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

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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

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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 lipoproteinaemia 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 cause 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