DISCOVERY OF POLYMORPHISMS IN THE VITAMIN D RECEPTOR GENE AMONG POST-MENOPAUSAL WOMEN SEEN AT THE PHILIPPINE ORTHOPEDIC CENTER USING NEXT-GENERATION SEQUENCING

Mark Pretzel P. Zumaraga (MS Mol Bio & Biotech), Celeste C. Tanchoco (Dr. Pub Health) and Cynthia Palmes-Saloma (PhD Physio)

BACKGROUND

Osteoporotic fractures are associated with high mortality and morbidity rates incurring significant health and economic impact. The current standard of care, which includes surgeries, lifestyle modifications, anti-osteoporosis drugs, calcium and vitamin D supplementation, have been consistently shown to be dependent on racial profile.

OBJECTIVES

This study aimed to discover novel genetic variants in the entire 101 kB vitamin D receptor (VDR) gene for osteoporotic fractures in a group of post-menopausal Filipino women using targeted next generation sequencing (TNGS) approach comparing women with and without fragility fractures.

MATERIALS AND METHODS

Study participants were seen at the Philippine Orthopedic Center. Blood samples were collected for determination of serum vitamin D, calcium, phosphorus, glucose, blood urea nitrogen, creatinine, aspartate aminotransferase, alanine aminotransferase and as primary source of genomic DNA for VDR gene sequencing. About 0.10 - 10 ng genomic DNA was used for enrichment of the VDR target gene by applying a custom VDR AmpliSeq™ panel. DNA libraries were constructed using the Ion Ampliseq Library Kit v2.0 and sequenced on the Ion Torrent Personal Genome Machine. Sequencing reads were aligned against the hg19 reference genome. The variant calling was based on the Genome AnalySee Tool Kit (GATK) best practice workflow using HaplotypeCaller. Variants were annotated using Annovar tool.

RESULTS

The frequencies of the detected variants were compared with dbSNP database. The diseased and healthy groups were comparable in terms of age, Body Mass Index (BMI), fasting plasma glucose, blood urea nitrogen, creatinine, serum vitamin D, serum calcium, serum phosphorus, number of hours of sun exposure per week and physical activity level. Calcium and vitamin D supplements were taken by 8% of case patients compared to 4% of control patients. A total of 1,496 unique variants in the whole 101 kb VDR gene were identified. Novel sequence variations not registered in the dbSNP database were found among cases and controls at a rate of 23.1% and 16.6% of total discovered variants, respectively. Noteworthy is the discovery of two disease-associated novel heterozygous frameshift deletions (Pearson chi square p-value <0.05). One regulatory SNP showed statistically significant association to low serum 25OHD vitamin D levels (Pearson chi square p-value = 0.009).

CONCLUSION

Taken together, these findings show the power of using TNGS in identifying sequence variations in a very large gene and the surprising results obtained in this study greatly expand the catalogue of known VDR sequence variants that may represent an important clue in the emergence of osteoporotic fractures. Such information will also provide the additional guidance necessary towards personalized nutritional advice to reach sufficient vitamin D status.