ABSTRACTS

15th International Conference on Preimplantation Genetic Diagnosis

Bologna, Italy

8th-11th May 2016

reasonable demand of embryos produces in in vitro fertilization (IVF). Karyomapping is a genome wide parental haplotyping using a high density single nucleotide polymorphism (SNP) array that allows the diagnosis of any single gene defects and provides information of meiotic trisomies and monosomies.

Methods: A couple with an affected child with primary congenital glaucoma (PCG) attended at our clinic. They were both previously dignosed as heterozygous carriers of PCG. Following genetic consultation, IVF combined with PGD using Karyomapping was recommended. Blood samples we re obtained from both parents and affected child prior to the IVF cycle for reference for Karyomapping. Twenty year old female patient received ovarian stimulation with antagonist protocol. Partner was a 24 year old male with normal semen parameters.

Results: Thirty six hours before oocyte collection an hCG trigger was administered and six oocyte - cumulus-complex was retrieved. Intracytoplasmic sperm injection was used as insemination method on the three mature oocytes. At day 1, one zygote showed the signs of normal fertilization and was cultured for five days. Trophectoderm biopsy was carried out 120 hours post -fertilization The obtained sample was sent to Karyomapping analysis and the embryo was vitrified. Result showed that the embryo is a heterozygous carrier for PCG. In the following cycle the embryo was thawed and transferred. A positive hCG result was obtained two weeks after embryo transfer and a single heart beat was detected at week six. A 2970 g healthy girl was delivered by ceasarian section at w eek 39.

Conclusions: Here we report the first live birth following IVF combined with PGD using Karyomapping in Hungary. Previously, it has been shown that genome wide karyomapping is able to accurately detect single gene disorders from a limited amount of samples. It can also provide information about meiotic aneuploidies that responsible about two third of all aneuploidies. Furthermore, it can be used without a significant preclinical workup.

Whole Exom Sequencing Enables Preimplantation Genetic Diagnosis For Couples With Undiagnosed Disorders

Ozer L[1], Unsal E[2], Aktuna S[2], Celikkol P[1],Kocak I[3], Duman T[4], Kutlu NK[1], Atik A[5], Baltaci A[5]Baltaci V[2]
(1) Mikrogen Genetik Tanı Laboratuvarı-Ankara Turkey, (2) Yeni Yüzyıl University Faculty of Medicine - İstanbul Turkey, (3) 19 Mayıs University, Faculty of Medicine-Samsun Turkey, (4) Ankara University School of Medicine- Ankara Turkey, (5) Genart Woman Health and Reproductive Biotechnology Center - Ankara Turkey

Introduction: Initial step of preimplantation genetic diagnosis for single gene disorders is the investigation of a pathogenic mutation associated with the clinically diagnosed disorder in parents.
In some cases clinical diagnosis of the baby with disease symptoms is elusive. Another scenario is the failure in detecting a pathogenic mutation associated with diagnosed disease. These situations prevent the possibility of parents to benefit from prenatal or preimplantation genetic diagnosis.
WES has been applied in different areas of research and diagnostics esspecially for families who had babies witohut any molecular diagnosis or in cases with heterogenous disorders (Rabbani et al 2014). This conditions include complex neurological disorders.

Band like calcification with simplified gyration and polymicrogyria (BLC-PMG) is a rare autosomal-recessive neurological disorder and known to result from mutations in the Occludin(OCLN) gene.
Intracranial calcification (ICC) and polymicrogyria (PMG) can be seen in a large and heterogeneous group of neurological disorders with diverse etiologies so differential diagnosis is complicated. Due to rareness of disease and complexity of differential diagnosis for ICC and polymicrogyria, WES was the best appropriate method for those patients.

Material & methods: The consanguineous couple referred to our center for genetic counseling of their two affected daughters. First patient was 5,5 years old and the second patient was 4 years old. Epileptic seizures, mental retardation, muscle weakness, delayed motor milest ones, delayed speech, development regression, dysmorphic findings, growth retardation, microcephaly, muscle weakness were noted in both girls. Cranial MRI findings of second child were microcephaly, bifrontal ploymicrogyria, ventricular dilatation, genera lized calsifications, calsifications at thalamus, dental nuclues and pons. Whole exome sequencing (WES) was performed for patients and parents. After couple's consent, PGD was designed specificly for detected mutation and flanking informative markers

to exclude allele drop out and false positive /negative results. Multiplex Nested PCR followed by RFLP or Minisequencing to gether with STR analysis was performed.

Results: WES performed on both siblings and parents revealed homozygous previously unreported variant c.173_194del (p.Trp58Phefs*10)in OCLN gene. Both parents are heterozygous carrier of the detected variant. In the light of this results PGD was suggested to this family and they have consented to PGD application.

PGD results revealed absence of mutation in 2 embryos (designated as normal) . Two embryos were heterozygous and 2 embryos were homozygous for c.173_194deletion.
According to PGD results 2 embryos were normal, 2 and 2 were found to be homozygously mutant. After transfer of normal embryo to mother succesfull pregnancy was observed.

Conclusions:WES identified the novel OCLN gene mutation in a family. Due to rareness of disease and complexity of differential diagnosis for ICC and polymicrogyria, WES was the best appropriate method for those patients. Our case presentation emphasizes the importance of WES analysis in families with undiagnosed genetic disorder to enable PGD. To our knowledge this is the first PGD application for Band like calcification with simplified gyration a nd polymicrogyria (BLC-PMG).

References :
1. Rabbani et al Journal of Human Genetics (2014) 59, 5–15; doi:10.1038/jhg.2013.114

Suggested indications of preimplantation genetic screening (PGS)

Park J[1], Chun S[1], Shim Y[1], Kim S[1]
(1) Gachon University Gil Hospital, Incheon, KOREA

Introduction: Although there have been improvements in a process of in vitro fertilization-embryo transfer (IVF-ET), implantation rates are reported to be approximately 30% in women less than 35 years old and less than 10% in women more than 40 years old. Approximately 50-75% of spontaneous miscarriages are due to numeric chromosomal abnormalities of embryos. Recently, preimplantation genetic screening (PGS) seems to be one of the methods in screening chromosomally abnormal embryos. Therefore, in this study, we are to study on indications of women and embryos undergoing PGS for successful pregnancy outcomes.

Methods: Total 51 cases of infertile couples who visited, underwent IVF and agreed to do PGS at Gil hospital in between August 2014 and January 2016 were included in this study. Data were collected by reviewing charts retrospectively. PGS was recommended to couples under following 4 indications; woman's age ≥ 38 years old, abnormal serum karyotyping in at least one parent, history of recurrent pregnancy loss or recurrent implantation failure. From a good quality embryo (grade I, II), embryo biopsy was done on day 3 and whole genomic amplification (WGA) with CMA were done on the same day. Percentages of euploidy status were c alculated and analyzed according to each indication.

Results: Total 234 embryos were sent for PGS and 188 (80.34%) embryos were found to be aneuploidy. We analyzed whether each indication has statistically significant effect on euploidy status. A percentage of euploidy status within embryos undergoing PGS among women's age ≥ 38 years old was 13.6 % and it had statistically significant impact on euploidy status (P -value = 0.001). Secondly, a percentage of euploidy status among couples who at least have abno rmal serum karyotyping was 11.7 % and it had statistically significant impact on euploidy status (P -value = 0.032). However, having history of spontaneous miscarriages ≥ 2 times did not have significant impact on euploidy status (P-value = 0.30). Lastly, a percentage of euploidy status among couples having history of repeated implantation failure ≥ 2 times was 10.6 % and it had statistically significant impact on euploidy status (P-value = 0.004).

Conclusion: Although embryos are graded as good qualities morphologically, couples having risk factors of advanced maternal age, abnormal serum karyotyping or history of repeated implantation failure ≥ 2 times were found to have statistically significant impact on aneuploidy status. Since these embryos would lead to low potentials of resulting successful deliveries, PGS should be emphasized and recommended especially to these couples for better pregnancy outcomes.