Assessing the analytical validity of SNP-chips for detecting very rare pathogenic variants: implications for direct-to-consumer genetic testing

Dear Dr. Wright,

Thank you for sending us your revised paper. I am afraid I write with some bad news. After careful consideration, our statistician, Professor Jon Deeks, has major reservations about the paper. These are explained in his review.

I recognise that this is particularly disappointing news considering the amount of work that has gone into the revisions of this paper, but we cannot publish papers that do not meet with the approval of our chief statistical consultant. I do hope that his comments might allow you to rework the paper and submit it to another journal.

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Very truly yours,

Dr Elizabeth Loder

eloder@bmj.com

Reviewer: 1

Comments:
To authors and editors

Better, but not there yet.

The revision makes quite clear that the study deals with the analytical validity of SNP-chip testing for rare pathogenic variants. Its continued use of the terms “accuracy” and precision” still obfuscate the major point of the paper. The problem with both of these terms is that they have different and overlapping definitions throughout the literature. If the authors would define “analytical validity” in the Introduction they can eliminate using accuracy and precision altogether. For instance, on p. 5, line 45, they could follow the sentence that ends “…how well SNP-chips detect rare genetic variants” with a sentence like: “Analytical validity measures how well they do so.” They could then define “analytical validity” or refer to the Definitions Box. In the Definitions Box, their definitions of sensitivity and specificity, and if they like, precision, can be subsumed under the definition of Analytical Validity. It would also be helpful to physician readers to contrast analytical with clinical validity in which sensitivity can be defined as “the proportion of people that are found by the index test who have or will get the disease” and likewise for clinical specificity. Throughout the paper, “analytical validity” can then be substituted for “accuracy” and “precision.”

The authors introduce the term “minor allele frequency (MAF)” on p. 6. But only in Table 1 does it become clear that it is used to distinguish common from rare variants. Without further elaboration,
the paper generates confusion between analytic and clinical testing. The presence of a “minor” allele indicates a heterozygote at the locus; there would be no minor allele in true homozygotes. Yet for recessive disorders, heterozygotes are not at risk of disease. For the non-BRCA disorders mentioned on P. 8, par. 1, the authors should explicitly indicate whether or not heterozygotes get the disease.

Specific comments

P. 5. Line 21. After suggesting that SNP-chips can be used to discover predispositions to complex multifactorial diseases...[but] “perform poorly for genotyping rare genetic variants,” they should insert the clause: “such as those incriminated in Mendelian (single gene) disorders,” before completing the sentence.

P. 6, lines 21-26. These sentences should be moved to the supplement. The acronym “UCSC” had not been defined.

___, lines 35-38. Sentence should be moved to supplement. The acronym “gVCF” has not been defined.

___, line 34 and 36. Using “directly” before “genotyped” adds nothing.

P. 8, lines 40-45. These sentences are not germane and should be deleted. The next sentence should start with “Most DTC companies...”

___, line 50, “third part” should be defined.

P. 9, line 12. The following should be added to the last sentence: “but they should have a gene sequencing test first.”

___, line, 16. “sequencing” should replace “standard diagnostic”

Title. I don’t think the revised title will grab clinicians’ attention. How about “Limitations of SNP-chip technologies used by companies offering detect-to-consumer genetic testing to detect rare pathogenic genotypes”?

Additional Questions:
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In their response, the authors have argued against the relevance of many of the points in my previous report. I can identify that there has been a degree of misunderstanding between us in the use of terminology – particularly around the term “analytical accuracy”, but the main arguments in my previous report relating to disease prevalence remain important and I would respectively request that the authors reconsider my points and revise their manuscript. In its present form I do not think that the paper is not suitable for publication in the BMJ.

1. The authors are clear that they see their study as one of “analytical validity” and not “clinical validity”. These phrases are used in different ways for different types of tests. In my report I was using the terms as they are most commonly used for laboratory tests, particularly from clinical chemistry, where “analytical validity” is a term used for early stage in the assessment which often uses atypical specimen sets rather than samples from real patients, and may use the tests in the hands of laboratory technical experts rather than health service staff. The results of these studies provide evidence of the maximal value of a test, and not how well it would perform in a real world setting. They are not suitable for publication in the BMJ as they do not provide an answer that is applicable to clinical practice.

However, my understanding is that for genetic test the phrase “analytical validity” is used in a different way, relating to identification of the gene or genetic change and not necessarily the presence of a specific disease. This is a different usage of the term than for many other diagnostic tests - that
it relates to the target condition being a genetic change rather than to a disease. It would be helpful if the authors directly clarified this in their paper that this is how they are applying this term.

If I am correct in the above assertion, there has possibly been some misunderstanding in several of my previous comments, particularly comment 9. However, the main thrust of the arguments of my report apply, and the authors need to reconsider my points.

2. Renaming positive predictive value "precision" is not helpful. Precision has a very clearly stated meaning in measurement science. The concept which I understand the term is used for is exactly the PPV, but of the gene or genetic change and not disease. I do not understand why the authors do not think that this term is appropriate. It gives the probability of the target condition in those with a positive test result, which is exactly the phenomenon you are using it to describe. The fact that this is a genetic analytical validity study rather than clinical validity study seems irrelevant to this issue, particularly when you are discussing the use of the tests for "clinically actionable" purposes. Likewise the term "likelihood of a true positive result" is non standard, undefined and ambiguous, and should be avoided.

3. I am somewhat baffled by the authors response to the issue of prevalence – particularly seeing that their responses to points 1 and 11 appear to contradict each other. Point 11 indicates that the challenge is the high number of false positives, which occurs because of the very low prevalence of the disease despite high specificity. This is the same argument I make in Points 1 and 8.

4. The view taken in the response to my previous point 1 that prevalence is a concept which is irrelevant in this paper. As I explained in point 8 of my report, there are two reasons why the tests perform poorly – the first is that they fail to be positive in patients with the condition, which is measured by test sensitivity. The second is that the prevalence of the condition is so low, that despite the specificity of the test being exceptionally high, most test positives will be false positives rather than true positives. If the authors are only interested in the poor sensitivity issue, then I would suggest that this paper has no place in the BMJ. If they include discussion of the meaning of the test results in a population screening application (which they can do as they have Biobank data reflecting the very low prevalence) then the paper will provide useful information to the debate concerning the clinical meaning of test results, and could continue to be considered for the BMJ.

I would suggest that the editor elicit the intent of the authors as to whether they wish to follow, and if they are willing to revise it for further consideration by the BMJ please return it to me so that I can review the adapted manuscript.

Additional Questions:
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