Mutation Analysis of Phospholipase C Zeta (PLCζ) in Patients With Low Fertilisation Rate

Ayşegül İkkan, Süleyman Aktuna, Canan Hürdağ, Yasemin E. Çanilloğlu, Türker Duman, Özge Üner, Leyla Özer, Volkan Baltac, Evrim Ünsal

INTRODUCTION
PLCζ, sperm soluble factor, has crucial roles in oocyte activation reported by various studies. Our objective was to investigate PLCζ mutations in patients who were globozoospermic, total-fertilization-failure, nuclear anomaly, total immotility to delineate if PLCζ mutations have the potential to alter gene function and lead to low fertilization. Investigation of 15 PLCζ exons with sequence analysis highlighted various variants (p.Q94X, p.R197H, p.L440R, p.T303A, p.S500L). Some of these variants were not reported in the literature before and were only detected in patients with low fertilization rate so they may have a significant effect on PLCζ function.

WHAT IS KNOWN ALREADY
PLCζ is a known regulator of intracellular Ca2+ oscillations. In some cases of male infertility, disruption of PLCζ function or expression leading to oocyte activation deficiency was reported. Studies have focused on expression profiling of the protein but there are limited number of studies investigating mutations in the gene itself. Detection of p.H398P and p.H233L mutations which modify 3D structure of the protein and cause altered Ca2+ oscillations suggested a correlation between PLCζ mutations and infertility.

STUDY DESIGN & SIZE
19 patients, globozoospermic (7), total-fertilization-failure (8), Total pinhead (2), total immotility (2), that had fertilization failure or low fertilization rate were included in the study.

MATERIALS & METHODS
DNA was extracted from peripheral blood and primers were designed to encompass all 15 exons of PLCζ gene. Sequence analysis was performed using mutation surveyor. Sperm samples were collected from patient for future expression profiling studies.

MAIN RESULTS
Sequence analysis revealed two variants that were already reported: p.Q94X (rs138801851), p.S500L (rs1050530) and three variants that were not reported before: p.R197H, p.L440R, p.T303A. p.Q94X causes an early termination of the transcription and can lead to a truncated protein and abolish the function of protein. Other missense variants can alter 3D structure of the protein. Investigation of population frequency of these variants is required in order to provide further support for this study and exclude role of chance. Family studies of these patients should also be designed to make sure that the variants are inherited.

TFF patients are especially key patients in correlating PLCζ variants with infertility. We had limited number of TFF patients so sequence analysis of additional TFF patients should be performed. This was a preliminary study and future studies will focus on these detected variants to perform population screening and family studies.

LIMITATIONS
There is only one reported PLCζ mutation in the Human Gene Mutation Database. PLCζ function is crucial for oocyte activation and delineating key variants in PLCζ gene will help us to correlate in PLCζ gene with male infertility. This will enable us to perform molecular screening of patients with total fertilization failure or low fertilization rate. Further investigation of detected variants will also enlighten us about the effects of variants on PLCζ structure and function.

CONCLUSIONS
TFF patients are especially key patients in correlating PLCζ variants with infertility. We had limited number of TFF patients so sequence analysis of additional TFF patients should be performed. This was a preliminary study and future studies will focus on these detected variants to perform population screening and family studies.