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Original Research

Pre-Implantation Genetic Testing for Oculocutaneous Albinism Type 1 Using Karyomapping

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Abstract

Background: Oculocutaneous albinism type IA (OCA1) is the most severe form of albinism, an autosomal recessive inherited deficit of the pigment melanin causing distinctive alterations of skin, hair, and visual system. Pre-implantation genetic testing (PGT) is a substitution for prenatal diagnosis. **Methods**: This study accomplished SNP array with karyomapping for PGT of OCA1 and validated the results with PCR-based PGT. **Results**: One family with a risk of having OCA1 c.819+3insATATGCC and c.896G>A (p.R299H) offspring chose to go through karyomapping PGT. Novel PCR protocols employing fluorescent PCR and mini-sequencing were developed, tested, and applied. In the clinical PGT cycle, two blastocyst stage embryos were subjected to PGT. Karyotyping PGT results of OCA1 revealed both of the embryos to be normal. PCR analysis confirmed haplotyping results. However, copy number variation (CNV) analysis exhibited an additional chromosome 14 and segmental loss of 7q in embryo No. 1, i.e., 47, XY,+14,-7q, and an additional chromosome 22 in embryo No. 2, i.e., 47, XY,+22. Therefore, there was no appropriate embryo for transfer. The patient will return for the next PGT cycle. **Conclusions**: Karyomapping PGT for OCA1, including insertion c.819+3insATATGCC and point mutation c.896G>A (p.R299H), was performed alongside PCR techniques. Karyomapping gives benefits of CNV information to avoid the transfer of chromosomally unbalanced embryos.

Keywords: oculocutaneous albinism type IA (OCA1); embryo selection; haplotyping; karyomapping; pre-implantation genetic testing for monogenic disease (PGT-M)

1. Introduction

Albinism is a genetic complex class of hypopigmentation conditions that have a complete or partial inherited deficit of the pigment melanin and results in distinctive alterations in the skin, hair, and visual system (eyes and optic tracts) [1]. Melanin is synthesized and stored inside melanosomes, intracellular organelles that can be found in the stratum basale of the epidermis, hair bulbs, and intraocular epithelia [2]. The major form of albinism is oculocutaneous albinism (OCA) characterized by partial or complete deficiency of melanin pigmentation of the skin, hair, foveal hypoplasia, nystagmus, photophobia, and low visual acuity [3]. The prevalence of albinism varies and is estimated at 1:17,000. The prevalence of gene carrier is about 1.4% [4].

1.1 Molecular Basis

Oculocutaneous albinism type I (OCA1, MIM#203100), an autosomal recessive disorder, is the most severe form of albinism. OCA1 is caused by homozygous or compound heterozygous mutation in tyrosinase (TYR, MIM#606933) gene on chromosome 11q14.3 [5], leading to the deficiency of tyrosinase enzyme, i.e., tyrosinase-negative OCA. Over 60 different mutations have been addressed [6]. The parents of an affected child are obligate carriers and the chance of having another affected offspring is 25%. The offspring of an affected patient are obligate carriers. Carriers are asymptomatic. The life expectancy of OCA patients is not reduced. Development and intellectual capabilities are normal. Patients with OCA have normal fertility [1].

TYR consists of five exons spanning 65 kb and encoding a 529 amino acid protein [7]. Tyrosinase is a copper-containing enzyme that plays a role in several crucial steps in melanin biosynthesis [8]. The most important symptom of OCA1 is severely reduced vision. The lack of melanin pigment in eyes during embryogenesis causes developmental anomalies, including hypoplasia of macula, misrouting of optic nerve fibers to the visual cortex and translucent iris, and visibility of choroidal vessels in the pigment-lacking retina. These lead to nystagmus, decreased visual acuity,

lack of stereoscopic vision, and severe photophobia. Refractive errors, i.e., myopia, hyperopia, astigmatism, and strabismus are commonly present. The second symptom is a consequence of sunlight on the skin. Frequent sunlight exposure leads to burn, degenerate changes, and an increased risk of skin cancer [1]. Severe visual handicap in OCA1 results in great daily living difficulties and limited professional options. Albinism is still incurable. Families at risk of having children with albinism will seek genetic counseling and prenatal diagnosis (PND).

1.2 Prenatal Diagnosis

PND of OCA by electron microscopy examination of fetal skin samples taken during fetoscopy at 20 weeks of gestation was first reported in 1983 [9]. A total of 12 cases of PND for OCA using fetal skin biopsies were reported in 2005 from the same group [10]. Thirty-one cases of PND by fetal scalp biopsies using fetoscopy with histological examination were reported in 1999 [11]. Fetal skins were then examined for the presence of absence of melanin in the epidermis and/or hair bulb melanocytes under light microscope. Subsequently, defining the stages of melanosomal differentiation to evaluate the course of melanogenesis was carried out using an electron microscope. Normal melanogenesis was revealed in 26 biopsies.

PND using DNA analysis from fetal cells, i.e., chorionic villous sampling or amniocentesis, was also performed by direct mutation analysis (one case) and linkage analysis (two cases) in the same report [11]. PND of OCA by analysis of the fetal tyrosinase gene was reported in 1994 [12]. Following amniocentesis, PCR with dot-blot was successfully performed on fetal cells. In cases of unidentified mutation, an electron microscopic 3,4-dihydroxyphenylamin reaction test of fetal skin biopsy was employed for PND [13,14]. A total of 55 PND of OCA using molecular genetic analysis in 37 families (32 families with OCA1) were reported from the same group in 2009 [15]. Half of the PND was performed by first trimester CVS and the other half by mid-trimester amniocentesis. Fetal samples underwent mutation analysis using either restriction fragment length polymorphism or direct sequencing and haplotyping analysis using 4–6 microsatellite linked markers.

DNA-based PND of a Korean family with the risk of having compound heterozygous OCA1 offspring was successfully performed in 1997 [16]. A combination of single-stranded conformation polymorphism/heteroduplex screening, restriction fragment length polymorphism, and allele-specific oligonucleotide hybridization analysis of DNA from chorionic villous sampling was employed. Two cases of molecular diagnosis using denaturing high-performance liquid chromatography and Sanger's sequencing from fetal blood samples were successfully carried out in 2006 [17]. Ten PND using fluorescent sequencing were reported in 2014 [18]. It is noted that sampling techniques have moved from fetal skin biopsy to either chorionic villous sampling or amniocentesis and diagnostic techniques moved from electron microscope to molecular genetic analysis. However, PND of OCA1 is sometimes controversial.

PND provides fetal samples for genetic analysis. Selections of invasive PND include chorionic villous sampling in the first trimester and amniocentesis and fetal blood sampling in the second trimester. Negative results assure the couples that their baby is healthy. Nevertheless, positive results provide difficult decisions for the couples regarding dismissing or continuing the pregnancy and preparing for postnatal treatment [18]. Additionally, some pregnancies may abort following the PND procedures.

1.3 Pre-Implantation Genetic Testing

Pre-implantation genetic testing (PGT) [19] is a substitution for the conventional PND giving the couples an opportunity to start a pregnancy knowing that the baby will be unaffected. PGT for monogenic disorder (PGT-M) in ten families at risk of having OCA offspring was reported in 2018 [20]. A total of 28 PGT cycles were performed giving rise to six unaffected children. However, there are no details of molecular techniques for reproduction. A PGT-M of OCA was successfully carried out in a family for three clinical cycles [21]. One healthy infant resulted. Sequencing following whole genome amplification (WGA) was employed. However, molecular techniques were briefly described and analysis results were not demonstrated.

Karyomapping is a sophisticated molecular technique employing single nucleotide polymorphism array (aSNP) for simultaneous haplotyping and copy number variation (CNV) testing [22]. Haplotyping-based PGT-M cycles have been reported [23,24]. However, clinical use of karyomapping is still uncommon. This study applied aSNP and karyomapping for PGT-M of OCA1 and PGT for aneuploidy (PGT-A) in one clinical PGT cycle and compared the results with those of PCR techniques.

2. Materials and Methods

2.1 Patient Details

One family with a risk of having OCA1 child entered the project after thoroughly counseling and written, informed consent was obtained. The study was conducted in accordance with the Declaration of Helsinki, and approved by the Research Ethics Committee of the Faculty of Medicine, Chiang Mai University, Thailand (protocol code OBG-2563-07801, December 30th, 2020). The proband was a 38 years old housewife. She and her 39 years old husband were symptomless for OCA. Her daughter was healthy. She experienced a spontaneous miscarriage in her second pregnancy. Her third child had OCA type I and died soon after birth. Her fourth and fifth pregnancies were terminated due to positive PND for OCA1. She and her daughter carried c.819+3insATATGCC mutation (splicing) within Intron 1 of the TYR gene. Her husband carried c.896G>A (p.R299H) mutation within the TYR gene, which was re-

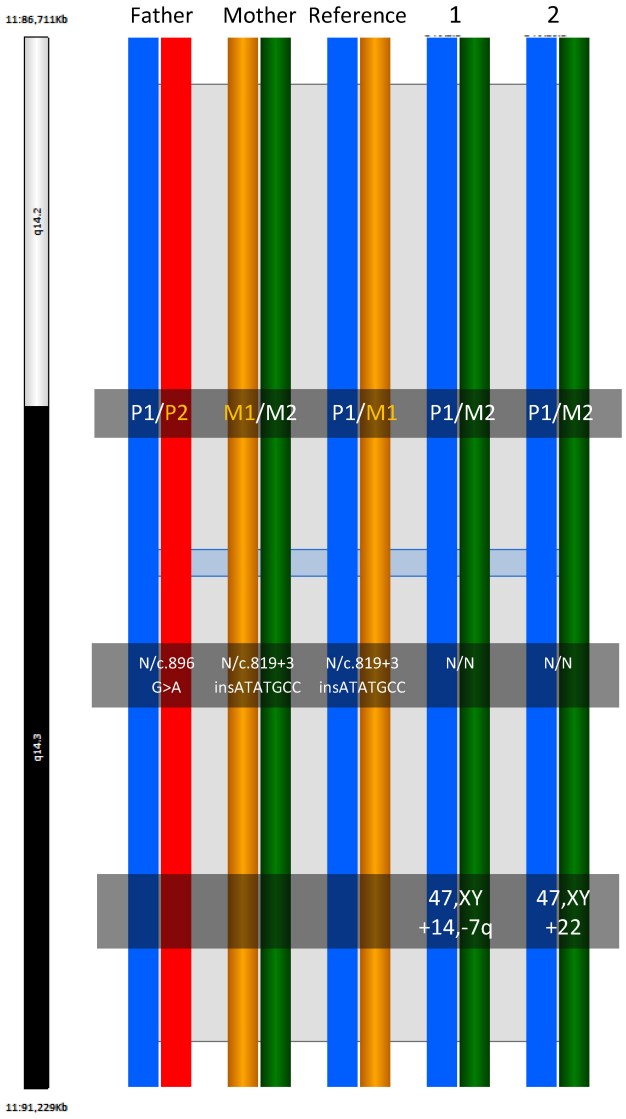

Fig. 1. Haploblock chart of TYR from karyomapping (BlueFuse Multi software) using SNP array data (Illumina HumanKaryomap-12 DNA Analysis Kit) and mutation analysis using multiplex fluorescent PCR (F-PCR) and mini-sequencing for TYR c.819+3insATATGCC and TYR c.896G>A mutations for the couples at risk of having OCA1. DNA of the healthy daughter of the proband who is a carrier was used as the reference. Haplotyping of the TYR gene was exhibited with PCR and chromosome analysis results. OCA1 mutations were absent in both embryos, however, embryo No. 1 showed an additional chromosome 14 with segmental loss of chromosome 7q and embryo No. 2 showed an additional chromosome 22.

ported to be pathogenic [25]. Her healthy daughter's DNA was employed as a reference in haplotyping study.

2.2 In Vitro Fertilization and Embryo Biopsy

The patient went through *in vitro* fertilization procedures. Ovarian stimulation was carried out. Intracytoplasmic sperm injection was done to eliminate the chance of sperm DNA contamination. Blastocysts were biopsied with a laser on day 5 post-fertilization. Five cells were biopsied for WGA and then molecular analysis, i.e., karyomapping and DNA analysis. Embryos vitrification was done following the biopsy.

2.3 Cell Lysis and Whole Genome Amplification

Biopsied trophectoderm were washed thoroughly in phosphate-buffered saline (Cell Signaling Technology, Theera Trading Co. Ltd. Bangkok, Thailand) with 0.1% polyvinyl alcohol (Sigma-Aldrich, Chiangmai VM Co., Ltd., Chiang Mai, Thailand) before transferring to microcentrifuge tubes with a total volume of 4 μL. DNA extraction was performed using alkaline lysis buffer protocol [26]. Three μL of denaturation buffer (0.25 μL of 1 M DTT and 2.75 μL of buffer DLB (REPLI-g® Single Cell Kit, Chiangmai VM Co., Ltd., Chiang Mai, Thailand)) was added, and mixtures were incubated at 65 °C for ten minutes. Then neutralization buffer (3 μL of stop solution) was added. WGA by multiple displacement amplification (REPLI-g® Single Cell Kit) was done according to the manufacturer's instructions. A mixture of 9 μL of water, 29 μL of reaction buffer, and 2 μL of DNA polymerase (REPLI-g® Single Cell Kit) was added to the extracted DNA, resulting in a total volume of 50 μL. Mixtures were incubated at 30 °C for eight hours and at 65 °C for three minutes to inactivate the reaction. SNP array with karyomapping analysis was performed for haplotyping and CNV [22].

2.4 SNP Array and Karyomapping Analysis

Amplified MDA samples were tested with SNP array using Illumina HumanKaryomap-12 DNA Analysis Kit (Bio-Active Co. Ltd., Bangkok, Thailand) according to the manufacturers' instruction [22,26]. SNP genotyping information was analyzed using BlueFuse Multi software version 4.5 (Illumina, Inc. California, USA) for karyomapping analysis and molecular cytogenetics. Haplotyping analysis from SNP genotyping information of the couples together with an offspring or an informative relative as references reveals inheritance of unaffected or affected genes in the embryos. This allows the diagnosis of a monogenic disorder of the embryos. Additionally, SNP genotyping provides CNV details of every chromosome. The results were compared with PCR results.

2.5 Multiplex Fluorescent PCR

Molecular mutation analysis using PCR was performed to confirm karyomapping results. Aliquots of amplified WGA products were subjected to multiplex fluorescent PCR and mini-sequencing analysis. 0.5 μL of amplified WGA products were subjected to PCR by primers encompassing TYR c.819+3insATATGCC (OMIM: AH003020.2) and TYR c.896G>A (OMIM: AH003020.2) mutations and HUMTH01 [27] polymorphic markers for extraneous DNA identification (Table 1). PCR

Table 1. Primers details for multiplex fluorescent PCR and mini-sequencing in the PGT-M protocol for OCA1. Primers covering TYR 819+3insATATGCC and TYR c.896G>A mutations and polymorphic markers were exhibited.

Primers	Location on TYR gene		Sequences	Fragment length (bp)	Labeling	References
TYR 819+3insATATGCC	Intron 1	forward	5′-CCA TGA AGC ACC AGC TTT TC-3′	292	6'FAM® (blue)	OMIM: AH003020.2
		reverse	5′-CCC TGC CTG AAG AAG TGA TT-3′			
TYR c.896G>A	Exon 2	forward	5′-CCA ACA TTT CTG CCT TCT CC-3′	268	HEX® (green)	OMIM: AH003020.2
		reverse	5′-GCA GCT TTA TCC ATG GAA CC-3′			
		mini-sequencing	5′-CCG AGG GAC CTT TAC GGC-3′			
HUMTH01		forward	5′-AGG GTA TCT GGG CTC TGG-3′	115–140	NED® (yellow)	[27]
		reverse	5′-CTT CCG AGT GCA GGT CAC-3′			

Table 2. PGT-M results of Oculocutaneous Albinism (OCA1) from karyomapping analysis (BlueFuse Multi) using SNP-Array information (Illumina HumanKaryomap-12 BeadChip) and mutation analysis using multiplex fluorescent PCR (F-PCR) for TYR c.819+3insATATGCC mutation and short tandemly repeat (STR) markers (HUMTH01) for contamination identification and mini-sequencing for TYR c.896G>A mutation (antisense in this table as C (black) > T (red)). Trophectoderm cells were biopsied from two day-5 embryos of the couples at risk of having compound heterozygous OCA1 offspring and went through whole genome amplification using multiple displacement amplification (MDA). Samples from both embryos were analyzed using karyomapping and multiplex F-PCR and mini-sequencing.

Embryo No.	F-PCR (TYR c.819+3insATATGCC) and Mini-Sequencing (TYR c.896G>A) Analysis				HUMTH01 STR (bp)		PCR Results Conclusion	Karyomapping Analysis		Karyomapping Conclusion	Concordant Results	Chromosome Analysis
	c.819+3insATATGCC		antisense c.896C>T		Alleles							
	Normal	**Mutant**	Normal	**Mutant**	Paternal	Maternal		Paternal	Maternal			
Father	Y	N	Y	**Y**	131/131		Ht	P1/**P2**				
Mother	Y	**Y**	Y	N		127/134	Ht		**M1**/M2			
Daughter	Y	**Y**	Y	N	131	134	Ht	P1	**M1**			
Embryo 1	Y	N	Y	N	131	127	Normal	P1	M2	Normal	Yes	47,XY +14,-7q
Embryo 2	Y	N	Y	N	131	134	Normal	P1	M2	Normal	Yes	47,XY +22

*Y, present; N, absent, Ht, heterozygous; Normal, homozygous normal.

mixture consisted of 0.2 μM of each primer, 2.5 μL of 2x QIAGEN® Multiplex PCR Master Mix (QIAGEN® Multiplex PCR Kit, Chiangmai VM Co., Ltd., Chiang Mai, Thailand) and was made up to a total volume of 5 μL with distilled deionized water. The amplification was performed with the following conditions: 94 °C for 30 s, annealing at 60 °C for one minute 30 s, and extension at 72 °C for one minute 30 s for 37 cycles. These were preceded by primary denaturation at 95 °C for 15 minutes to activate the HotStarTaq DNA Polymerase (QIAGEN® Multiplex PCR Kit) followed by a final extension at 72 °C for 10 min. The multiplex amplified products were each tagged with different fluorochromes using labeled primers [28]. This allowed analysis to be performed on an automated laser fluorescent sequencer ABI Prism® 3130 (Thermo Fisher Scientific, Massachusetts, USA.). TYR 819+3insATATGCC, TYR c.896G>A and HUMTH01 fragments were labeled with 6'FAM® (blue), HEX® (green), and NED® (yellow/black) fluorescent dyes, respectively. Mini-sequencing [26] was carried out for mutation analysis of TYR c.896G>A mutation. Details of the primers are summarized in Table 1.

2.6 Fragment Analysis on ABI Prism® 3130

A mixture of 1 μL multiplex fluorescent PCR product, 10 μL deionized formamide (GenePlus Co., Ltd.) and 0.1 μL Genescan™-500LIZ® size standard (GenePlus Co., Ltd.) was prepared and denatured at 95 °C for five minutes. The denatured sample was subjected to capillary electrophoresis using Performance-Optimized Polymer 7 (POP-7®, GenePlus Co., Ltd.; 5 s injection time, 15,000 V, 60 °C, 20 min) on an automated laser fluorescent sequencer ABI Prism® 3130. The data was analyzed by GeneMapper® software version 4.0 (Thermo Fisher Scientific, Massachusetts, USA).

2.7 Mini-Sequencing

Mini-sequencing techniques were employed for mutation analysis of TYR c.896G>A, antisense in this study as C>T using the PCR products. Amplified PCR products were treated with Exonuclease I/Alkaline Phosphatase using ExoProStar™ 1-Step (Bang Trading 1992 Co., Ltd., Bangkok, Thailand) to remove unincorporated primers and dNTPs from PCR reactions before DNA sequencing. 1.07 μL of PCR products were added into 0.2-mL microcentrifuge tubes containing 0.43 μL of ExoProStar™ 1-Step and incubated at 37 °C for 30 minutes, followed by 80 °C for 15 minutes. Mini-sequencing reaction mixture was set up on ice and comprised 2.5 μL of SNaPshot® Multiplex Kit (GenePlus Co., Ltd.), 0.5 μL of mini-sequencing primer (0.1 μM stock) (Table 1), 1.5 μL of the purified template and distilled deionized water in a total volume of 5 μL. The PCR was carried out with the conditions of 96 °C for 10 s, 50 °C for 5 s, and 60 °C for 30 s for 25 cycles.

A mixture of 1 μL of purified mini-sequencing product, 0.1 μL of GeneScan™-120LIZ® (GenePlus Co., Ltd.) size standard and 10 μL of deionized formamide (GenePlus Co., Ltd.) was prepared and heated to 95 °C for five minutes. Denatured samples were subjected to capillary electrophoresis using POP-7® (5 s injection time, 15,000 V, 60 °C, 24 min). Data were analyzed by GeneMapper® software version 4.0. The color of individual peaks was interpreted as A (Green, dR6G dye), C (Yellow/Black, dTAMRA™ dye), G (Blue, dR110 dye), and T (Red, dROX™ dye) [26].

3. Results

One clinical PGT-M cycle for OCA was carried out in this study. Maternal (TYR c.819+3insATATGCC carrier) and paternal (TYR c.896G>A carrier) haplotypes were assigned as M1/M2 and P1/P2, respectively. Two embryos were subjected to PGT-M using aSNP for karyomapping and CNV analysis. DNA of the healthy daughter of the proband who was a carrier of maternal OCA1 c.819+3insATATGCC (P1/M1 haplotypes) was employed as the reference for the haplotyping analysis. Karyomapping results revealed the absence of c.819+3insATATGCC (M1 haplotype) and c.896G>A (P2 haplotype) mutations in both embryos (read as P1/M2 haplotype in both embryos) (Fig. 1).

Novel multiplex fluorescent PCR with mini-sequencing protocols specifically for TYR c.819+3insATATGCC and TYR c.896G>A mutations were developed and tested. Applying the PCR protocols to 20 samples of WGA products resulted in 100% amplification efficiency and 0% allele drop out (ADO) rate. Allele drop out (ADO) is the event when one of the alleles of a heterozygous sample with low copy number of the templates fails to amplify in the PCR reaction. A heterozygous sample may show as homozygous normal or homozygous affected in the presence of ADO. PCR-based PGT-M of OCA from WGA products gave results in all samples without ADO. PCR-based mutation analysis revealed both embryos to be absent of both TYR c.819+3insATATGCC and TYR c.896G>A mutations, confirmed normal haplotyping results (Table 2). Mini-sequencing and fluorescent PCR results were demonstrated in Table 2. Polymorphic marker (HUMTH01 STR marker) analysis demonstrated that there was no contamination.

In addition to haplotyping results, high-resolution SNP microarray in this study provided CNV information with parental origins information of chromosomal gain or loss. aSNP analysis exhibited an additional chromosome 14 (maternal gain) and segmental loss of 7q (paternal loss) in embryo No. 1, i.e., 47,XY+14,-7q (Fig. 2a) and an additional chromosome 22 (maternal gain) in embryo No. 2, i.e., 47,XY+22 (Fig. 2b). Therefore, both embryos were unsuitable for transfer. The patient will return for the next PGT-M cycle.

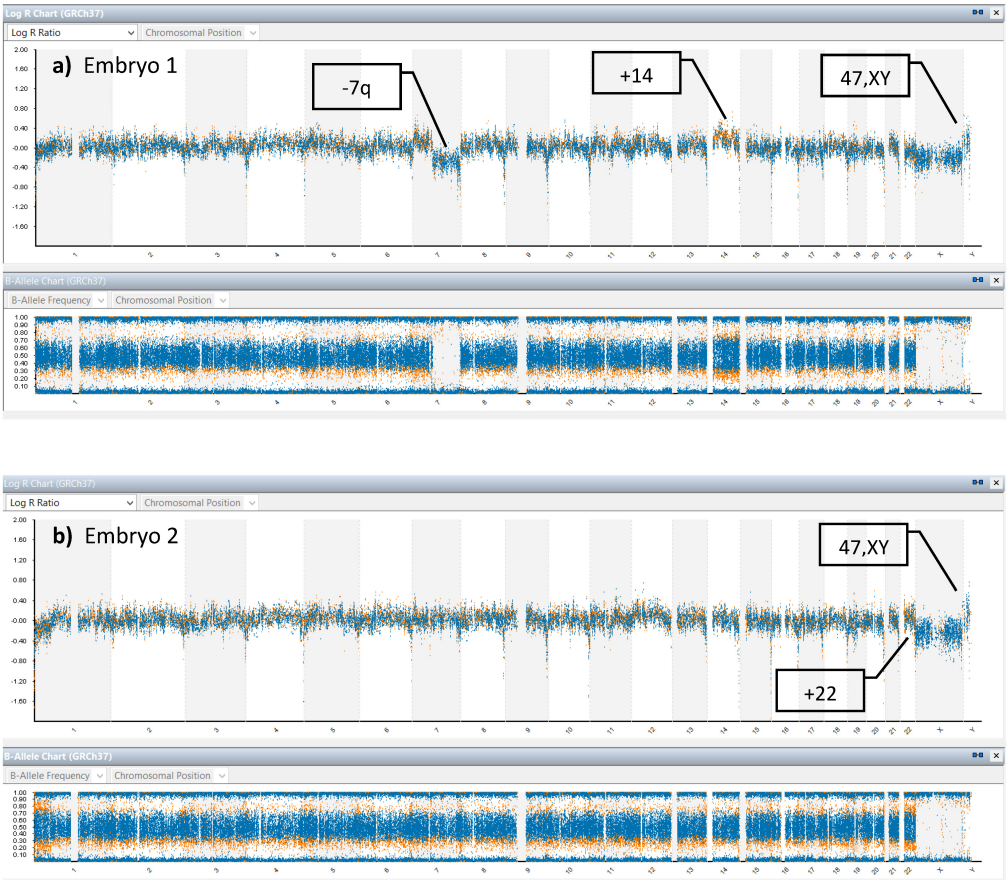

Fig. 2. Chromosome balance analysis from aSNP results. (a) shows copy number variation (CNV) of embryo 1, 47,XY+14 (maternal gain),-7q (paternal loss). (b) shows CNV of embryo 2, 47, XY+22 (maternal gain).

4. Discussion

Homozygous or compound heterozygous mutations within the tyrosinase (TYR) gene on chromosome 11 causes OCA1 which is inherited in an autosomal recessive manner [5]. Severely reduced vision is the most crucial disability to the patients. There are a wide variety of different mutations, over 60 mutations have been reported [6]. Compound heterozygous subjects are common. PND of OCA in the early age employed electron microscopy examination of fetal skin biopsy in utero [9] and later using DNA analysis techniques [11]. Even with a lot of recent advanced molecular techniques, the nature of a wide variety of mutations causing OCA1 among the population makes molecular diagnosis complicated. Developing specific molecular analysis protocols for each OCA1 family is labor-intensive and time-consuming. The success of PGT-M of OCA attempts has been reported but the details of the techniques and the results were untold [20,21]. Identification of specific OCA mutations and the development of PCR protocol for PGT-M for each family is labor-intensive and challenging. This study is the first to perform PGT-M for OCA1 employing advanced aSNP with karyomapping algorithm for haplotyping and simultaneous CNV analysis. Additionally, molecular analysis using fluorescent PCR and mini-sequencing was done alongside to validate karyomapping results. The details of PCR protocol and results analysis were also exhibited.

Karyomapping in this clinical PGT-M cycle exhibited both embryos to be absent of both paternal and maternal mutations by haplotyping based analysis from aSNP information. Novel PCR protocols using multiplex fluorescent PCR with mini-sequencing techniques were developed and applied for PGT-M of OCA1 (TYR c.819+3insATATGCC and TYR c.896G>A mutations) in this study to verify karyomapping results. Fluorescent PCR with fragment analysis was employed to analyze TYR c.819+3insATATGCC mutation which is seven base pair insertion and mini-sequencing to analyze TYR c.896G>A mutation which is a single nucleotide substitution mutation. Genotyping results from PCR-based PGT-M confirmed karyomapping based PGT-M in both embryos. Therefore, this study showed that haplotyping results from karyomapping analysis can be used for PGT-M accurately not only for small insertion, i.e., TYR c.819+3insATATGCC mutation but also for single nucleotide substitution mutations, i.e., TYR c.896G>A mutation. Karyomapping can be used as a widely applicable genetic testing method.

Additional advantage of karyomapping which is demonstrated in this study is the CNV and parental origins information of the embryos. aSNP reveals chromosomal unbalance and parental origins in both embryos, i.e., 47, XY,-7q (paternal loss),+14 (maternal gain) in Embryos 1 and 47, XY,+22 (maternal gain) in Embryos 2. Both embryos were not suitable for transfer due to chromosomal imbalance. Due to the COVID-19 pandemic, the couples will return for the next PGT-M cycle later. It was shown that karyomapping can be used as a precise, time-saving for protocol development, widely applicable PGT-M protocol for single gene disorders of different natures of mutations (i.e., small deletion and single base substitution mutations) and provides the additional CNV and parental origin details which are common during pre-implantation development of human embryos.

Before performing aSNP there is a need to have a high precision 0.5 μm microarray scanner, necessary equipment for hybridization, and an experienced molecular biologist in addition to the standard PGT laboratory. This is quite a big sum of extra investment for a PGT laboratory. The bead chip and their consumables come with a price. In the first chip, there is a need to spare 3–4 samples for the parental and 1–2 references' DNA. Therefore, 8–9 embryos can be analyzed on a 12-sample chip. In case of more embryos, the second chip will be able to analyze 12 embryos without the need to repeat the parental and references' DNA. However, the cost of the chip and consumables will be double without cheaper.

Most PGT laboratory already has all the necessary equipment and consumables for PCR-based PGT which are a lot cheaper than those the microarray-based. Designing primers and PCR protocols for new monogenic diseases or new mutations is difficult and labor-intensive. However, the additional expenses of the PCR-based PGT are just the cost of the additional primers for each new mutation which is insignificant. For the regions with monogenic diseases of common mutations, i.e., alpha- and beta-thalassemias, the running cost of PCR-based PGT for the laboratory is not expensive at all because one stock of primers can be used for several cases. Therefore, microarray-based PGT may be suitable for those laboratories with monogenic diseases of various mutations, i.e., OCA, Marfan syndrome, etc., and with adequate funding.

5. Conclusions

This study presents a clinical PGT-M cycle using karyomapping for a family at risk of having a compound heterozygous OCA1 offspring. The novel PCR protocols using fluorescent PCR and mini-sequencing were described, tested and confirmed karyomapping results. Both embryos in this clinical PGT-M cycle were absent of both paternal and maternal mutations. However, aSNP revealed chromosomal unbalance in both embryos and excluded them from transfer. This study demonstrates that karyomapping can be used as a precise, time-saving for protocol development, widely applicable PGT-M protocol for single gene disorders of different natures of mutations (i.e., small insertion and single base substitution) and provides the additional CNV and parental origin details which are common during pre-implantation development of human embryos.

Data Availability Statement

No datasets were generated or analyzed during the current study.

Content

This study presents pre-implantation genetic testing of monogenic disorders (PGT-M) for oculocutaneous albinism type IA (OCA1) including insertion c.819+3insATATGCC and point mutation c.896G>A (p.R299H) employing single nucleotide polymorphism microarray (aSNP) with karyomapping algorithm for haplotyping and copy number variation (CNV) analysis. In addition, novel multiplex fluorescent PCR with mini-sequencing was developed and performed alongside to validate karyomapping results. The details of PCR protocols were exhibited.

Message

This study presents pre-implantation genetic testing (PGT) for oculocutaneous albinism type IA (OCA1) employing aSNP with karyomapping for haplotyping and copy number variation (CNV) analysis. In addition, PCR-based PGT-M was performed to validate karyomapping.

Abbreviations

ADO, allele drop-out; aSNP, single nucleotide polymorphism microarray; CNV, copy number variation; ICSI, intracytoplasmic sperm injection; IVF, *in-vitro* fertilization; MDA, multiple displacement amplification; OCA1, oculocutaneous albinism type I; PCR, polymerase chain reaction; PGT-A, pre-implantation genetic testing for aneuploidy; PGT-M, pre-implantation genetic testing for monogenic disorders; PND, prenatal diagnosis; WGA, whole genome amplification.

Author Contributions

Study conception and design were performed by SP, SM and WP. Ovarian stimulation, oocytes collection, and embryology laboratory were performed by TP and SM. aSNP and karyomapping analysis were performed by RS, SM, SP and WP. PCR analysis and mini-sequencing were performed by WS, SP and WP. Data collection and analysis were performed by SP, SM and WP. WC took care of clinical diagnosis and assessment. Prenatal and postnatal diagnoses were performed by TT and WP. SM and SP contributed equally to this work. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

The study was approved by the Research Ethics Committee of the Faculty of Medicine, Chiang Mai University, Thailand (protocol code OBG-2563-07801, December 30th, 2020).

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Conflict of Interest

The authors declare no conflict of interest.

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