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Award Winners

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The study of embryonic causes of recurrent pregnancy loss in couples with consanguineous marriage and normal karyotype with a specific approach to determine the importance of single genes

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Background: Pregnancy loss is a significant health concern, especially in developing countries. Nearly 3-5% of couples trying to have children experience recurrent pregnancy loss (RPL). Unfortunately, advancing maternal age is highly associated with miscarriage while the available reproductive years are shortened, therefore determining why miscarriage occurs and how to prevent further miscarriages has become a major clinical and research focus. It has been estimated that the cause remains unexplained in more than 50% of cases, which strongly suggests that genetic factors may contribute towards the phenotype. This might be considered the fact that hundreds of genes and several critical pathways are involved in each physiological step necessary for guaranteeing reproductive success.

Objective: Mendelian forms of embryonic lethality offer a window into the essential genetic components of early embryonic development in humans. The study hypothesis was that exome sequencing can identify genetic causes of idiopathic recurrent pregnancy loss (RPL), which will further our understanding of human development at a molecular level.

Materials and Methods: This study involved 10 consanguineous couples having suffered at least 3 consecutive embryonic losses. Patients having risk factors such as abnormal karyotype, infectious disease during pregnancy, metabolic, autoimmune, endocrine disease, and uterine anomalies were excluded from the study. DNA was extracted from the tissue sample of ten aborted fetuses (probands) from ten different families with a history of idiopathic recurrent pregnancy loss. Parental peripheral blood samples were collected for confirmatory analysis and follow-up testing. Whole exome sequencing (WES) was performed using illumine HiSeq 2000 platform. Cytoscape 3.7.2 and BINGO were used for pathway and biological enrichment analysis of putatively pathogenic variants in miscarriages. All variants identified by exome sequencing were verified by Sanger sequencing in all parents.

Results: We were able to identify 32 variants (7 pathogenic, 9 likely pathogenic, and 16 VOUS) of which three genes are already known to be involved in lethal recessive disorders. These genes were compatible with the clinical phenotypes. In cases 1, 2,4 pathogenic variants were identified in know genes (CHRNG, RYR1) responsible for disorders with a clinical description that correlated with the phenotypic description. Of fetuses, in case 3,8,6 novel variants were identified in MYH3, ERBB3, FRAS1 responsible for muscular disorders and Fraser syndrome. Bioinformatics analysis of genes with mutation showed enrichment in biological processes of importance for embryonic development e.g. Fibrin clot formation complement, coagulation cascades, and Striated muscle contraction/muscle contraction, actin-myosin filament sliding. Variants with potential diagnostic value were reported to the patients referring physicians for genetic counseling and further diagnostic and reproductive action.

Conclusion: Next-generation sequencing (NGS) has been reported as being a useful tool for identifying variants in genes related to rare disorders leading to RPL. It has also helped to identify variants related to

fetal molecular pathways in pregnancies having unexplained embryonic lethality or unexplained fetal malformations. This approach thus helps, genetic counseling regarding lethal fetal disorders of high-risk families and preimplantation genetic diagnosis. Future efforts should be directed towards increasing the number of sequencing families with RPL.

Key words: *Multiple pterygium syndromes, Whole-exome sequencing, Recurrent pregnancy loss, Thrombophilia.*