Reviewer’s report

Title: Use of a Targeted, Combinatorial Next-Generation Sequencing Approach for the Study of Bicuspid Aortic Valve

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Reviewer: Maria Grazia Andreassi

Reviewer’s report:

This paper described an interesting approach using combinatorial pooling and targeted multi-gene sequencing in a large cohort of patients with Bicuspid aortic valve (BAV).

This design resulted able to identify 33 rare, non synonymous exonic variants predicted damaging by in silico analysis. Traditional Sanger sequencing methods confirmed 31 of these 33 changes (94%). The study is clearly presented and the findings provide insights into a rapid and cost effective NGS method based on sample pooling and targeted capture strategy.

I have only several questions/comments which should be clarified by Authors

Minor Essential Revisions

How was the ascertainment of BAV confirmed in each patient? How many patients had a family history of BAV? Please provide more details on the clinical characteristics of study population.

The authors state that of two variants were de novo, both present in the same individual with a family history of coarctation of the aorta. The authors should better clarify these variants. The table 1 is not clear because it reports two de novo and two unknown variants, but only p.T545M variant in MCTP2 gene seems to not have a minor allele frequency in 1000G. Please clarify.

The authors discussed a cost analysis of this pooling technique compared to both whole exome sequencing and whole genome sequencing, but what about a comparison of cost respect to targeted gene sequencing done on individual pools wherein each sample is labeled with a unique genetic “barcode”?

Level of interest: An article of importance in its field

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