Combined Preimplantation Genetic Diagnosis for a Monogenic Disease and Aneuploidy Screening with Array Comparative Genomic Hybridisation — First Live Birth in Hong Kong and a Review of the Approach

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Preimplantation genetic diagnosis (PGD) is an alternative of reproductive options of couple with genetic diseases. Alpha-thalassaemia is one of the most common indications for PGD in our locality. In the past, only target gene location could be detected during PGD treatment. However, after the recent advances in the technique of whole-genome amplification, the addition of aneuploidy screening in the PGD treatment cycles may provide further advantage. It can avoid aneuploidy or other chromosomal abnormalities of the newborn after PGD. We reported our first live birth in Hong Kong after this combination approach. It illustrated that it can be an attractive option for couples with genetic diseases or predisposition to genetic diseases.
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Case Report

A 33-year-old woman was referred to the reproductive genetic counselling clinic of Queen Mary Hospital in December 2012 with a request for preimplantation genetic diagnosis (PGD) for alpha-thalassaemia. She had previously had three spontaneous pregnancies, one ending in early miscarriage and the other two complicated by haemoglobin Bart's hydrops fetus with consequent termination in the second trimester. The couple were found to carry the heterozygous --Southeast Asian (SEA) deletion in alpha-globin genes. After genetic counselling, the couple was very keen to undergo PGD for alpha-thalassaemia in order to avoid further pregnancies with haemoglobin Bart's hydrops. They also raised their concern about trisomy pregnancies and requested aneuploidy screening in the same setting of PGD. After extensive counselling, combined PGD for alpha-thalassemia and aneuploidy screening was offered.

The patient underwent one in-vitro fertilisation treatment cycle in April 2013 in an agonist protocol. Forty-four oocytes were retrieved and 42 were mature for intracytoplasmic sperm injection; of which 35 were normally fertilised and 16 good-quality embryos were available for blastomere biopsy on day 3. Whole-genome amplification (WGA) was performed on a single blastomere[1]. After gap polymerase chain reaction for the alpha-globin gene and microsatellite markers flanking alpha-globin gene loci, 12 embryos were determined to be either normal or a carrier of the alpha-thalassaemia --SEA deletion. Aneuploidy screening was performed on the same WGA product with array comparative genomic hybridisation (aCGH) according to the manufacturer's protocol (24sure V3; Illumina, Cambridge, United Kingdom). No aneuploidy was detected in nine embryos, but seven embryos had various chromosomal abnormalities, including trisomies 4 and 18, monosomy 22, and segmental aberrations (Figure 1). In summary, of the 12 embryos that were either normal or a carrier of alpha-thalassaemia, only seven had no aneuploidy detected on aCGH (Figure 2). These seven

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blastocysts were of good quality and vitrified on day 5. The patient underwent frozen-thawed blastocyst transfer in September 2013 with one blastocyst transferred resulting in a singleton pregnancy. She delivered a full-term baby boy weighing 3.17 kg by Caesarean section on 9 June 2014.

Discussion

Since the development of PGD, more than 10,000 treatment cycles have been performed worldwide[2]. We developed our PGD service in 2001 and the first birth after PGD was in 2002. As thalassaemia is prevalent in our locality, it was the first monogenic disease to be targeted by our PGD service. For alpha-thalassaemia, an autosomal recessive disease, the most common type of alpha-globin gene mutation is deletion of two alpha-globin genes, the --SEA deletion. The most common cause of haemoglobinopathy hydrops in South-East Asians is being homozygous for the --SEA mutation, i.e. no alpha-globin gene present. These fetuses will exhibit abnormal ultrasound features in early pregnancy including cardiomegaly and placentomegaly, making early diagnosis possible. As this abnormality is not compatible with life, the couple will generally elect to abort the fetus. For couples with heterozygous --SEA deletions, 25% of their offspring can be expected to suffer from haemoglobin Bart's disease.

Our patient had two consecutive pregnancies affected by the disease. Because of the possibility of psychological trauma related to the previous abortions, she was very reluctant to accept the risk of an aneuploidy pregnancy that could result in a miscarriage or another abortion. Although the risk of aneuploidy, especially trisomy 21, in human pregnancies rises after maternal age above 35 years, 70% of children with Down syndrome are born to women below 35 years. Morphological assessment of blastocysts under the microscope alone cannot distinguish aneuploid blastocysts from euploid ones. Transfer of aneuploidy blastocysts most likely ends up as either implantation failure, miscarriage, or delivery of a congenitally abnormal baby[3]. A strategy of screening for aneuplodies can thus potentially shorten the time to successful delivery of healthy babies.

With the development of WGA and comprehensive chromosomal screening (CCS), combined PGD for monogenic diseases and aneuploidy screening is possible with one biopsy. We have previously demonstrated the feasibility of simultaneous PGD for alpha-thalassemia and aCGH on the same biopsy sample[1]. In this cohort of embryos, five (41.7%) genetically replaceable embryos on alpha-globin locus were found to be abnormal after aneuploidy screening. The benefits of using preimplantation genetic screening (PGS) with PGD include a significant reduction in pregnancies with chromosomal abnormalities and a possible reduction in miscarriages. Nonetheless, this strategy requires a good number of embryos for selection and may only benefit women with good ovarian reserve.

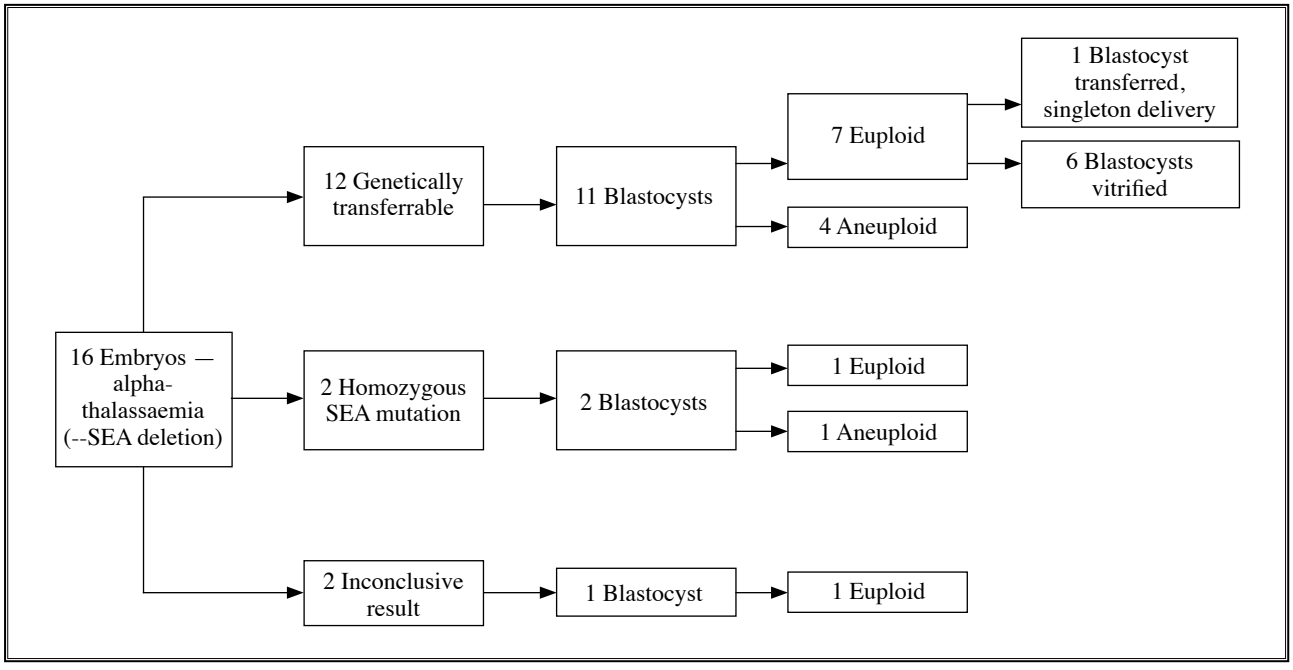


Figure 1. Results of preimplantation genetic diagnosis and aneuploidy screening

Abbreviation: SEA = Southeast Asian

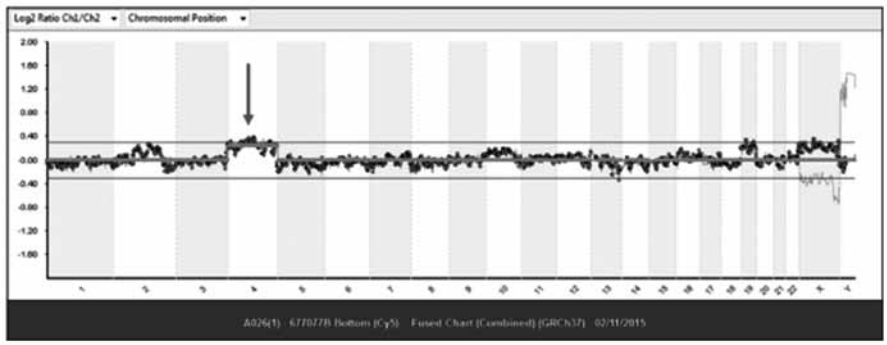
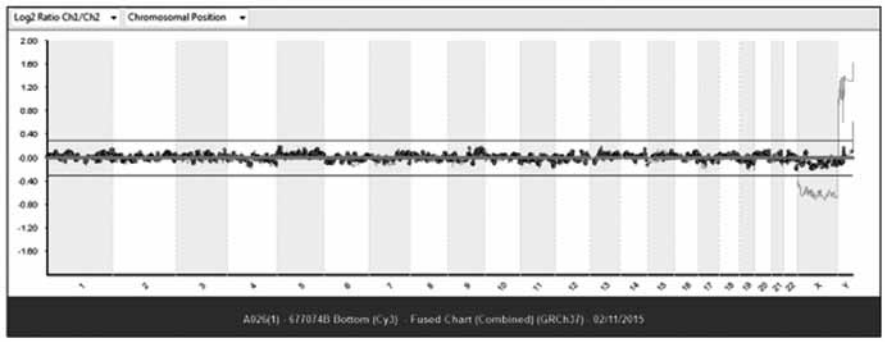
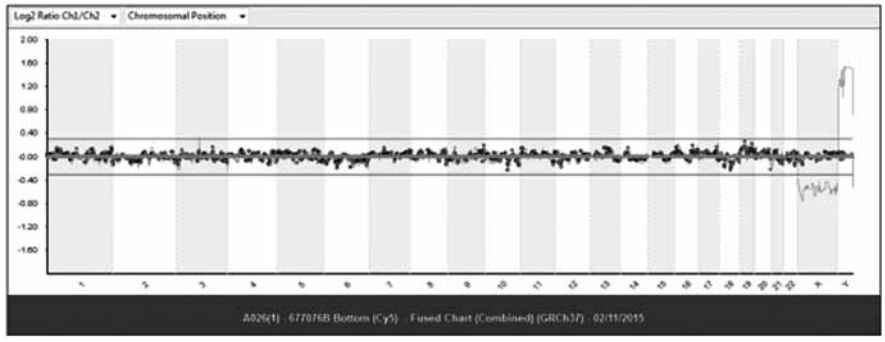
Embryo (quality on day 5)	Genotype	PGS result
7 (5BB)	Normal or alpha-thalassemia-1	Trisomy 4 
19 (5CB)	Homozygous for alpha --SEA deletion	No aneuploidy detected 
42 (5AA)	Normal	No aneuploidy detected 

Figure 2. Examples of preimplantation genetic diagnosis (PGD) and preimplantation genetic screening (PGS) result on good-quality blastocysts

Genetically replaceable but aneuploidy (embryo 7); the arrow depicted the gain in chromosome 4. Genetically non-replaceable but no aneuploidy detected (embryo 19). Genetically replaceable and no aneuploidy detected (embryo 42). The embryos have been replaced in frozen-thawed cycle and result in normal live birth of a baby boy

We offer this strategy to all couples undergoing PGD for monogenic diseases, and detailed counselling is provided to them before making a decision.

The use of PGS with CCS in assisted reproductive technology treatment cycles has been a topic hotly debated in the last couple of years. The potential advantages of this strategy include improved efficacy, shortened interval to live birth, reduced miscarriage rate and implantation failure. Nonetheless, there is debate about the potential problems arising from this use, similar to the enthusiasm about using PGS with fluorescent in-situ hybridisation (FISH) about two decades ago. Subsequently, a meta-analysis of 11 randomised trials demonstrated that this approach of using PGS with FISH did not improve the pregnancy rate at all[4]. The problems of using FISH lie in

the fact that only five chromosomes can be tested in one round and repeated rounds to test more chromosomes can hamper diagnosis accuracy. In addition, aneuploidies can arise from all chromosomes[5], and could explain partially the reason why PGS using FISH failed to improve the success rate. Also, in early cleavage embryos, mosaicism is very common as 70% of good-quality embryos are mosaic in nature. The percentage of mosaicism in blastocysts would be lower than in cleavage stage embryos, so using the trophectoderm biopsy with CCS in PGS can hopefully achieve the potential benefits. Nonetheless, before we fall into the same trap similar to 20 years ago[6], large randomised trials are urgently needed prior to the routine use of PGS with CCS in artificial reproductive technology settings.

Our case showed a potential but clear benefit of combining PGS with CCS in couples undergoing PGD for monogenic diseases. Careful patient counselling is vital. The combined screening approach should not be the routine treatment for couples undergoing PGD for monogenic diseases.

Declaration

All authors have disclosed no conflicts of interest.

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