Article details: 2021-0272
Title: Comparing genome sequencing technologies to improve rare disease diagnostics: a protocol for the evaluation of a pilot project, Genome-wide Sequencing Ontario
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Reviewer 1: Douglas Wilson
Institution: Obstetrics and Gynecology, University of Calgary, Calgary, Alta.
General comments (author response in bold)

1. Interpretation section needs more detail and process explanation with rules for evaluation
Thank you for this comment. We are hopeful that the revision now provides adequate detail on our approach to evaluation and the anticipated implications of our findings.

Reviewer 2: Dr. Martin Dawes
Institution: UBC
General comments (author response in bold)

1. There was a clear description of GS and ES for people not used to these technologies. Examples of both would have been useful in distinguishing the two. I would put in a reference for CNV's and include an example (CYP2D6) in the GS description as many readers may not be aware of the CNV.
Thank you for this comment. We have provided a reference for structural variants in the Introduction. While CYP2D6 is a well characterized gene related to enzyme metabolism, our preference would be to avoid including a specific example since the range of relevant variants is vast and not all are highly relevant to the study population

2. Page 5 Line 42 – it would be useful to include a statement that the Ontario process is similar to many provinces to ensure readers understand that this research would apply to any province doing GS.
Thank you for this comment. We have included a statement that reflects upon access to GWS (and alludes to related exceptional access programs in other provinces) in the third paragraph of the Introduction. This text reads, "As a result of policy efforts and the evolution of exceptional access programs in Ontario and other Canadian provinces in recent years, approximately 1500 Canadian patients with rare disease, per year, receive access to clinical ES performed outside the country."

3. It would be useful to give a ball park figure of the prevalence of people tested and the incidence either just in Ontario or across Canada.
Thank you for this comment. We have now included an estimate of the number of Canadian patients who have had access to GWS in recent years. As above, "As a result of policy efforts and the evolution of exceptional access programs in Ontario and other Canadian provinces in recent years, approximately 1500

Canadian patients with rare disease, per year, receive access to clinical ES performed outside the country."

Methods

4. P6 L8 Spell out CHEO before using the acronym
Thank you for this comment. We have made this correction.

5. Stratified randomization – is there a block level within this process and if so what number of patients? Is the randomisation concealed from the clinicians prior to allocation?
Thank you for this comment. We have clarified our approach to randomization; the text now reads as follows, "Patients are randomly assigned to ES or GS using a 1:1 ratio and an un- blinded stratified permuted block randomization design. Cases are stratified by clinical site, phenotype, and molecular testing history. Depending on the expected recruitment in each stratum, block sizes (i.e. number of patients in each strata) are 2, 4 or 6."

6. Statistical analysis: You will study diagnostic utility but what do you mean by that. Are you looking at likelihood ratios and comparing those? What is the statistical approach you will be using for that?
Thank you for this comment. Diagnostic utility is defined as the rate of causative, pathogenic, or likely pathogenic genotypes in known disease genes. It will be reported as the proportion of cases for whom diagnostic, partially diagnostic, and medically-actionable secondary variants are identified. We have clarified the Statistical Analysis section of the Methods. It now reads as follows, "We will analyse our data using descriptive statistics (mean, median, range and standard deviation). Point estimates for diagnostic utility and timeliness will be compared statistically for ES and GS. We will examine the relationship between clinical characteristics (e.g. age, age of onset, sex, phenotype, prior testing history) and diagnostic yield using parametric or non-parametric univariate statistics as appropriate. If indicated, we will explore explanatory variables of diagnostic utility using a regression model."

7. It looks like the study will only be looking at direct costings and not include non-direct? A reason for this approach, needs to be provided. It is not entirely clear how you will compare costs – is it per patient or is it per diagnosis? Given that both tests can provide information over a patient's lifetime will you do markov or other modelling of that to assess lifetime costs and gains? In terms of outcomes, I was expecting to see DALYs or QALYs as one assumes there will be a difference in clinical outcome between the two technologies. It maybe you are keeping that for further work with the data at a later date? I saw no plan to produce incremental cost effectiveness ratios? I think the whole costing analysis needs more description and rationale for the approach you are taking
Thank you for these comments. We have re-written the section related to our approach to costing and to cost effectiveness analyses. The text now reads as follows, "A cost per trio ES and cost per trio GS will be determined. Costs for each input related to the ES and GS laboratory workflows will be calculated by multiplying resource volume by unit price. For labour, time in minutes for each task will be multiplied by wage rates. The most recently available price units will be used (2021). Otherwise, the prices as reported in previous microcosting studies will be assumed to be stable based on consultation with lab managers.

The costs of laboratory workflow inputs for each sequencing approach will be summed to determine a per sample cost. A cost-effectiveness analysis will be undertaken from an institutional payer perspective in which the difference in costs between GS and ES will be calculated and divided by the difference in diagnostic yield to calculate an incremental cost- effectiveness ratio expressed as the incremental cost of GS compared to ES per additional patient with a pathogenic variant detected. As an implementation project, this CEA is limited to laboratory costs and outcomes. Given the complexity of diagnostic outcomes and the heterogeneity of the patient sample, it will not be possible to model patients' health states and the range of treatment options that might ensue from a given diagnostic result to generate health benefits, such as QALYs, over a lifetime. All costs will be reported in 2021 Canadian dollars (CAD). SQUIRE reporting guidelines will be used."

8. In the sub-group analysis what precautions will you take to ensure that findings are not due to the chance, due to the number of subgroups examined
Thank you for this comment. In our revised Statistical Analysis section, we have removed references to sub-group analyses.

9. I was surprised that as you will be evaluating two tools and randomising patients that there is no ethics requirement. That said I know every IRB is different.
Thank you for this comment. The absence of the ethics requirement was tied to the implementation cohort, which has been removed from the revised manuscript. As described in the Ethics Approval section, ethics approval is in place for those who participate in randomization.

10. This is a great project, and an interesting well written paper. I am keen to see the results. I started enthusiastically and then came away a bit disappointed that there was not more description of the analytic approaches as these are so challenging. This is not a straightforward comparison of two tests for one disease. I think the paper needs more work on describing the statistical analysis and the reasons for that. I think you need to lead the reader through the logic of the stats and the costing comparison approach and even include some examples.
Thank you for these constructive comments. We hope that our revision addresses these concerns.