HELLENIC REPUBLIC
National and Kapodistrian University of Athens
EST. 1837

P01.045.C. Investigation of single nucleotide variants (SNVs) in a fertile population in association with oocyte and embryo quality

G. Kakourou[1*], T. Mamas[1], C. Vrettou[1], E. Tsorva[2], S. Zacharia[2], C. Oraiopoulou[3], E. Chatzi[4], G. Kourlaba[5], D. Christopikou[2], M. Mastrominas[2], M. Moisidou[3], A. Chatziparasidou[3], I. Georgiou[4], J. Traeger-Synodinos[1]

[1]Department of Medical Genetics, National & Kapodistrian University of Athens, St. Sophia's Children's Hospital, Athens, Greece, [2]Embryogenesis, Assisted Reproduction Unit, Athens, Greece, [3]Embryolab SA, IVF Unit, Thessaloniki, Greece, [4]Genetics and IVF Unit, Department of Obstetrics and Gynecology, Faculty of Medicine, University of Ioannina, Ioannina, Greece, [5]Center for Clinical Epidemiology and Outcomes Research (CLEO), Athens, Greece.

*contact email: gkakourou@med.uoa.gr

BACKGROUND

A number of studies in the field of Assisted Reproductive Technology (ART) have focused on identifying biomarkers of gamete and embryo quality, to detect embryos with the highest chance of leading to live birth and improve IVF success. Several approaches have been reported for this purpose (assessment of chromosomal status, genomic, transcriptomic, proteomic or metabolomic analysis of follicular fluid, oocytes, cumulus cells, culture media and embryos, time-lapse imaging or gamete/embryo mitochondrial DNA analysis). Amongst these, the genetic profile of prospective parents has been shown to have an impact on oocyte and embryo quality and information on genes of interest has been provided by both human and animal studies.

In most studies, single nucleotide variations (SNVs) have been investigated mostly on infertile couples undergoing IVF. Recently, maternal SNVs in infertile women (rs1801133 and rs1801131 in *MTHFR* and rs2305957 on chromosome 4 linked to *INTU, SLC25A31, HSPA4L, PLK4, MFSD8, LARP1B* and *PGRMC2*) were associated with embryo quality or chromosomal status[1, 2].

OBJECTIVES

1. To evaluate whether the associations of variants rs1801133, rs1801131 and rs2305957 with embryo quality are applicable to a population of fertile women
2. To investigate whether SNVs in 26 selected genes confer a risk to oocyte quality and embryo development in fertile women

PATIENTS

85 fertile women undergoing IVF with the purpose of Preimplantation Genetic Testing for Monogenic Disease (PGT-M). The study involved PGT-M cycles performed between 2013-2019 and took place at the UoA Laboratory of Medical Genetics

METHODOLOGICAL APPROACH

- Maternal genotyping was performed by NGS (Qiaseq™ Targeted Custom Panel, Qiagen,Miseq Reagent Nano kit v2, Illumina) for the exonic regions of 18 selected genes (*AIRE-AMH-AURKA-AURKB-AURKC-FSHR-HSPA4L-HUWE1-INTU-KHDC3L-LARP1B-MFSD8-MTHFR-PGRMC2-PLK4-SENP7-SLC25A31-WBP1*) and 9 selected SNVs in a further 8 genes: rs175080(*MLH3*), rs1799963(*F2*), rs6025(*F5*), rs5918(*ITGB3*), rs5985(*F13A1*), rs1805087(*MTR*), rs1801394(*MTRR*), rs28756992(*MLH3*) and rs2305957(*HSPA4L*). Gene selection was based on bibliographic search supporting their potential role in gamete quality and/or preimplantation development. Qubit Fluorimeter (Invitrogen) and Bioanalyzer 2100 (Agilent) were employed for DNA and library quantitative and qualitative assessment.
- NGS analysis was performed with Qiaseq DNA V3 panel analysis και VarAFT 2.13. Statistical analysis was performed by the Center for Clinical Epidemiology and Outcomes Research. The Mann-Whitney and Kruskal Wallis tests were used to check the existence of association between a continuous variable and the different genotype. The level of statistical significance was set to a=5%. All analyses were conducted using the statistical software STATA SE v.13.
- The study was approved by the University of Athens Bioethics Committee and National Authority of Assisted Reproduction

MAIN OUTCOME MEASURES

Maternal genotypes were analysed in association with number of oocytes collected, fertilization rate, percentage of blastocysts developed per MII oocyte and percentage of blastocysts per 2PN embryo

RESULTS

- A 20x coverage was achieved in all gene exons and SNVs investigated, identifying 121 variants.
- Significant associations were revealed for 16 SNVs in *AMH, AURKA, AURKB, HSPA4L, KHDC3L, MTHFR, PLK4* and *SENP7*.
- No significant associations were revealed for rs1801131 and rs2305957 in our study group.
- Additional associations were revealed for rs3746964, rs878081, rs1800521 (*AIRE*), rs10407022 (*AMH*) and rs175080 (*MLH3*), when stratifying for age and stimulation protocol.
- *HSPA4L* and *PLK4* are the only genes out of seven, linked to the previously identified polymorphism rs2305957 on chromosome 4, that have revealed significant associations with the number of oocytes and oocyte fertilization rate in our study population[3, 4]
- Other studies have previously supported a role of *AURK*s, *SENP7*, *AMH* and *KHDC3L* in reproduction [5, 6, 7, 8]

Tables I, II, III, IV:
Summary of results for SNVs that revealed significant associations for the four studied parameters

I. Number of Oocytes				
Gene	**SNV (Minor Allele)**	**Compared Genotypes**	**Association revealed**	**p value**
AURKA	rs2273535 (T)	AA, AT+TT	↑ number in AT+TT	0.014
KHDC3L	rs564533 (C)	GG, GC	↑ number in GG	0.023
SENP7	rs7616677 (C)	TC, TT	↑ number in TC	0.001
	rs939443 (A)	AA, GA, GG	↓ number in AA	0.006

II. Fertilization rate				
Gene	**SNV (Minor Allele)**	**Compared Genotypes**	**Association revealed**	**p value**
AMH	rs61736572 (A)	GA, GG	↑ in GA	0.016
	rs61736575 (A)	GA, GG	↑ in GA	0.016
AURKB	rs1059476 (A)	GG, AA+AG	↑ in GG	0.008
	rs2241909 (G)	AA, GA, GG	↑ in AA	0.015
PLK4	rs56043017 (C)	GC, GG	↑ in GC	0.009

III. Blastocyst number per MII oocyte				
Gene	**SNV (Minor Allele)**	**Compared Genotypes**	**Association**	**p value**
MTHFR	rs1801133 (A)	GG, AA+AG	↑ in AA+AG	0.052 (borderline)
SENP7	rs7616677 (C)	CC, TC, TT	↑ in TT	0.02
	rs939443 (A)	AA, GA, GG	↑ in GG	0.021
	rs2433031 (A)	TT, AA+TA	↑ in AA+TA	0.0003

IV. Blastocyst number per 2PN embryo				
Gene	**SNV (Minor Allele)**	**Compared Genotypes**	**Association**	**p value**
HSPA4L	rs35518193 (C)	TT, CC+TC	↑ in TT	0.038
SENP7	rs7616677 (C)	TC, TT	↑ in TC	0.009
	rs939443 (A)	GA, GG	↑ in GA	0.006
	rs2433031 (A)	TT, AA+TA	↑ in AA+TA	0.001

CONCLUSION

Investigating genetic factors of the maternal genome, such as maternal SNVs, has been of great interest in the search of biomarkers of embryo development, as human embryos rely on maternal resources to ensure their survival especially during the early post-zygotic stages. Many studies have already linked common polymorphisms with several stages of the IVF procedure.

Our understanding of genes that may impact the preimplantation stage and the search for genomic biomarkers predicting IVF success may benefit by investigating a fertile population, minimizing many confounding variables, and potentially facilitating the identification of genomic biomarkers predictive of gamete/embryo quality.

Present data should be considered preliminary and taken with caution, setting the path for further investigation in a larger group of fertile women to confirm our findings.

The main limitations of the study include its retrospective nature, whereby IVF practices have changed considerably over the period of the study (2013-2019).

FUTURE PERSPECTIVE

The polymorphisms identified with likely significant impact, require prospective validation in other PGT-M cycles, as well as on embryo samples of variable quality.

Embryos of poor quality, not suitable for transfer or cryopreservation, and embryos of good quality diagnosed as affected following PGT-M, could be evaluated for genotype assessment of selected SNVs of interest. It would also be interesting to investigate the paternal genotypes of selected SNVs for potential associations with embryo development.

❖ This study was supported by the State Scholarships Foundation (IKY), co-funded by national grants and the European Union (ESPA 2014-2020)