A Successful Strategy for Preimplantation Genetic Diagnosis of beta-Thalassemia and Simultaneous Detection of Down's Syndrome Using Multiplex Fluorescent PCR

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Objectives: *Preimplantation Genetic Diagnosis (PGD) is an alternative to prenatal diagnosis providing couples the chance to start a pregnancy with an unaffected fetus. The objective of the present study was to develop and apply quick, sensitive and accurate single cell PCR protocols for PGD of beta-thalassemia and Down's syndrome detection.*
Material and Method: *Two couples carrying beta-thalassemia codon41-42 mutation underwent routine IVF procedures. Embryo biopsy was performed on Day-3 post-fertilisation and single cell multiplex fluorescent PCR was employed for mutation analysis, contamination detection and diagnosis of trisomy 21 cases.*
Results: *Seventeen embryos were tested in two clinical PGD cycles. This resulted in the first birth following PGD for a single gene disorder in Thailand and South East Asia, confirmed by prenatal testing. Two embryos were shown to be affected by Down's syndrome.*
Conclusion: *Successful strategy for PGD of beta-thalassemia and Down's syndrome detection using multiplex fluorescent PCR was introduced.*

Keywords: *beta-thalassemia, Down's syndrome, Embryo selection, Preimplantation genetic diagnosis (PGD), Prenatal diagnosis (PND), Multiplex fluorescent single cell PCR, Trisomy 21*

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Thalassemia, the world's most common single gene disorder[1], is prevalent in Thailand. Babies with the most severe form of beta-thalassemia develop anemia from their transfusion dependency within 6 months after birth. These regular blood transfusions may also lead to infection with HIV, Hepatitis B or C, or iron overloading. The only mean of curing this condition is through bone marrow transplant and this is expensive and risky. Therefore, the most widely applicable strategy for dealing with thalassemias has been to control the incidence of new cases by offering screening for heterozygotes, genetic counseling, and Pre-Natal Diagnosis (PND) with termination of pregnancy in affected cases[2].

An alternative to routine PND is Preimplantation Genetic Diagnosis (PGD), which allows selection of unaffected embryos prior to establishment of a pregnancy, thus providing couples with the chance to start a pregnancy knowing that the fetus is unaffected and

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eliminating the need for pregnancy termination[3]. PGD involves the sampling and testing of a single cell from cleavage stage embryos generated using IVF technology. For the diagnosis of single gene disorders, it is necessary to amplify DNA from the cell using the Polymerase Chain Reaction (PCR). A variety of modified PCR techniques have been developed, however, amplification failure, Allele Drop-Out (ADO) and contamination are still major problems encountered during PCR at the single cell level[4]. Beside the limited amount of material available for testing (only 1-2 cells), the diagnoses must be completed within 24 hours. Therefore, the diagnostic techniques need to be quick, sensitive and accurate. PGD of single gene disorders have been done in Western countries[5,6], but there has not been a report from any country in South East Asia yet.

The objective of the present study was to develop single cell PCR protocols for PGD of beta thalassemia codon 41-42 mutation and perform clinical PGD cases for couples at risk. Additionally, chromosome 21 microsatellite markers were utilized in order to detect cases of Down's syndrome and extraneous DNA contamination.

Material and Method

Patient details

Fourteen couples at risk of having an affected fetus with beta-thalassemia were counseled regarding the project, two decided to join the project and signed consents were obtained. The mother and father of family 'A' were 32 and 35 years old respectively. The mother and father of family 'B' were 24 and 25 years old respectively. The parents of both families were carriers of beta-thalassemia codon 41-42 mutation and experienced one termination of pregnancy following positive prenatal diagnosis of beta-thalassemia.

ICSI procedure and cleavage stage embryo biopsy

The patients underwent routine superovulation using recombinant FSH (Puregon Pen, Organon Thailand Co., Ltd., Bangkok, Thailand) and synthetic decapeptide ganirelix (Orgalutran , Organon Thailand Co., Ltd., Bangkok, Thailand) and oocytes were fertilized using Intra Cytoplasmic Sperm Injection (ICSI). ICSI is used as a precaution to reduce the risk of sperm DNA contamination in subsequent PCR amplifications. Laser biopsy was performed on Day 3 post-fertilization (4–9 cell stage), allowing two blastomeres to be removed from embryos consisting of 6+ cells and one blastomere from embryos with 4-5 cells (Fig. 1). Cleavage stage embryos were graded 1, 2^-, 2, 2^+ and 3 where grade 1 had the best morphology and grade 3 was a highly fragmented, poor quality embryo[7].

Single cell isolation

Buccal cells, isolated by micromanipulation, and biopsied blastomeres were transferred into droplets of phosphate-buffered saline (PBS) (GibcoBRL , Gibthai Co., Ltd., Chiang Mai, Thailand) with 4% bovine serum albumin (BSA) (Sigma , S.M. Chemical Supplies Co., Ltd., Chiang Mai, Thailand) on a 5 cm Petri dish in a laminar flow cabinet. Cells were washed in a minimum of four fresh PBS droplets, while visualizing under a dissecting microscope, and were then transferred to thin-wall microcentrifuge tubes. A 2 l aliquot of the last washing drop was also taken as a blank for each single blastomere. Cell lysis was carried out as described previously[8].

Multiplex fluorescent PCR

Extracted DNA from single cells was amplified using a combination of PW007 primers (forward 5'-ATT TTC CCA CCC TTA CCG TG-3', reverse 5'-GCA GCT CAC TCA GTG TCC G-3') covering beta-thalassemia codon 41-42 mutation and a microsatellite D21S1411[9] or D21S11[10] primers (Gene Systems Co., Ltd., Bangkok, Thailand). The PCR mixture consisted of 200 M of each primer, 0.2 mM deoxynucleoside triphosphates (dNTPs) (Eppendorf , DynaScience Co., Ltd., Chiang Mai, Thailand), 1 x GeneAmp Buffer (10 x GeneAmp Buffer contains 100 mM Tris-HCl pH 8.3, 500 mM KCl, 15 mM $MgCl_2$) (Gene Systems Co., Ltd., Bangkok, Thailand) and 1.5 U AmpliTaq Gold (Gene Systems Co., Ltd., Bangkok, Thailand) and was made up to a total volume of 25 l with distilled deionized water. The amplifications were performed with the conditions: 94 C 45 s (96 C for the first ten cycles), annealing at 60 C 45 s and extension at 72 C 1 min for 40 cycles. These were preceded by denaturation at 94 C for 12 min to activate the AmpliTaq Gold enzyme. The multiplex amplified products from single cells were each tagged with two different fluorochromes using labeled primers. This allowed analysis to be performed on an automated laser fluorescent sequencer ABI Prism 310 (Gene Systems Co., Ltd., Bangkok, Thailand). PW007 and D21S11 fragments were labeled with the blue fluorescent dye (6-FAM), D21S1411 fragments were labeled with the yellow fluorescent dye (NED).

Fragment analysis on ABI Prism 310

A mixture of 1 l fluorescent PCR products, 12 l deionized formamide and 0.5 l size standard

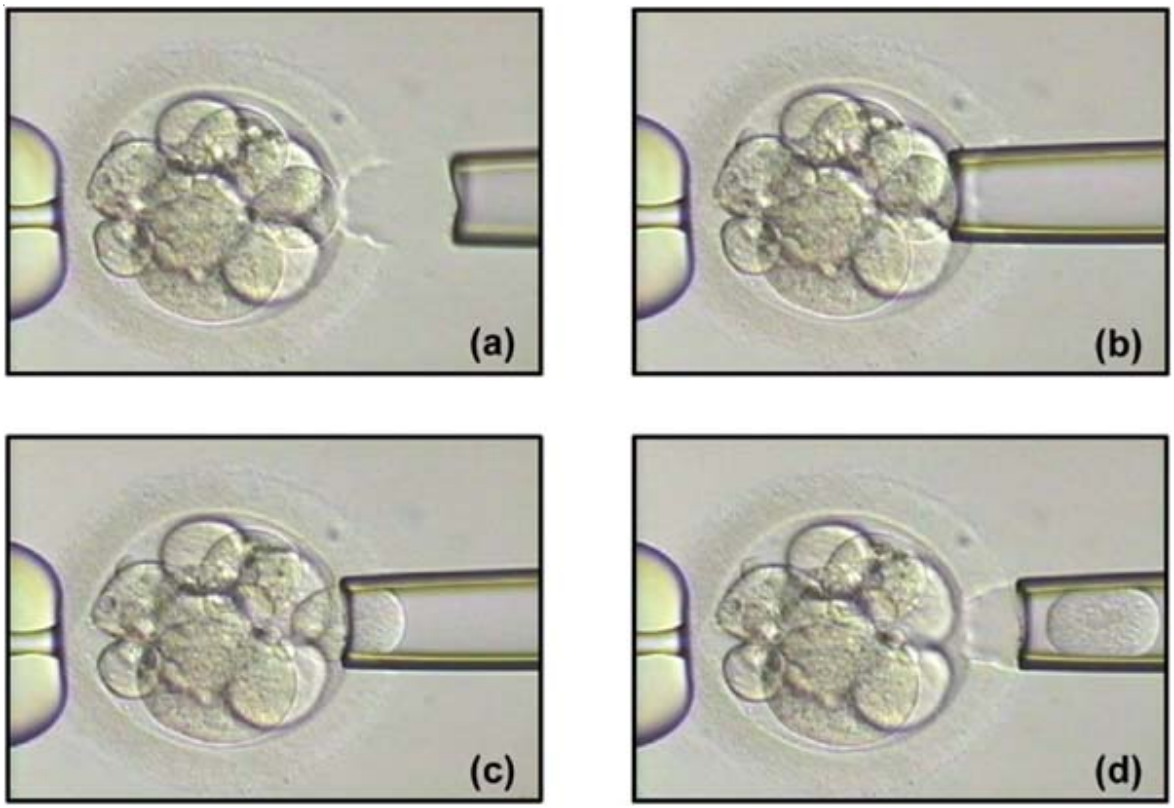

Fig. 1 Cleavage stage embryo biopsy in the present study: (a) the embryo is stabilized by a holding pipette (left) with negative pressure, a hole is created in the zona pellucida (zona drilling) using laser, (b), (c) and (d) a blastomere is gently pulled away from the embryo using a biopsy pipette (right) with negative pressure through the hole in the zona pellucida. Procedure performed by TV

(Genescan™-500 (ROX); Gene Systems Co., Ltd., Bangkok, Thailand) was prepared and denatured at 95 C for 5 min. The denatured sample was subjected to capillary electrophoresis using Performance Optimized Polymer 4 (POP-4 , Gene Systems Co., Ltd., Bangkok, Thailand; 5 s injection time, 15,000 V, 60 C, 24 min). The data was analyzed by GeneScan™ analysis software (Gene Systems Co., Ltd., Bangkok, Thailand).

Results

Single cell PCR protocols for beta-thalassemia condon 41-42 mutation were developed and tested specifically for each couple. A new set of PCR primers (PW007) was designed for mutation analysis of beta-thalassemia codon 41-42 mutation. In addition to amplifying the beta-globin gene region, a polymorphic marker, D21S1411 or D21S11, was incorporated into the PGD protocol as multiplex PCR to serve as a very basic form of DNA fingerprint. Both couples were fully informative for each marker used, in other words the parents of each couple shared no alleles in common. Therefore their embryos could only inherit one of four possible genotypes. Any deviation from these combinations of alleles, such as the detection of alleles not derived from either parent, was indicative of contamination. The compatibility of each pair of primers was assessed and optimized using single buccal cells derived from each patient and single blastomeres from embryos donated for research prior to clinical application. Genotyping of the couples 'A' (PW007 and D21S1411 primers) and 'B' (PW007 and D21S11 primers) are demonstrated in Fig. 2.

Preclinical assessment of methodology

From 60 single buccal cells of couple 'A', multiplex fluorescent PCR protocol gave an Amplification Efficiency (AE) of 86.7% for PW007 and 83.3% for D21S1411 primers and ADO rates of 21.2% for PW007 and 26% for D21S1411 primers. The application of this single cell PCR protocol to 45 spare single human blas-

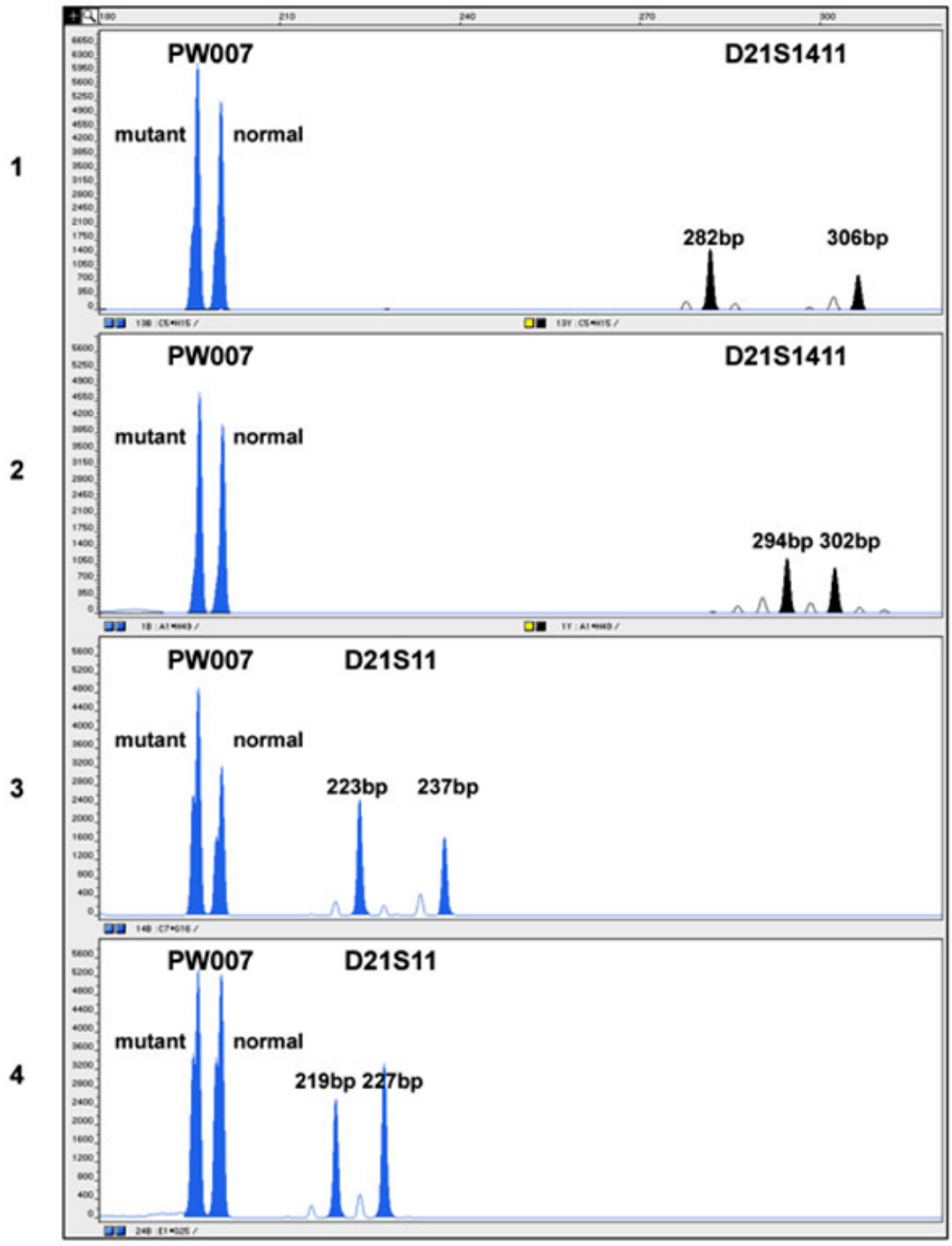

Fig. 2 Results from GeneScan™ analysis on ABI Prism 310 for PW007, D21S1411 and D21S11 primers multiplex amplified from single buccal cells of the mother (lane 1) and the father (lane 2) of the family A and the mother (lane 3) and the father (lane 4) of the family B. The left blue peaks in lane 1, 2, 3 and 4 show mutant and normal beta thalassemia codon 41-42 mutation (PW007) alleles, the black peaks in lane 1 and 2 show microsatellite (D21S1411) alleles and the right blue peaks in lane 3 and 4 show microsatellite (D21S11) alleles. The x and y axes are base-pairs and fluorescence units, respectively

tomeres donated for research exhibited acceptable AE rates of 86.7% for PW007 and 88.9% for D21S1411 primers. The optimized protocol for family 'B' showed AE of 87.5% for PW007 and 90% for D21S11 primers and ADO rates of 2.9% for PW007 and 13.9% for D21S11 primers from 40 single heterozygous buccal cells. This protocol demonstrated AE of 92.3% for PW007 and 88.5% for D21S11 primers on 26 single human blastomeres.

Preimplantation diagnosis results

PGD cycle 1 for family 'A' gave 14 oocytes, 10 of those were sperm-injected. Eight embryos were of sufficient quality for biopsy on Day 3 post-fertilization, yielding 13 cells. Molecular analyses using multiplex fluorescent PCR revealed three embryos (embryos A1, A5 and A6) to be affected, two normal (embryos A4 and A7, Fig. 3), one heterozygous (embryo A8), one with ambiguous result (embryo A2) and one with no results (embryo A3) (Table 1). No DNA contamination was detected. Follow-up analyses were performed on the embryos that had been diagnosed affected, or were unsuitable for transfer for embryological reasons. For this purpose whole embryos were transferred to PCR tubes and subjected to the same protocol as used for the actual PGD. The initial diagnoses of heterozygous and affected results were confirmed in all cases, but one (embryo A5). Embryo A5 was found to be heterozygous rather than affected as originally thought. The most likely explanation for this error is ADO affecting the normal allele in both analyzed blastomeres. Embryo A2 had given ambiguous results, one of the blastomeres tested showing only a mutant allele, while the second blastomere analyzed displayed only a normal allele. The analysis of the rest of the embryos showed a heterozygous genotype, suggesting that the disparity in genotype of the two cells was due to ADO affecting a different allele in each cell. The blastomere A3.1 that failed to give results was from a heterozygous embryo. Both normally diagnosed embryos were transferred and one clinical pregnancy (singleton) was obtained. Prenatal diagnosis by fetal blood sampling at 19 weeks of gestation confirmed the homozygous normal beta-globin gene genotype of the fetus. A disease-free baby boy was born in June 2005.

During PGD cycle 1 for family 'B' 20 oocytes were collected, 11 were successfully sperm-injected. After oocyte retrieval the patient encountered ovarian hyperstimulation syndrome, therefore nine embryos with good development were kept frozen on Day 2. Two months after freezing, the embryos were thawed and culture resumed. All nine embryos were of sufficient quality for biopsy on Day 3 and yielded 16 cells. Multiplex fluorescent PCR protocol for family 'B' showed three embryos (embryos B1, B5 and B9) to be normal, three heterozygous (embryos B2, B6 and B7), two with ambiguous results (embryos B4 and B8) and one with tri-allelic of D21S11 marker (embryo B3, Fig. 3). No DNA contamination in negative control samples was detected. Follow-up study on un-transferred embryos confirmed the initial diagnoses in all normal and heterozygous embryos. Embryos B4 and B8 with initially discordant results between two blastomeres appeared to be heterozygous from the un-transferred embryos analyses, like embryo A2. Interestingly, the tri-allelic result of D21S11 marker from the initial diagnosis was also found when the remainder of the embryo was analyzed (Fig. 3), indicating that the additional allele was due to trisomy 21 rather than contamination. Moreover, tri-allelic result of D21S11 marker on chromosome 21 was also found in embryo B4, suggestive of trisomy 21 embryo. These made the prevalence of trisomy 21 embryos be 22.2% (2/9) in this PGD cycle, surprising given that the mother was just 24 years old. Most of the embryos were arrested on Day 4, therefore one normal (embryo B1) and one heterozygous (embryo B2) were transferred. Unfortunately, no pregnancy resulted on this occasion.

Discussion

Thalassemia is prevalent and causes an enormous financial burden in Thailand and throughout South East Asia. Carrier screening, providing PND for couples at risk and termination of affected pregnancy is the most common solution at present[2]. For parents who are carriers of thalassemia mutations a quarter of all pregnancies will, on average, be affected. Sadly, some couples have multiple consecutive affected fetuses. PGD is an alternative to PND and provides couples with a chance to start a pregnancy knowing that the baby will be unaffected, eliminating the need for Termination Of Pregnancy (TOP). The application of PGD for single gene disorders represents an integration of modern Artificial Reproductive Technology (ART) techniques with molecular genetic diagnosis techniques. The development of such protocols is known to be challenging and labor intensive, often necessitating the collaboration of several organizations.

The first successful clinical PGD pregnancy for a single gene disorder in Thailand and South East Asia was reported here. Specific single cell multiplex fluorescent PCR protocols were developed for these

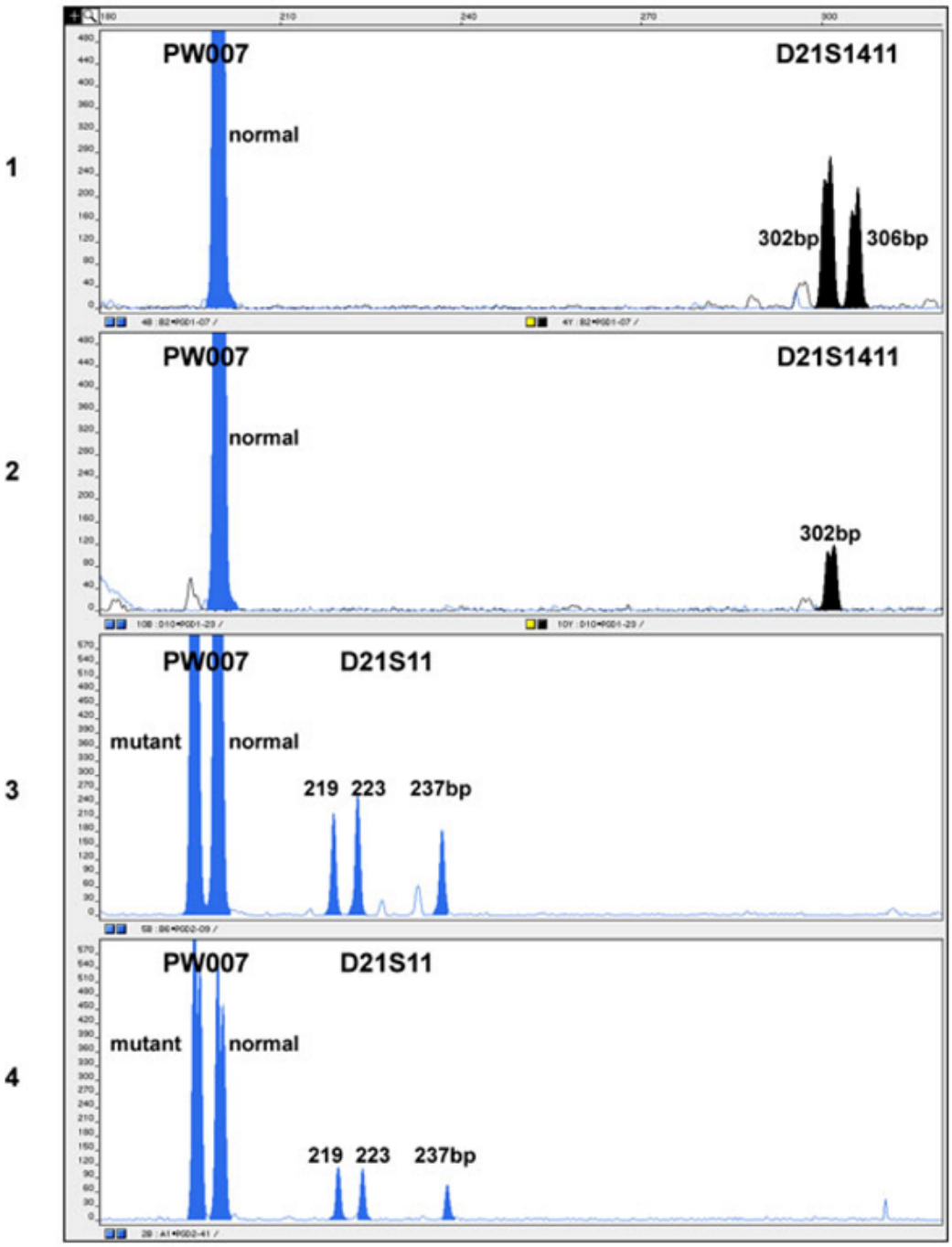

Fig. 3 Results from GeneScan™ analysis on ABI Prism 310 for PW007, D21S1411 and D21S11 primers multiplex amplified from single blastomeres and embryo of clinical PGD cycles. Lanes 1 and 2 are results of normal beta-thalassemia codon 41-42 mutation allele (PW007, blue peaks) and microsatellite alleles (D21S1411, black peaks) from the normal blastomeres A4.1 and A7.1 respectively, which were chosen for embryo transfer. Lane 3 and 4 shows results of mutant and normal beta-thalassemia codon 41-42 mutation alleles (PW007, left blue peaks) and microsatellite alleles (D21S11, right blue peaks) of the trisomy 21 blastomere B3.1 and un-transferred embryo B3 respectively. The x and y axes are base-pairs and fluorescence units, respectively

Table 1. Preimplantation genetic diagnosis results of family 'A', cycle 1 (embryos A1-A8) and family 'B', cycle 1 (embryos B1-B9)

Embryo no.	Embryo grade (no. of cells) before biopsy (Day 3)	Cells taken (*n*)	Cell no.	beta-thalassemia results	Microsatellite markers results (D21S1411 for family 'A' and D21S11 for family 'B')	Diagnosis	Outcomes	Confirmatory results
A1	2^{-}(8)	2	A1.1	Affected	NC*	Affected	Untransferred	Affected
			A1.2	Affected	NC			
A2	2^{-}(8)	2	A2.1	Affected	No result	Ambiguous, likely to be heterozygous	Untransferred	Heterozygous
			A2.2	Normal	No result			
A3	2^{-}(5)	1	A3.1	No result	No result	No result	Untransferred	Heterozygous
A4	2^{-}(8)	2 (1 lysed)	A4.1	Normal	NC	Normal	Embryo transferred	-
A5	2(8)	2	A5.1	Affected	NC	Affected	Untransferred	Heterozygous
			A5.2	Affected	NC			
A6	1(8)	2 (1 lysed)	A6.1	Affected	NC	Affected	Untransferred	Affected
A7	2(8)	2	A7.1	Normal	NC	Normal	Embryo transferred	-
			A7.2	No result	No result			
A8	2(8)	2	A8.1	Heterozygous	NC	Heterozygous	Untransferred	Heterozygous
			A8.2	Heterozygous	NC			
B1	2^{+}(7)	2	B1.1	Normal	NC	Normal	Embryo transferred	-
			B1.2	Normal	NC			
B2	2^{+}(8)	2	B2.1	Heterozygous	NC	Heterozygous	Embryo transferred	-
			B2.2	Heterozygous	NC			
B3	2^{+}(8)	2	B3.1	Heterozygous	C*	Heterozygous	Untransferred (arrested)	Heterozygous with trisomy 21
			B3.2	Heterozygous	NC			
B4	2^{+}(6)	2	B4.1	Affected	No result	Ambiguous, likely to be heterozygous	Untransferred	Heterozygous with trisomy 21
			B4.2	Heterozygous	No result			
B5	2(7)	2	B5.1	Normal	NC	Normal	Untransferred (arrested)	Normal
			B5.2	Normal	NC			
B6	2^{+}(7)	2	B6.1	Heterozygous	NC	Heterozygous	Untransferred (arrested)	Heterozygous
			B6.2	Heterozygous	NC			
B7	2^{+}(5)	1	B7.1	Heterozygous	NC	Heterozygous	Untransferred (arrested)	Heterozygous
B8	2^{+}(6)	2	B8.1	Heterozygous	NC	Ambiguous, likely to be heterozygous	Untransferred	Heterozygous
			B8.2	Normal	NC			
B9	2^{+}(6)	1	B9.1	Normal	NC	Normal	Untransferred (arrested)	Normal

*NC = No contamination detected
*C = Contamination or aneuploidy detected

families. Compared to previous reported PGD protocols for beta-thalassemia, i.e. Restriction Fragment Length Polymorphism (RFLP) technique[6,11,12], DGGE[5], the use of multiplex fluorescent PCR protocols in the present study offers the advantage of increased sensitivity. Fewer PCR cycles are necessary for PCR product detection and the size of the fragments generated can be determined with high accuracy using fluorescent

DNA sequencing apparatus. This means that the speed of diagnosis is an accelerated key factor for PGD. Moreover, a new set of primers was designed for a shorter amplified DNA product specific for the diagnosis of beta-thalassemia codon 41-42 mutation[13], to have a more efficient set of primers than the previously developed protocol[14]. In one clinical PGD cycle (PGD cycle 1 for family 'B'), the amplification efficiency of PW007 met 100% in a duplex single cell PCR. The use of sequencing[15] and whole genome amplification[16] protocols are expensive, labor-intensive, time consuming and sometimes unnecessary.

Contamination of a homozygous affected blastomere with maternal DNA or paternal DNA (derived from cumulus cells or surplus sperm attached to the zona pellucida respectively) can lead to a heterozygous (unaffected) diagnosis, and subsequent transfer of an affected embryo. Therefore, in addition to direct amplification of the causative mutation, the protocol described included a highly polymorphic marker for contamination detection (D21S1411 for family 'A' and D21S11 for family 'B'). The embryos produced by a given couple can only inherit a limited combination of alleles (one allele from each parent). If biopsies of blastomeres do not give one of these predictable combinations, it is likely that they are contaminated. In previous PGD cases this strategy has allowed us to detect contamination restricted to a single PCR tube[17]. The use of a microsatellite marker on chromosome 21 also permits evaluation of the copy number of this chromosome, revealing monosomy and trisomy due to mal-segregation during meiosis I. The three alleles detected for the D21S11 marker in embryo B3, which had no contamination detectable in the corresponding negative control blank, indicated the presence of trisomy 21. Three copies of chromosome 21 were also evident during analysis of embryo B4. Discordant results of chromosome 21 markers in blastomeres B3.1, B3.2, B4.1, B4.2 and embryos B3, B4 might be from amplification failure, ADO or chaotic division of the embryos[18]. Given that most of the patients seek PGD in order to avoid a pregnancy termination, the detection of Down's syndrome prior to initiation of a pregnancy is highly desirable. Furthermore, many aneuploid pregnancies (e.g. monosomy 21) spontaneously abort, thus the pregnancy rates following PGD can be improved by identifying and preferentially transferring embryos with a normal chromosome number. To date few PGD tests have provided a combination of single gene analysis and chromosomal ploidy information[19].

From the confirmatory analysis of the untransferred embryos, the ambiguous (Embryo A2, B4 and B8) and incorrectly genotyped (Embryo A5) results in the present study were due to ADO. ADO of a heterozygous cell leads to a homozygous normal or homozygous affected result. Therefore, the presence of ADO in beta-thalassemia analysis, where both parents carry the same mutation (as in the cases presented here) cannot lead to the transfer of a homozygous affected embryo. However, incorrect diagnosis of unaffected heterozygous embryos as affected leads to a reduced number of embryos available for transfer as demonstrated in Embryo A2 and A5 of the present study. This, in turn, leads to reduced pregnancy rates. On the other hand, a homozygous normal result of a single blastomere may be from a heterozygous blastomere with ADO of the mutant allele. Therefore, it is possible that the transferred Embryos A4, A7 and B1 might be normal or heterozygous; however, these cases were clinically unaffected and therefore acceptable for PGD.

In conclusion, novel PGD protocols for beta-thalassemia codon 41-42 mutation and Down's syndrome detection using multiplex fluorescent single cell PCR were developed and optimized. A new set of primers for detecting beta-thalassemia codon 41-42 mutation was designed for effective mutation detection and coupled with co-amplification of an informative microsatellite marker (D21S1411 for family 'A' and D21S11 for family 'B') for detection of contamination and instances of Down's syndrome caused by mal-segregation of chromosome 21 during meiosis I. The protocols were applied in two clinical PGD cycles and resulted in the first clinical pregnancy following PGD for a single gene disorder in Thailand and South East Asia. Two preimplantation embryos diagnosed as Down's syndrome were also reported, supporting the benefit of the successful strategy in simultaneous detection of beta-thalassemia and Down's syndrome using multiplex fluorescent single cell PCR.

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ความสำเร็จของหลักการวินิจฉัยก่อนการฝังตัวของตัวอ่อนสำหรับโรคบีต้าธาลัสซีเมียพร้อมกับกลุ่มอาการดาวน์โดยเทคนิคปฏิกิริยาลูกโซ่ชนิดเรืองแสง

วีรวิทย์ ปิยะมงคล, ธีระพร วุฒยวนิช, ศิริวิภา ปิยะมงคล, เดแกน เวลส์, ชัยรัตน์ คุณาวิกติกุล, ธีระ ทองสง, สมศักดิ์ เชาว์วิศิษฐ์เสรี, รัตน์ติกา แซ่ตั้ง, ต่อพงศ์ สงวนเสริมศรี

วัตถุประสงค์: *การวินิจฉัยก่อนการฝังตัวของตัวอ่อนเป็นทางเลือกใหม่ในการวินิจฉัยก่อนคลอดที่ช่วยให้คู่สมรสมีโอกาสเริ่มต้นการตั้งครรภ์ด้วยทารกที่ปราศจากโรค การศึกษานี้มีวัตถุประสงค์ที่จะพัฒนาโปรโตคอลในการวินิจฉัยโรคบีต้าธาลัสซีเมียร่วมกับกลุ่มอาการดาวน์ที่มีความไวสูง รวดเร็ว และแม่นยำ และทำการวินิจฉัยก่อนการฝังตัวของตัวอ่อนให้กับผู้ป่วย*

วัสดุและวิธีการ: *คู่สมรส 2 คู่ที่เป็นพาหะของโรคบีต้าธาลัสซีเมียชนิดโคดอน 41-42 ตัดสินใจเข้าร่วมโครงการและผ่านขั้นตอนการทำทารกหลอดแก้ว การตรวจเซลล์ตัวอ่อนกระทำในวันที่ 3 หลังการปฏิสนธิ โดยใช้เทคนิคปฏิกิริยาลูกโซ่ชนิดเรืองแสงจากเซลล์เดียวที่พัฒนาขึ้นมาใหม่สำหรับการวินิจฉัยโรคบีต้าธาลัสซีเมียชนิดโคดอน 41-42 พร้อมกับการตรวจการปนเปื้อนและการวินิจฉัยกลุ่มอาการดาวน์*

ผลการศึกษา: *ตัวอ่อนจำนวน 17 ตัวได้รับการตรวจจากการวินิจฉัยก่อนการฝังตัวของตัวอ่อน 2 ราย ประสบความสำเร็จได้การตั้งครรภ์จากการวินิจฉัยก่อนการฝังตัวของตัวอ่อนสำหรับโรคพันธุกรรมชนิดยีนเดี่ยวเป็นครั้งแรกในประเทศไทยและภูมิภาคเอเชียตะวันออกเฉียงใต้ ผลการตรวจได้รับการยืนยันโดยการวินิจฉัยก่อนคลอดและหลังคลอด นอกจากนี้ยังพบว่ามีตัวอ่อน 2 ตัวเป็นกลุ่มอาการดาวน์*

สรุป: *หลักการในการวินิจฉัยก่อนการฝังตัวของตัวอ่อนสำหรับโรคบีต้าธาลัสซีเมียพร้อมกับกลุ่มอาการดาวน์ โดยใช้ปฏิกิริยาลูกโซ่ชนิดเรืองแสงได้ประสบความสำเร็จและถูกนำเสนอไว้ในรายงานนี้*