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ORIGINAL ARTICLE

On the future of genetic risk assessment

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Abstract Next-generation sequencing (NGS) techniques have greatly accelerated the molecular elucidation of Mendelian disorders, and affordable NGS-based diagnostic tests are around the corner that promise to detect or rule out mutations in specific subsets of the known disease genes. Whole exome sequencing and shortly afterwards whole genome sequencing (WGS) will become an even more comprehensive alternative to such targeted tests. In view of the current enthusiasm to implement these methods, but also given their rapidly dropping costs, it is quite possible that WGS will soon be adopted as universal intake test in Clinical Genetics. Central databases and large-scale genotype–phenotype comparisons will be required to progressively identify the clinically relevant sequence variants and to distinguish them from neutral polymorphisms in the human genome, and these databases will become indispensable for the interpretation of individual genome sequences. In this scenario, there will be massively growing demand for genetic counselling, but the need for experienced syndromologists will not increase proportionally, as the success of the diagnostic process will become far less dependent on the ability of clinical geneticists to reliably recognize genetic syndromes.

Introduction

Since the introduction of high-throughput DNA sequencing technologies, the costs of sequencing the (non-repetitive portion of the) human genome have plummeted, from approximately US$ 100 million for sequencing Craig Venter's genome in 2007 (Levy et al. 2007) to US$ 1 million in 2008 (Wheeler et al. 2008) to US$ 5,000 in June 2011 (Complete Genomics, Illumina, and other commercial sequencing services.), and there is reason to believe that in a few years, whole genome sequencing (WGS) will be offered for less than US$ 1,000. For sequencing only the protein-coding portion of the human genome, or exome, which carries most of the disease-causing mutations, costs are expected to break through the US$ 1,000 barrier much sooner, and diagnostic tests for X-linked or severe recessive childhood disorders are under development that will be even more affordable (Bell et al. 2011). Therefore, the question is no longer whether, but when deep sequencing will become routine in the diagnosis of genetic disorders (Bainbridge et al. 2011; Kingsmore and Saunders 2011). In this article, I will discuss the technological aspects of these developments, their practical implications for the diagnosis and prevention of genetic disease, as well as the consequences for the future organisation of Genetic Services.

Next-generation sequencing: technological aspects

The high throughput of all next-generation sequencing (NGS) techniques is due to the simultaneous analysis of several thousand to millions of DNA sequences instead of analysing one DNA fragment at a time, and most next-generation sequencers use sequencing-by-synthesis protocols. However, different signal detection methods are employed: Roche FLX sequencers measure pyrophosphate that is released when single nucleotides are added to the nascent DNA chain, in a light-generating reaction known as

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pyrosequencing; whereas Ion Torrent measures the release of protons during this process, using miniaturized pH metres. Otherwise, these two procedures are very similar: prior to sequencing, the DNA is fragmented, amplified and rendered single stranded on the surface of microbeads. Individual beads are then sequestered into microwells on a plate, where the opposite DNA strand is synthesized by stepwise incorporation of the four nucleotides A,T, G and C.

Illumina, the market leader, employs an ingenious procedure to generate millions of clonally amplified, single-stranded DNA fragments on the surface of a slide, or flow cell, and sequencing is based on the simultaneous incorporation of all four nucleotides, each carrying different fluorescent tags as well as terminators, into the nascent opposite strands in a stepwise manner. After each step, fluorescence signals of all clones are monitored and stored as images, and fluorescent tags and terminators are removed to prepare the growing DNA strands for the next cycle. Life Technology's SOLiD sequencer, the commercial NGS system with the second highest market share, is based on oligonucleotide hybridization and ligation (for details, see Mardis 2008). Output- and cost-wise, the novel SOLiD 5500xl and Illumina's most advanced HiSeq 2000 system are almost comparable, but the latter has a higher read length which facilitates the alignment of sequence reads to the human genome.

For Roche's FLX sequencer, operating costs per base are about ten times higher, and both this system and the conceptually similar 'Personal Genome Machine', marketed by Life Technology's subsidiary Ion Torrent, have problems with sequences containing long homopolymer stretches; on the other hand, the superior read length of the Roche FLX sequencer renders it particularly suitable for the de novo sequence assembly and for certain diagnostic applications. As to Ion Torrent, its highly parallel pH detection system is integrated into a semiconductor platform, which renders sequencing very fast and inexpensive, and further price reductions can be expected as the costs for semiconductor chips continue to decline. At present (September 2011), the capacity of the 'Personal Genome Machine' is still too low for mutation screening of whole human genomes or all protein-coding sequences, but it looks like a perfect choice for sequencing smaller targets, such as all genes implicated in deafness, blindness or mitochondrial diseases (Tucker et al. 2011), which can be enriched efficiently by hybrid capture or droplet PCR (Hu et al. 2009a, b; Mamanova et al. 2010; Mondal et al. 2011).

The latest addition to the growing list of high-throughput sequencers is the PacBio RS, the first real-time, single-molecule sequencing system on the market. In principle, its technology does not depend on prior DNA amplification, and it requires very little input DNA. Other distinguishing features are the superior read length which even exceeds that of Sanger sequencing, as well as its enormous speed. With this technology, individual DNA polymerase molecules, immobilized at the bottom of nanowells, can be monitored while synthesizing double-stranded DNA, using single-stranded target DNA as template. A problem of this approach is the high error rate which necessitates 'rolling circle sequencing' (Eid et al. 2009), as well as the high price of the system. Despite the low operating costs, it is therefore too early to tell whether this technology will find its niche in a clinical setting, and when.

Other, even more advanced and potentially far less expensive methods are under development. Indeed, the first commercial nanopore sequencer should be on the market before the end of 2012 (see http://www.nanoporetech.com/news/press-releases/view/39/), but it remains to be seen when its performance will be superior to already existing sequencing technologies, which also undergo constant improvement.

A breakthrough for the elucidation of genetic disorders

Since the first description of NGS systems (Bentley et al. 2008; Margulies et al. 2005) and the development of methods for genome partitioning, including genome-wide exon capture and droplet PCR-based DNA enrichment (Gnirke et al. 2009; Hodges et al. 2007; Hu et al. 2009a, b; Mondal et al. 2011), numerous groups have successfully combined these methods to identify the molecular causes of monogenic disorders, and whole exome sequencing has been established as a potent and affordable strategy to identify disease-causing mutations in the 1 % of the human genome that codes for protein (reviewed by Gilissen et al. 2011). It is becoming clear, however, that this approach is not successful in a significant proportion of families with monogenic disorders (e.g. see Shendure 2011). Apart from mutations in non-coding sequences, uneven enrichment and insufficient sequencing depth, as well as difficulties to recognize causative mutations in a sea of polymorphic sequence variants may play a role, as suggested by the high mutation yield of more focused approaches combining linkage mapping, targeted exon enrichment and NGS (Najmabadi et al. 2011). In view of rapidly diminishing costs, WGS will soon replace whole exome sequencing (WES) as the method of choice for identifying disease-causing mutations. This will shed more light on the neutral variability in the human genome, and in turn, together with the ongoing large-scale sequencing of healthy controls (e.g. see http://www.1000genomes.org/), it will greatly facilitate the identification of causative mutations, even in non-coding sequences.

There are probably far more than the ~7,000 monogenic conditions described to date (Cooper et al. 2010; Ropers 2007). Their molecular elucidation has to be a priority of genome research because it is a prerequisite for diagnosis, prevention and therapy of these disorders which are mostly

severe and have a high recurrence risk. Moreover, leading genome researchers are now convinced that the systematic elucidation of monogenic disorders will also be a clue to understanding the pathogenesis of complex diseases and that it is a better option than genome-wide association studies (Check Hayden 2009).

NGS in molecular diagnosis and preconception care

The introduction of NGS techniques offers great promise for the molecular diagnosis and prevention of genetic diseases. NGS-based tests have already proven very useful for the diagnosis of the many specific gene defects underlying deafness, retina degeneration, mitochondriopathies and other genetically heterogeneous disorders, as discussed above. Diagnostic tests of this kind have also been developed for the >90 gene defects known to give rise of X-linked intellectual disability, and they are even offered by commercial suppliers (e.g. Ambry Genetics, USA, http://www.ambrygen.com/). Going one step further, researchers at the (US) National Center of Genome Resources, Santa Fe and the Children's Mercy Hospitals in Kansas City, MO have developed a test to rule out mutations in (the coding portion of) almost 500 genes implicated in severe recessive childhood diseases (Bell et al. 2011; Kingsmore and Saunders 2011). After validation, this test should be available in spring 2012 and will be competitively priced (D. Dinwiddie and S. Kingsmore, Kansas City, personal communication). Future versions of this test are expected to cover an even wider range of severe childhood disorders, including genes that have been implicated in patients with dominant de novo mutations (e.g. see Hamdan et al. 2009; Vissers et al. 2010). A similar test that covers all exonic sequences on the X-chromosome has been employed for mutation screening in >250 families with X-linked forms of intellectual disability (Kalscheuer et al., presented at the Annual ESHG meeting, Amsterdam 2011); in principle, it can be used as a comprehensive diagnostic test for all X-linked disorders.

Another potential application of these tests is the identification of carriers for recessive gene defects. Indeed, the preconception identification of couples at risk for having children with Batten disease and other recessive disorders had been the original aim of Kingsmore and colleagues before they decided to first employ their test as a diagnostic tool (see http://www.ncgr.org/preventing-rare-genetic-diseases). In Western populations with their small family sizes, most patients with recessive disorders are isolated cases, and therefore, recessive inheritance or even a genetic etiology will often not be considered. Moreover, even if the correct diagnosis is made and the molecular basis of the disorder is known, very few cases can be prevented by prenatal diagnosis in subsequent pregnancies.

Assuming that in our population, the birth prevalence of severe recessive disorders is between 0.25 and 0.5 %, 1 to 2 % of all couples will be at risk for having affected children, and for consanguineous parents, the proportion of couples at risk will be even much higher. To put this into perspective, the Down syndrome risk for children of 37 years old mothers is also about 0.5 %, and there is consensus in our society that this justifies prenatal diagnosis, in spite of the small, but measurable risk that this intervention will induce an abortion (reliable non-invasive prenatal tests are under development, but so far, only for excluding Down syndrome, e.g. see Chiu et al. 2011; Papageorgiou et al. 2011). In contrast, preconceptual carrier testing for severe recessive disorders is a non-invasive procedure that can be done on blood, hair roots or even buccal smear of the parents. Therefore, it is likely that before long, diagnostic tests for recessive disorders will be employed for voluntary preconception carrier testing, not only in Near and Middle East countries where parental consanguinity is common, but also elsewhere. In the long run, the inherently more difficult prenatal detection of heterozygous disease-causing mutations can also be envisaged, as a reproductive option to rule out disorders that are due to dominant de novo mutations, which may be a relatively common in outbred populations (Hamdan et al. 2009; Vissers et al. 2010)

The introduction of such tests as novel diagnostic tools will significantly shorten the time required to establish the molecular diagnosis in patients and families which rare diseases (for more details, see below). Since these tests will provide little or no information about other, unrelated genetic risks, their introduction should meet no opposition, neither from health care professionals nor from the public. Moreover, because of their small target size, it is likely that for a number of years, these tests will remain more affordable than WES and WGS, also because data analysis and storage will be less costly (e.g. see Sboner et al. 2011).

On the other hand, these tests are generally confined to the protein-coding (or exonic) regions of the relevant genes, which have to be enriched prior to NGS. Therefore, they will miss a (hitherto unknown) proportion of the disease-causing sequence variants. For the same reason, WES is not an ideal diagnostic option, even though it is becoming very popular in research. Compared to procedures focusing on specific disease genes, the larger output of WES renders it more difficult to identify specific disease-causing changes, and WES will generate more unsolicited genetic information. As sequencing costs continue to drop, the price difference between WES and WGS is rapidly diminishing, and due to the additional costs of DNA enrichment, WES will eventually become even more expensive than WGS. Thus, it seems likely that as a diagnostic procedure, WGS has a brighter future than WES, also because the former has the potential to detect all genetic variants in the human genome.

Implementation of NGS as a diagnostic tool: array CGH leads the way

According to a recent compilation, present net costs of WGS amount to US$ 6,500, without depreciation and labour costs and without identification of potentially causative genetic variants (Sboner et al. 2011), whereas sequencing services offer WGS including sequence analysis for US$ 5,000 or even US$ 4,000, depending on the number of samples. Reduced prices are the result of fierce competition between commercial service providers, but with the existing technology it is doubtful whether costs will break through the US$ 1,000 barrier any time soon. Still, sequencing costs are no longer a decisive argument against the introduction of WGS as diagnostic tool, given the much higher costs of current 'try and error' strategies to find the causative defect by sequential exclusion of more or less plausible candidate genes. At present, WGS is barely more expensive than the exclusion of mutations in single large genes by conventional Sanger sequencing (e.g. see Heger, M., http://www.genomeweb.com/print/979677.)

Established NGS technologies are at least as reliable as conventional sequencing, because they involve much less error-prone human intervention. For example, in a recent study that led to the identification of 50 novel candidate genes for intellectual disability (Najmabadi et al. 2011), virtually all discrepancies between the results of NGS and subsequent Sanger sequencing performed to validate these findings could be traced back to the validation step. Even the problem that is common to WGS, WES or more targeted NGS-based mutation detection tests, i.e. the difficulty to distinguish clinically relevant mutations from neutral sequence variants, is not a valid argument against the clinical introduction of these techniques. After all, the same complication arises every time when Sanger sequencing reveals a novel, not previously reported sequence variant in a disease gene, and indeed, comprehensive NGS-based re-analyses have recently found that 12 % of the previously reported mutations are not disease-causing (Bell et al. 2011).

In principle, this problem can be solved by large-scale WGS, as shown by Pelak et al. (2010), who found that sequencing only 20 unrelated individuals reduced the number of novel variants from 3.4 million to a mere 150,000 per genome. Therefore, sequencing 100,000 individuals and comparing the results with their complete medical records, as previously proposed by G. Church and co-workers (http://www.personalgenomes.org/), would identify the vast majority of changes that do not give rise to disease, thereby greatly facilitating the identification of the disease-causing ones. However, it is doubtful whether this proposal will ever get off the ground, and the same aim can also be reached by large-scale diagnostic application of targeted NGS, WES or WGS, as illustrated by the implementation of array comparative genomic hybridization (CGH) as a diagnostic test.

Long before array CGH was officially recognized as a reliable technology for detecting unbalanced rearrangements in the human genome, numerous research laboratories adopted it because of its superior resolution and ease, and when used in a diagnostic context, conspicuous array CGH results were validated by targeted FISH studies. Many novel and recurrent genomic imbalances were found, both in patients and healthy individuals, and collecting these data as well as the relevant clinical findings in central databases like Decipher (http://decipher.sanger.ac.uk/) or the Database of Genomic Structural Variations (http://www.ncbi.nlm.nih.gov/dbvar) was instrumental in distinguishing between disease-causing and apparently neutral polymorphic variants. When tiling path BAC arrays were replaced by commercial very high-resolution oligonucleotide arrays, this technology became widely accessible, and at present, array CGH is an established diagnostic procedure which is about to replace conventional karyotyping worldwide.

The analogies with NGS are striking: already now, only a few years after its introduction, leading manufacturers of NGS systems observe a shift of genome sequencing from research to clinical application (see Heger, M, op. cit.), which implies that NGS is already being used in the clinic even though none of these systems have been certified as diagnostic tools. This is not problematic because—and as long as—conspicuous findings are always validated by established technologies such as Sanger sequencing. In contrast, a huge and as yet unsolved problem is the identification of clinically relevant mutations in a sea of functionally neutral single nucleotide polymorphisms. Common single-nucleotide polymorphisms (SNPs) can be filtered out through comparison with genomes and exomes from healthy individuals (e.g. http://www.1000genomes.org/; see also Li et al. 2010) or dbSNP (see http://www.ncbi.nlm.nih.gov/projects/SNP/, despite its increasing contamination with clinically relevant mutations), but this approach is not possible for the many rare SNPs in the human genome. The only practical solution for this problem is to submit all conspicuous sequence variants, as well as the corresponding clinical findings, to central databases, such as the Human Gene Mutation Database (HGMD, see http://www.hgmd.org/), a counterpart of the previously mentioned databases for diseases-associated copy number variants. It is currently not clear whether the HGMD will be able to function as a clearing house for this purpose or whether other bodies such as the Human Variome Project (http://www.humanvariomeproject.org/) or the Human Genome Organization (http://www.hugo-international.org/) will step in, but without central databases for apparently disease-causing mutations, the clinical significance of many genetic variants and the role of the relevant genes in disease may remain in

limbo for a long time. Even so, there cannot be any reasonable doubt that rather sooner than later, NGS will become a standard diagnostic procedure in Medical Genetics and beyond, even though the prediction may be premature that by 2020, all newborns will be routinely checked by WGS (J. Flatley, CEO Illumina, see Julia Karow, Clinical Sequencing News, June 15th 2011).

On the future organisation of genetic services

In Germany and several other European countries, it usually takes several years to establish the correct diagnosis in a family with a rare genetic disorder, and in 40 % of the cases, the first diagnosis will be wrong (source: EURORDIS, http://www.eurordis.org/). At least in part, this seems to be related to the organisation of genetic health care, i.e. the absence of comprehensive Clinical Genetic Centres that are interconnected and integrated into large, preferably academic hospitals, as in the UK and the Netherlands where the standards of genetic health care are particularly high. At present, an even more critical factor is the availability of experienced genetic syndromologists, which are particularly rare in countries where the quality of genetic health care lags behind. In view of the renewed focus of genome research on monogenic disorders (Check Hayden 2009; Ropers 2010b), it is likely that during the next few years, thousands of novel disease genes will be identified, and this will temporarily aggravate the lack of genetic syndromologists. In the long run, however, the introduction of large-scale WGS will neutralize this effect, and it will have major implications for the organisation of genetic health care, as discussed below.

There is no doubt that the molecular elucidation of many more genetic diseases and the growing possibilities for their diagnosis and prevention will lead to massively increasing demand for genetic tests and for advice. Given the high throughput of NGS-based screening tests and the eager adoption of these technologies, not only by large genetic centres but also by clinical geneticists working in a private practice, it is unlikely, however, that the capacity to conduct these tests will become a bottleneck.

In contrast, handling the vast amounts of sequencing data and extracting the relevant genetic information requires substantial bioinformatic and genetic know-how, and this is a strong argument for concentrating NGS-based diagnostic tests at large centres with the necessary infrastructure and expertise. Another compelling reason for this is that WGS, WES, but also more targeted NGS-based diagnostic tests will generate information that patients and their families may want to treat as confidential. At the same time, submission of such sequence information and the corresponding clinical data to central databases is indispensable for making sense of the many sequence variants that are hitherto unknown, as discussed above. It is difficult to reconcile these objectives if NGS-based tests are performed at each and every genetic laboratory, because this will increase the risk that either the confidentiality will be breached or that data will be lost. Concentrating these tests—and if possible, genetic health care in general—at few large Clinical Genetic Centres may be the only way to avoid this dilemma.

In view of the very limited diagnostic and prognostic relevance of most genetic risk factors, and in spite of unsolved problems related to health and life insurance of individuals at risk, there is growing public awareness that in general, little is to be feared or will be gained by sequencing the genomes of healthy adults. This, and the adoption of NGS-based tests in the diagnostic routine, will likely demystify genome sequencing and will defuse fierce discussions about the privacy of the own genome and related property issues. Given the expectation that with the introduction of nanopore sequencing, the costs of WGS will drop far below thousand or even hundred US$, it is then not difficult to imagine that in a decade from now, several hundred thousand or even millions of human genomes will have been sequenced, analysed and compared. This will be a breakthrough for the identification of disease-causing mutations, and it may even reveal clinically relevant risk factors for common diseases that large-scale genome-wide association studies failed to detect.

In this scenario, WGS data will become an indispensable source of information for genetic counsellors, even more important than the family history and any physical examination by experienced syndromologists. It can be envisaged that by then, efficient computer programmes will be available that are hooked up to central databases and will flag the relatively few sequence variants in individual genomes that entail disease risks, either for the proband or for his or her offspring. In view of the low costs, it is likely that that this kind of analysis will become routine, even as a preconception test, and that eventually, most people will undergo genetic testing and counselling, not only those with clearly elevated genetic risks.

Thus, many more counsellors will be needed that are trained to interpret DNA sequences, translate the results of WGS into genetic risks and to provide appropriate advice—but not necessarily more experienced syndromologists, as their expertise will only be required for those relatively few cases that cannot be solved by computer-assisted interpretation of WGS results. This is good news for those countries, like Germany, were genetic syndromologists are in short supply. However, these developments will also be embraced elsewhere, as even today, no single genetic expert alive is experienced enough to recognize all known genetic conditions—apart from the fact that many different gene defects can give rise to clinically indistinguishable disease phenotypes.

Conclusions

The time is ripe for the introduction of NGS-based diagnostic tests replacing costly and often inefficient attempts to identify the causative gene defects by iterative mutation screening of candidate genes for rare diseases. Several research laboratories and companies have described or are developing tests to detect or rule out mutations in most known genes for deafness, retinitis pigmentosa, X-linked intellectual disability and mitochondrial disorders, and more comprehensive tests to rule out most severe recessive childhood disorders or even all X-linked gene defects will be introduced soon.

Preconception carrier testing for recessive disorders has been offered to certain high-risk communities since almost 40 years, including Mediterranean populations or Ashkenasim with elevated risks for thalassemia and Tay-Sachs disease, respectively. Voluntary parental carrier testing for all known recessive gene defects will also reduce the risk for offspring with serious childhood disorders in outbred Western populations. Therefore, the development and implementation of such tests should be a priority of genetic health care, and parents should be free to decide for themselves whether or not to use them.

Much earlier than expected, WGS is becoming affordable, and many groups working at the interface of molecular diagnosis and research have already started using this technology to identify disease-causing mutations in families or in sporadic patients where other approaches failed. Thus, despite unsolved issues related to data protection and the old conflict between the right to know and the right not to know, the clinical introduction of WGS is already underway.

Very likely, large-scale application of WGS in health care will also identify genetic variants that predispose to, but do not cause disease. Array CGH has revealed several copy number variants that raise the odds for common diseases up to tenfold, such as CNVs on chromosome 16p that are major risk factors for intellectual disability and related disorders, as discussed elsewhere (Ropers 2010a). Finding such changes and assessing their clinical impact will be a fertile field of research for many years to come. Since the actual risk conferred by these variants may depend on the genetic background, they promise to be a considerable challenge for genetic counselling.

Taking the introduction of array CGH as an example, it is tempting to speculate that after the implementation of WGS as a diagnostic standard test in genetic health care, the analysis of the first hundred thousand or million genomes will be a matter of only a few years. If most of the relevant data will find their way into central databases, this will allow very fine-grained genotype–phenotype comparisons, and as a result, these databases will become an invaluable source of information for genetic counsellors interpreting the results of diagnostic WGS.

Eventually, WGS will be routinely performed as an entry test, even before the counsellor will be involved and the family history is taken. In this—very plausible—scenario, the absolute demand for counselling will massively increase, but not necessarily, the need for highly qualified and experienced clinical geneticists that are able to diagnose most clinically recognizable disorders. Since such experienced syndromologists are a rare species, this is good news for the worldwide quality of future genetic health care.

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