Introducing diagnostic applications of ‘3Gb-testing’ in human genetics

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Technical Roadmap for diagnostic implementation of Whole genome Sequencing (WGS)

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# TABLE OF CONTENTS

Introduction ................................................................................................................................................................... 3  
1. NGS Wet-lab Innovations .......................................................................................................................................... 4  
2. Gap analysis on tools, methods and approaches to introduce WGS into routine diagnostic practice ............ 13  
3. Statement on diagnostic reporting pipeline ........................................................................................................... 15  
4. Variant validation .................................................................................................................................................... 20  
5. Training requirements ............................................................................................................................................. 23  
6. Ethical proposals for the implementation of whole genome sequencing ............................................................... 26  
7. Guidelines for WGS EQA: issues and requirements .................................................................................................. 33  
8. Requirements for HTA: clinical and economic evidence ........................................................................................... 35  
9. 3Gb-TEST Policy Statement on New Technologies and Their Clinical Utility in Genome Diagnostics .......... 43  
10. Conclusions ........................................................................................................................................................... 45  
Summary ...................................................................................................................................................................... 51  
Acknowledgements ..................................................................................................................................................... 52
INTRODUCTION

By B. Janssen, E. Thomassen, and E. Bakker

The rapid development of new high-throughput and massively parallel DNA sequencing technologies has substantially reduced both the cost and the time required to sequence an entire human genome. These so-called ‘next-generation’ sequencing (NGS) technologies are currently causing drastic changes in the field of molecular diagnostics. It will soon be easier, quicker and cheaper to sequence an entire genome than to sequence a single gene (fig 0.1). In the near future, Whole genome sequencing (the ‘3Gb-Test’) will be a more effective and a financially viable alternative for genetic diagnostic services.

![Fig. 0.1: The price per genome according to the NIH (www.genome.gov/sequencingcosts).](image)

In this technical roadmap we provide a forecast on these developments. The future of molecular testing will pose a challenge to both laboratory and clinical geneticists, due to the wealth and complexity of the sequence information obtained. We will discuss the clinical utility and anticipated difficulties of whole genome sequencing. It is important that patients and families receive correct advice and appropriate management. It is also important to ensure that the introduction of such new technologies are an effective use of healthcare budgets, requiring supporting clinical and economic evidence produced as part of robust health technology assessments. Therefore, technical issues, quality issues, and ethical and health technology assessment issues are discussed with equal priority. Therefore, technical issues, quality issues, and ethical and health technology assessment issues are discussed with equal priority.

The information provided here has been collected in the context of the European FP7 project ‘3Gb-TEST’ (Nr. 602269). The 3Gb-TEST project aimed to support the implementation of whole genome sequencing throughout Europe and to help society prepare for this change. In the period 2013-2015, the project partners have organised symposia, workshops, courses and an EQA (External Quality Assessment). This roadmap summarises the final conclusions of the project and may serve as a guideline document for the health care community and the European Committee.
Technical Roadmap for diagnostic implementation of WGS

1. NGS WET-LAB INNOVATIONS

By H. Buermans and J. den Dunnen

Impressive progress has been made in the field of Next Generation Sequencing (NGS). Through advancements in the fields of molecular biology and technical engineering, parallelisation of the sequencing reaction has profoundly increased the total number of produced sequence reads per run. Here, we review the technical background of the different commercially available NGS platforms with respect to template generation, the sequencing reaction, and their potential use in whole genome sequencing (WGS). Finally we take a small step towards what the upcoming NGS technologies will bring.

Introduction

The growing power and reducing cost has sparked an enormous range of applications of Next generation sequencing (NGS) technology. Gradually, sequencing is starting to become THE standard technology to apply, certainly at the first step where the main question is "what is involved", "what is the basis". It should be realised that for many applications whole genome sequencing would always have been THE method of choice, yet it was science-fiction, technically unthinkable and later possible but far too costly. Until recently we performed genome-wide association studies (GWAS) using SNP-arrays simply because we could not afford to perform whole-genome sequencing in thousands of individuals. This is changing rapidly and sequencing will become our molecular microscope; the tool to get a first look. Although replication, transcription, translation, methylation and nuclear DNA folding are completely different processes, they can all be studied using sequencing.

An important advantage of sequence data is its quality, robustness and low noise. It should be noted that a successful NGS project requires expertise both at the wet lab as well as the bioinformatics side in order to warrant high quality data and data interpretation. The sequence itself is hard evidence of its correctness. A sequencing system will not produce "random" sequences and when it does this becomes evident immediately from QC calls obtained from spike-in controls. Furthermore random sequences will have no match and can be easily discarded during data analysis and when their number exceeds a certain threshold it is evident there is a serious problem somewhere in the study.

Sequence library preparation

All currently available sequencing platforms require some level of DNA pre-processing into a library suitable for sequencing. In general, these steps involve shearing of high molecular weight DNA into an appropriate platform-specific size range, followed by an end polishing step to generate blunt ended DNA fragments. Specific adapters are ligated to these fragments by either A/T overhang or direct blunt ligation. A functional library is required to have specific adapter sequences to be compatible with the further steps of the process (Figure 1.1).

Following adapter ligation Life Technologies (Solid, PGM, Proton) libraries require a nick translation step to get functional molecules while for the other technologies the sample is in principle ready for loading immediately after ligation. One may then choose to sequence these libraries directly as amplification free libraries or introduce a pre-amplification step prior to sequencing. It is important to realize that any step during pre-processing which involves amplification of the molecules [Dabney and Meyer(2012)] or which has been shown to be sequence biased, like ligations [1]
et al. (2011)], will impose a selection on molecules that end up in the sequenceable libraries.

seqencing, being the Illumina and LifeTechnologies Semiconductor sequencing.

**Illumina Technology**

All of the enzymatic processes and imaging steps of the Illumina technology take place in a flow cell. Depending on the specific Illumina platform it may be partitioned into 1 (MiSeq), 2 (HiSeq2500) or 8 (HiSeq2000, HiSeq2500) separate lanes. The Illumina platform uses bridge amplification for polony generation and a sequencing by synthesis (SBS) approach (Figure 2A). Forward and reverse oligos for amplification (one with a cleavable site), complementary to the adapter sequences introduced during the library preparation steps, are attached to the entire inside surface of the flow cell lanes. The first step for loading the library onto the flow-cell is denaturation of the dsDNA fragments into individual ssDNA molecules. When on the flow-cell, these hybridize to the oligo nucleotides on the surface which are used as primers to form an initial copy of the individual sequencing template molecule. The initial library molecules are removed and the copied, flow cell-attached fragments are used to generate a cluster of identical template molecules using isothermal amplification. This is done through cyclic alternations of three specific buffers that mediate the denaturation, annealing and extension steps at 60 °C. During these steps the 3' end of the copied library molecules can hybridise to the complementary oligos on the flow cell, thus forming a bridge structure. The final step is to remove one strand of the dsDNA fragments using the cleavable site in the surface oligo and to block all 3' ends with ddNTP to prevent the otherwise open 3' ends to act as sequencing primer sites on adjacent library molecules [Bentley et al. (2008)].

With optimal loading of library molecules one flow-cell lane will yield approximately 800-1000 K clusters per mm2. Optimal amounts depend not only on the concentration of the library, but also on the length of the molecules. Short molecules yield clusters with a small area that
are denser and therefore generate more intense signals. Loading a wide fragment size distribution will generate clusters varying widely in size and signal strength which may impair the number of passing filter reads.

### Table 1.1

<table>
<thead>
<tr>
<th>Sequence by Detection Run types</th>
<th>Run time</th>
<th>Read length (bp)</th>
<th># reads per run</th>
<th>output per run</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roche GS FLX Titanium XL+</td>
<td>single end</td>
<td>23h</td>
<td>700</td>
<td>1 million</td>
<td>700 Mb</td>
</tr>
<tr>
<td>GS Junior System</td>
<td>synthesis</td>
<td>Pyrophosphate detection</td>
<td>single end</td>
<td>10h</td>
<td>400</td>
</tr>
<tr>
<td>Life Ion torrent</td>
<td>synthesis</td>
<td>Proton release</td>
<td>single end</td>
<td>4h</td>
<td>200-400</td>
</tr>
<tr>
<td>Proton</td>
<td>synthesis</td>
<td>Proton release</td>
<td>single end</td>
<td>4h</td>
<td>125</td>
</tr>
<tr>
<td>Abi/solid ligation</td>
<td>Fluorescence detection of di-base probes</td>
<td>single &amp; paired-end</td>
<td>10 days</td>
<td>75+35</td>
<td>2.7 billion</td>
</tr>
<tr>
<td>Illumina/solexa HISeq2000</td>
<td>synthesis</td>
<td>Fluorescence; reversible terminators</td>
<td>single &amp; paired-end</td>
<td>12 days</td>
<td>2x100</td>
</tr>
<tr>
<td>HiSeq2500</td>
<td>synthesis</td>
<td>Fluorescence; reversible terminators</td>
<td>single &amp; paired-end</td>
<td>6 days</td>
<td>2x125</td>
</tr>
<tr>
<td>MiSeq</td>
<td>synthesis</td>
<td>Fluorescence; reversible terminators</td>
<td>single &amp; paired-end</td>
<td>65h</td>
<td>2x300</td>
</tr>
<tr>
<td>NextSeq 500</td>
<td>synthesis</td>
<td>Fluorescence; reversible terminators</td>
<td>single &amp; paired-end</td>
<td>29h</td>
<td>2x150</td>
</tr>
<tr>
<td>Pacific biosciences RSII</td>
<td>Single molecule synthesis</td>
<td>Fluorescence; terminally phospholinked</td>
<td>single end</td>
<td>2 days</td>
<td>50% of reads &gt; 10 kb</td>
</tr>
</tbody>
</table>

Bridge amplification is not a very efficient method for clonal amplification, i.e., the 35 cycles of isothermal amplification yield a mere 1000 copies of the initial molecule. Moreover, there will be predominantly outward growth of the clusters, there is a high probability of the template strands to re-hybridise instead of annealing to a new primer site on the glass surface and there is both an upper and a lower limit to the length of the template molecules that can be reliably amplified. In addition, DNA polymerases, which are known to have biases towards specific DNA templates, are used during the amplification processes. The bridge amplification scheme that Illumina exploits yields a high number of clusters, i.e., with good loading of the flow cell, the total number of reads generated per HiSeq2000 lane may reach 180 million. With a paired-end 2x100 bp read format the total output of one flow-cell lane is up to 36
Gb. A full run of 2 flow cells sequencing in parallel may yield 600 Gbase of data.

During sequencing, the polonies on the flow cell are read one nucleotide at a time in repetitive cycles. During these cycles, fluorescently labeled dNTPs are incorporated into the growing DNA chain. Each of the four dNTP species (A, C, T, G) has a single different fluorescent label which serves to identify the base and act as a reversible terminator to prevent multiple extension events. After imaging the fluorescent group is cleaved off, the reversible terminator is de-activated and the template strands are ready for the next incorporation cycle. The sequence is read by following the fluorescent signal per extension step for each cluster. Under ideal circumstances, all bases within a cluster will be extended in phase. However, a small portion of the molecules are not extended properly and fall either behind (phasing) or advance a base (pre-phasing). Over many cycles, these errors will accumulate and decrease the signal to noise ratio per cluster, causing a decrease in quality towards the ends of the reads.

The cycle time for the HiSeq2000 is approximately 1 hour. The major contributor is the imaging of the flow-cell. The enzymatic reactions take very little time at all. By reducing the imaging time, the whole sequencing process can be sped up considerably. This is implemented in the MiSeq and HiSeq2500 platforms by providing the option to decrease the total surface area to be imaged. In rapid mode, the cycle time can thereby be reduced to 5 and 10 minutes for the MiSeq and HiSeq 2500, respectively. Furthermore, with optimized reagents kits for these short cycle times it is possible to achieve a 2x300 bp paired end run on the MiSeq, with 85% of data points above Q30 and run times of 65 hours. However, the increased sequencing speed does come at a price. With the decreased surface area, the total number of data points that can be generated per run will reduce, increasing sequencing cost per nucleotide significantly.

Early 2014, Illumina has announced the release of two new sequencer models, i.e., the NextSeq 500 and the HiSeq X Ten. The former system was designed to be a highly flexible, smaller version of the HiSeq2500, providing both medium (40Gb) and a high output (120Gb) modes both with run times under 30 hours. The HiSeq X Ten was designed for one main purpose: enabling whole human genome sequencing and reaching the $1,000 genome in run costs. The main advancement enabling this is the introduction of the patterned flowcells. In contrast to the spatial random cluster generation of the HiSeq and MiSeq flowcells, the X Ten flowcells contain a pre-formatted grid of nanowells, which can each produce one sequence polony. This allows for optimised cluster densities and in combination with faster scanning protocols, specifically tailored software and new sequencing chemistry this system can produce 600 gigabases of data in a single day or 1.8 terabases within three days, which is enough for 5 and 15 whole human genomes, respectively.

**Ion Torrent Technology**

For the PGM/Proton sequence platforms the sequence templates are generated on a bead or sphere via emulsion PCR (emPCR) [Nakano et al.(2003)] [Dressman et al.(2003)]. An oil-water emulsion is created to partition small reaction vesicles that each ideally contain one sphere, one library molecule and all the reagents needed for amplification. Two primers that are complementary to the sequence library adapters are present, but one is only present in solution while the other is bound to the sphere. This serves to select for the library molecules with both an A and a B adapter while excluding those molecules with two A or B adapters from loading on the beads during emPCR. In addition, this ensures a uniform orientation of the sequence library molecules on the sphere. During the emPCR steps, individual library molecules get amplified to millions of identical copies that are bound to the beads to allow ultimate detection of the signal.

Although one emPCR reaction can generate billions of templated spheres, some aspects inherent to the emPCR method, in addition to the general biases during PCR amplification, prevent optimal output. Due to the double Poisson distribution behavior, it is impossible to
achieve optimal loading of one library molecule into all individual vesicles. In fact one third of the vesicles will have the one molecule to one vesicle ratio, the remaining two thirds will be either without a molecule or have more than one. In addition, breaking of the emulsion and recovery of the spheres is inefficient even with the latest automated systems. In the final step spheres containing amplified DNA are selected in an enrichment step from empty spheres and the loaded spheres are deposited into the sequencing chip.

The Ion Torrent chip consists of a flow compartment and solid state pH sensor micro-arrayed wells that are manufactured using processes built on standard CMOS technology. The detection of the incorporated bases during sequencing is not based on imaging of fluorescent signals but on the release of an H+ during extension of each nucleotide. The release of H+ is detected as a change in the pH within the sensor wells. Due to the lack of the time consuming imaging a sequencing run can be completed within 4 hours. Since there is no detectable difference for H+ released from an A, C, G or T base, the individual dNTPs are applied in multiple cycles of consecutive order. If upon delivery of a dNTP no change in pH is detected in a specific well, that nucleotide is not present in the template at the next available position. Alternatively, if a change in pH is detected, that base is in the template. In contrast to Illumina’s SBS method the dNTPs used are not blocked and when the template contains a series of a nucleotide after each other (a homo-polymer stretch), the entire stretch of identical bases will be extended, leading to an accordingly stronger pH change which is directly proportional to the number of identical bases incorporated. Relative to a single ‘A’ a stretch of ‘AA’ will give a 2 fold increase the pH, while an ‘AAA’ template will yield a 1.5 fold (3/2) increase in pH relative to ‘AA’ and for 6 vs 5 identical bases this relative increase is just 1.2 fold. This decrease of the relative increase of the change in pH as the homopolymer length increases reduces the probability by which a homopolymer region is called correctly.

The dNTPs are added in a predefined flow order. At the first release of the system, this order was a repetitive T-A-C-G sequence. As with Illumina sequencing, not all of the template molecules on a templated sphere get extended in perfect synchrony. On average 0.5-1% of the molecules deviate from the flow either because they lag behind due to improper extension or they advance ahead due to carry over of dNTPs from a previous cycle. In order to minimise this de-phasing, the flow order was changed to a more sophisticated sequence that incorporated A-T-A “catch-up” type flows. This scheme allows incomplete extension of the A nucleotide to catch up after the T base. Although this does come at a cost of decreased overall read length, the overall quality of the read does improve. Still, the quality of the reads gradually decreases towards the ends of the reads. By taking into account the flow order, it is possible to make flow-aware base caller algorithms and flow-space aware aligner software and variant detection tools that take the actual flow order into account when processing the data in order to generate higher accuracy data [Golan and Medvedev(2013)] [Merriman et al.(2012)]. The present error rate for substitutions is 0.1% [Merriman et al.(2012)] which is very similar to that of the Illumina systems. The main point of criticism the system endures are the homo-polymer errors. Despite many improvements the 5-mer homo-polymer error rate is still at 3.5% [Merriman et al.(2012)].

Since the initial release of the Ion torrent platform, this technology has evolved at a very rapid pace. The output specification of the first Ion-314 chip was a mere 10 Mb. Through increasing the total surface area of the chips and the sensor wells density, all on the 350 nm CMOS technology, in addition to increasing the average read length from 100 up to 400 bp, the newest Ion-318 chips produce 1 Gb. For the Ion Proton System 110 nm CMOS technology was used to manufacture the Proton-I chips. The diameter of the spheres and the sensor wells decreased which allowed the number of wells to increase to 165 million per chip. The Proton-I chips currently yield 60-80 million reads per run, reaching 10 Gbase. This is enough to sequence two human exomes at 50x coverage. The announced Proton-II chip will have 4x the number of sensor wells, with an expected output of 32 Gb per chip promising to generate a
whole human genome at 10x coverage, still within the 4 hours run time. This output puts it at par with a paired-end run on single HiSeq2000 lane. 

Life Technologies have developed an alternative method to generate polonies called Wildfire [Ma et al.(2013)]. The process generates clusters on a solid surface using isothermal amplification without denaturation or amplification cycles. Although initially designed for the Solid 5500 system, it is likely that this method could also be applied to the Ion Torrent semiconductor sequencing. This may involve spheres as an intermediate carrier or clusters may be generated directly into the sensor wells. A Proton-III chip has been announced that will double the number of wells to 1.2 billion, leading to an expected output of 64 Gbase per run. With these output levels, the Ion Proton will become a competitor to the current Illumina HiSeq systems.

Future sequencing technology
The advancements made on sequencing technology over the last years have been impressive. However, the ultimate sequencing platform would work on single DNA or RNA molecules without any (pre-) amplification, without use of optical steps, reads of Mb to Gb in length, no GC bias, high read accuracy and would be flexible enough to generate as many sequence reads as are necessary for the specific research question at hand. In addition, it should be both cheap to acquire and run, easy to operate, have short run times and simple or no library pre-preparation steps. Needless to say, this sequencing platform does not exist, yet. In the next section we will discuss one emerging sequencing platform which may have the potential to make the next step towards these ultimate sequencers.

Oxford Nanopore Sequencing
Expected characteristics for the nanopore sequencers are single molecule, amplification free, base detection without labels, long reads, low GC bias and scalable in data output. The basic principle behind the technology is tunneling of (polymer) molecules through a pore that separates two compartments. Physical presence of the molecule passing through the pore causes a characteristic temporary change in the potential between the two compartments which allows for identification of the specific molecule [Ashkenasy et al.(2005)]. Two version of nanopore DNA sequencing are being developed, i.e., using the natural pore forming protein alpha-hemolysin [Clarke et al.(2009)] or manufactured solid state pores [Li et al.(2003)] [Fologea et al.(2005)].

Oxford Nanopore Technologies (ONT) is one of the companies working on building nano-pore sequencing devices. Although ONT has put their focus on nucleic acid sequencing, in principle this technology could be applied to any (bio-) polymer as long as the molecules yield distinguishable changes in the current between the compartments. In Q4 2012 Oxford Nanopore announced the early access release of their MiniION system. This is a “palm sized” sequencing device that facilitates real-time analysis of single molecules such as DNA and RNA. However, specifics on read length, accuracy and run times are difficult to obtain.

Diagnostic NGS Applications
Below we will briefly describe a range of diagnostic NGS applications in clinical genetics. The applications discussed are non-invasive prenatal testing, disease gene identification (including WGS), and human disease and health.

Non-invasive prenatal testing
To obtain DNA from a fetus, prenatal diagnosis generally involves the costly and risky sampling of either chorionic villi or amniotic fluid. It is long known that DNA of the fetus can be found in maternal blood (cell free serum), yet it has low abundance and low quality and it is not easy to discriminate fetal from maternal DNA. These characteristics prevented wide-spread implementation of prenatal tests performed on maternal blood. However, the enormous power

2 Current reports show that the base-by-base accuracy of the MiniON is still far below the accuracy of e.g. Illumina sequencers [Quick et al., 2015; Loman et al., 2015]. According to these reports the MiniON base calling has an accuracy of 72%-85%.
of NGS technology seems particularly attractive for non-invasive prenatal testing (NIPT). To detect trisomies, in particular trisomy-21 or Down’s syndrome, a very simple but effective brute-force method was developed: sequence, map, count. DNA isolated from maternal serum is sequenced, reads are mapped to the human genome and counted per chromosome. When 5-10 million reads are mapped, trisomies will reveal themselves by giving a significantly too high number of reads mapping to a particular chromosome [Norton et al.(2012)]. A recent study showed that when genome sequencing of both parents, genome-wide maternal haplotyping and deep sequencing of maternal plasma DNA are combined even the genome sequence of an 18.5 weeks human fetus can be determined [Kitzman et al.(2012)].

**Disease gene identification**

A combination of genome-wide association studies (GWAS) and specific targeting by sequence capture of the genomic regions detected is now used extensively trying to identify the variants that functionally link the DNA with the phenotype. Similarly, genome sequencing can be used as a tool to characterise genetic variation in a specific population, determine haplotype structure and use this knowledge to impute alleles and boost the outcome of GWAS analysis [GoNL consortium, 2014, in press].

One of the most impressive applications of NGS lies in the field of human genetics and disease gene identification. In the past, larger families were an absolute requirement for a successful approach. Without being able to first map a disease gene to a specific position and then zooming in on the genes in that region, the human genome was simply too big and analysis too costly. Some successes were obtained using candidate disease gene approaches but generally these only worked when for a specific disease a new gene was discovered making similar genes or neighboring genes in a certain pathway obvious candidates. NGS studies were much more successful, even when only one or a few cases are available [Hoischen et al.(2010)]. Parent-child trio analysis turned out to be very effective to reveal dominant de novo diseases [Vissers et al.(2010)], while recessive diseases can be revealed when several unrelated cases are available or when clearly damaging variants are present [Ng et al.(2010)]. The latter successes could already be obtained with exome sequencing, i.e. a method to zoom in on the 1-2% protein coding sequences of the human genome only. Needless to say that, when cost drops further, full genome sequencing (WGS) will be used to detect also deleterious variants that are not in the protein coding regions. Early steps towards the ultimate application of genome-based medicine/ personalised medicine have been set by the UK and Saudi Arabia which last year both announced projects to sequence the genome of 100,000 individuals.

**Human disease and health**

So far studies have been mostly performed on the level of cell cultures, whole tissues or sorted cell populations. Although the yield per cell, 30%-70% of all RNA or DNA present, can still be improved, recent NGS developments have now made genome-wide single cell analysis feasible. Individual cells turn out to be quite different showing extensive genomic and transcriptomic heterogeneity in both normal development and disease [Bernards(2014)]. This turns out to be especially true for cancer tissue being a complex mixture of many different cell populations each carrying a range of genomic rearrangements driving its unrestricted growth. Dissecting these using (very) deep sequencing of the cancer genome / transcriptome as a whole or from single cell analysis should give us a tool to identify the so-called driver mutations. These will be different in different tumors and instrumental to direct treatment and prescribe the best (set of) drugs to be used, personalised cancer treatment. Similarly NGS approaches will be used to study drug resistance, identify their mechanism and provide strategies to combat resistance [Macaulay and Voet (2014)]. Coordinated by the International Cancer Genome Consortium (ICGC) a large project is ongoing trying to resolve the genomic changes present in many forms of cancers by analysing 50 cancer/normal tissue pairs [Zhang et al.(2011)]. In due time NGS developments will start to impact our daily life. While it will initially be used as a molecular
microscope to diagnose disease, ultimately it will also be used to monitor our personal health. Our genome sequence will be read once, but e.g. blood-derived RNA analysis, completed with proteomics and metabolomics measurements, will be used on a regular basis to study the status of our body, the Whole-body-BIOscan.

References


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2. GAP ANALYSIS ON TOOLS, METHODS AND APPROACHES TO INTRODUCE WGS INTO ROUTINE DIAGNOSTIC PRACTICE

By T. Smets and S. van Vooren

Introduction

In this section, we provide an overview of the gaps identified in tools, methods and approaches to introduce WGS test into routine diagnostic practice. Further detail is then provided in the next section (chapter 3). For this survey, the following methods were used:

- Extensive literature survey
- Questionnaire survey
- Online research
- Discussions with 3Gb-TEST Work Package leaders

The following key gaps and challenges were identified:

a) discrepancies in ways of working across labs, lack of uniform approach to variant assessment and reporting; building consensus among labs (identification, standardisation, publication of guidelines)
   - consensus documentation, education
   - community guidelines are being developed (cfr. ACMG)

b) identification of tools (quality, clinical use), lack of tools fit for clinical use; especially
   - tools geared at whole genome sequence
   - splice site prediction tools
   - missense and effect prediction algorithms; especially added value, utility, usability in a routine clinical setting

c) lack of high-throughput tools, automation for large volumes of tests and large numbers of variants (3Gb)
   - need for automation of lab SOPs
   - realisation that diagnostic assessment process in lab does not scale well (single gene -panel - exome - genome: no longer manual review)

d) clinical, diagnostic trustworthiness of prediction and assessment tools issue rather than availability and range of options

e) lack of tools that use the clinical context, phenotype, clinical annotation of unaffected individuals, variant effect prediction tools that use phenotype, standardisation on nomenclatures such as HPO

f) community wide, domain specific, open and compliant sharing of variant assessment and variant frequency statistics

Table 2.1 Conclusions from the survey

<table>
<thead>
<tr>
<th>Clinical community adopting 3Gb Test needs:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Mindset switch to adopt automation to cut out manual review</td>
</tr>
<tr>
<td>2. right tools to allow for high throughput and automation of SOPs</td>
</tr>
<tr>
<td>3. community guidelines on best practices and tools</td>
</tr>
<tr>
<td>4. clinically validated assessment algorithms</td>
</tr>
<tr>
<td>5. data sharing</td>
</tr>
</tbody>
</table>

Conclusions

After an extensive consultation of the literature, together with the responses of our survey, we have identified certain gaps in bioinformatics tools that still need to be overcome to make the introduction of NGS in routine diagnostics easier.

Firstly, we have noticed that finding the appropriate bioinformatics tools within the large amount of tools available remains a challenge, especially in regard to quality and trustworthiness. Therefore, it seems to be of great importance to know which tools other labs are using, hence data sharing could make an enormous difference at this level. Data sharing could pave the way towards a consensus, benefit mutual learning and promote knowledge distribution.

The ACMG guidelines have already set an example in the right direction, a necessary action given the broad diversity of bioinformatics tools and unique workflow different labs deal with.

Secondly, the need for good splice site prediction
tools in clinical practice is still high. The amount of splice site prediction tools mentioned in our survey is low, although this could be due to the question being not specific enough.

The prediction of splice sites is a complex and difficult task since it is species-specific and because several mechanisms of splicing do exist, based on enzymatic mechanisms. The state-of-the-art tools such as HSF, NNSPLICE, MaxEntScan, GeneSplicer, and SpliceSiteFinder-like are based on algorithms developed already some time ago. These are black box solutions and not suitable for high throughput.

Thirdly, we have remarked that there is a good consensus regarding the missense prediction tools, almost 50% of the respondents said to use Polyphen-2, SIFT and MutationTaster for variant effect prediction. But at the same time, these tools are receiving the highest scores for untrustworthiness. This points towards a gap in trustworthy tools for missense prediction and the need for clinical validation.

Fourthly, the high throughput of the large number of variants in the whole genome forms a challenge by itself. Not only at the technical level (tools that can deal with this task) but also for the interpretation and reporting SOPs of a laboratory.

Therefore, automation of interpretation SOPs and an associated mindset switch are required. Labs need to face the fact that at the single gene level, a handful of variants can be reviewed manually but at the exome and even the genome level, 10 or 100 000s of variants are not manually reviewable anymore. In addition to this, we will also need powerful and validated tools in order to make laboratories feel comfortable and acquire confidence in an automated approach to their workflows. The Cartagenia Bench Lab platform is an example of a software solution that allows automation of laboratory variant assessment SOPs and go from many variants to few, construct a laboratory database of findings, and draft clinical reports. An implementation of the ACMG guidelines was automated on this platform by an independent clinical genetics laboratory as proof of principle (see below).

In the fifth place, we still need to work on better strategies to deal with large amounts of data and data integration. Gaps present at this level are reliable gene and variant ranking methods based on reason for referral and patient's phenotype. There is still a lack of tools dealing with family information, inheritance hypotheses and tools that incorporate phenotype information in missense algorithms. However, progress is being made, tools like The Exomizer, PhenIX, and Endeavour are some good examples.

Sixth, for WGS not many annotations are available yet for evaluating nucleotide variations in intergenic regions. It is important to obtain databases focusing on these intergenic regions and to share this information. Therefore, the generation of bioinformatics tools and databases focusing on regulatory, intergenic and non-coding regions is a key attention point for the future.

Last but not least, the importance of sharing genotype-phenotype information cannot be under-estimated as it is necessary to make phenotype-genotype correlations possible. The major challenges that are associated with it is to overcome the issues at laboratories obtaining IRB approval and consent, the ethical issues and responsibility but also the organizational complexity. Today we already have the possibility for patients to opt-in and there are ongoing initiatives for patients to share their phenotype information online with others. However, too many factors are still in the way for introducing the sharing of genotype and phenotype information into the routine diagnostic practice.

5https://www.sanger.ac.uk/resources/software/exomizer/
6 http://compbio.charite.de/PhenIX/

4 See chapter 3
3. STATEMENT ON DIAGNOSTIC REPORTING PIPELINE

By T. Smets and S. van Vooren

Introduction
For this chapter, the following methods were used:
- Extensive literature survey
- Questionnaire survey
- Online research
- Discussions with 3Gb-TEST Work Package leaders

To identify the requirements and possible gaps within a typical clinical genomics diagnostic pipeline, we have formulated a questionnaire to see which tools expert labs are currently using. Specifically, we have asked which tools they use for upstream analysis of WGS raw data (i.e. alignment, FASTQ, BAM, vcf,...), which variant assessment tools they use (i.e. clinical significance, annotation, classification, databases, etc.)

How do they validate these tools and which of these are considered trustworthy or not and why? We were also interested in the generation of laboratory reporting (manually, guided or automated) and if this process was linked with a LIMS/EHR. Finally, we asked the participants to identify the bottleneck in their current workflow.

Below, we provide a summary overview of the tools, methods and approaches commonly used in routine diagnostic practice, based on the survey we sent out.

Conclusions - detailed description
Regarding upstream analysis tools, the majority of the responses were recorded for BWA and GATK, followed by Torrent Server, MiSeq and SeqNext (table 3.1).

At the level of variant assessment tools the answers vary widely with highest scores ranging from dbSNP and the 1000 Genomes database to ClinVar and ExAC (table 3.2). A clear agreement however can be found in the validation of tools, where SANGER sequencing is the golden standard for most labs. Many respondents also noted that it is very important to compare and combine multiple tools and a minority referred to family/segregation analysis and literature as a means for validation (table 3.3). Remarkably, the tools said to be used most often for variant effect prediction are Polyphen-2, SIFT and MutationTaster, while these tools are also mentioned first when people think of non-reliable tools (tables 3.4 and 3.5). Most respondents mention the importance of taking into account the combined evidence from different sources for variant assessment and interpretation (table 3.6). Regarding the generation of lab reports, the majority of lab reports is said to be generated in a guided manner, using templates or in a semi-automated fashion, only a minority reports to have an automated reporting system (table 3.7). The largest bottleneck in the complete NGS workflow seems to be the interpretation and/or clinical assessment of variants followed by sample preparation/lag time. The high score for variant interpretation and clinical assessment is in accordance with the broad diversity of tools used for this purpose and points towards the need of certain guidelines within the community.

To conclude, we find that many different tools are out there for variant assessment and interpretation support. A host of population frequency databases, variant assessment algorithms with and without phenotype information, and annotations exist.

We also find that there is some level of community consensus arising on which sources are trustworthy. Luckily, professional bodies are stepping in and providing guidelines for clinical adoption of NGS and best-of-breed approaches for diagnostic use.

One very important set of guidelines was recently published by the ACMG (Richard S. et al., Genetics in Medicine, 2015). These guidelines provide best practices for assessing and reporting on the clinical significance of NGS variants in a routine clinical setting. The...
guidelines mention a wide range of resources, tools and practices and the level of evidence they provide towards benign or pathogenic classification.

Cartagenia Bench Lab NGS provides a framework for labs to bring together the tools, content sources and filtration approaches of their choice, and provides an ideal platform to bring together the sources and strategies put forth by these ACMG guidelines. Together with an independent clinical diagnostic lab, Cartagenia has created and published a paper in which this lab takes those ACMG guidelines and translates them into an automated pipeline that implements this lab’s take on the guidelines [Richards et al., (2015)] (i)). This white paper illustrates how community-validated and established resources, databases and prediction tools can be brought together in a robust, versioned workflow to reflect the state of the art in variant assessment and reporting put forth by the ACMG guideline publication.

Table 3.1: usage of upstream analysis tools

<table>
<thead>
<tr>
<th>Upstream analysis tools</th>
<th>Nr of responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>BWA</td>
<td>15</td>
</tr>
<tr>
<td>GATK</td>
<td>15</td>
</tr>
<tr>
<td>Ion PGM™/Torrent Server (Life Technologies)</td>
<td>8</td>
</tr>
<tr>
<td>MiSeq (Illumina™)</td>
<td>6</td>
</tr>
<tr>
<td>SeqNext (JSI Medical systems)</td>
<td>6</td>
</tr>
<tr>
<td>Platypus</td>
<td>4</td>
</tr>
<tr>
<td>NEXTGENe® (Softgenetics®)</td>
<td>3</td>
</tr>
<tr>
<td>SureCall (Agilent Technologies™)</td>
<td>3</td>
</tr>
<tr>
<td>Novoalign (Novocraft™)</td>
<td>2</td>
</tr>
<tr>
<td>Bowtie</td>
<td>1</td>
</tr>
<tr>
<td>CLC workbench (Qiagen™)</td>
<td>1</td>
</tr>
<tr>
<td>Variant studio (Ingenuity, Qiagen™)</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 3.2: Variant assessment tools used by participating laboratories

<table>
<thead>
<tr>
<th>tool</th>
<th>Nr. of responses</th>
<th>tool</th>
<th>Nr.</th>
<th>tool</th>
<th>Nr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>dbSNP</td>
<td>13</td>
<td>Variant Studio</td>
<td>4</td>
<td>GATK</td>
<td>2</td>
</tr>
<tr>
<td>Alamut</td>
<td>11</td>
<td>Mutalyzer</td>
<td>3</td>
<td>ENSEMBL Var. Effect</td>
<td>2</td>
</tr>
<tr>
<td>1000 Genomes</td>
<td>10</td>
<td>VEP</td>
<td>3</td>
<td>OMIM</td>
<td>2</td>
</tr>
<tr>
<td>ClinVar</td>
<td>8</td>
<td>UCSC</td>
<td>3</td>
<td>dbNSFP</td>
<td>2</td>
</tr>
<tr>
<td>ExAC</td>
<td>8</td>
<td>EVS</td>
<td>3</td>
<td>Other tools</td>
<td>1</td>
</tr>
<tr>
<td>Annovar</td>
<td>7</td>
<td>ESP6500</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HGMD</td>
<td>7</td>
<td>SeqNext</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIC</td>
<td>5</td>
<td>Human Splicing Finder</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyphen-2</td>
<td>5</td>
<td>Ion PGM/Torrent Server</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOVD</td>
<td>5</td>
<td>COSMIC</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SIFT</td>
<td>4</td>
<td>LuCAMP</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MutationTaster</td>
<td>4</td>
<td>Var. Analysis (Ingenuity)</td>
<td>2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table 3.3: Means for validation

<table>
<thead>
<tr>
<th>Validation of tools</th>
<th>Number of responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>SANGER</td>
<td>30</td>
</tr>
<tr>
<td>Comparison of different tools/samples</td>
<td>10</td>
</tr>
<tr>
<td>Segregation/family analysis</td>
<td>4</td>
</tr>
<tr>
<td>Literature</td>
<td>5</td>
</tr>
<tr>
<td>Sophia Genetics®</td>
<td>1</td>
</tr>
</tbody>
</table>

### Table 3.4:

<table>
<thead>
<tr>
<th>Tools for effect prediction and phenotype correlation</th>
<th>Nr of responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyphen-2</td>
<td>18</td>
</tr>
<tr>
<td>SIFT</td>
<td>18</td>
</tr>
<tr>
<td>MutationTaster</td>
<td>13</td>
</tr>
<tr>
<td>Alamut</td>
<td>4</td>
</tr>
<tr>
<td>Align GVGD</td>
<td>4</td>
</tr>
<tr>
<td>SNPEff</td>
<td>4</td>
</tr>
<tr>
<td>ClinVar</td>
<td>2</td>
</tr>
<tr>
<td>FATHMM</td>
<td>2</td>
</tr>
</tbody>
</table>

### Table 3.5:

<table>
<thead>
<tr>
<th>Tools not considered trustworthy</th>
<th>Number of responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>MutationTaster, Polyphen-2, SIFT</td>
<td>17</td>
</tr>
<tr>
<td>No stand-alone tool; combination is required</td>
<td>10</td>
</tr>
<tr>
<td>All bioinformatics tools</td>
<td>4</td>
</tr>
<tr>
<td>SeqNext CNV mode</td>
<td>1</td>
</tr>
<tr>
<td>CLC Bio - threshold too high for indels</td>
<td>1</td>
</tr>
<tr>
<td>dbSNP</td>
<td>1</td>
</tr>
<tr>
<td>dbNSFP</td>
<td>1</td>
</tr>
<tr>
<td>Splice site predictors</td>
<td>1</td>
</tr>
<tr>
<td>None/ No answer</td>
<td>16</td>
</tr>
</tbody>
</table>
### Table 3.6: Tools considered to be trustworthy

<table>
<thead>
<tr>
<th>Tools considered to be trustworthy</th>
<th>Nr of responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined evidence needed</td>
<td>6</td>
</tr>
<tr>
<td>BWA, GATK</td>
<td>4</td>
</tr>
<tr>
<td>No answer</td>
<td>5</td>
</tr>
<tr>
<td>Galaxy</td>
<td>3</td>
</tr>
<tr>
<td>Ion PGM™/Torrent Server (Lifechnologies)</td>
<td>4</td>
</tr>
<tr>
<td>NEXTGENe® (Softgenetics®)</td>
<td>1</td>
</tr>
<tr>
<td>Sanger</td>
<td>2</td>
</tr>
<tr>
<td>Alamut</td>
<td>2</td>
</tr>
<tr>
<td>Mutalyzer</td>
<td>1</td>
</tr>
<tr>
<td>SeqNext</td>
<td>2</td>
</tr>
<tr>
<td>Sophia Genetics</td>
<td>2</td>
</tr>
<tr>
<td>SIFT, Polyphen-2, MutationTaster</td>
<td>2</td>
</tr>
<tr>
<td>Population and mutation databases</td>
<td>1</td>
</tr>
<tr>
<td>HGMD®, BIC, LOVD</td>
<td>1</td>
</tr>
<tr>
<td>All</td>
<td>4</td>
</tr>
<tr>
<td>Literature</td>
<td>2</td>
</tr>
</tbody>
</table>

### Table 3.7: The generation of lab reports

<table>
<thead>
<tr>
<th>Generation of lab reports</th>
<th>Number of responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guided (e.g. with templates, semi-automated)</td>
<td>35</td>
</tr>
<tr>
<td>Automated (reporting system, automated prefilling of text, tables, links, literature)</td>
<td>5</td>
</tr>
<tr>
<td>Manual (e.g. written in Word)</td>
<td>10</td>
</tr>
</tbody>
</table>
Table 3.8: Bottlenecks in the NGS workflow

<table>
<thead>
<tr>
<th>Bottleneck in NGS workflow</th>
<th>Nr. of responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variant interpretation and/or clinical assessment</td>
<td>34</td>
</tr>
<tr>
<td>Bioinformatics</td>
<td>4</td>
</tr>
<tr>
<td>Human resources</td>
<td>1</td>
</tr>
<tr>
<td>Sample preparation/ lag time</td>
<td>10</td>
</tr>
<tr>
<td>Lab reporting</td>
<td>1</td>
</tr>
</tbody>
</table>

References

4. VARIANT VALIDATION

By J. Traeger-Synodinos, T. Smets, S. Van Vooren and S. Patton

Variant validation network: A network of bio- and medical- informatics tools and databases with potential to support variant validation.

Molecular diagnostics is evolving towards the routine incorporation into the clinic of high-throughput and massively parallel DNA sequencing methods (otherwise known as “next generation sequencing, NGS”). It is expected that soon the analysis of the complete genome of a patient/individual (whole genome sequencing, WGS, or “3Gb-test”) will be more cost-effective for many diagnostic applications compared with most current molecular genetic tests. Within the context of clinical interpretation of WGS data, the aim of the 3Gb-TEST project is to identify those tools with potential and/or proven clinical utility to create a network of bio- and medical- informatics tools and databases with potential to support variant assessment and clinical interpretation.

The entire WGS procedure involves an initial “wet-lab” side, followed by extensive data analysis. The wet-lab aspects of sequence generation are almost entirely automated. Sequence data output takes the form of millions to billions of short sequence reads. This data is then subjected to rigorous computational and bioinformatics analysis workflow. The short sequence reads have first to be mapped and aligned to a reference human genome sequence. After mapping and alignment, variant calls are made at locations where nucleotides differ from the reference sequence. All called variants are then annotated, which includes identifying the build of the reference genome, the chromosomal position, the gene, the gene region (exonic, splicing, intronic, intergenic, etc.), the coding change information all with various quality scores. The final and most challenging step is to filter the variants, validate them and evaluate them with respect to their relevance to the patients’ clinical phenotype. The final clinical interpretation requires support by robust bio- and medical- informatics tools and databases.

One of the purposes of the 3Gb-test collaboration was to identify the current “gaps” in all aspects of WGS in a clinical diagnostic setting. When the project was first initiated, there were many gaps in the bioinformatics analysis process, such as standards and mechanisms for consistency & accuracy (so-called data analysis “pipelines” or workflows), good reference databases, (preferably publicly available), systematic data sharing and finally standardized approaches for variant validation, assessment and clinical interpretation.

There are now many concerted efforts towards filling these gaps and the situation is progressing fast. There are many recent publications of integrated data-analysis pipelines and many data-sharing initiatives e.g.

- HGVS, HVP and IUPAC to develop standards for diagnostic grade Variant Call File (vcf) and standards on pipelines for vcf analysis
- HVP, LOVD and InSIGHT for variant classification review
- DECIPHER- integrating shared data on CNV’s, sequence variations and phenotypes in developmental disorders.
- ClinGen Resource (NHGRI) (http://www.iccg.org/about-the-iccg/clingen/)

Guidelines are emerging to support standardised approaches for variant validation and interpretation for clinical WGS applications. Amongst the published recommendations and best-practice strategies for variant assessment are those of Eurogentest, the American College of Medical Genetics and Genomics (ACMG) and the Association for Clinical Genetic Science (ACGS) (see reference list below).
A more researched-focused paper by MacArthur et al, Nature 2014, identifies two levels on which to assess evidence for variant(s) implicated:

a) Gene level
   • Genetic: affected gene shows statistical excess of rare (or de novo) probably damaging variants segregating in cases versus controls or null models
   • Experimental: Protein interactions, Biochemical function, Expression, Gene disruption, Model systems, Rescue.

b) Variant level
   • Genetic: Association, Segregation, Population frequency
   • Informatic: Conservation, Predicted effect on function.
   • Experimental: Gene disruption (in patient cells or well-validated in vitro model), Phenotype recapitulation (following introduction of variant into cell-line or animal model), Rescue.

The experimental level is not possible in a clinical diagnostic setting for the vast majority of cases, for practical reasons of cost and time, but if the labs working in the research-clinical interface follow standards suggested, then it can be expected that useful data will emerge to reinforce the robustness of interpreting variants of clinical relevance.

Another important aspect to support clinical interpretation are the locus (gene), disease and phenotype databases such as LOVD, HGMD and Clinvar. Currently many of these repositories are rather “patchwork”, non-standardized and not easily accessible or user-friendly for a clinical setting. They will need constant populating with validated information through concerted data-sharing efforts. (Vihinen et al, 2012; den Dunnen et al, in press, 2015).

The ideal is to develop a dynamic and comprehensive reference database of clinically important variants that is easily cross referenced to exome and genome sequence data and allows for an accumulation of expert opinion. Many concerted efforts are underway to address the “gaps” and fulfill the ideal aims. It is expected that these repositories will soon become an indispensable resource for clinical WGS (Johnston et al, 2013; Stanley et al, 2013; Ramos et al, 2014; Lerner-Ellis et al, 2015).

Finally to facilitate the routine use of data analysis using validated resources, databases and prediction tools there is a need to incorporate them within in a user-friendly, robust, versioned workflow which is continuously updated with the latest variant assessment and reporting tools. One such data analysis “pipeline” or workflow has been developed by Cartagenia in collaboration with developed by an independent Molecular Genetics Lab in Sweden in collaboration with Cartagenia. This The laboratory’s variant assessment protocol was implemented on the Cartagenia Bench Lab NGS platform was used for based on the guideline document of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (Richard et al, 2015).

To conclude, under the current circumstances it is not possible to propose a single network of bio- and medical- informatics tools and databases with potential to support variant validation. However, the fast rate of progress towards the incorporation of standards in bio- and medical- informatics tools and databases used in the clinical interpretation of WGS data is encouraging. To achieve the ultimate goals it is important that all stake-holders are encouraged to participate in all collaborative efforts underway.

References


Johnston JJ, Biesecker LG. Databases of genomic variation and phenotypes: existing resources and future needs Human Molecular Genetics, 2013, Vol. 22R27–R31

Lerner-Ellis J, Wang M, White S, Lebo MS; Canadian Open Genetics Repository Group. Canadian Open Genetics Repository (COGR): a unified clinical genomics database as a community resource for standardising and


5. TRAINING REQUIREMENTS

By J. Traeger-Synodinos

The drafting of training programs for geneticists and physicians in the use of integrated bio- and medical-informatics tools.

In the context of clinical human genetics, whole genome sequencing (WGS, or “3Gb-test” as named in this project) has wide potential application, including definitive diagnosis of patients, carrier-detection to support informed reproductive choices, pre-symptomatic testing for certain monogenic diseases (e.g. familial hypercholesterolemia), susceptibility testing for common multifactorial diseases, cancer diagnosis and management, pharmacogenomics and eventually all levels of “personalised medicine”.

However, there are many issues to address before WGS can be optimally incorporated into medical practice. WGS involves a wet-lab step, followed by intense bioinformatics analysis and evaluation of findings according to the clinical phenotype of the case/patients. Assuming that quality aspects of clinical WGS tests can/will be addressed and that WGS becomes cost-effective, big challenges remain with respect to the clinically useful evaluation and interpretation of a large number of sequence variations potentially identified in each sample. Certainly, over- or misinterpretation of data needs to be minimised.

To support effective use of WGS in a clinical context, education of health scientists and practitioners is fundamental. Thus there is a need for comprehensive training programs for laboratory and clinical geneticists, as well as non-geneticist physicians, especially in the use of bio- and medical-informatics tools and databases. This spans from raw WGS data analysis through data evaluation, validation and interpretation, preferably within structured pipelines that have been validated for the quality level and accuracy that is acceptable for clinical use. Furthermore, as patient phenotypes are key to correct WGS interpretation, patient characteristics will need to be thoroughly evaluated and clearly recorded using standardized terms (ICD-10), for example by use of the Human Phenotype Ontology (HPO) and Phenomizer (Köhler S et al. Nucl. Acis Res. (2014). Health practitioners will need guidelines for counseling patients pre- and post-WGS testing. Additionally health scientists and practitioners will need to become familiar with the concept and possibly direct use of and “big” data analysis, storage and data sharing tools.

Short training courses, are probably sufficient for those health practitioners already specialised in genetics (laboratory and/or clinical) to support their familiarization and competences in the use of clinical WGS. These courses could be part of their professional education program. The typical content of such courses are summarised in Table 5.1 and some existing short courses are summarised in Table 5.2.

Examples of other more comprehensive courses in Europe that are relevant to genomic medicine and clinical bioinformatics include:

- Full-time and part-time Master’s in Genomic Medicine, offered by 9 universities in the UK so far (University of Birmingham, University of Cambridge, University of Manchester, University of Newcastle, University of Sheffield, University of Southampton, Imperial College London, Queen Mary’s London, and St. Georges, London)
- Clinical Science Master’s degree (3yrs) run by NGRL, Nowgen Centre & University of Manchester: a Scientist Training Programme (STP) in Clinical Bioinformatics.
**Table 5.1. Typical content of short training courses for genetics healthcare practitioners**

<table>
<thead>
<tr>
<th>Topic</th>
<th>What is there</th>
<th>Gaps to address</th>
<th>Suggested action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Basics of NGS technology</td>
<td>Information &amp; support from NGS system providers</td>
<td>None obvious</td>
<td>None obvious</td>
</tr>
<tr>
<td>2. Bioinformatic Tools</td>
<td>Teaching about many algorithms and pipelines for data handling.</td>
<td>Currently no consensus on bio- and medical-informatics tools that can fulfill appropriate criteria for quality assurance in the clinic. Based on best Practice Guidelines (*see below for currently available guidelines)</td>
<td>Recommend training content for bioinformatic tools, data storage and security using relevant excerpts of deliverables from 3Gb-TEST. Promote Best Practice based on guidelines (see below) and outcomes of EQA schemes run by EMQN, with support from Eurogentest under the ESHG.</td>
</tr>
<tr>
<td>3. Data handling</td>
<td>Many in-house and remote data storage options</td>
<td>Absence of standard practice to ensure that users of clinical NGS comprehend all aspects of data storage and sharing (*see below for currently available guidelines)</td>
<td>As above</td>
</tr>
</tbody>
</table>


**Table 5.2 Existing short courses on WGS**

<table>
<thead>
<tr>
<th>What is there</th>
<th>Gaps to address</th>
<th>Suggested actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Many on-line or short courses (up to several days) run by academic centres, institutes or companies. Regular courses include: - EBI and EMBL (UK, Germany), <a href="http://www.embl.de/training/events/index.php">http://www.embl.de/training/events/index.php</a> - European Genetics Foundation (Italy) - Netherlands Bioinformatics Centre</td>
<td>Many courses are directed for research purposes, so adjustment of current courses with respect to clinical application and interpretation.</td>
<td>Suggest guidelines for quality in training courses/workshops in Europe appropriate for clinical applications E.g. the Education Committee of the ESHG.</td>
</tr>
</tbody>
</table>
Generally, in certain European countries, for example the UK and the Netherlands, where the integration of WGS into clinical diagnostics is progressing at speed, there are concerted and systematic efforts towards establishing training programs for geneticists and physicians in the use of integrated bio- and medical-informatics tools.

For clinicians who are not specialised in genetics but whose patients will benefit by the use of WGS, we will need to foresee education/training in more basic concepts of genetic medicine and genomics, as well as issues related to the most appropriate routes and protocols for patient referral, as well as an evaluation of the limits of such tests. This should support the optimum value of performing WGS in clinical diagnostics. Such trends are apparent in the U.S. where there appears to be a coordinated consensus on many aspects for clinicians training in aspects of genomic medicine. For clinicians in the US, policy and training in genomic medicine includes:

- “Genetics Education and Training” [Report of the Secretary’s Advisory Committee on Genetics, Health, and Society, (HHS). Feb 2011].
- Integration of genomic medicine into pathology residency training: the Stanford open curriculum. [Schrijver et al. (2013)].
- Framework for development of physician competencies in genomic medicine: report of the Competencies Working Group of the Inter-Society Coordinating Committee for Physician Education in Genomics. [Korf et al. (2014)].

In conclusion, in Europe, many aspects for the training of geneticist health scientists and practitioners in the use of integrated bio- and medical-informatics tools would probably benefit from co-ordinated efforts of academic organizations and scientific societies. For example, an ideal candidate to facilitate such co-ordinated efforts could be the Education Committee and Eurogentest, under the auspices of the European Society of Human Genetics (ESHG). Furthermore, the ESHG could also liaise with European and International Societies for other medical specialities to support more general training in aspects of genomic medicine.

References


6. ETHICAL PROPOSALS FOR THE IMPLEMENTATION OF WHOLE GENOME SEQUENCING

By S. Leonard and A. Cambon-Thomsen

Introduction

In the context of 3Gb-TEST, an in-depth literature search was carried out to identify the principal ethical issues being discussed with regards to whole genome sequencing and to uncover areas of convergence and dissent. “Return of incidental findings” was identified as the primary area of dissent, particularly with regards to returning incidental results for which no prevention or cure currently exists. More fundamentally, there was divergence in opinion as to how the benefits of the technology should be maximised. The American College of Medical Genetics (ACMG) advocated the use of WGS as a screening opportunity in addition to its role in seeking the answer to a specific clinical question (ACMG 2013). In Europe the European Society for Human Genetics recommended using targeted testing wherever possible (ESHG 2013). Following the publication of a number of articles arguing against opportunistic screening10, a statement published by the ACMG changing their former stance that opportunistic screening should be obligatory each time that WGS is performed for any diagnostic purpose (ACMG 2014). Surveys showed that opinion generally among professional stakeholders was divided with regards to a number of issues, including the return of unsought incidental findings (Lohn et al 2013, Lemke et al 2013).

The available data regarding the attitudes of professional stakeholders came from North America. For 3Gb-TEST, a web-based questionnaire was therefore developed to investigate the attitudes of European professional stakeholders. This revealed convergence in some areas, such as that opportunistic screening should be offered but not be mandatory, and that incidental findings for preventable or treatable disorders should be shared with both minor and adult patients. Return of other incidental findings and re-contact were areas where opinion diverged.

The aim of this chapter is to make proposals for future guidelines, following consideration of the different ethical challenges posed by the offer of diagnostic whole genome sequencing. This was achieved through an ethical analysis of the issues identified through the literature search and based on experience with other genetic testing technologies, and will incorporate consideration of the different positions reflected in the questionnaire study.

Informed consent

Whole genome sequencing offers the possibility of a wide ranging set of information about the person’s genetic status and future health. However the test is generally performed in order to answer a specific clinical question. Medical consent procedures aim to give sufficient information to the patient to allow them to make an informed decision. Generally this involves talking through potential results of testing. It is expected that the number of incidental findings will be higher with WGS than with other forms of genetic testing, and it is important to determine the level of detail that is optimal for WGS consent. Counselling in detail regarding all of the possible predispositions for medical conditions that could potentially be detected by WGS is not feasible. One possible approach is that categories of disorders rather than individual disorders should be discussed.

Experience with genetic counseling has shown that preparation of patients for predictive tests poses particular challenges. Such tests are carried out in the context of a known family history of a particular condition for which the patient is at increased risk. The condition is therefore generally familiar to the patient, and they have often had some time to reflect on the meaning of that condition to themselves and to decide whether to take the test. Counselling

10 Opportunistic screening: WGS without indication
prior to WGS for all of the potential clinically significant results that may arise that are predictive of serious and in some cases incurable disorders is potentially very complex and time consuming. A study counseling patients in a research setting for a diagnostic WGS test took several hours to counsel patients. The patients reported that they found the process overly long (Tabor et al 2012). There is therefore a need to develop a concise pre-test counseling procedure that gives patients sufficient information to make an informed choice, without overwhelming them to the point that making an autonomous choice is unreasonably difficult. Zeiler has argued that too much choice actually harms, rather than promotes, autonomy (Zeiler 2004).

The survey carried out for 3Gb-TEST indicated that 85% of participants were in favour of a more in-depth consent procedure for WGS than for current untargeted genetic tests such as array-CGH. Of those that were in favour of a more in-depth procedure, 88% felt that the explanation of possible results should be longer and 64% felt that there should be a more detailed consent form. Around a third of participants would consider incorporating more than one counselling session into a pre-test consent process and one third would consider using a web-based decision-aid tool.

Proposal: Care should be taken to allow for ample time for reflection about the test decision without subjecting the patient to an overly lengthy information-giving session. Patients should ideally be offered a two-step counseling process, with written information to be given after the first appointment. It is noted that some patients may find a two-appointment system burdensome, for example if they live far from the genetics centre or have a heavy schedule of appointments for a disabled child. In such cases, alternative approaches might be used such as telephone contact by a genetic counselor before or after the appointment, or the sending out of written material prior to the appointment. A further possibility is the use of web-based information and educational material to help patients to go to a single appointment with a basic understanding of the process and to allow them to begin thinking of the questions that they would like to ask their clinician.

Opportunistic screening vs whole genome sequencing vs targeted testing

Although there appears to be consensus that if opportunistic screening will be offered it should be optional, we will here consider each of the four possibilities: WGS with compulsory opportunistic screening, WGS with optional opportunistic screening, WGS without opportunistic screening and targeted testing. Aside from the ACMG, all organisations whose guidelines were included, were in favour of targeted sequencing rather than whole genome sequencing when feasible. The argument in favour of performing opportunistic screening is that “reporting some incidental findings would likely have medical benefit for the patients and families of patients undergoing clinical sequencing” (ACMG 2013, p.3). The reasons for not offering the possibility of opting out of this additional screening were listed as: 1. It would be too great a burden to perform the depth of genetic counseling necessary to respect patient preferences 2. It would be “unwieldy” for the laboratories to mask or ignore these medically significant results according to patient preference 3. Healthcare professionals have a duty to prevent harm by warning patients and their families about certain incidental findings and that this principle supersedes concerns about autonomy just as it does in the reporting of incidental findings elsewhere in medical practice” (ACMG 2013, p. 11). The ACMG noted that this “may seem to violate existing ethical norms regarding the patient’s autonomy and “right not to know” genetic risk information” (ACMG 2013 p.11).

Arguments against this compulsory testing include the high cost of the additional pre and post-test counseling in both financial and personnel terms, and the laboratory costs of analysing additional data. From an ethical point of view, generally only the incapacity of the patient justifies overriding autonomy for his own benefit. Furthermore, Allyse and Michie (2013)
argue that the offer of WGS on the condition that opportunistic screening is accepted “borders on the coercive” (Allyse and Michie 2013 p.440). The concerns with such an approach were reflected in the survey of professionals working in clinical genetics and related fields, which showed that only 2% of participants were in favour of opportunistic screening without opt out.

Opportunistic screening with the possibility of opting out was supported by 55% of the survey respondents. In a 3Gb-TEST workshop11, a number of reasons not to offer such screening, even with an opt-out were highlighted. These included the difficulties of interpreting variants and emerging evidence that the penetrance of pathogenic mutations may be dependent on multiple genetic and environmental factors and that we cannot yet be certain of the impact of identified variants on patients’ health. In addition, if no mutations are identified, there is a risk that patients may be inappropriately reassured and cease to follow basic health advice such as not smoking, or participating in established national screening programs. A final argument was made that if opportunistic screening represents such great benefit for the patient, it is difficult to justify offering it exclusively to those undergoing WGS for other reasons, since members of the general public are equally likely to have significant secondary findings. The ensuing debate revealed mixed views but was generally not in favour of opportunistic screening in any form. Further arguments that have been advanced include the lack of a screening infrastructure (and the perceived lack of financial capacity to put in place such a structure) to support and follow up patients who have results predictive of future illness, and concerns regarding the insurance status of individuals with positive findings. In favour of opportunistic screening with opt out was the possibility of ensuring a healthy life for longer. If genetic opportunistic screening is thought of in a similar way to opportunistic screening in other fields of medicine, it seems desirable to derive the maximum possible benefit from a test that is taking place, in the same way that a radiologist will examine the whole film and not solely the area of interest.

Whole genome sequencing without opportunistic screening could be seen as an option that tries to balance the risks and benefits of opportunistic screening and masked testing. It would allow for some additional benefits to be derived from WGS but would avoid some of the problems associated with systematic searches for additional variants.

There are a number of reasons for favouring targeted testing. These include the complicated nature of interpreting whole genome sequencing results, and the potential problems engendered by uncovering susceptibilities to disorders for which preventative measures might either not exist or are unavailable for practical and financial reasons. In addition there is a justice argument that testing of the genome beyond the regions known to be related to the clinical indication (other than where it is judged to be necessary to increase diagnostic capacity) is not being done for medical need, and yet is not available to all equally, and so it cannot be justified in a state-subsidised healthcare system.

Proposal: In view of the difficulties of interpretation and the lack of an infrastructure for long-term follow up of healthy patients with predictions of future disease (and particularly in light of the fact that in such healthy patients

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11 The workshop “When Opportunity Knocks: Understanding the ethical, legal and social aspects of clinical Whole Genome Sequencing” (3Gb-TEST/Genotoul societal Meeting May 4 2015 in Toulouse, France)
without a family history penetrance of the known pathogenic mutations may well be decreased owing to selection bias of initial cases) it is proposed that tests should be targeted wherever possible, and that opportunistic screening not be offered at this time.

The offer of results to adult patients

Classification of findings: There are a range of potential results which could be offered to adult patients, from results relating solely to the clinical indication for testing to all of the information generated by the test. Results can be classified into categories:

- Results related to clinical indication for testing
- Results related to a treatable or preventable disorder unrelated to the clinical indication for testing (sometimes referred to as ‘clinically actionable’ results)
- Results related to a medically significant but non treatable or preventable disorder
- Carrier status
- Susceptibility loci
- Variants of uncertain significance (in any of the above categories)
- ‘Social’ findings such as the ‘warrior gene’\(^\text{12}\).

What is clinically actionable? In a number of articles written about whole genome sequencing, incidental findings have been classified into ‘clinically actionable’ and ‘not clinically actionable’ or some variation thereof. A ‘clinically actionable’ finding could be one such as the discovery of a BRCA 1 mutation, for which screening or prophylactic surgery may be available. A ‘not clinically actionable’ mutation might be one that is predictive of an incurable disease for which there are no preventative measures, such as Huntington’s disease.

However, although there may be no clinical action available, such information might be of ‘actionable’ value to some patients, who would plan their lives differently if they were aware of this risk. Others would not want to be given this kind of information under any circumstances. Experience with predictive testing shows that some patients want this kind of information whereas others choose to avoid it. This is in the context of a known disorder within the family and a high individual risk, where a careful staged pre-test counselling programme is generally offered. It is not known what the consequences might be of revealing this kind of predictive information to a previously low risk patient might be.

A number of conflicting concerns arise. On the one hand, it might be considered that patients have a right to know all of their medical information. On the other hand it might be considered burdensome or unnecessarily complex to return certain types of results, such as those of no known medical value. For certain categories of results there is an almost unanimous opinion that results should be returned. Such is the case for clinically significant preventable and treatable disorders in adult patients, and those that would be clinically actionable during childhood for paediatric patients. There is less accord over returning results showing an increased risk of a non-actionable but serious disorder such as a neurodegenerative disorder. Few participants in our survey were in favour of returning results regarding variants of uncertain significance or benign variants, or social findings such as paternity or tone deafness.

Proposal: Each laboratory and clinical department will need to decide (if there are no national guidelines) which results will be offered. Communication is essential here so that the patient is aware of which results will be made available. Ideally, the patient should be given options for which categories they would like to receive results if more than just the “results related to clinical indication” will be given.

The offer of results to parents of minor patients

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\(^{12}\) ‘Warrior gene’: MAOA gene variants associated with low monoamine oxidase A activity and high aggression levels
This is an issue that poses particular problems, and one which divided opinion in the survey. There is a strong tradition in clinical genetics of not testing children for adult onset conditions, the reason being the importance placed on the autonomy of the future adult. By not testing for adult-onset conditions, the future adult’s choice to know or not to know their status is protected. However, in WGS the child is not deliberately tested for adult-onset conditions (unless opportunistic screening is being carried out), but the results are generated by untargeted WGS performed for a specific clinical indication. Arguments have been made that it is in the interests of the child for the clinician to share the information with their parents, firstly because it may get lost or forgotten by the time the child reaches adulthood, and secondly because their parents may carry the same mutation and therefore be at risk of illness. It is in the best interests of the child to have healthy parents. Against this it could be argued that the child is not directly benefiting from sharing the results in childhood, and that the risk of not protecting their future autonomous choice outweighs any future benefit. In the workshop held in the context of 3 Gb-TEST in Toulouse in May 2015, this topic was debated. The general (but not unanimous) consensus was that the problem of finding predictive adult-onset results in children is so serious and difficult to resolve that the best option is to mask genes known to be associated with adult-onset disorders so that these results are never produced. It was felt that, at least with the current state of medical knowledge, this action best protects the interests of the child.

Proposal: Until such time as the ability to interpret variants and to prevent adult onset conditions advances significantly, it is proposed that when children undergo WGS, results for genes known to be associated with adult-onset conditions but unrelated to the condition being tested for are masked. Parents need to be made aware of this so that they do not think that the child has been tested for those conditions.

Recontact

There are a number of logistical as well as ethical constraints on the process of recontacting patients following whole genome sequencing when the state of knowledge regarding a variant has evolved. The logistical constraints are related to whether the data will be stored for years after the initial test or whether it is cheaper and more effective to re-sequence a new sample, and to the resources required to follow up patients each time that information regarding a variant has changed. It is likely that requiring laboratories to recontact patients for every change in status of every variant found during their analysis would currently be too burdensome. However if a change in status leads to a change in the report issued relating to the indication for testing, it may be argued that the laboratory has a duty to update their report. In the 3 Gb-TEST survey 59.4% of the participants working in clinical genetics-related fields felt that the clinical service ordering WGS has a duty to review previous results in the light of new information and to recontact patients (assuming consent has been obtained). However several participants expressed concerns about the practical challenges of such a procedure, and others commented that such duties should be limited to the original indication for testing, not for incidental findings. It remains to be assessed whether recontact is practically possible.

The possibility of recontact in the other direction should also be foreseen. Patients may benefit from being able to re-access their genomic data at a future point, either for the original test indication or for unrelated indications, for example for personalised pharmacogenetic data. A clinician wishing to prescribe a particular drug could, with the consent of the patient, request a re-analysis of the original data to look at up-to-date pharmacogenetic data, or ask that, in light of new discoveries, a sample is re-examined.

Proposal: Laboratories should not be expected to issue new reports related to variants outside of the indication for testing. However if the change is related to a variant reported in connection with the indication for testing then the laboratory could potentially be considered to have a duty to update the report. Whether such updating is practically feasible or not will depend on such factors as the capacity of the laboratory
and their relationship with the prescribing clinicians. A decision therefore needs to be taken by each laboratory (where such a procedure is not already dictated by local law or professional guidelines) as to whether the laboratory will return results as a one-off report based on the state of current knowledge with no intention to update, or whether results could be subject to updating in the future. Whichever procedure is chosen, the patient must be informed at the time of testing whether their result is a ‘snapshot’ which reflects knowledge at the time of the test or whether it is an ongoing, evolving process liable to give rise to changing interpretations as knowledge advances. If possible data should be kept as part of the patient record so that the patient has the opportunity to benefit from his/her genomic data in the future.

Solidarity and data sharing

Whole genome sequencing is expanding the paradigm of genetic testing. It was already the case that in order to provide answers for a patient, it was necessary to gather information about family members, and in some cases invite them for testing to better interpret results generated through tests on the patient. With whole genome sequencing we often need to look much further for answers. With so many variants being detected, and, for the moment, relatively limited knowledge of their significance in many cases, our ability to be able to make the fullest use of this data will depend on a cooperative endeavour on a global scale. This cooperation can occur at a number of different levels, from patient participation in research projects, to entry of rare variants into databases and sharing of appropriately anonymized and securised data between researchers and clinicians.

Proposal: Opportunities for cooperation should be sought for and used as part of routine practice, as only this kind of large-scale effort will help to achieve more rapid understanding and translation of variant findings. Whilst care must be taken to avoid patients feeling under pressure to agree to participate in research protocols and databases, an explanation of the aims and nature of available cooperative opportunities whenever appropriate will allow more patients to benefit more quickly from genomic testing. Steps should be taken to facilitate sharing of data between research groups and between research and clinical groups wherever this can be done without compromising the boundaries of consent and confidentiality. Work should be done on the design of consent and confidentiality in new research projects and shared databases to consider the possibility of future collaborative efforts.

Conclusion

Genetics has long been a rapidly evolving field, but with the advent of diagnostic whole genome sequencing, new frontiers of knowledge are before us. We are not yet ready to be able to reap the full range of benefits of all that the genome has for us. Before we can arrive at such a point, we need to go through a steep and probably laborious learning process, in which shifting interpretations of results may mean that incorrect reassurance or unnecessary investigations are put in place, or simply that we may be faced for some time with the possibility of regularly finding ourselves in a situation of returning uncertain results. It is important that progress is not hindered, and that we can continue to advance towards these new horizons with a sense of curiosity and anticipation.

Such anticipation should however be balanced by a realistic view of the challenges to overcome before we can access all of this anticipated benefit. For this reason these proposals remain cautious, and call attention to the realities of our abilities to interpret and understand genomic information. We must focus on the means of accomplishing the goal of mastering this interpretation and understanding: being open and clear with patients about what can be accomplished at present with this technology, and inviting them, when appropriate to participate in this acquisition of knowledge and experience by permitting data to be shared. As researchers and clinicians, we can help move towards our goal faster by taking the time to
share data, clinical findings and experiences with the implementation and use of different policies for these issues whenever it is possible and appropriate to do so. Cooperation, both doctor-patient and between research teams will be the key to achieving successful use of this powerful tool.

References


ACMG-American College of Medical Genetics. (2014) ACMG updates recommendation on “opt out” for genome sequencing return of results. [downloaded from https://www.acmg.net/ on 01/09/2014]


Several guidelines for the implementation and use of NGS in a diagnostic setting have already been published. EQA (also called Proficiency Testing, PT) are most covered in the American guidelines. These guidelines encourage the laboratories to participate in EQA to get an independent measure of performance. Ideally, EQA should take place twice yearly and involve the analysis of at least three blinded samples. Both analytical results and interpretation are expected to be returned. After participating in EQA, laboratories should receive a summary of the results of all the EQA participants, a comparison of results and performance as well as the identification of analytical and interpretative errors. In 2012, Gargis et al. suggested that laboratories perform Alternate Assessment (AA) since no EQA was available for NGS. AA involves the exchange of blinded samples between laboratories, the re-testing of patient samples or the testing of split samples by two laboratories. However AA suffers from the small number of exchange partners, the absence of anonymity of the results, the risk of low diversity in methods and technologies, the difficulties to resolve discrepancies and the risk of not identifying systematic errors. Due to the cost of NGS tests, Gargis et al. (2012) finally proposed that labs perform one EQA and one AA, each of two samples, each year. In 2013, Rehm et al. mentioned that they were developing a pilot EQA scheme. Finally, both American guidelines gave recommendations for setting up an EQA scheme. They specifically required that the EQA scheme should be method based (technical wet laboratory and informatics). It should be independent of a gene panel and a genetic disorder and that the verification of analytical accuracy must be done independently. Several kinds of samples were suggested including reference materials, DNA derived from human cell lines, patient samples (gDNA of blood sample), synthetic DNA or electronic data.

A document on the diagnostic use of NGS has been written on behalf of EuroGentest. Three quality control regions (large exons already included in exome capturing kits, and which could be added to any target panel) are proposed. These regions would allow comparison and monitoring between different assays, enrichment methods, sequencing platforms, etc and could thus be used for EQA. The guidelines recommend that laboratories regularly exchange samples and participate in EQA once their test has been validated.

References


8. REQUIREMENTS FOR HTA: CLINICAL AND ECONOMIC EVIDENCE
By M. Eden, N. Davison, K. Lee, and K. Payne

Introduction

Health technology assessment (HTAs) has evolved in an environment of finite and constrained healthcare budgets where difficult choices at regional and national levels must be made regarding how best to use available resources. The basic premise behind the use of HTAs is that they use a multidisciplinary approach to provide a systematic and structured summary of the available evidence base to allow informed decision making as part of subsequent appraisal processes, such as those used by the National Institute for Health and Care Excellence [1].

Current HTAs tend to focus on a particular technology for a specific disease or condition. From the outset, it was clear that to conduct a HTA for whole genome and next generation sequencing technologies will require careful definition of the 'technology', which is a complex combination of: a test; a process for generating a test result; a mechanism of communicating the result and interpretation and use of the result to inform future clinical care. The challenges regarding HTA of a genomic-based diagnostic test are now described based around the five key HTA stages and an exemplar application [2].

1. Define the policy question
It is important to be clear what particular health technology is being assessed. Given the number and breadth of potential applications of whole genome sequencing, this is where we employed key criteria to inform the selection of a relevant case study. It became clear that whole genome sequencing was still only available in the research setting and actual clinical applications had not yet emerged. Therefore, we used an example of a NGS gene-panel test as an indicator of the potential requirements for HTA for genomic-based diagnostic tests using new genomic sequencing technologies.

Two other key definitions were also necessary to plan the scope of a HTA: (1) the nature of the patient population to be tested and (2) the definition of the phenotype being identified. The eligible patient population was defined as adult patients with suspected inherited retinal dystrophies (IRD) that had been referred to a clinical genetics service. The phenotype being identified was a clinically based impression of an inherited eye condition. The patient may (or not) have had additional ophthalmic diagnostic tests.

2. Collate background information
This step involves choosing which aspects of the problem need to be focused upon given the planned use of the final HTA report. The focus of this use of HTA was to provide a systematic and robust summary of the available evidence base to support the reimbursement of genomic-based diagnostic tests. Using this overarching remit the focus of the HTA report was to summarise the epidemiology, clinical effectiveness, safety and economic data to support whether the NGS gene panel test should replace current Sanger Sequencing technologies for the diagnosis of IRD.

3. Define research objectives
This step defines the research objectives to be addressed that must align with the pre-defined policy question and scope of the HTA and subsequent methods used to address the identified objectives. For this HTA, the scope should be defined in terms of five distinct but related research objectives for comparing the NGS gene panel with the current single Sanger Sequencing diagnostic tests for IRD:

- What is the evidence defining the nature and size of the relevant eligible patient populations?
- What is the evidence to support the clinical validity (i.e. is there an association between genotype/phenotype and outcome?)
- What is the evidence to support the clinical utility (i.e. does NGS gene panel test lead to differences in treatment (and outcomes) for patients depending on their genotype/phenotype?)
- What is the evidence to support any potential risks or harms?
- What is the economic impact (i.e. what are the resource use, costs and outcomes
potentially influenced by changing the sequencing technology)?

4. **Conduct clinical & economic review**

This step generally forms the main component of the final HTA report and involves identifying and summarising the clinical, safety and economic literature and if appropriate, or feasible, building and populating an economic model. Standard systematic review methods are generally advocated in which a structured and systematic search of pre-defined library databases are used to identify published randomised controlled trials (RCTs) that are then assimilated, if appropriate given the extent of heterogeneity in the evidence base, using meta-analysis to provide an overall measure of effectiveness [3]. For the purpose of HTA used in the context of whether to reimburse new types of sequencing technologies, this will mean identifying the relevant literature to support clinical validity and clinical utility of the resulting genomic-based diagnostic test. Similar to other diagnostic tests [4], the clinical evidence base for genomic-based diagnostic tests for inherited rare disorders is unlikely to come from RCTs but may come from observational cohort studies. Using evidence from non-randomised study designs can introduce selection and other biases into the evidence base [5] and decision-makers using this evidence base need to take these inherent biases into account. Another key practical difficulty in using observational data is likely to be the observed lack of consistency and clarity in published studies. There is also likely to be differences in the actual technology given that most genomic-based diagnostic tests will be a bespoke laboratory developed test [6]. The lack of consistency may make it difficult to assess the overall clinical sensitivity, specificity and the predictive values of testing since these may differ between laboratories.

A systematic review performed in the context of 3Gb-TEST confirmed there was no existing economic evaluation of a NGS gene panel test for IRD. This inferred a need to develop a de novo economic model. The need for a de novo economic model is likely to be required for subsequent HTAs of genomic-based diagnostic tests as the application will be specific for a particular decision problem. Any observed heterogeneity in the clinical evidence base will have serious implications for a planned economic model. An early stage in structuring an economic model is to develop a clear understanding of the decision problem including the technology, comparators in current clinical practice and study population. This process is done as part of model conceptualisation. Then it is necessary to take account of the available clinical evidence base as the absence of any clinical utility studies, it will be unclear what the impact of the new test and alternatives and possible extent of test uptake will be on the patient population. Early economic modelling studies do still have a role in HTAs and this is likely to be the most promising use of economic evidence in the context of genomic-based diagnostic tests. However, decision-makers will need to recognise the ‘early’ nature of this evidence base when making reimbursement decisions and make sure the HTA process includes a time-scale and plan for reassessment as, and when, new evidence comes available.

5. **Produce final HTA report**

As with any HTA the final report needs to be written taking into consideration the intended audience. A HTA report in the context of new genomic-based diagnostic tests will likely be written for specialist service commissioners and practising geneticists and molecular scientists deciding if and how to introduce new genomic technologies into clinical practice. The lack of evidence on clinical effectiveness (clinical validity and clinical utility) and cost effectiveness, rather than evidence of a lack of clinical and cost effectiveness, is likely to be the short-term challenge for such decision-makers deciding whether to recommend routine use of NGS gene panels in clinical practice.

**Evidence requirements for HTAs of genomic-based diagnostic tests**

The PICO mnemonic provided a framework to define well-specified clinical and economic questions and subsequent evidence requirements for HTA of genomic-based diagnostic tests that use new sequencing technologies, in general.
**Population**
For most HTAs the population of interest can be specified easily by taking account of the licensed indication of a medicine or the defined condition to be diagnosed. It is more problematic to define the population of interest for genomic-based diagnostic tests that use either a specified panel of (some hundreds) genes (NGS) or aim to find any inherited condition (WGS). The population may not be an easily defined group of patients. The definition of the patient in the genetic context is also complicated by the need to extend beyond a single individual to consider a family of individuals as the ‘patient’ unit. The potential for genetic test information to impact upon family members must be acknowledged when determining the appropriate population in each HTA.

For practical reasons, it is suggested that the population to be considered in a HTA of a genomic-based diagnostic test should be initially defined by the presenting population of probands who have developed symptoms suggestive of an underlying genetic cause and have been referred to a clinical genetic service for a diagnosis. It some instances it may be necessary to expand the population to include family members and this should always be explicit in the planned HTA. The population component for a genomic-based diagnostic test is then logically informed by the primary condition of interest, which is in turn informed by the presenting phenotype. However, this approach still places the HTA of a genomic-based diagnostic test within the model of a single-disease focus rather than taking account of the (stated but not proven) applications using a personalised approach to medicine that recognises the patient as a whole rather than a collective of single conditions.

The selected case study of a NGS gene panel test for the diagnosis of IRD illustrated how the size and nature of the population eligible for testing was dependent on the available genomic technology. NGS and WGS could potentially expand the population eligible for testing as current single Sanger sequencing tests limit the population who can be tested. The need to define the nature and size of the relevant population calls for robust epidemiological data to understand the current prevalence of genetic mutations. However, such epidemiological data can only come with better genomic-based diagnosis, which makes proving the value of a new technology into a circular conundrum.

The ability of a new sequencing technology to change the nature and size of a relevant population is a key component that should be captured in a HTA. Model-based economic analyses of healthcare interventions tend to assume that the relevant patient population is in a steady state. This infers that the volume of the ‘intervention’ available is irrelevant and capacity constraints in terms of the intervention are set at zero. In some circumstances ignoring capacity constraints will have a detrimental effect on the relevance of an economic analysis for a decision maker [7]. The impact of increased demand for a genomic-based diagnostic test for IRD was shown when using a NGS gene panel test that allowed all patients with IRD to be offered testing rather than the current proportion of 10% who could be tested using existing Sanger sequencing genetic tests. This increased volume of testing was shown to potentially affect the turnaround time and need for associated genetic counselling services to meet the new demand for offering the NGS gene panel test.

**Intervention**
The intervention for the HTA is generally defined by the manufacturer and product license. For a diagnostic test, either genomic or non-genomic, the nature of the intervention is complicated by being clear whether it is the test or technology that is of primary interest. In addition, a diagnostic test will be embedded within a model of service delivery in which a test must be ordered and then the result read, understood and used to inform a clinical decision. Figure 8.1 illustrates the relationship between the technology, diagnostic test and service. Recognition of this relationship can complicate the focus of the intervention for HTA.

The obvious starting point is to understand the relevant policy question and whether the focus should be what test is used to obtain a diagnosis of an inherited condition or on what technology is used to produce the test to obtain a diagnosis. The difference between these two options may
seem trivial but it is necessary to be clear as the decision reached will affect the scope and design of the HTA. In the exemplar model-based economic evaluation of the NGS gene panel test for the diagnosis of IRD the patient pathways included in the intervention started from referral to a clinical genetic services, through consent for testing to analysing the sample and producing and using the test result. This allowed a clear specification of the decision problem being addressed and a decision-maker using the resulting evidence base can assess if, and how, this patient pathway aligns with their own practice.

**Figure 8.1: The link between the technology, test and clinical service**

Defining the intervention in this way means that patient pathways of care and also methods for how incidental findings will be managed and reported must be specified. This considered approach to defining the exact nature of the intervention under assessment aligns closely with the MRC Framework for the evaluation of complex interventions that involves a transparent step in the process in which the intervention is clearly specified[8].

**Comparator**

The relevant comparator for a HTA of a new intervention should be informed by current clinical practice. In some instances, this will mean that doing nothing is the most relevant comparator. The comparator, as with the intervention, is defined by the relevant decision problem being addressed and the population of interest.

For genomic-based diagnostic tests, the choice of the relevant comparator shares the same challenges as the specification of the intervention. For NGS gene panel-tests the relevant comparators are likely to be defined by identifying current laboratory protocols that specify the sequence of detecting mutations in single genetic tests using Sanger sequencing. It is also important to take account of current diagnostic pathways that methods other than existent molecular tests, such as taking a family history, to inform the genetic diagnosis. In the exemplar model-based economic analysis of using the NGS gene panel test for IRD the relevant comparators were specified by an existing laboratory protocol.

The 3Gb-TEST results highlighted the current lack of national pricing tariffs for genomic-based diagnostic tests. This is a fundamental challenge for conducting a robust HTA and is closely aligned with how both the intervention and comparators are specified and described. Frank et al. [14] suggested that the human genome is made up of 3000 Mb and suggested that the number of Mb is a useful metric to use to compare the cost of sequencing technologies. This is not consistent with current approaches used by laboratories to provide a price charged for a single genetic test. Though, the current process of identifying the cost of a genetic test based on the number of ‘genetic units’ (GenUs), and the absence of national pricing tariffs, is not sustainable or suitable for the purpose of conducting robust HTAs of new genomic sequencing technologies to inform reimbursement decisions.

**Outcome**

The primary outcome of interest for a HTA is different for the clinical and economic components of the evidence base. In general, the clinical outcome of interest will be informed by the target condition or disease being treated. For a diagnostic technology the most relevant clinical outcome will be the number of cases diagnosed.

The primary clinical outcome of interest for a HTA of a new sequencing technology needs to recognise the key aim performing the genomic-based diagnostic test and also capture the number of different end-points possible. The relevant clinical endpoint for a patient is
obtaining a genetic diagnosis for their condition. A good (desired) outcome is getting a positive test result that indicates the relevant genetic cause for the presenting condition (phenotype). A test result that does not offer a diagnosis (a negative test result) would be viewed as a bad (unwanted) outcome. For a genomic-based diagnostic test there is also the potential endpoint of an equivocal test result. To conduct a robust HTA of a new genomic sequencing technology it is necessary to have evidence to inform how many patients fall into each of these ‘end-points’ to provide meaningful data on the relevant clinical outcome.

The clinical outcome used in RCTs and hence a summary of the clinical evidence base in an HTA is generally an intermediate, or proxy, outcome measure. The relevant economic outcome used to measure patient benefit should be guided by the jurisdiction for the economic analysis and the specified decision problem [20]. In general, most model-based economic analysis aim to inform how to spend a healthcare budget, which indicates that changes in health should be identified and measured using a metric such as the quality adjusted life year (QALY). A notable feature of certain diagnostic technologies is that there is sometimes limited scope for health improvements to follow directly from the provision of test results. This may be particularly relevant for genomic-based diagnostic tests used for inherited rare conditions for which no treatment options are available. There are examples in the literature [21,22] where it has been shown that genetic information is valued and can contribute to the outcome ‘capability to make an informed decision’. There is ongoing debate within the fields of health economics and genetics regarding the optimal means of establishing the patient benefits for use in economic evaluations of genomic sequencing technologies [23–25]. In the short term, the most practical solution is to focus on using the appropriate clinical outcome measure within a HTA.

Towards HTAs of genomic-based diagnostic tests

The literature is scattered with publications identifying the challenges associated with conducting HTAs and economic evaluations of genomic-based diagnostic tests [23,26]. These challenges centre on methodological, technical, practical and organisational barriers.

Towards HTAs of genomic-based diagnostic tests: some practical suggestions

Identifying such barriers is necessary but not sufficient in terms of offering a solution for decision makers who need to assess if, and how, to introduce new sequencing technologies for the genomic-based diagnosis of inherited conditions. Further methodological work is clearly required and this needs to be supported by technical developments in decision-analytic modelling. This requires substantial and sustained input from research funding bodies and academic health economists working alongside molecular scientists, clinicians and patients. Changing healthcare system organisational structures and budgets to allow a patient-centred, personalised approach to medicine, rather than the current disease-focussed model, to allow healthcare funding to follow the patient needs is a long-term challenge. In the interim, therefore, it is logical to focus on finding some practical solutions that EU Member States could work together to provide.

- Set national transparent pricing tariffs for genomic-based diagnostic tests. Health economics can offer micro-costing methods to provide some insights into the type of resources used when providing a genomic-based test (obtaining the sample; running the test; computational and manual interpretation of the results; data storage). Such micro-costing studies can provide a clear specification of the resource use required. Laboratories need to agree a metric to use as the unit (for example: Mb of data) and a system to set the price. This requires laboratories to work together within a healthcare system and, given the European movement of samples, across EU MS.
Set clear recommendations on how to define the relevant intervention and comparator for use in a HTA of genomic-based diagnostic tests. 3Gb-TEST has used the PICO framework to outline the key steps and evidence requirements for conducting a robust HTA. Health economists and other health service researcher working collectively with molecular scientists, geneticists, genetic counsellors and patients, should be able to define a clear set of criteria to use when specifying the description of an genomic-based diagnostic test using sequencing technologies and the relevant comparators for use in clinical and economic evaluation studies to feed into the HTA process.

Continue to collate epidemiological data, using robust databases such as Orphanet, to describe the prevalence of inherited rare conditions. Epidemiological data are a key requirement to specify the relevant population for a HTA of a genomic-based diagnostic test. Furthermore, a model-based economic evaluation will need data on the proportion of particular genotypes within a population to generate the predictive value of the sequencing technology.

Set criteria to define when standard HTA that use model-based cost-effectiveness analysis to quantify the added value in terms of health gain (QALYs) are not sufficient. Genomic-based tests that aim to provide a diagnosis in non-cancer, rare inherited diseases are not generally used to inform treatment options, as no treatments are available. The lack of availability of a treatment option could be a simple criterion to use to set aside a HTA process that may need to extend beyond health gain alone as the economic measure of patient benefit. In England, NICE has already set a precedent for a distinct appraisal system and evidence base requirement for specific technologies such as the highly specialised technologies programme that only considers drugs for very rare conditions [29]. A similar system could be set aside for genomic-based diagnostic tests for conditions with no treatment options.

Establish a database of decision-analytic model structures. The use of model-based economic analysis is challenging for technical reasons but a simple practical solution would be to start a quality assured and quality controlled system of collating available decision-analytic models to use as a resource for analysts such that a de novo model does not have to be created for each HTA of a new sequencing technology. This approach has been suggested in the literature [30] for decision-analytic models in general but, to date, not implemented in practice.

Conclusion
The report has suggested some practical solutions to move towards conducting HTA of genomic-based diagnostic tests that use different sequencing technologies. These practical solutions are needed now to provide a starting point to conduct robust HTA of new genomic sequencing technologies so that patient populations and clinicians and healthcare systems can realise the promised benefits. All of these practical solutions are achievable but require the continued efforts of clinicians, patients, scientists, methodologists, reimbursement agencies and healthcare funders and payers, to work collectively and with a common purpose.

References
Technical Roadmap for diagnostic implementation of WGS


On Friday May 30, 2014, 76 scientists met in Milan, Italy, for an expert meeting on the latest developments in genome diagnostics. There was a clear focus on clinical and quality issues related to the introduction of next generation sequencing. The meeting was organised by the European Society of Human Genetics (www.eshg.org), the FP7 funded consortium 3Gb-TEST (www.3Gb-test.eu), and the ESHG EuroGentest committee (www.eurogentest.org).

Policy statement

The 3Gb-TEST policy is based on expert opinions expressed and discussed during the above mentioned expert meeting. Also taken into account were discussions during an expert meeting held in November, 2013, Nijmegen, in the context of the preparation of NGS Guidelines by the Eurogentest consortium. Furthermore, a policy paper on whole genome sequencing in clinical practice (van El et al., 2013) and an opinion paper on the clinical use of exome sequencing (van Zelst et al., 2014) have also been used.

Main Conclusions:

1. NGS techniques are a major step forward in the clinical diagnosis of complex monogenic disorders (with high locus and allelic heterogeneity), and are in principle suitable to substitute parallel Sanger sequencing of multiple genes

2. Several approaches are now emerging:
   • Gene panel approaches (capturing of coding DNA sequences of genes known to be potentially causative for the particular entity)
   • Exome sequencing with filtering at the data analysis and interpretation level for variants in potentially causative genes
   • Whole genome sequencing (WGS) with filtering at the data analysis and interpretation level for variants in potentially causative genes, copy number variant analysis, and identification of balanced structural rearrangements.

Exome sequencing and WGS have the advantage that it has a flexible design, i.e. once a further gene associated with a particular clinical entity has been identified, it is relatively easy to adapt the filter settings, whereas a gene panel approach requires adaptation of the capture array. Exome sequencing has additional advantages: after the analysis of the predefined set of disease genes the data can be further analysed for (1) additional gene panels or (2) for all remaining genes. The first approach may result in unanticipated findings, i.e. if the clinical condition is not clear or actually undiagnosed. The second approach output may be the identification of candidate causative genes. Results from approach (2) cannot be reported as a clinical diagnosis and have to be further studied in a research setting. If the clinical diagnosis allows this it is preferable to use a targeted approach first in order to avoid unsolicited findings.

3. Informed consent is mandatory. Patients should be diagnosed in the context of a medical setting, i.e. diagnostic results should be communicated and explained by a medical specialist such as a clinical geneticist.

4. Written procedures have to be in place on the reporting of unsolicited findings. In case such a variant could cause serious health problems in the person tested or his or her close relatives and the condition is actionable by treatment or prevention, in principle, a health-care professional should report such genetic variants.

5. Guidelines have to be developed by genetic laboratory scientists on how to report variants that may be clinically relevant. Variants of unclear significance should not be reported.

6. Confirmation of the identified variants by Sanger sequencing is not mandatory. Laboratories are advised to validate their analytical strategies and decide on the necessity for confirmation by an alternative sequencing method based on the false negative mutation detection rate. This requires adequate quality
parameters including standards for minimal coverage and required percentages of reads for heterozygous and homozygous variant calling. For the identification of sample swaps a validated procedure should be in place.

7. For more straightforward diagnostics such as a clear-cut monogenic disorder the gold standard can still be Sanger sequencing. This is cost effective and circumvents the problem of unsolicited findings. For this purpose, diagnostic labs are required to develop analytical approaches on when and how to apply which technology.

8. The analytical approach for a complex monogenic condition should at least for an established core list of genes also be capable to exclude at the DNA level that a clinical entity has been caused by genomic alterations in the genes analysed. This requires a clinical sensitivity and reportable range at least equal to Sanger sequencing.

9. In the near future it can be foreseen that whole genome sequencing will be the ultimate generic technique.

References:


van Zelst-Stams W, Scheffer H, Veltman J (2014) Clinical exome sequencing in daily practice: 1,000 patients and beyond. Genome Medicine, 6:2
10. CONCLUSIONS

By E. Bakker

Introduction

Molecular diagnostic testing in human clinical genetics emerged about 30 years ago when the first genes causing monogenic disorders were identified. Novel genetic technologies, e.g. the many PCR based screening methods, often got implemented instantaneously, while proper validation only followed in performing the “diagnostic” tests. Which often took longer than anticipated, due to the limited number samples, in case of rare disorders, and therefore complicated proper separation between research and routine diagnostics. International collaborations such European Molecular Genetic Quality Network (EMQN) and EuroGentest (FP6 Network of Excellence, 2004) helped significantly to assure the quality of molecular testing. This was respectively done by organising external quality assessment since 1996 and, Harmonising clinical genetic services (as of 2004). Also due to converging standard techniques such as PCR and Sanger sequencing, quality issues were properly mastered and reliable diagnostic services were set up throughout Europe. As expertise of the researchers involved was sufficient to introduce most new tests in the diagnostic laboratories, services were up-to-date and quality was maintained at the highest levels.

With recent NGS (next generation sequencing) developments, again overlap between research and diagnostics appeared. Both equipment and kits (capture kits, targeted gene panels and whole exome kits) were introduced instantly and used as diagnostic tools... A new collective action was needed and found in Eurogentest and TechGene (FP7-project). Together, guidelines for diagnostic NGS (Matthijs et al. in press) were prepared and after consultation by the professionals in the field the guidelines were accepted.

These NGS guidelines will help to uniformly introduce both targeted gene panels, and whole exome sequencing (WES) into diagnostic labs.

Also for future introduction of whole genome sequencing (WGS) in the diagnostics these NGS guidelines form a good basis. As it is anticipated that it will soon be easier, quicker and cheaper to sequence an entire genome than to sequence a single gene. Whole genome sequencing will then be a more effective and a financially viable alternative for many genetic diagnostic tests. Sequencing the total human genome (WGS), or a 3Gb-Test as we coined it, is coming close (probably within 5 years) to be implemented as a tool in healthcare. Here in our 3Gb-TEST project we have identified what is missing and is needed (‘gaps and needs’) to implement WGS in the diagnostic setting.

Wet lab innovations; Technological developments, current status.

The so-called “next generation sequencing” NGS, covers a spectrum of sequencing approaches used nowadays, which all have in common massive parallel sequencing, often of multiple individuals and or multiple targets. These different strategies and the technical background of the different commercially available NGS platforms have recently been reviewed with respect to template generation, the sequencing reaction, and their potential use in whole genome sequencing (WGS).

The advancements made on sequencing technology over the last years have been impressive. We expect that advancements will continue and that ultimately (in 5-10 years) sequencers will allow single molecule amplification free base detection, probably without fluorescent labels. These sequencers will yield long reads with low GC bias. They will be scalable in data output and will be cost efficient. With this in mind we did formulate the following need:

Need 1: A regular (at least yearly) check on the advancement of the sequencing platforms.

This first need is advised to foresee upcoming changes and their potential for the diagnostic setting.
Eliminating enrichment procedures

For the time being WES is still the preferred approach (Sun et al. 2015). However, as soon as sequence costs and technology allows, WGS will replace WES and the larger targeted panels when investigators start using WGS-derived exome data without analyzing the intragenic sequences. Further, as it will be possible not only to read out the exome variation (SNP’s, indels) from the WGS data but also the genome variation (structural variants and CNVs), WGS will ultimately replace the Arrays. Later, the increased knowledge on intragenic sequences will be used to analyse these regions as well. This gradual approach will be an ideal way to bridge the gap in knowledge identified between WES and WGS. The existing lack of information about the non-coding regions in the genome, which hamper proper interpretation of these non-coding regions of the genome, can gradually be filled. By building up statistical (genotype-phenotype) data on the non-coding regions of the genome.

Need 2: Filling the gap between WES and WGS gradually, by using WGS - WES read-out and collecting genotype - phenotype information on the non-coding regions in the genome.

BioIT tools for data analysis, variant detection, variant prioritization and variant effect prediction.

Within the 3Gb-TEST project a catalogue was prepared of the vast number of software programs available for analysis and interpretation of data, by collecting information and comparison of these programs. By use of an extensive literature survey, an on-line questionnaire, a web search, and discussions within and between 3Gb-TEST Work Packages, information was collected and sorted. This yielded an overview of gaps for bioinformatics tools for use in NGS and especially in WGS. Noted was that clinical, diagnostic trustworthiness of prediction and assessment tools for WGS are an issue rather than the availability and range of options. In this overview, we found that many of the different tools (over 180) currently available, are for variant assessment and interpretation support. Similar data was recently published by the ACMG (Richard S. et al., 2015) as an important set of guidelines. Our roadmap together with the ACMG guidelines provide best practices for assessing and reporting on the clinical significance of NGS variants in a routine clinical setting. The ACMG guidelines mention a wide range of resources, tools and practices and the level of evidence they provide towards benign or pathogenic classification.

Many population frequency databases, variant assessment algorithms with and without phenotype information, and annotations exist. However, there are so many options to choose from that stratification must be done by comparing outcomes of different pipelines. The high throughput of the large number of variants in the whole genome forms a challenge by itself. Not only at the technical level (tools that can deal with this task) but also for the interpretation and reporting SOPs of a lab. Therefore, automation of interpretation procedures and an associated mind-set switch are required. Labs need to face the fact that at the single gene level, a handful of variants can be reviewed manually but at the exome and even the genome level, 10 or 100 000s of variants are not manually reviewable anymore.

Need 3: Standard data sets (reference sets) for variant prediction and testing bioinformatics tools to come to some level of community consensus on which sources are trustworthy and useful for diagnostic WGS.

Clinical input is needed to stratify the genes to be analysed and detected variants to be interpreted. Standardisation of patient phenotypes are key to correct WGS interpretation, patient characteristics will need to be thoroughly evaluated and clearly recorded using standardized terms (ICD-10), for example by use of the Human Phenotype Ontology (HPO) and Phenomizer.

Need 4: Introducing - next to genotype information - also phenotype information into the routine diagnostic practice by automation, by
use of phenotype standardisation and nomenclatures such as HPO.

Gene-panel-based approaches as well as exome sequencing-based and filtering of potentially relevant gene variants at the bioinformatics level can be distinguished. Advantages and disadvantages were reviewed in detail. In brief, exome sequencing has the advantage of flexible adaptation of the genes that can be included in the clinical interpretation phase, e.g. when a novel causative gene for a particular condition has been identified. Also, if consent has been given by the patient, also the remainder of the exome can be analyzed. This opens up the possibility to identify in a clinical setting hitherto unidentified causative genes, i.e. unanticipated findings can be made. As a result novel candidate genes may result from a clinical test, but the finding needs further confirmation in a research setting, either by bioinformatics analysis or by functional testing.

This underlines the need for improved variant data sharing between clinical and research genetic laboratories. The drawback of clinical exome sequencing is that also unsolicited findings can be made, though this risk can be limited by a good design of the bioinformatics filtering step. In gene panel-based approaches this disadvantage is more limited, though also not fully excluded.

Need 5: Further automation of interpretation of variants is needed.

Also evident is the need for a “Variant validation network: a network of bio- and medical-informatics tools and databases with potential to support variant validation” from the original “Variant validation net-work: A network of European laboratories with potential to support functional studies for variant validation”. Functional tests, in vitro and/or in vivo might be needed to ultimately proof the effect of a given variant, this could be a collaborative effort with research laboratories.

Need 6: Organized functional tests, in vitro and/or in vivo to proof the effect of a given variant.

To conclude, under the current circumstances it is not possible to propose a single network of bio- and medical- informatics tools and databases with potential to support variant validation. However, the fast rate of progress towards the incorporation of standards in bio- and medical- informatics tools and databases used in the clinical interpretation of WGS data is encouraging. To achieve the ultimate goals it is important that all stake-holders are encouraged to participate in all collaborative efforts underway.

Indications for WGS and its clinical utility.

While WGS eventually could replace most of the current genetic tests, clinical utility should be proven before implementation is started, as highlighted in the NGS guidelines by Matthijs et al3.

The analytical approach for a complex monogenic condition should at least be performed for an established list of core genes, capable to exclude at the DNA level that a clinical entity has been caused by genomic alterations in the genes analysed. This requires a clinical sensitivity and reportable range at least equal to Sanger sequencing and thereby a higher diagnostic yield. Currently, there is no consensus when to use which test, a single (or multiple) gene test, a targeted test (panel of genes) by use of NGS, or to perform a WES, let alone a WGS. It all will depend on the costs to run a certain number of samples, e.g. throughput, turn- around-time requested or needed and the overall mutation detection rate.

Need 7: Discussion and guidance on types of diagnostic tests to be used, per indication.

With respect to clinical utility when testing for an inherited disease, the evidence required for the tests depends on the indication e.g. confirmation of clinical diagnosis and if the disease management will be influenced by the result of the genetic test. Also cost effectiveness of alternative diagnostic methods is to be judged. Which in this respect often depends on the clinical entity tested. WGS will turn out to be
more cost efficient due to the significantly improved mutation detection rate.

**Training of professionals.**

To support effective use of WGS in a clinical context, education of health scientists and practitioners is fundamental. Comprehensive training programs are needed for laboratory and clinical geneticists, as well as non-geneticist physicians, especially in the use of bio- and medical-informatics tools and databases. This spans from raw WGS data analysis through data evaluation, validation and interpretation, preferably within structured pipelines that have been validated for the quality level and accuracy that is acceptable for clinical use, storage and data sharing tools. Additionally health scientists and practitioners will need to become familiar with the concept and possibly direct use of and analysis of “big” data.

Need 8: Comprehensive training programs for laboratory and clinical geneticists, as well as non-geneticist physicians, especially in the use of bio- and medical-informatics tools and databases.

Furthermore, as patient phenotypes are key to correct WGS interpretation, patient characteristics will need to be thoroughly evaluated and clearly recorded using standardized terms (ICD-10), for example by use of the Human Phenotype Ontology (HPO) and Phenomizer7, (need 4) and health practitioners will need guidelines for counselling patients pre- and post-WGS testing.

**Ethics.**

Like all NGS applications, WGS is to be implemented according to the Guidelines for diagnostic NGS2. WGS data can be analysed stepwise for a specific disorder or by filtering with focus specific genes (panel). This has additional advantages: after the analysis of the predefined set of disease genes the data can be further analysed for additional gene panels or for all remaining genes (Exome). These latter approaches may result in unanticipated findings, i.e. if the clinical condition is not clear or actually undiagnosed. The output may also be the identification of candidate causative genes, which of course cannot be reported as a clinical diagnosis and has to be further studied in a research setting. If the clinical diagnosis allows this it is preferable to use a targeted approach first in order to avoid unsolicited findings. A concise pre-test counselling procedure is needed that gives patients sufficient information to make an informed choice, without overwhelming them to the point that making an autonomous choice is unreasonably difficult. Zeiler et al., (2004) has argued that too much choice actually harms, rather than promotes, autonomy7.

Need 9: Counselling procedure for counselling patients pre- and post-WGS testing.

Genetics has long been a rapidly evolving field, but with the advent of diagnostic whole genome sequencing, new frontiers of knowledge are before us. We are not yet ready to be able to reap the full range of benefits of all that the genome has for us. Before we can arrive at such a point, we need to go through a steep and probably laborious learning process, in which shifting interpretations of results may mean that incorrect reassurance or unnecessary investigations are put in place, or simply that we may be faced for some time with the possibility of regularly finding ourselves in a situation of returning uncertain results. It is important that progress is not hindered, and that we can continue to advance towards these new horizons with a sense of curiosity and anticipation. Such anticipation should however be balanced by a realistic view of the challenges to overcome before we can access all of this anticipated benefit. For this reason these proposals remain cautious, and call attention to the realities of our abilities to interpret and understand genomic information. We must focus on the means of accomplishing the goal of mastering this interpretation and understanding: being open and clear with patients about what can be accomplished at present with this technology, and inviting them, when appropriate to participate in this acquisition of knowledge and experience by permitting data to be shared.
As researchers and clinicians, we can help move towards our goal faster by taking the time to share data, clinical findings and experiences with the implementation and use of different policies for these issues whenever it is possible and appropriate to do so. Cooperation, both doctor-patient and between research teams will be the key to achieving successful use of this powerful tool.

Need 10: Open approach when using these novel technologies, were it is important that progress is not hindered, and that we can continue to advance towards these new horizons with a sense of curiosity and anticipation.

Health technology assessment, Costs reimbursement Healthcare

At present the costs for WES and WGS are not reimbursed in most European countries. A sustainable reimbursement system is required before these technologies can become a routine test. For value-based reimbursement, payers demand evidence of improved clinical outcome, efficacy, effectiveness and cost efficiency.

The roadmap suggests some practical solutions to move towards conducting HTA of genomic-based diagnostic tests that use different sequencing technologies. These practical solutions are needed now to provide a starting point to conduct robust HTA of new genomic sequencing technologies so that patient populations and clinicians and healthcare systems can realise the promised benefits. All of these practical solutions are achievable but require the continued efforts of clinicians, patients, scientists, methodologists, reimbursement agencies and healthcare funders and payers, to work collectively and with a common purpose. Set national transparent pricing tariffs for genomic-based diagnostic tests. Health economics can offer micro-costing methods to provide some insights into the type of resources used when providing a genome-based test (obtaining the sample; running the test; computational and manual interpretation of the results; data storage).

Such micro-costing studies can provide a clear specification of the resource use required. Laboratories need to agree on a metric to use as the unit (for example: Mb of data) and a system to set the price. This requires laboratories to work together within a healthcare system and, given the European movement of samples, across EU member states.

Need 11: Transparent pricing tariffs for genome-based tests.

Capacity building within Europe

It will be necessary to increase the test capacity in Europe swiftly and to make WGS cost efficient. European scientists will have to be trained to perform the new diagnostic tests and to analyse the genome data. Regional test centers will have to be established. The current practice to involve American and Chinese laboratories for wet-lab NGS analyses is to be discontinued. Instead, one should focus on building sufficient capacity in the European regions, establishing national centers.

Need 12: Increase cost-efficient testing capacity locally.

Conclusion

The overall consensus for the short term is that WGS is not ready for diagnostic use yet, but soon will be. Therefore we suggest to first expand the current WES analysis with CNV detection. This will replace Array analysis as a first line tool and is for the time being be the best (cost efficient) option. Meanwhile the advancement of the sequencing platforms will have to be checked regularly because, as soon as the price allows WGS should be used to read out the exome as well as all genomic CNVs. This latter option would be an ideal way to bridge the gap between WES and full WGS, and
add info to build up statistical (genotype) data on the non-coding regions of the genome.

Whilst the technology of genome sequencing is now almost a sufficiently mature and affordable technology for it to be implemented clinically, significant challenges around interpretation and implementation remain. The 3Gb-TEST project assessed these challenges, discussed them and did produce an extensive technology roadmap (i.e. this document) highlighting the gaps involved. Here, we have listed 12 of the identified needs that need to be in place before the full diagnostic implementation of WGS will be/can be called successful.

References


SUMMARY

In a two year EU funded (FP7) coordination support action (CSA) project: 3Gb-TEST, we explored the current status of whole genome sequencing to identify gaps and needs for its implementation in routine diagnostics. As history learned, novel genetic technologies, e.g. the many PCR based screening methods, often got implemented instantaneously, while proper validation only followed in performing the “diagnostic” tests. These blurring lines between research and diagnostics are usually caused by the limited number of samples in case of rare disorders. Similarly with all recent NGS (next generation sequencing) approaches a similar trend was observed, the myriad of equipment and available capture kits, targeted gene panels up to whole exome kits are being introduced instantly as diagnostic tools all with limited in-house validation. Sequencing the total human genome (WGS), or a 3Gb-Test as we coined it, is coming close to be implemented as a tool in healthcare. Here we were early enough to identify what needs to be in place before implementation. Workshops and expert meetings were organized to address the following topics: Wet lab innovations, Bioinformatics’ tools, Indications for WGS and Clinical utilization, Training of professionals, Ethics and last but not least Health technology assessment.

 Twelve major gaps and needs were identified:

1. A regular check on the advancement of the sequencing platforms.

2. Filling the gap between WES and WGS gradually, by using WGS - WES read-out and collecting genotype - phenotype information on the non-coding regions in the genome.

3. Standard data sets (reference sets) for variant prediction and testing bioinformatics tools to come to consensus on which sources are trustworthy and useful for diagnostic WGS.

4. Introducing - next to genotype information - also phenotype information into the routine diagnostic practice by automation, by use of phenotype standardization and nomenclatures such as HPO.

5. Further automation of interpretation of variants.

6. Organized functional tests, in vitro and/or in vivo to proof the effect of a given variant.

7. Discussion and guidance on types of diagnostic tests to be used, per indication.

8. Comprehensive training programs for laboratory and clinical geneticists, especially in the use of bio- and medical-informatics tools and databases.

9. Counselling procedure for counselling patients pre- and post-WGS testing.

10. Open approach when using these novel technologies, were it is important that progress is not hindered, and that we can continue to advance towards these new horizons with a sense of curiosity and anticipation.

11. Transparent pricing tariffs for genome-based tests.

12. Increase cost-efficient testing capacity locally.

Concluding, the overall consensus for the short term is that WGS is not yet ready for diagnostic use, but soon will be. We suggest to first expand the current WES analysis with CNV detection. This can be achieved by performing low-pass WGS (<0.5x coverage) in addition to WES, or by using novel WES capture kits with the capacity to also interpret genomic CNVs. This will replace Array analysis as a first line tool and is for the time being be the best (most cost efficient) option. Alternatively, if the price allows, WGS (at e.g. 30x coverage) could be used to read out the exome in an unbiased manner as well as all genomic CNVs. This latter option would be an ideal way to bridge the gap between WES and full WGS, and add info to build up statistical (genotype) data on the non-coding regions of the genome.
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