Implementation of genomic medicine in Sri Lanka: Initial experience and challenges

Nirmala D. Sirisena, Nilaksha Neththikumara, Kalum Wethasinghe, Vajira H.W. Dissanayake *

Human Genetics Unit, Faculty of Medicine, University of Colombo, Sri Lanka

Abstract

The recent advances in next generation sequencing technologies have made it possible to implement genomic medicine in developing countries such as Sri Lanka where capacity for utilization is limited. This paper aims to describe our initial experience and challenges faced in integrating genomic medicine into routine clinical practice. Using the Illumina MiSeq Next generation sequencing (NGS) platform and an in-house developed bioinformatics pipeline/workflow, we successfully implemented clinical exome sequencing for rare disorders, complex disorders with unusual coexisting phenotypes, and multigene cancer panel testing for inherited cancer syndromes. The advantages of implementing these tests, the challenges for bioinformatics analysis and reporting, the ethical, legal, and social implications of moving from genetic to genomic counseling, and special policy issues related to implementing these tests are further discussed. The implementation of genomic medicine into our routine clinical practice has facilitated improved care for our patients, attesting to the ability of resource limited countries to improve care using advanced genomic technology.

1. Introduction

In recent years, genomic medicine has been hailed as an important tool in the implementation of predictive and personalized medicine (Biesecker and Green, 2014). The utility of clinical exome sequencing (CES) for the rapid and accurate identification of known and novel disease genes in families segregating rare Mendelian forms of disease is now well established (Williams et al., 2016). Since its inception, genomic medicine has continued to gain momentum across the entire clinical spectrum from risk assessment in healthy individuals, through diagnosis and prognosis to genome-guided treatment in patients with rare and complex diseases (Manolio et al., 2013; McCarthy et al., 2013). CES, where exome sequencing is focused only on the genes for which the function is known, has enabled small labs with limited bioinformatics capabilities to practice genomic medicine (Lee et al., 2014).

Research in the field of cancer genetics has demonstrated that all cancers arise as a result of variants which confer a growth advantage upon the cells in which they have occurred, giving rise to tumors (McCarthy et al., 2013). Identifying the genes involved in predisposition to cancer is known to have potential utility in risk management (Rahman, 2014). Genetic testing is not so easy because there are more than 100 cancer predisposing genes and an individual with a family history of cancer would need to be tested gene by gene to identify the mutation. However, this limitation has now been overcome through the availability of a multigene cancer gene panel test where multiple genes that are known to cause inherited cancer syndromes can be tested at once using the next generation sequencing (NGS) platforms faster and cheaper than testing a single gene. It provides a rapid and economical solution to single-gene tests as it can analyse multiple genes simultaneously saving both time and money.

Although Sri Lanka is a developing country, advances in other parts of the world are reaching its shores faster than ever before due to widespread penetration of the internet, which is accessed by a highly literate and technology savvy population. As a result of increasing public awareness, the demand for genetic testing to end the diagnostic odysseys of undiagnosed conditions as well to identify hereditary predisposition to cancer is growing rapidly. Therefore, we felt the urgent need to implement NGS services in Sri Lanka. In order to achieve this, we had to improve the existing laboratory infrastructure, train our staff, and focus especially on developing our in-house bioinformatics capabilities.

In addition, we had to convince technology suppliers that we were committed to implementing NGS services. The main challenge we expected to encounter was the issue of who pays for the tests and establishing the test pricing. These continue to be challenges because the cost of service contracts, reagents, and related consumables etc. are constantly escalating, and a service dependent on payment by the customer cannot survive on narrow profit margins. In spite of these however, by
implementing NGS in the country, we have made available a service that otherwise patients with a spectrum of genetically heterogeneous rare Mendelian disorders and providers could have only dreamt of in a third world country.

Implementing CES and a multigene cancer panel have enabled us to diagnose and successfully manage patients with rare disorders, complex disorders with unusual coexisting phenotypes, and inherited cancer syndromes, all of whom hitherto lacked a precise genetic diagnosis and appropriate treatment. We plan to continue providing these services in the country as we continually seek potential solutions to the existing challenges for sustainability through increased public-private partnerships, research funding, international networking, and locally generated revenue from consultancy services. In this paper we describe novel missense mutations that were identified using NGS. These have not been reported in scientific literature and were also absent in our existing database of de-identified Sri Lankan exome/genome sequences.

2. Implementation

Patients are referred to our unit to obtain a genetic diagnosis by the Specialists managing them. As a routine practice at our centre, pre-test counseling is provided and written informed consent obtained from all patients prior to genetic testing.

CES was performed using the TruSight One® exon enrichment technology on the Illumina MiSeq NGS platform for undiagnosed, suspected genetic conditions. The CES kit contains 4836 clinically relevant genes. CES was conducted as trio-CES (both parents and their affected child sequenced simultaneously) to effectively detect de novo and compound heterozygous variants or as proband-CES (only the affected individual sequenced) when parental samples were not available. The trio-CES test has the potential benefit of ruling out many heterozygous rare variants as causal in the affected individual because transmission is observed from an unaffected parent (Lee et al., 2014).

Sequencing was followed by bioinformatics analysis. Single nucleotide polymorphisms (SNPs) and Indels were identified from the paired-end sequenced data using an in-house developed variant calling and annotation pipeline (vide infra). Benign variants were filtered out using a virtual gene panel, consisting of genes with confirmed association to the underlying conditions. Resultant variants were further scrutinized for their functional impact on the protein, availability in public databases and the level of conservation in the resided region.

The cancer gene panel we implemented tests 94 genes associated with both common (e.g. breast, ovarian, uterine, colorectal, prostate, and thyroid) and rare hereditary cancers. It was performed using the TruSight Cancer® sequencing kit produced by Illumina, USA. Sequencing was followed by analysis of the data on a bioinformatics pipeline. Paired end sequencing data was first aligned to the GrCh37 human reference sequence using BWA-mem algorithm. Thereafter, the resultant sam file was converted to a binary formatted alignment file (bam) using sam tools. Duplicate reads were removed from the bam file using picard tools. Resultant reads were re-aligned around indels and variants were discovered using GATK. Annotation was done using SNP- eff with dbSNP. 1000 Genomes, Exome Variant Server, Exome Aggregation Consortium, phastCons100way, ClinVar, locus specific databases, and in-silico analysis using Mutation Taster, SIFT, Polyphen2, and Provean was carried out to determine the functional significance of the variants identified. In addition, other affected and non-affected family members were tested for further confirmation when required.

3. Results

So far, we have successfully implemented CES and the multigene cancer panel test to identify pathogenic mutations in >30 probands with a spectrum of genetically heterogeneous rare Mendelian disorders. Their ages ranged from 8 months to 70 years, with some individuals having spent several decades without a precise diagnosis. Among those tested, some of the novel pathogenic variants and their associated phenotypes discovered are reported here. They include the following: Type1 hyper lipoproteinemia caused by LPL c.808C > G, p.Arg270Gly; progressive distal peripheral neuropathy in a patient with non-insulin dependent diabetes mellitus caused by MYH14 c.795C > A, p.Asp265Glu; and familial adenomatous polyposis caused by APC c.7781C > G, p.Ser2594Cys. Detailed genotypic-phenotypic features of these 3 cases are described below.

3.1. Case 1

An eight month old male child born to consanguineous parents, who was clinically diagnosed with hyperlipidemia and hepatosplenomegaly was referred for genetic testing. His lipid profile showed: serum triglycerides: 1500.2 mg/dl (10–200 mg/dl), serum cholesterol: 196.4 mg/dl (140–239 mg/dl), HDL cholesterol: 12.3 mg/dl (35–85 mg/dl), and VLDL cholesterol: 300.04 mg/dl (10–41 mg/dl). Parents were normal with no family history of dyslipidemic conditions. A missense mutation in the Lipoprotein lipase (LPL) gene, NM_000237: c.808C > G [NP_000228: p.Arg270Gly] causing autosomal recessive Type 1 hyperlipoproteinemia was detected. The patient was a homozygote for the mutation while both parents were heterozygote carriers.

NM_000237: c.808C > G [NP_000228: p.Arg270Gly] in the LPL gene, is a novel missense mutation which has never been reported in the scientific literature. It was also absent in our existing database of de-identified Sri Lankan exome/genome sequences. The Human Gene Mutation Database (HGMD) records a variant in the same position with a different alternative allele (T) coding for a different amino acid (Cys), which is pathogenic for Lipoprotein lipase deficiency, NM_000237: c.808C > T [NP_000228: p.Arg270Cys]. This mutation resides in a highly conserved region (PhastCons score = 1), and was predicted to be pathogenic on bioinformatics functional analysis. MutationTaster classified this variant as ‘disease causing’ with a probability of 0.999 whilst PolyPhen-2 predicted this variant as ‘probably damaging’ with the highest available score (1.00) using both HumDiv and HumVar prediction models. Provean also predicted this mutation to be ‘deleterious’ with a score of −6.46 (cutoff < −2.5) and SIFT predicted it to be ‘damaging’ with 0.000 score (cutoff < 0.05). This finding is beneficial not only in providing appropriate therapeutic management for the proband but also in offering genetic counseling for the family with regard to the risk associated with intermarriage within the parents’ families (a practice which was common in the community to which the family belonged), the detection of carrier/presymptomatic relatives and prenatal diagnosis.

3.2. Case 2

A 47-year-old male who was clinically diagnosed with non-insulin dependent diabetes mellitus (NIDDM) and grade I distal sensory peripheral neuropathy of the lower limbs was referred for genetic testing. Both parents had NIDDM. Two of his male siblings and his father were also affected with distal sensory peripheral neuropathy. A missense mutation in the MYH14 gene, NM_001145809: c.795C > A [NP_001139281: p.Asp265Glu] that is associated with sensory peripheral neuropathy was detected. The patient was a heterozygote for the mutation. This is a novel missense mutation, which has never been reported in the scientific literature and was also absent in our existing database of de-identified Sri Lankan exome/genome sequences. This mutation resides in a highly conserved region of the gene (PhastCons score = 0.996), and was predicted to be pathogenic on bioinformatics functional analysis. MutationTaster classified this variant as a ‘disease causing’ mutation with a probability of 0.999 while PolyPhen-2 predicted it as ‘probably damaging’ with a score of 0.996. Provean also predicted this mutation to be ‘deleterious’ with a score of −3.76 (cutoff < −2.5) and SIFT predicted it to be ‘damaging’ with a 0.028 score (cutoff < 0.05). This case highlights the value of exome sequencing in elucidating the exact
aetiology of the peripheral neuropathy in this diabetic patient that may have all along been misattributed to the microvascular complications of NIDDM arising from poor glycemic control rather than the underlying hereditary neuropathy. Further functional studies, however, will be required to ascertain the pathogenicity of this variant.

3.3. Case 3

A 70 year old female who was clinically diagnosed with familial adenomatous polyposis (FAP) was referred for genetic testing. She had a strong family history with 3 of her first degree relatives affected with FAP. A missense mutation in the APC gene, NM_000038.4: c.7781C>G; [NP_000029.2: p.Ser2594Cys] was detected. This mutation resides in a highly conserved region of the gene (PhastCons score = 0.995), and was predicted to be likely pathogenic by bioinformatics functional analysis. The patient was a heterozygote for the germline mutation. MutationTaster classified this variant as a ‘disease causing’ mutation with a probability of 0.973 while PolyPhen-2 predicted it as ‘benign’. Provean also predicted this mutation to be ‘tolerated’ with a score of $-1.05$ (cutoff $< -2.5$) but SIFT predicted it to be ‘damaging’ with a 0.029 score (cutoff $< 0.05$). This is a rare mutation that has not previously been reported in association with FAP in the scientific literature. The identification of this familial mutation is beneficial for the clinician in decision making regarding specific management strategies for improving clinical outcome in the patient and also has implications for both affected and unaffected members of the family who are at risk.

4. Discussion

The cases above highlight the importance of implementing NGS technology to diagnose difficult to diagnose cases as well as to discover novel pathogenic mutations. The implementation of genomic medicine into our routine clinical practice has facilitated the rapid, accurate and affordable diagnosis of rare and complex genetic diseases in a comprehensive, cost effective and timely manner in Sri Lanka. It further lead to important new discoveries to add to our de-identified Sri Lankan database. It is important to mention that ideally, in the case of novel mutations, functional studies are needed to thoroughly understand the pathogenicity of these variants.

The genotypic confirmation of these diseases allows for targeted treatment, where therapies are available, leading to a positive impact on patient care and a holistic understanding of the disease conditions. In addition, it provides accurate estimates of recurrence risk and facilitate preconception intervention or prenatal diagnosis for the affected patient and/or affected or at-risk relatives. In adult-onset disease, one of the most useful outcomes of successfully identifying the causative variant is the subsequent detection of pre-symptomatic, at-risk relatives for whom screening or preventive therapy might improve the clinical outcome. Examples include enhanced surveillance or prophylactic risk reduction measures for patients found to have a genetic susceptibility to cancer (Biesecker and Green, 2014).

We have encountered several challenges in implementing genomic medicine into our routine clinical practice. Some of these issues are discussed below. The challenge for bioinformatics and reporting, includes the interpretation of variants in the context of the phenotypic data, dealing with variants of uncertain clinical significance, non-representation of variants found in the Sri Lankan population in public databases, and dealing with incidental findings. In the case of incidental findings, our current practice is to use the recently published guidelines of the American College of Medical Genetics (ACMG) to determine which genes, variants and specific conditions should be considered for reporting (Green et al., 2013; Richards et al., 2015). We are constantly fine tuning and optimizing our in-house bioinformatics pipeline and workflow to address the other bioinformatics related challenges.

The ethical, legal, and social implications of moving from genetic to genomic counseling, managing patient privacy in an atmosphere of collaborative, open-access public data sharing, and working with families encountering legal prohibition of selective termination of pregnancies based on genetic information are some of the other challenging concerns related to genomic medicine we have had to tackle. As a routine practice at our centre, all those undergoing genetic testing are offered comprehensive pre- and post-test counseling and informed consent obtained prior to testing. Genetic counseling allows individuals an opportunity to learn how heredity contributes to disease risk, understand their personal risk of developing the disease, understand their options for managing their disease risk and encourages adoption of risk reducing behaviors that are appropriate for them.

Special issues related to implementing these tests stemming from policy issues related to financing tests and perception about the usefulness of these tests among policy makers and administrators posed additional challenges. Currently, there is non-coverage of all genomic tests by the insurance companies in Sri Lanka and no government subsidies have been put in place to assist patients undergoing genetic testing. Thus, patients have had to bear the burden of financing these tests all by themselves. To make these tests affordable to patients we have priced CES at the equivalent of approximately USD 1000/- [LKR 150,000] and the multigene cancer panel at USD 550/- [LKR 75,000]. Further cost reductions in the future and physician education would make it possible for us to ensure more widespread use of our NGS services. We have discussed the challenges we face in physician education elsewhere (de Abrew et al., 2014).

In addition, a robust means of integrating genomic data into existing health records is needed, with consideration of not only data storage formats and privacy issues but also appropriate clinical decision support tools for prompting point of care use of genomic information and delivering it to healthcare providers in an easily interpretable format (McCarthy et al., 2013). Alongside this, there is also the need for all health care professionals in the country to be trained on specific core competencies regarding knowledge, skills, and attitudes in order to effectively implement genomic medicine at different levels of healthcare delivery. Professional practice guidelines also need to be developed (Korf et al., 2014; Manolillo et al., 2015).

It is believed that in the foreseeable future, as these challenges are gradually overcome, more routine implementation of CES and cancer gene panel tests will improve molecular diagnostic success rates in patients across the spectrum of genetically heterogeneous disorders (Lee et al., 2014). In addition, routine implementation of other gene panel tests for indications such as inherited cardiac disorders, inherited neuropathies, and inherited myopathies to name a few will also lead to better diagnostic success rates and appropriate treatment and possibly prevention strategies.

Developing the researchers’ skills is one of the most important aspects in advancing genome research in the country, which is necessary to advancing genomic medicine nationally. This development should be in both experimental and computational skills. We believe that education and training in the basics of genomics and bioinformatics should begin at the undergraduate level with more advanced training at graduate levels. Furthermore, we hope to generate research funding, encourage international collaborations, and organize specialized training programs including workshops and postgraduate courses as possible potential solutions for the sustainable future development of genomic medicine services and research in the country.

In conclusion, despite the challenges faced, successful implementation of genomic medicine into our routine clinical practice has facilitated the rapid and accurate diagnosis of rare and complex genetic diseases in a comprehensive, cost effective and timely manner and, of note, in the context of a developing country. Future plans for enlarging the current efforts and reaching out to a larger populace are currently being considered through increased public-private partnerships, research funding, international collaborations, and generation of local revenue through provision of consultancy and training services.
Competing interests

The authors declare that they have no competing interests.

Sources of funding

Human Genetics Unit Development Fund, University of Colombo.

Authors’ contributions

NS acquired the data and drafted the manuscript. NN carried out the analysis and interpretation of the data. KW carried out the molecular genetic studies. VHWD conceived the study, critically revised the final manuscript for important intellectual content and approved it. All authors read and approved the final version of the manuscript to be published.

Acknowledgements

Nil.

References

Biesecker, L.G., Green, R.C., 2014. Diagnostic clinical genome and exome sequencing. N. Engl. J. Med. 370, 2418–2425.