Meeting report series

Report of the 4th DSC Working Group on Sequencing teleconference

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Organization

Organized by: IRDiRC Scientific Secretariat
Teleconference

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Agenda

- Sequencing for diagnostics vs. sequencing for research and the grey zone between the two
- Accreditation/certification of the NGS sequencing process (ISO or other)
- Reporting of variants – previously known or unknown
- How do different countries deal with exome sequencing for RD – an inventory?
The purpose of this teleconference was to discuss and form an opinion on topics relevant to this WG that often mentioned in informal discussion.

**Sequencing for diagnostics vs. sequencing for research and the grey zone between the two**

In the context of research, the objective of sequencing experiments in rare diseases is not always clear: research only or research and diagnostic, particularly in case of undiagnosed patients. The issue of return of incidental finding should be clarified before the start of the experiment. What should be done when going back to results years later when the necessary knowledge is finally available?

Members of the WG agreed that:

- Separation of clinic and research in sequencing is necessary. As an example: participants of studies for biobanks do not think they are going to enter a genetic testing.
- When doing research, the responsibility is toward the research question. Research studies should not be expected to return incidental findings, as researchers may or may not have the expertise to really evaluate the results.
- If researchers are close to the clinical and wish to report findings, they should be sure that it is going to improve the observation.
- However if patients join a research project to find a diagnosis and the results provide a clear explanation for the disease, results should be returned back to the patients.

There are cases where the situation is clear:

- At the University of Leuven, discussion led to the agreement that researchers cannot be aware of clinical situations of patients, and that they are not expected to look at incidental finding when doing research. This is only valid if the aim of the project is research, but if not the purpose is to help individual patients.
- In France, research rules indicate that data is only for research purpose, and that researchers don’t have to go back to patient. In case of gene discovery during research, researchers can inform the clinician who can decide to investigate the specific gene for diagnostics in clinical setting. Most of the time, researchers will not give the information in case of incidental finding.

Part of the topic is tackled in the EuroGentest guidelines, which specify that:

- Research results have to be confirmed in an accredited laboratory before being transferred to the referring clinician and patient
- Distinction between research and diagnostics has to be respected at all times, even if thanks to these novel technologies, the borders between them are blurred.
Other comments:
- Too many patients should not be sequenced in research when they belong to the diagnostic field as there is not enough research funding to sequence all clinical case (clinicians tend to include unsolved case into research projects).
- Clinic to Research needs more guidance as there is no Institutional Review Board governing that area of work, in contrast with Research to Clinic area of work.

Accreditation/certification of the NGS sequencing process (ISO or other)

Laboratories/institutes are moving towards ISO certification (such as ISO 9001 - Quality Management Systems; ISO 17025 - General requirements for the competence of testing and calibration laboratories; ISO 15189 - Medical laboratories: Requirements for quality and competence) for diagnostics or collaboration with Industry for example.

Two main issues were reported by the WG:
- It is difficult to find standards for sequencing, particularly in Europe. CAP (College of American pathology) have standards for sequencing, but only semi-hard standards as user still has to set its own standards when analysis materials from CAP.
- Accreditation/certification or at least the same quality as for diagnosis sequencing is difficult to impose to research laboratories. The goals of projects will be highly variable (looking to common variants where sequencing massive batches with limited coverage is different from RD studies) and it is thus challenging to come up with those types of standards that are related to coverage and content.

However, members of the WG agreed that basic quality controls should be implemented - using control, avoiding contamination, etc. - that everyone should follow when doing clinical or research.

In research, there is no best practice standard and the quality of sequencing is difficult to evaluate as there if often not enough details in the publication. It is better to balance the quality with the attended goal of the project. However, every project has a different set of priorities, questions to address, budget, etc. so it is really hard to control beyond the peer-review process (grant application and publication).

⇒ This topic might be interesting to pursue for this WG, such as defining some measures in order to be able to give a couple of matrix that can be measure easily, or launching an email to journal editors to inquire if they are putting enough emphasis on quality of exome as they are putting emphasis on other parts of studies (statistics, etc.).

Reporting of variants – previously known or unknown

This topic was already partially tackled in the previous discussion on incidental findings.
With regard to variant of unknown pathogenicity, members of the WG agreed that:
- From a diagnostic standpoint, variant of uncertain pathogenicity should be reported to enable follow up.
From a research standpoint, only variant of certain pathogenicity should be reported. At research level, discovery of a new variant of unknown significance is the beginning of the project. It should be then studied to demonstrate the involvement of this specific variant.

**How do different countries deal with exome sequencing for RD – an inventory?**

From the discussion of the WG, it appears that different countries approach the topic in different ways:

- In France, they are trying to establish a network at national level for diagnosis by building an inventory of all variants for one disease (National Exome variant database); inventory available to researchers. This database is restricted to RD and family members.
- In Belgium, there is an initiative where people doing exome sequencing in genetics can share at least exome variant files from unaffected family members to create a national database (referent exomes).
- In the US, the Exome Aggregation Consortium (ExAC) has announces the public release of allele frequency data from a massive data-set of over 61,000 exomes.
- Netherlands has given the example with the Genome of the Netherlands project leading to a Dutch Variant databases.
- At the international level, MatchMaker project - in development - aims to increase gene discovery by enabling databases to link up through API to work on genomic matches of phenotype and genotype.
- In Spain, they have generated a 300 exome reference database.

Two members of the Global Genomic Medicine Collaborative (G2MC) initiative where the same questions were raised and which is trying to catalog what is happening in different countries were invited to join the teleconference but there were not available.

The Scientific Secretariat informed the participant of the teleconference of the recent establishment of the WG on Population Controls Variant Datasets, supervised by the Diagnostics Scientific Committee, working on the creation of a database for population controls.

- One person of this WG should join the new WG on Population Controls.
- IRDiRC should put pressure for the accessibility of these national DBs.
- WG could prepare some recommendations to people who want to build such registries or national registries (specificity about the data quality) as the problem in all these databases or national registries is the methods of frequency extraction that depends on the kit used and so forth. Without this level of information, frequency calculation will be underestimated and it is necessary try to capture the information about what have been sequenced, what are the bed files, what you expect, what is the pipeline used, in order to identify bias or artefact of a specific platform bioinformatic process.
Deliverables

Members of the WG have to think about how to move forward in contributing to IRDiRC’s objectives what they want to achieve. This will be first define by email exchange in November before discussion it on a teleconference. Possibilities of topics: quality criteria, or sequencing per se, quality on sequencing data