Preimplantation Genetic Testing for Thalassemia Through Next- Generation Sequencing of Affected-embryos

Zhanhui Ou
Guangzhou Women and Children's Medical Center, Guangzhou Medical University
Yu Deng
Guangzhou Women and Children's Medical Center, Guangzhou Medical University
Yunhao Liang
Guangzhou Women and Children's Medical Center, Guangzhou Medical University
Zhiheng Chen
Guangzhou Women and Children's Medical Center, Guangzhou Medical University
Ling Sun (sunling6299@163.com)
Guangzhou Women and Children's Medical Center, Guangzhou Medical University

Research Article

Keywords: Preimplantation genetic testing, α-thalassemia, β-thalassemia, SNP linkage, Next- generation sequencing, Affected embryo, Monogenic diseases

Posted Date: December 29th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1202545/v1

Abstract

Background: To evaluate the ability of next-generation sequencing (NGS) to conduct preimplantation genetic testing (PGT) for thalassemia using affected embryos.

Methods: This study included data from 36 couples who underwent PGT for thalassemia without proband and relative pedigrees. NGS results were compared with prenatal diagnosis results.

Results: Thirty-six couples (29 α-thalassemia and 7 β-thalassemia) underwent 41 PGT cycles (31 α-thalassemia and 10 β-thalassemia). All biopsied blastocysts received conclusive results from NGS analysis (100%, 217/217). One hundred and sixty (73.7%, 160/217) were determined to be unaffected by thalassemia. PGT-A (PGT for aneuploidy) results showed that 112 (70.0%, 112/160) were euploid. Thirty-four couples were transferred with a single blastocyst (53 frozen embryo transfer (FET) cycles). Thirty-two cycles resulted in clinical pregnancies, and the clinical pregnancy rate was 60.1% (32/53) per FET cycle. Twenty-two cycles (22 couples) resulted in 23 live births and the live birth rate was 43.4% (23/53, 3 cycles were ongoing pregnancy). All 25 cycles' prenatal diagnosis results and/or thalassemia gene analysis after the delivery were concordant with the NGS-PGT results. Seven cycles were miscarried before 12 weeks' gestation, and the abortion villus in four cycles showed a normal karyotype and thalassemia results consistent with the NGS-PGT results. Aborted fetus samples from 3 cycles were not available because the pregnancy was less than 5 weeks.

Conclusion: NGS can be used to conduct PGT for thalassemia using affected embryos as a reference.

Trial registration: Retrospectively registered.

Introduction

Thalassemia, one of the most common monogenic diseases, is an inherited blood disorder characterized by a reduction in the synthesis of hemoglobin (HB) subunit α or β (HB α or β chain). In southern China, the prevalence of α-thalassemia and β-thalassemia are 8.53% and 2.54%, respectively (1). This genetically inherited disease has threatened the lives of millions of people for decades and no effective treatments are available. Homozygotes with the Southeast Asian (SEA) deletion develop Hb Bart's hydrops fetalis syndrome and they usually die either late in gestation or a few minutes after birth (2). Infants with severe forms of β-thalassemia can now survive but require much medical intervention, resulting in a rising global economic and healthcare burden (3).

Prenatal diagnosis is advocated in China to prevent the birth of babies with severe thalassemia. However, it requires an invasive procedure that may induce miscarriage, and may result in the mental burden of terminating an affected pregnancy. At present, preimplantation genetic testing for monogenic disease (PGT-M) is a method that can effectively prevent thalassemia in the children of couples who are at risk of transmitting this genetic condition to their offspring (4, 5).

Since the early 1990s, PGT-M has been used for X-linked genetic diseases (6), with PCR-based methods being used in past decades. However, allele dropout (ADO) is a main cause of misdiagnosis in PGT-M so a direct PCR approach cannot be used as the sole method for diagnosis and detection of the target pathogenic mutation sites (7). In recent years, linkage analysis has been widely used to increase PGT-M accuracy (8). This method relies on short tandem repeat (STR), or single nucleotide polymorphism (SNP) markers linked to the mutations, but in some cases with de novo mutations or lack of proband, haplotypes cannot be constructed.

This has made some inherited genetic diseases, like α-thalassemia difficult to detect with PGT. This is a serious issue as babies with this condition usually die immediately after birth. Sometimes couples have discovered their status as carriers in the preoperative examination before in vitro fertilization (IVF) treatment. Thus, there is a particularity in thalassemia concerning the haplotypes. In recent years, constructing the haplotypes through both parents has been a common approach, but this method is tedious and expensive. Another method is through single sperm and polar body diagnosis using NGS when lacking relatives or proband, which is also used in clinics (9, 10). However, this method requires an extra biopsy to collect polar bodies, and often needs multiple single sperms, which is tedious and expensive. Recently, some studies have explored constructing the haplotypes through affected embryos with NGS (4, 11, 12). But since these studies only had one or two cases, this method requires further validation.

In this study, we conducted PGT for thalassemia using SNP haplotyping with affected embryos as a reference. To the best of our knowledge, this is the largest study to use PGT-M for thalassemia without the relatives and proband in a clinic.

Materials And Methods

Patients

This study was approved by the Reproductive Medical Ethics Committee of Guangzhou Women and Children's Hospital. Thirty-six couples where both parents carried genes for either α-thalassemia (29 couples) or β-thalassemia (7 couples) were selected at the Guangzhou Women and Children's Hospital between June 2017 and June 2021. Written informed consent was obtained from each couple.

Blastocyst biopsy and vitrification

We performed standard ovarian stimulation, intracytoplasmic sperm injection (ICSI), embryo culture, and blastocyst vitrification for each of the 36 couples as previously reported (12, 13). Biopsy was performed on day five or six depending on the blastocyst grade on the day of biopsy (7).

Whole- genome amplification (WGA)

Multiple displacement amplification (MDA) using a REPLI-g Single Cell Kit (Qiagen, Germany) was performed according to the manufacturer's protocol. The WGA products were purified and then sequenced. All PCR amplifications were performed on a 96 Well Thermal Cycler Veriti DX (Life Technologies). All procedures were carried out in accordance with the manufacturer's protocol and, as previously reported (12).

NGS sequencing and haplotype construction

Mutation and SNP sites were submitted to Ion Ampliseq Designer (https://www.ampliseq.com/) for primer design. Overall, 138 SNPs within 1 Mb upstream and 132 SNPs within 2 Mb downstream of the mutation gene (chr16:215400-234700 NM_000517.4 (HBA2) and NM_000558.4 (HBA1)) were selected for NGS-based α-thalassemia SNP haplotyping. Eighty-five SNP markers located either 1 Mb upstream or downstream of the mutation gene (chr11:5246696-5248301 NM_000518.4 (HBB)) were selected for NGS-based β-thalassemia SNP haplotyping. Only the SNPs that were heterozygous in one parent and homozygous in their parent were considered as informative SNPs. The genomic DNA of the couple and the WGA products were amplified with these primers for haplotype construction. Sequencing libraries were prepared using the sequencing library kit (NEXTflex Rapid DNA-seq Kit 96rxns, BIOO), and the libraries were sequenced on an Illumina MiseqDX platform (Illumina) using a MiSeq Dx Reagent Kit V3 (Illumina). All procedures were carried out in accordance with the manufacturer's protocol. The sequencing data were analyzed by Peking Jabrehoo Med Tech., Ltd. Copy number variations (CNV) analysis for aneuploidy testing was performed as previously described (13).

Frozen embryo transfer and follow up

Hormone replacement therapy was used to prepare the uterus endometrium. A frozen non-pathogenic blastocyst with euploid karyotype was thawed and cultured for 2 hours before being transferred into the uterus as previously described (14). Clinical pregnancies (CP) were defined after observation of a gestational sac with or without a fetal heartbeat on ultrasound evaluation 4 weeks after frozen embryo transfer (FET). Clinical miscarriage was determined to occur when a pregnancy failed to progress after an intrauterine gestational sac had been detected with pelvic ultrasonography. Amniocentesis was performed at about 17 weeks of gestation and/or gene detection of the blood was performed after birth to verify the consistency with the PGT.

Statistical analysis

Statistical analysis was performed using SPSS software v. 19 for Windows (SPSS Inc., Chicago, USA), applying parametric and nonparametric tests when appropriate. Continuous variables were expressed as mean±standard deviation (SD) and analyzed using Student's t-test. Categorical variables were expressed as percentages and analyzed using χ^2 or Fisher's exact test depending on the sample size. Statistical significance was defined as p-values less than 0.05.

Results

Trophectoderm biopsy and WGA

The 36 couples (29 α-thalassemia and 7 β-thalassemia) underwent a total of 41 PGT cycles (31 α-thalassemia and 10 β-thalassemia) (Table 1 and Supplementary Table 1) (Figure 1). Seven hundred and seventy-seven oocytes were retrieved. Of these 631 were fertilized with intracytoplasmic sperm injection (ICSI), and 217 were cultured to blastocysts of good enough quality to perform trophectoderm (TE) biopsy (better than □CC). The average number of blastocysts for each couple was 6.0 (217/36). WGA was successfully performed for all TE cells.

NGS-based SNP haplotyping and mutation detection

Due to these families lacking related relatives and proband, all analyses were based on the blastocysts. Three couples (families 8, 31, and 35) only had two biopsied blastocysts and did not receive conclusive PGT results from NGS. A second PGT cycle produced conclusive results. Therefore, all of the biopsied blastocysts received conclusive PGT results (100%, 217/217) (Table 1).

Using family 13 as an example to analyze α- thalassemia (Figure 2), 138 SNPs within 1 Mb upstream and 132 SNPs within 2 Mb downstream from the HBA1 and HBA2 gene were adopted with sequencing depth >30X. We could deduce whether the mutation allele was present in the embryo by analyzing these SNPs. For instance, we found that embryos 5 and 7 carried the disease allele from the couple according to the sequencing depth of the SEA area (Figure 2a, part of the SNP results). We also found that these two embryos inherited both maternal and paternal disease-associated haplotypes. Next, information SNPs in the SEA area were used to construct the haplotype. In brief, the mother was heterozygous A/C, and the father was A/A on SNP in position 119006. This SNP was considered as the maternal information SNP. While the affected embryos (5 and 7) were homozygous A/A (Figure 2b, part of the SNP results), we could easily deduce that alleles with the base A from the mother were pathogenic, and this was the disease-associated haplotype.

At least two upstream and two downstream markers closely linked to the gene underlying the mutation were analyzed, and the disease-associated and non-disease-associated maternal haplotype was successfully distinguished. Hence, we concluded that embryos 1, 5, and 7 carried the disease-associated maternal haplotype. Similarly, in position 207611, the maternal was C/C and the paternal was C/T, this SNPs was considered as the paternal information SNPs (Figure 2b). While the affected embryos (5 and 7) were homozygous C/C. We could easily deduce that alleles with base C from the father were pathogenic, and this was another disease-associated haplotype. Hence, we concluded that embryos 2, 3, 4, 5, and 7 carried the disease-associated paternal haplotype. So, embryos 5 and 7 were homozygous, embryos 1, 2, 3, and 4 were heterozygous, and embryos 6, 8, and 9 were wildtype.

The analysis method for β- thalassemia (using family 30 as an example) is shown in Figure 3. Ninety-five SNPs within 2 Mb upstream and downstream respective from the *HBB* gene were adopted with sequencing depth >30X. At first, we could deduce embryos 4 and 6 carried the disease allele according to the sequencing depth of the βCD41-42 (Figure 3a). Thus, we deduced that these two embryos inherited both maternal and paternal disease-associated haplotypes (Figure 3b). Next, information SNPs in the *HBB* were used to construct the haplotype as described above. We could also conclude that embryos 1, 4, 5, and 6 carried the disease-associated maternal haplotype and that embryos 2, 4, and 6 carried the disease-associated paternal haplotype. So, embryos 4 and 6 were homozygous, embryos 1, 2, and 5 were heterozygous, and embryo 3 was wild-type.

PGT results

After NGS-based SNP haplotyping and mutation detection, 160 (73.7%, 160/217) blastocysts were found to be unaffected by either α- thalassemia or β-thalassemia (Table 1). PGT-A results of these blastocysts showed that 112 (70.0%, 112/160) were euploid, which were defined as transferable blastocysts (Table 1). And the average number of transferable blastocysts for each couple was 3.1 (112/36).

Table 1

The preimplantation genetic testing outcomes of the 36 families.

	Cycles		Embryo state			PGT results		Pregnancy results		Genetic testing		
	PGT	FET	Oocyte	M□	Biopsy blastocyst	Unaffected[1]	Transferrable[2]	CP	LB	PGT-M	Amniocentesis	After born
Family 1	1	1	26	25	9	6	3	1	1	Wild type	Wild type	Wild type
Family 2	1	1	22	17	8	6	4	1	1	Heterozygote	Heterozygote	Heterozygote
Family 3	1	1	13	12	3	2	1	1	1	Wild type	NA	Wild type
Family 4	1	1	11	10	5	2	2	1	1	Wild type	NA	Wild type
Family 5	1	1	12	12	9	6	3	1	1	Heterozygote	Heterozygote	Heterozygote
Family 6	1	2	15	14	7	6	2	1	1	Heterozygote	Heterozygote	Heterozygote
Family 7	1	1	24	20	4	4	1	1	1	Heterozygote	Heterozygote	Heterozygote
Family 8[3]	2	2	44	31	8	6	4	1	1	Heterozygote	NA	Heterozygote
Family 9	1	2	35	27	6	6	6	1	0	Wild type	Miscarriage	NA
Family 10	1	1	28	16	4	1	1	1	0	Wild type	Miscarriage	NA
Family 11	1	1	15	11	5	3	2	1	1	Wild type	Wild type	NA
Family 12	1	3	21	16	4	4	3	1	1	Heterozygote	NA	Heterozygote
Family 13	1	2	26	20	9	7	5	1	1	Wild type	Wild type	NA
Family 14	1	2	7	5	3	1	1	0	0	Heterozygote	NA	NA
Family 15[4]	2	1	48	36	11	7	2	1	2	Wild type	NA	Wild type
Family 16	1	1	19	12	4	3	2	1	1	Heterozygote	Heterozygote	NA
Family 17	1	0	6	4	2	0	0	0	0	NA	NA	NA
Family 18	1	1	19	10	3	2	2	1	1	Heterozygote	Heterozygote	NA
Family 19	1	3	40	33	15	11	11	1	1	Heterozygote	NA	Heterozygote
Family 20	1	2	25	21	4	4	3	1	1	Heterozygote	NA	Heterozygote
Family 21	1	1	19	14	8	8	4	1	1	Wild type	Wild type	NA
Family 22	1	1	19	13	5	5	3	1	1	Heterozygote	Heterozygote	NA
Family 23	1	1	29	27	6	4	4	1	0	Heterozygote	NA	NA
Family 24	1	1	16	15	7	4	4	1	1	Heterozygote	Heterozygote	NA
Family 25	1	1	19	19	5	3	2	1	0	Wild type	Miscarriage	NA
Family 26	1	2	8	7	4	4	4	1	0	Wild type	Miscarriage	NA
Family 27	1	2	11	9	6	5	3	1	0	Wild type	Miscarriage	NA

Family 28	1	1	11	11	3	2	1	0	0	Wild type	NA	NA
Family 29	1	2	17	13	5	3	3	0	0	Wild type	NA	NA
Family 30	2	4	47	40	10	7	6	2	0	Wild type	Miscarriage	NA
Family 31[3]	2	3	27	26	7	6	5	1	1	Heterozygote	Heterozygote	Heterozygote
Family 32	1	2	21	15	6	5	5	1	1	Heterozygote	Heterozygote	Heterozygote
Family 33	1	0	15	14	3	1	0	0	0	NA	NA	NA
Family 34	1	1	12	11	9	7	7	1	1	Wild type	NA	Wild type
Family 35[3]	2	1	28	28	4	4	1	1	0	Heterozygote	Heterozygote	NA
Family 36	1	1	22	17	6	5	2	1	0	Heterozygote	Heterozygote	NA

PGT: preimplantation genetic testing, FET: Frozen embryo transfer, CP: clinical pregnancy, LB: live birth, NA: not applicable

1 Unaffected embryos, including non-carrier and carrier embryos

2 Transferable embryos diagnosed as unaffected and euploid

3 Not enough biopsied blastocysts for analysis and a second oocyte pick-up was needed.

4 Single blastocyst transfer which developed to monochorionic diamniotic.

Clinical outcomes

Two couples had no transferable blastocysts after the first PGT cycle, and did not perform another PGT cycle. The other 34 couples were transferred with a single blastocyst (53 FET cycles). Thirty-two cycles resulted in clinical pregnancy and the clinical pregnancy rate was 60.1% (32/53) per FET cycle. Family 15 developed a monochorionic diamniotic twin pregnancy after a single blastocyst transfer and resulted in the birth of two healthy babies.

Twenty-two cycles (22 couples) resulted in 23 live births and the live birth rate was 43.4% (23/53, 3 cycles were ongoing pregnancy). The prenatal diagnosis results and/or thalassemia gene analysis after the delivery were concordant with the NGS-PGT results for all 25 cycles. Seven cycles resulted in miscarriage before 12 weeks' gestation (7/32, 21.9%), and the abortion villus from 4 of the cycles showed normal karyotype and thalassemia results consistent with the NGS-PGT results. However, samples from the aborted fetuses' in 3 cycles were not available because the pregnancy lasted less than 5 weeks.

Discussion

The small amount of DNA taken from biopsied trophectoderm cells and amplification bias based on WGA can lead to ADO (15). And PCR—based methods for PGT are inevitably affected by ADOs. Although haplotype analysis with short tandem repeat (STR) may reduce the effects of ADO, the number of STR loci is limited. Further, recombination between STR loci and target genes may affect the diagnosis accuracy (16, 17). Hence, single-nucleotide polymorphisms (SNPs) linked to the mutated genes are being used more and more to establish haplotype linkages in clinical practice (18). However, this technique requires proband or the pedigrees of the parents to construct the haplotype for linkage analysis. This makes performing PGT difficult for detecting some inherited genetic diseases like α-thalassemia.

At present, the most widely used method for PGT-M without pedigrees is single-sperm for haplotyping, namely, isolating a single sperm and analyzing the genotypes of SNP alleles via NGS (10, 19). However, polymorphic markers need to be identified before linkage analysis and this requires multiple steps and extra laboratory work. This method is usually suitable for dominant inherited genetic diseases from the paternal parent, such as Osteogenesis imperfecta (10). However, both of α-thalassemia and β-thalassemia are recessive inherited genetic diseases. Thus, using single-sperm for haplotyping would be tedious and cannot identify the maternal haplotype unless polar bodies are also biopsied.

NGS-based PGT can simultaneously detect target mutation sites and linked SNPs, making it possible to provide multiple diagnostic results with the advantages of high accuracy, and throughput (20). Thus, we chose to use affected embryos as a reference to perform haplotype construction in order to avoid the multiple steps. All biopsied blastocysts received conclusive PGT results. In fact, this method is very useful in a clinic as many monogenetic inherited genetic diseases lack intact pedigrees, such as our previous case report PGT-M for Marfan syndrome (12). A previous study by Ren et al. (11) successfully carried out PGT-M based on a mutated allele by sequencing with aneuploidy and linkage analyses for two carrier families with children

affected with spinal muscular atrophy. They found that this method can correctly diagnose embryos by using affected embryos as the probands. Another study by Chen et al. (4) also found that NGS-based haplotyping can be performed by directly detecting mutation sites and using affected embryos as probands for PGT-M. Li et al. (21) successfully applied the linked-read sequencing method to construct parental haplotypes without recruiting additional family members in two families with alpha thalassemia and the other with NDP gene disorder. However, these studies only used one or two couples. Thus, it is necessary to conduct studies with more samples to validate the use of affected embryos as the probands in a clinic.

In this study, 29 couples with α-thalassemia and 7 couples with β-thalassemia successfully underwent PGT using affected embryos as a reference. All 217 biopsied blastocysts received conclusive PGT results. After a single blastocyst was transferred in 53 cycles, 32 cycles resulted in clinical pregnancy, and 22 cycles (23 babies) resulted in a live birth. All prenatal diagnosis results and/or thalassemia gene analysis after delivery were concordant with the NGS-PGT results. Therefore, we successfully conducted PGT for thalassemia using SNP haplotyping with affected embryos as a reference. This technique is very useful for some couples with other monogenetic diseases who need to perform PGT without proband and his/her parents' pedigrees.

Although there are challenges associated with ADO, haplotyping linkage analysis with more informative SNPs could help to avoid this. NGS-based PGT can detect the mutated gene directly and construct haplotypes with SNPs close to the mutated gene using PGT to determine ADO and prevent misdiagnosis. Moreover, haplotyping can also be used to distinguish the chromosome of the pathogenic gene from normal chromosomes and find monosomies of chromosomes to avoid misdiagnosis. In our study, more than 100 SNP markers within 1Mb upstream and downstream of the pathogenic mutation site were used to establish the haplotype. By analyzing these SNPs, we could determine the disease-carrying allele state of each embryo.

The main limitation of this method is that patients may not obtain sufficient embryos as a reference, requiring another oocyte pick-up cycle. Additionally, marriages in proximity may not lead to enough informative SNPs to establish haplotyping.

Conclusions

NGS can be used to conduct PGT for thalassemia using affected embryos as a reference. Additionally, this method could also be used to perform PGT for other monogenic diseases in the absence of proband and the parents' pedigrees.

Declarations

Ethics approval and consent to participate

The study has been approved by the Ethics Committee of our Institution (2021167A01). Written consent was obtained for all willing participants prior to registering for this study. Patient Informed consent to participate in this study.

Consent for publication

The authors have consented for publication.

Availability of data and materials

Data were obtained from the referenced publications. Further information is available by contacting Dr. Ou at zhanhui-ou@hotmail.com.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was supported by institutional funding of Health Science and technology project of Guangzhou (20201A011029 and 20211A011026).

Authors' contributions

ZHO developed the concept of the study; All authors contributed to data accumulation; ZHO, YD, YHL and ZHC contributed to data analysis; ZHO wrote the manuscript. All authors contributed to revisions of the manuscript, and approved of the final submission. ZHO takes responsibility for the accuracy of the data analysis.

Acknowledgments

Thanks to all the peer reviewers for their opinions and suggestions. We also thanks to the company of Peking Jabrehoo Med Tech., Ltd. from China for some technologic helps.

Author details

1 Center of Reproductive Medicine, Guangzhou Women and Children's Medical Center, Guangzhou Medical University, 510623, Guangzhou, Guangdong, P.R. China.

References

1. Xu XM, Zhou YQ, Luo GX, Liao C, Zhou M, Chen PY, et al. The prevalence and spectrum of alpha and beta thalassaemia in Guangdong Province: implications for the future health burden and population screening. Journal of clinical pathology. 2004;57(5):517-22.
2. Taher AT, Weatherall DJ, Cappellini MD. Thalassaemia. Lancet. 2018;391(10116):155-67.
3. Weatherall DJ. Thalassemia as a global health problem: recent progress toward its control in the developing countries. Annals of the New York Academy of Sciences. 2010;1202:17-23.
4. Chen D, Shen X, Wu C, Xu Y, Ding C, Zhang G, et al. Eleven healthy live births: a result of simultaneous preimplantation genetic testing of alpha- and beta-double thalassemia and aneuploidy screening. Journal of assisted reproduction and genetics. 2020;37(3):549-57.
5. Chen D, Shen X, Xu Y, Ding C, Ye Q, Zhong Y, et al. Successful four-factor preimplantation genetic testing: alpha- and beta-thalassemia, human leukocyte antigen typing, and aneuploidy screening. Systems biology in reproductive medicine. 2021;67(2):151-9.
6. Handyside AH, Kontogianni EH, Hardy K, Winston RM. Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification. Nature. 1990;344(6268):768-70.
7. Harton GL, De Rycke M, Fiorentino F, Moutou C, SenGupta S, Traeger-Synodinos J, et al. ESHRE PGD consortium best practice guidelines for amplification-based PGD. Human reproduction. 2011;26(1):33-40.
8. Thornhill AR, Handyside AH, Ottolini C, Natesan SA, Taylor J, Sage K, et al. Karyomapping-a comprehensive means of simultaneous monogenic and cytogenetic PGD: comparison with standard approaches in real time for Marfan syndrome. Journal of assisted reproduction and genetics. 2015;32(3):347-56.
9. Wu H, Shen X, Huang L, Zeng Y, Gao Y, Shao L, et al. Genotyping single-sperm cells by universal MARSALA enables the acquisition of linkage information for combined pre-implantation genetic diagnosis and genome screening. Journal of assisted reproduction and genetics. 2018;35(6):1071-8.
10. Chen L, Diao Z, Xu Z, Zhou J, Yan G, Sun H. The clinical application of single-sperm-based SNP haplotyping for PGD of osteogenesis imperfecta. Systems biology in reproductive medicine. 2019;65(1):75-80.
11. Ren Y, Zhi X, Zhu X, Huang J, Lian Y, Li R, et al. Clinical applications of MARSALA for preimplantation genetic diagnosis of spinal muscular atrophy. Journal of genetics and genomics = Yi chuan xue bao. 2016;43(9):541-7.
12. Deng Y, Ou Z, Li R, Chen Z, Liang P, Sun L. Affected-embryo-based SNP haplotyping with NGS for the preimplantation genetic testing of Marfan syndrome. Systems biology in reproductive medicine. 2021;67(4):298-306.
13. Ou Z, Chen Z, Yin M, Deng Y, Liang Y, Wang W, et al. Re-analysis of whole blastocysts after trophectoderm biopsy indicated chromosome aneuploidy. Human genomics. 2020;14(1):3.
14. Wang H, Ou Z, Chen Z, Yang L, Sun L. Influence of different post-thaw culture time on the clinical outcomes of different quality embryos. Advances in clinical and experimental medicine : official organ Wroclaw Medical University. 2019;28(4):523-7.
15. Harper JC, Wilton L, Traeger-Synodinos J, Goossens V, Moutou C, SenGupta SB, et al. The ESHRE PGD Consortium: 10 years of data collection. Human reproduction update. 2012;18(3):234-47.
16. Natesan SA, Bladon AJ, Coskun S, Qubbaj W, Prates R, Munne S, et al. Genome-wide karyomapping accurately identifies the inheritance of single-gene defects in human preimplantation embryos in vitro. Genetics in medicine : official journal of the American College of Medical Genetics. 2014;16(11):838-45.
17. Gueye NA, Jalas C, Tao X, Taylor D, Scott RT, Jr., Treff NR. Improved sensitivity to detect recombination using qPCR for Dyskeratosis Congenita PGD. Journal of assisted reproduction and genetics. 2014;31(9):1227-30.
18. Chen L, Diao Z, Xu Z, Zhou J, Yan G, Sun H. The clinical application of NGS-based SNP haplotyping for PGD of Hb H disease. Systems biology in reproductive medicine. 2017;63(3):212-7.
19. Altarescu G, Brooks B, Kaplan Y, Eldar-Geva T, Margalioth EJ, Levy-Lahad E, et al. Single-sperm analysis for haplotype construction of de-novo paternal mutations: application to PGD for neurofibromatosis type 1. Human reproduction. 2006;21(8):2047-51.
20. Chamayou S, Sicali M, Lombardo D, Alecci C, Ragolia C, Maglia E, et al. Universal strategy for preimplantation genetic testing for cystic fibrosis based on next-generation sequencing. Journal of assisted reproduction and genetics. 2019.
21. Li Q, Mao Y, Li S, Du H, He W, He J, et al. Haplotyping by linked-read sequencing (HLRS) of the genetic disease carriers for preimplantation genetic testing without a proband or relatives. BMC medical genomics. 2020;13(1):117.

Figures

Figure 2a

		start	end	M	F	E1	E2	E3	E4	E5	E6	E7	E8	E9
THA_SEA	chr16	216815	216989	313	397	335	1098	782	696	2	1673	2	1278	1319
THA_SEA	chr16	216978	217122	299	556	444	1156	1135	1099	5	2363	11	1870	2136
THA_SEA	chr16	217111	217320	113	139	123	329	293	218	0	548	1	392	577
THA_SEA	chr16	217306	217443	2672	3691	1429	3435	2801	3170	8	6689	9	6568	5771
THA_SEA	chr16	217432	217659	91	151	150	391	407	283	0	623	0	514	660
THA_SEA	chr16	217639	217803	458	726	503	1286	1108	1071	6	1866	4	1869	1978
THA_SEA	chr16	217787	217882	423	432	314	615	488	635	6	1874	0	1268	1277
THA_SEA	chr16	218278	218461	2400	3767	2932	8284	5802	6790	24	12045	29	12269	11966
THA_SEA	chr16	218449	218636	56	95	122	310	267	202	1	450	4	382	507
THA_SEA	chr16	218625	218838	79	161	189	461	454	321	1	575	0	566	630
THA_SEA	chr16	218706	218932	106	178	154	425	413						

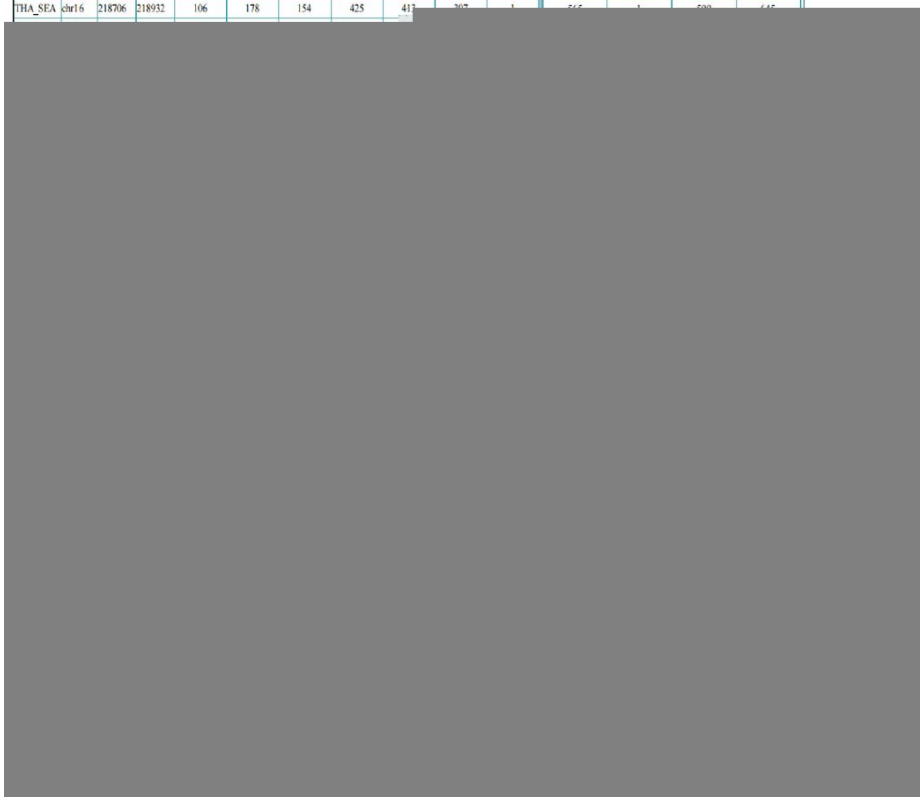

Figure 1

Flow chart and the pregnancy outcomes of the study.

Figure 2a

		start	end	M	F	E1	E2	E3	E4	E5	E6	E7	E8	E9	
THA_SEA	chr16	216815	216989	313	397	335	1098	782	696	2	1673	2	1278	1319	
THA_SEA	chr16	216978	217122	299	556	444	1156	1135	1099	5	2363	11	1870	2136	
THA_SEA	chr16	217111	217320	113	139	123	329	293	218	0	548	1	392	577	
THA_SEA	chr16	217306	217443	2672	3691	1429	3435	2801	3170	8	6689	9	6568	5771	
THA_SEA	chr16	217432	217659	91	151	150	391	407	283	0	623	0	514	660	
THA_SEA	chr16	217639	217803	458	726	503	1286	1108	1071	6	1866	4	1869	1978	
THA_SEA	chr16	217787	217882	423	432	314	615	488	635	6	1874	0	1268	1277	
THA_SEA	chr16	218278	218461	2400	3767	2932	8284	5802	6790	24	12045	29	12269	11966	
THA_SEA	chr16	218449	218636	56	95	122	310	267	202	1	450	4	382	507	
THA_SEA	chr16	218625	218838	79	161	189	461	454	321	1	575	0	566	630	
THA_SEA	chr16	218706	218932	106	178	154	425	413	307	1	565	1	509	645	
THA_SEA	chr16	218921	219129	738	1307	774	2090	1664	1757	1	2906	6	2860	2983	
THA_SEA	chr16	219111	219285	161	206	237	598	487	417	0	937	1	823	883	
THA_SEA	chr16	219274	219472	330	613	1017	2431	1875	1663	2	3105	11	3464	3236	
THA_SEA	chr16	219445	219598	173	267	283	613	518	520	1	1011	3	1001	1010	
THA_SEA	chr16	219524	219749	13	22	14	44	30	38	0	108	0	82	109	
THA_SEA	chr16	219738	219900	551	701	783	1721	1412	1241	5	2387	3	2016	2636	
THA_SEA	chr16	219856	220064	137	188	384	746	625	636	1	1077	1	903	1388	
THA_SEA	chr16	220061	220164	913	1085	1520	2123	1982	2093	6	4793	3	3244	3666	
THA_SEA	chr16	220152	220362	479	746	1006	2209	1885	1790	10	3049	1	2877	3091	
THA_SEA	chr16	220341	220490	458	603	811	1442	1241	1219	6	2391	7	1935	2106	
THA_SEA	chr16	220838	220923	544	602	511	774	878	1067	5	2461	2	1914	1872	
THA_SEA	chr16	220912	221126	539	908	1317	2751	2446	2418	6	4098	10	3604	4259	

Figure 2b

		position	M		F		E1		E2		E3		E4		E5		E6		E7		E8		E9	
			M0	M1	F0	F1	M0	F1	M1	F0	M1	F0:1	M1	F0	M0	F0	M1	F1	M0	F0	M1	F1	M1	F1
1	0_m	119006	A	C	A	A	A	A	C	A	C	A	C	A	A	A	C	A	A	A	C	A	C	A
2	1_m	152220	G	A	G	G	G	G	A	G	A	G	A	G	G	G	A	G	G	G	A	G	A	G
3	2_m	155966	C	G	C	C	C	C	G	C	G	C	G	C	C	C	G	C	C	C	G	C	G	C
4	3_m	170044	G	A	G	G	G	G	A	G	A	G	A	G	G	G	A	G	G	G	A	G	A	G
5	4_m	170328	C	T	C	C	C	C	T	C	T	C	T	C	C	C	T	C	C	C	T	C	T	C
6	5_m	175726	G	A	G	G	G	G	A	G	A	G	A	G	G	G	A	G	G	G	A	G	A	G
7	6_m	175727	C	T	C	C	C	C	T	C	T	C	T	C	C	C	T	C	C	C	T	C	T	C
8	7_m	176743	G	A	G	G	G	G	A	G	A	G	A	G	G	G	A	G	G	G	A	G	A	G
9	8_m	185102	C	G	C	C	C	C	G	C	G	C	G	C	C	C	G	C	C	C	G	C	G	C
10	9_p	207611	C	C	C	T	C	T	C	C	C	C	C	C	C	C	C	T	C	C	C	T	C	T
11	10_p	211105	G	G	G	T	G	T	G	G	G	G	G	G	G	G	G	T	?	?	G	T	G	T
12	11_m	211106	C	T	C	C	C	C	T	C	T	C	T	C	C	C	T	C	?	?	T	C	T	C
13	12	218532	?	C	?	T	?	T	C	?	C	?	C	?	?	?	C	T	?	?	C	T	C	T
14	13	224619	?	T	?	C	?	C	T	?	T	?	T	?	?	?	T	C	?	?	T	C	T	C
15	14_p	247888	A	A	A	G	A	G	A	A	A	A	A	A	A	A	A	G	A	A	A	G	A	G
16	15_p	250278	C	C	C	A	C	A	C	C	C	C	C	C	C	C	C	A	C	C	C	A	C	A
17	16_m	277458	G	A	G	G	G	G	A	G	A	G	A	G	G	G	A	G	G	G	A	G	A	G
18	17_m	285313	T	C	T	T	T	T	C	T	C	T	C	T	T	T	C	T	T	T	C	T	C	T
19	18_p	304514	C	C	C	T	C	T	C	C	C	C	C	C	C	C	C	T	C	C	C	T	C	T
20	19_m	336660	A	G	A	A	A	A	G	A	G	A	G	A	A	A	G	A	A	A	G	A	G	A
21	20_m	349293	A	G	A	A	A	A	G	A	G	A	G	A	A	A	G	A	A	A	G	A	G	A
22	21_m	358161	C	A	C	C	C	C	A	C	A	C	A	C	C	C	A	C	C	C	A	C	A	C
23	22_m	369413	G	A	G	G	G	G	A	G	A	G	A	G	G	G	A	G	G	G	A	G	A	G
24	23_m	396264	A	G	A	A	A	A	G	A	G	A	G	A	A	A	G	A	A	A	G	A	G	A

Figure 2

Case 13 presented as an example analysis of α- thalassemia (part of the SNP results). A. Sequencing depth of the SEA area. B. SNPs in the SEA area were used to construct the haplotype. The positions marked in dark green are paternal informative SNPs while those in light green are maternal informative SNPs. The yellow squares represent ADO sites. M0 and F0 represent an affected haplotype from mother and father, respectively. M1 and F1 indicate the normal inherited allele. A question mark represents the undetected site in the SEA area.

M: mother, F: father, E: embryo.

Figure 3a

No.	chr11:5247992 CD41/42delAAAG		
	Depth	Genotype	Base_Depth
M	1310	C/C-	CAAAG(649)C(661)
F	833	C/C-	CAAAG(427)C(405)
E1	3074	C/C-	CAAAG(1570)C(1503)
E2	573	C/C-	CAAAG(245)C(328)
E3	3452	C/C	C(3444)
E4	2463	C-/C-	CAAAG(12)C(2449)
E5	2965	C/C-	CAAAG(1002)C(1962)
E6	4942	C-/C-	CAAAG(25)C(4917)

Figure 3b

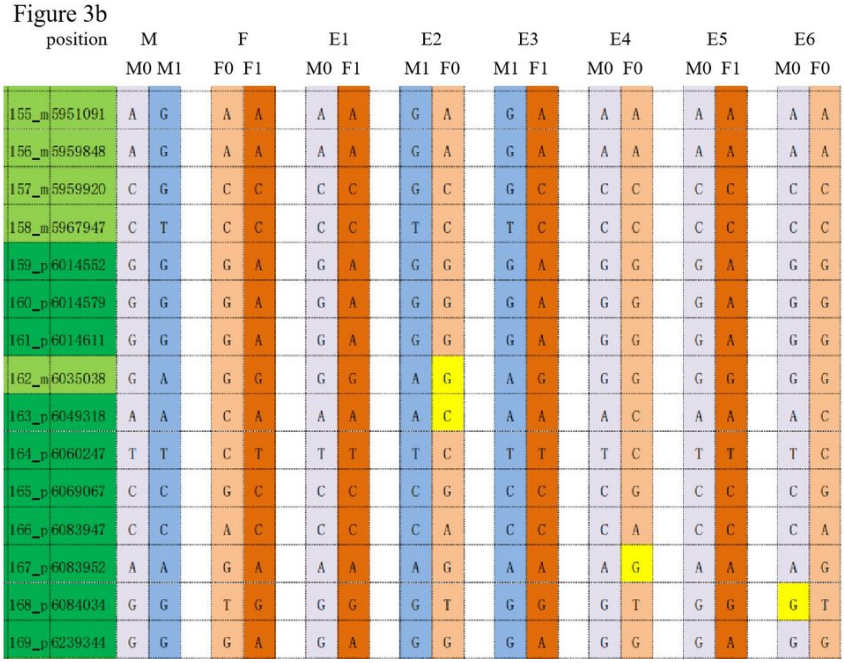

Figure 3

Case 30, presented as an example analysis of β- thalassemia. A. Sequencing depth of the CD 41-42 area. B. SNPs near the CD 41-42 area were used to construct the haplotype (part of the SNP results). The positions marked in dark green are paternal informative SNPs while the positions in light green are maternal informative SNPs. The yellow squares represent ADO sites. M0 and F0 represent an affected haplotype from the mother and father, respectively. M1 and F1 indicate the normal inherited allele.

M: mother, F: father, E: embryo.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaltable.doc