seems a real risk that unreliable input will give unreliable results. This statement should be removed or justified.

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, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests.