July 2014

Authors
Dr Leila Luheshi
Dr Sobia Raza

Contact
Dr Leila Luheshi
leila.luheshi@phgfoundation.org

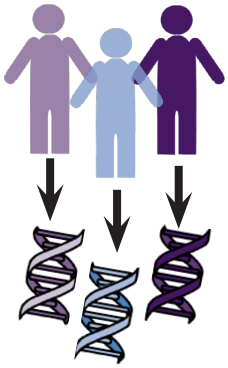
Setting the right standards for clinical genome analysis

As the proportion of the genome accessible for clinical interrogation increases from a handful of known disease-associated genes to encompass nearly all our 20,000 genes, the provision for genetic testing is changing rapidly. Are existing guidelines and standards of practice fit to meet the challenges - of scalability and accuracy of analysis and interpretation of the test and its results - presented by whole genome based testing?

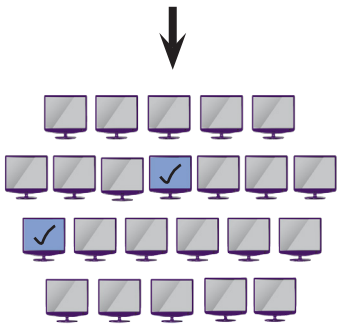
The development of whole genome analysis (WGA) is reaching a tipping point in the UK as it moves from being a tool used only by researchers to a potentially powerful clinical diagnostic technique. Yet the lack of consensus on the optimal method for WGA, makes variations in approaches used to analyse genomic sequence data inevitable. This variation can result in disparities in outcome - *i.e.* the same genome, analysed by two different methods, may yield two different diagnostic conclusions. Whilst it is constructive to allow WGA providers flexibility in their choice of methods and approaches (in order to drive innovation and work towards optimal solutions), it is nonetheless incumbent upon the health system to work to minimise variations in practice, to ensure, as far as reasonably practicable, that patients receive the same quality of care (and outcomes) regardless of the methodology used to achieve their diagnosis.

Standards and best practice guidelines are an essential part of the quality assurance framework within NHS genetics services. Ensuring such standards are fit for purpose and universally adopted will be particularly important as the number of providers for genetic testing and analysis services in the UK increases.

3 patient genomes



Genomes from 3 unrelated patients with inherited diseases and their families sequenced and analysed



2 / 23
correct

Only 2 out of 23 laboratories reported the likely disease causing variants for all three cases

Left: Results of the CLARITY challenge, a research contest for genome interpretation groups to identify disease causing mutations in three young patients[1]

Ensuring quality of genetic diagnostic services

In addition to conforming to best practise guidelines, any labs or services used by the NHS:

Must be accredited *(i.e.* possess formal recognition by a third party, of competence to perform a specific task).

Accredited laboratories are required to participate in external quality assurance (EQA) schemes, the accredited providers of which include national (UKNEQAS) and international (EMQN) services.

Together, standards, guidelines, accreditation and EQA are intended to ensure test results are accurate, reliable and comparable regardless of where they are performed.

Current guidelines for clinical genetic analysis

Existing best practice guidelines for clinical genetic testing in the UK cover the analysis and interpretation of genomic sequence data and sequence variants[2,3]. These guidelines have been developed by clinical scientists working in NHS genetics laboratories under the auspices of the Association of Clinical Genetic Science (ACGS), part of the British Society for Genetic Medicine. Guidelines have also been published by groups from the Netherlands, Australia and the US[4-7].

Current ACGS guidelines focus on 'targeted' genetic tests, *i.e.* where only a small (typically 5-100) set of genes related to a patient's phenotype are sequenced and analysed. As we enter the era of genome wide analysis, the entire genome will be sequenced and available for analysis. The most profound effect of moving from targeted sequencing to whole genome sequencing is the expansion in the search space for disease causing changes (known as pathogenic variants). This will result in a significant increase in the complexity of the analysis, the number of variants detected and, potentially, the uncertainty of the results obtained. The development of consensus best practice guidelines that help laboratories to manage these issues will be vital to ensuring high quality services can be provided[8].

Moving from genes to genomes - what additional guidance and regulations may be required?

Most steps in genome wide analysis are sufficiently similar to those undertaken in targeted NGS testing to be effectively regulated by existing standards and guidelines. However, as highlighted above, additional guidance on how to manage the analysis of the increasing volume and complexity of data that WGA produces will be required. Below we focus on two aspects of WGA for which best practice guidelines are needed prior to, or indeed during the gradual implementation of this approach, if unwarranted variation in the quality of the service is to be minimised.

3 million to 1 – variant filtering and prioritisation

3 million variants are typically detected during whole genome sequencing, compared with the reference genome. A clinical scientist looking for the molecular cause of a rare disease is typically looking for the one or two variants out of those millions that is the 'true' cause of their patient's disease.

The challenge faced by a scientist undertaking WGA is to develop a rational and robust search strategy that maximises the chance of finding the disease-causing variant and minimises the chance of finding and misreporting a variant that is not the 'true' cause of their patient's illness. This is further complicated by the need to focus their 'search space' to a number of variants small enough to be subjected to the detailed analysis required to reach a confident diagnostic conclusion by the small cohort of appropriately qualified clinical scientists.

Specific guidance will be required for:

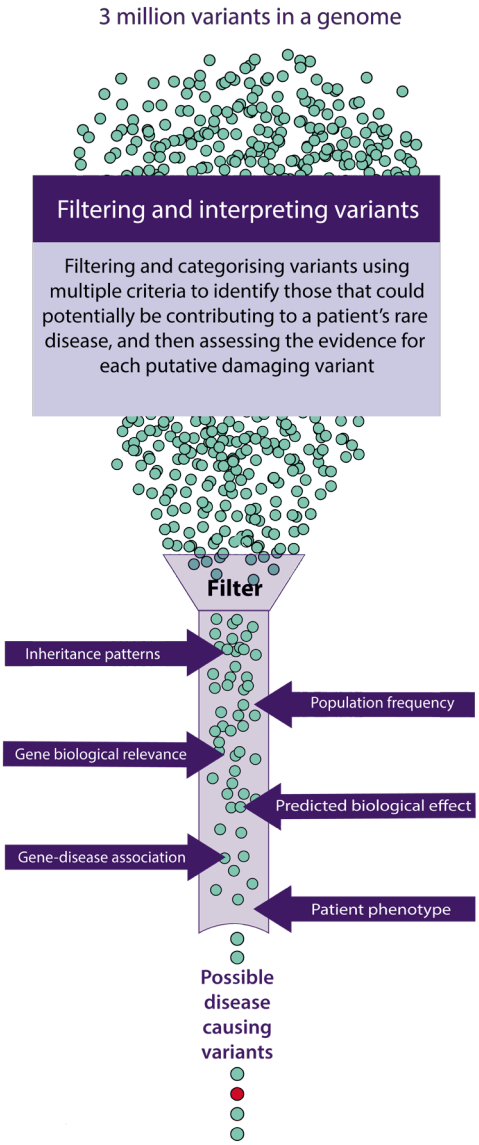
- Integrating methods for capturing standardised patient phenotype and family history to decide which parts of the genome to focus analysis upon.
- Developing and curating an agreed minimum gene set for each disease to facilitate targeted analysis.
- Defining procedures and parameters for identifying variants, annotating them, and filtering to find the possible disease causing ones, and including appropriate population data in these steps.
- Establishing rules used to generate prioritised lists of genes and variants for manual interpretation by clinical scientists.

The consequence of the current lack of consensus on how to tackle each of these issues will be that different laboratories, pursuing different whole genome analytical strategies may, when presented with the same patient, reach different, often incorrect, diagnostic conclusions[1].

Scaling up and automation – delivering more diagnoses

Interpreting the clinical significance of genomic variants is a labour intensive process where the evidence for each putative damaging variant is carefully assessed by clinical scientists. As the number of variants to be interpreted, and the number of patients being offered genomic testing increase, so too will the size and impact of this bottleneck. The solution to this is to automate a greater proportion of the WGA workflow, particularly the filtering of variants (so as to arrive to only a small number of candidate causative variants), and aspects of interpretation, in order to manage these changes in scale. It will therefore be vital that guidelines are in place to ensure such approaches are reliable and that they are acceptable substitutes for human judgement.

In particular it will be important to ensure that computational tools and automated 'scalable' workflows are subject to rigorous validation and that the limitations of the underlying sources of information on which they rely are well understood and do not adversely affect their performance.



The common assessment criteria for filtering and interpreting genomic variants. Depending on how cut-offs are set for these criteria, the output of possible pathogenic variants can change.

The policy challenge – balancing high quality care and innovation in the genomic era

Beyond the need to increase the scope of specific guidance on the conduct of genomic analysis, there are several general principles that should be applied to help ensure the management and delivery of genetic testing is fit to safeguard patient wellbeing and benefit in the genomic era:

- Standards and guidelines are essential and must be in place as genomic services undergo radical restructuring, including diversification in the providers of sequencing and analysis services as envisaged by the 100,000 Genomes Project.
- Standards must adapt to be cognisant of the challenges of genome scale analysis, the need for an increased degree of automation and the realities of the heightened uncertainties this will entail.
- The role of external quality assurance programmes must be emphasised, especially during this transitional phase of the implementation of WGA when variation in practice between laboratories is likely to be greatest. This includes quality assurance for each element of the diagnostic pathway, from pre-analytic to the provision of results and recording in health records.
- Existing professional groups are well placed to develop standards and oversee quality assurance processes but may need to include other stakeholders involved in the delivery of genomic medicine from within and beyond the NHS.
- A greater understanding of the potential pitfalls and limitations of WGA in the clinical diagnostic setting is important, to develop standards that promote high quality genetic testing but are not disproportionately burdensome such that they inappropriately restrict access.

The delivery of genomic medicine in this exciting but transitional period will require striking a difficult balance - between the need to retain a rigorous, high quality service that safeguards patient safety, and increasing accessibility to genomic medicine. Comprehensive guidelines, applied proportionately, will make a significant contribution to achieving this balance.

References

1. Brownstein CA *et al.*, Genome Biolo. 15(3) (2014). 2. Wallis Y *et al.* , ACGS (2013). 3. Ellard S *et al.*, ACGS (2014). 4. Weiss MM *et al.* , Hum Mutat. 34(10) (2014). 5. Website: http://pathwiki.rcpaqap.com.au/pathwiki/ 6. Rehm HL *et al.*, Genet Med. 15(9) (2013). 7. Richards CS *et al.*, Genet Med. 10(4) (2008). 8. MacArthur DG *et al.* Nature. 508(7497) (2014).