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Case Report

Live Birth Following Pre-Implantation Genetic Diagnosis for Detecting Oculocutaneous Albinism Type 1 (OCA1) Affected Embryos: A Case Report -

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ABSTRACT

We report a live birth of a normal male baby from a couple who are carriers of the genetic disease Oculocutaneous Albinism type 1 (OCA1) following pre-implantation genetic diagnostic testing. This is the first live birth in which the technique of trophectoderm biopsy was used for this disease screening.

Keywords: Oculocutaneous albinism type 1; Pre-implantation genetic diagnosis ; Genetic testing; pregnancy

INTRODUCTION

Albinism is a heterogeneous group of genetic disorders currently defined by abnormalities in visual system development and a variable hypopigmentation phenotype. There are two major types of albinism: non-syndromic and syndromic. Non-syndromic albinism includes X-linked Ocular Albinism (OA), caused by mutation in GPR143 (OA1) gene that is confined to the eyes, and Oculocutaneous Albinism (OCA), in which lack or reduction of pigment affects the eyes, skin and hair [1].

OCA 1, the most common type of albinism, is caused by bi-allelic mutations (missense, nonsense or frame shift) in the TYR gene on chromosome 11q14.3 and occurs in approximately 1:40,000 worldwide. This type of albinism, most common in Caucasians, is subdivided clinically into two groups: the most severe type of albinism, in which tyrosinase activity and melanin synthesis are undetectable, OCA 1A, or partially measurable, OCA 1B.

In 2011, a large series of cases (938 PGD cycles for 146 different monogenic conditions) was published describing the use of polar body based pre-implantation genetic testing with the application of microarray technology. OCA was mentioned as one of those conditions [2]. We report a successful pregnancy and delivery in a healthy male baby from a couple who are both carriers for the OCA 1 mutation who underwent intracytoplasmic sperm injection and Pre-Implantation Genetic Testing for Monogenic (single-gene) disorder (PGT-M) using (NGS) for trophectoderm biopsied blastocysts (as the PB biopsy can represent the maternal originated mutations which is not enough in our case).

CASE REPORT

Case data

Our couple, male aged 36 years and his wife 24 years (second cousins), had one affected child with Oculocutaneous Albinism type 1 (OCA1) and so they were referred to a clinical geneticist and both partners were diagnosed by NGS (Next Generation Sequencing) as trait carriers with a single copy of a pathogenic variant in TYR causing amino acid change R 116 as the genotype report revealed (the wife was: N/c.346 C>T and the husband was c.346 C>T/N). They were therefore scheduled for PGT. The couple was counseled about the procedure and risks of PGT. It was decided to take a trophectoderm biopsy from their embryos, so as to perform PGT.

Biological data

Two cycles of Intra-Cytoplasmic Sperm Injection (ICSI) were carried out in 2015 (Table 1 for the details). Embryo culture from one-cell to blastocyst stage was done in a continuous single culture medium (Global Total, Global, USA) within a labotect C 60 CO₂ incubator (labotect™, Germany) at 37°C, 7% CO₂, 5% O₂. All day-5 blastocysts were biopsied with further vitrification (each in a specific Rapid I device using Irvine vitrification medium). Trophectoderm

Table 1: A summary of the 2 ICSI cycles performed.

| | First ICSI cycle | Second ICSI cycle |
|--|---|--|
| Ovarian stimulation protocol | GnRH*-antagonist protocol with daily subcutaneous injections of 150 IU recombinant FSH** and 150 IU urinary gonadotropins | GnRH*-antagonist protocol with daily subcutaneous injections of 375 IU urinary gonadotropins |
| Stimulation days | 12 | 13 |
| Ovulation trigger | 0.2 mg GnRH*-agonist | 10,000 IU intramuscular hCG*** |
| Peak Estradiol concentration | 1,820 pg/ml | 1,020 pg/ml |
| Cumulus masses | 15 | 8 |
| Metaphase II | 10 | 8 |
| Sperm Source | Fresh Ejaculate | Frozen Ejaculate |
| Normal Fertilization | 8 | 6 |
| Day 5 Blastocysts | 3 | 3 |
| *GnRH: Gonadotrophin Releasing Hormone **FSH: Follicle Stimulating Hormone ***hCG: Human Chorionic Gonadotrophins. | | |

biopsy was performed on day 5, an opening of 6 to 9 um was made in the zona pellucida with several pulses from a non-contact 1.48 um Saturn 5 (Research Instruments, UK), and few Trophectoderm (TE) cells were aspirated into a biopsy pipette and separated from the blastocysts by applying multiple laser pulses between the trophectoderm cells at the stretching area. The biopsied TE cells were washed in 1xPBS (Phosphate Buffered Saline) and loaded into a PCR (Polymerase Chain Reaction) tube containing 2.5 µl 1x PBS and the tubes from both cycles were kept in a deep freezer till sent to the Genetic laboratory. The test was done concerning the location Ch. 11: 88911467 on NCBI Build 37 NM_000372.4 (TYR): c.346C > T (p.Arg1161er).

Next Generation Sequencing (NGS) analysis

Enzymatic shearing to get fragment sizes ~200 bp was followed by DNA fragments purification and stabilization by AMPure Bead. After binding to ion sphere particle, 1000s time's amplification was done by a PCR reaction emulsion. Dynabeads MyOne Streptavidin CI bead washes (Invitrogen, Carlsbad, CA) was used to get template positive ion sphere particles. Sequencing primers and Ion Hi-Q sequencing polymerase were added to the samples and loaded to a sequencing chip for entire genome analysis by a Personal Genome Machine, Torrent Browser Server/ Ion Reporter Server (Thermo Fisher Scientific, Waltham, MA) were used for comprehensive data analysis and interpretation.

Two embryos were subsequently shown to be normal for NGS signals (direct mutation analysis), another 2 proved to be carriers (N/c.346 C>T and c.346C>T/N) and other two with failed



amplification (no evidence of any DNA) (Table 2 for the details). For affected embryos (not in this case) the signal would be c.346C>T/c.346C>T. In May 2017, thawing by Irvine warming medium and transfer of one normal blastocyst was done: Full Blastocyst BB [3]. The pregnancy proceeded uneventfully with the delivery a healthy baby boy in February 2018.

DISCUSSION

Rare and undiagnosed autosomal recessive diseases frequently occur in the offspring of consanguineous couples. Consanguineous marriages occur in significant numbers around the world, accounting for 20%-50% in several regions of the Middle East and the Mediterranean basin but also increasingly affecting populations in Western European countries. Children born to consanguineous couples are at increased risk of presenting with congenital anomalies. Even for consanguineous couples with a negative family history, prospective carrier screening may be useful to minimize the increased basal risk of 6-10% for giving birth to a child with a congenital malformation or condition. Current routine diagnostic procedures often fail to identify the underlying genetic defect.

Pre-Implantation Genetic Testing for Monogenic (single-gene) disorders (PGT-M) as an established procedure for couples at risk of producing offspring with inherited disorders was applied for more than 200 different genetic conditions, resulting in the birth of thousands of unaffected children by the present time. With an increasing number of different genetic disorders for which PGD is being applied each year, it may presently be applicable for any inherited disorder for which sequence information or relevant haplotypes are available for the detection by direct mutation analysis or haplotyping in oocytes or embryos. The available experience provides over 99% accuracy in leading PGD centers [4].

Previous techniques established since the early 1990s such as FISH (Fluorescent In Situ Hybridization) to detect chromosome abnormalities and Sanger sequencing after PCR (Polymerase Chain Reaction) to detect specific point mutations were replaced by the Genome-wide aneuploidy screening methods, such as Comparative Genomic Hybridization (CGH) array, SNP (Single-Nucleotide Polymorphism) array multiplex quantitative PCR. They were used to select aneuploidy free embryos and can be applied in the field of embryo diagnosis for specific genetic anomalies [5]. In fact combined testing for monogenic disorder and aneuploidy is already considered a gold standard by leading PGT laboratories since embryos with a normal genotyping result for the disease mutation are not always euploid [6] even without the need for additional embryo biopsy or whole genome amplification [7]. Eliminating these errors is a major challenge for PGD. Linkage analysis has become a standard method of circumventing false positive and/or false-negative Single-Nucleotide Variations (SNVs), which could lead to wrong selection of embryos for transfer [8] by detecting Short Tandem Repeats (STR) or by karyomapping with an SNP array [9] to determine the disease allele.

Recently, next-generation sequencing has emerged as a PGT

strategy both for mutation target diagnosis and simultaneous whole genome analysis. Many studies have shown that NGS panel diagnostics or diagnostic exome sequencing can be a powerful tool for the detection of carrier status in consanguineous or even non-consanguineous couples with positive family history suggestive of a recessive disorder. The identification of a causative variants can be used for the estimation of the risk for affected offspring, for family planning and enables informed reproductive decision-making for the affected families [10]. NGS offers many advantages, including reduced costs, increased precision, and higher base resolution. NGS can be used to detect monogenic diseases, de novo mutations and mitochondrial mutation [11].

We recommended that the couple would have 2 cycles of ICSI and blastocyst vitrification (each blastocyst in a separate Rapid I device) as it would maximize the number of tested blastocysts to avoid cancellation of the embryo transfer procedure due to the absence of unaffected embryos. Meticulous labeling is important for the success of such complicated procedure of vitrification, genetic testing and later warming and embryo transfer as Rechitsky et al 2011 reported 3 cases of the wrong embryo transfer, which was obvious from the haplotypes of the resulting fetuses and children contrasting with the haplotypes of the embryos recommended for transfer [4].

The major difficulties in any PGT cycle are contamination, Amplification Failure (AF) and Allele Drop-Out (ADO). In our case we had 2 biopsies with failure of amplification. This is usually attributed to pre-analytical causes and account for up to 20% of the biopsies taken [12] and can be explained by DNA degradation, also it occurs in long amplified fragments more often than shorter fragments. Breaks in the DNA strand are expected to occur at random positions, consequently the longer the DNA fragment the greater the chance of a break occurring between the two PCR primers.

Controversy arises around the whole PGT technique specially about embryo sexing for non-medical reasons and Human Leukocyte Antigen (HLA) matching to enable future tissue donation from an unaffected child who is not yet born to a living affected child [13,14]. PGT was used in our case with the intension of saving the whole family a great trouble having another child with the same disease.

CONCLUSION

In conclusion, this case shows the beneficial role of NGS in achieving successful live birth (free from the tested disease) through testing embryos in a couple with carrier state of Oculocutaneous Albinism type 1 (OCA1). To our knowledge, this is the first report of such live birth using NGS on trophectoderm biopsied blastocysts.

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Authors' roles M.A. supervised the study and followed the patient during fertility management. M.H. was the gynecologist responsible

Table 2: The progress of the embryos biopsied in the 2 ICSI cycles.

| D5 Grading (Gardner et al 1998) | 1 st ICSI Cycle | | | 2 nd ICSI Cycle | | |
|------------------------------------|----------------------------|--------------------|----------------------|----------------------------|------------------------|----------------------|
| | Full Blastocyst BB | Full Blastocyst BB | Full Blastocyst BC | Expanded Blastocyst AB | Expanded Blastocyst BC | Full Blastocyst AA |
| NGS Result | Failed Amplification | Normal | Failed Amplification | Normal | Carrier N/c.346 C > T | Carrier c.346C > T/N |



for the ovarian stimulation; embryo transfer and pregnancy follow up. E.F., I.F. and H.E. from the IVF laboratory were concerned with embryo culture and blastocyst biopsy and vitrification. E.F. wrote the preliminary manuscript. E.E, W.K. and A.B. contributed to the Genetic counseling and Genetic testing. All authors were involved in writing the manuscript and have approved the final version.

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