Journal of Assisted Reproduction and Genetics (2019) 36:2515–2523
https://doi.org/10.1007/s10815-019-01595-7

GENETICS

Karyomapping in preimplantation genetic testing for β-thalassemia combined with HLA matching: a systematic summary

Jing Wang[1] · Bao-Min Lu[1] · Rong Li[1] · Jing Guo[1] · Yan Xu[1] · Jia-Fu Pan[1] · Yan-hong Zeng[1] · Can-Quan Zhou[1] · Yan-Wen Xu[1,2]

Received: 3 July 2019 / Accepted: 24 September 2019 / Published online: 22 November 2019

Abstract

Purpose To investigate the validity, accuracy, and clinical outcomes of Karyomapping in preimplantation genetic testing (PGT) for β-thalassemia combined with human leukocyte antigen (HLA) matching.

Methods A total of 128 cycles from January 2014 to December 2017 were identified, and 1205 embryos were biopsied. The case group included 88 cycles using Karyomapping for PGT-HLA, compared with 40 cycles using polymerase chain reaction-short tandem repeat (PCR-STR) as the control group.

Results There were significant differences in the HLA matching rate (21.34 *vs.* 14.37%), the matched transferable embryo rate (9.79 *vs.* 14.07%), the clinical pregnancy rate (65.08 *vs.* 41.86%), and the spontaneous miscarriage rate (2.44 *vs.* 22.22%) between the case and control groups. In the case group, nearly 1/3 (33.37%) of the embryos showed aneuploidy. According to the results of single nucleotide polymorphism (SNP) haplotype analysis, the recombination rates of *HBB* (hemoglobin subunit beta) and *HLA* were 11.46% and 5.61% respectively. *HLA* gene recombination was mostly distributed between HLA-A and HLA-B and the downstream region of HLA-DQB1. In addition, STR analysis could be considered in the case of copy-neutral loss of heterozygosity (LOH) in the region where the *HLA* gene is located.

Conclusion Karyomapping contributes to accurate selection of matched embryos, along with aneuploidy screening. However, STRs assist identification in cases of LOH in the target region.

Keywords HLA matching · Karyomapping · Preimplantation · Genetic testing · β-Thalassemia

Introduction

β-Thalassemia is an autosomal recessive inherited hemolytic disease. In south China, the prevalence of β-thalassemia carriers ranges from 1.96 to 6.43% [1–4]. β-Thalassemia major requires long-term blood transfusion and iron removal [5]. Currently, hematopoietic stem cell transplantation is considered to be the most effective therapeutic approach for β-thalassemia [6]. HLA-matched siblings are the best donors for hematopoietic stem cell transplantation [7].

Preimplantation genetic testing for monogenic diseases (PGT-M) combined with human leukocyte antigen (HLA) matching is a technology used to select embryos by target gene detection and HLA matching, eventually transferring matched and unaffected embryos. In addition to obtaining healthy offspring, the technique can provide sibling donors for hematopoietic stem cell transplantation to rescue the probands. Therefore, this technique is particularly helpful for families with probands suffering from β-thalassemia major who are in urgent need of stem cell transplantation. At present, over 600 PGT-HLA cycles have been reported, resulting in over 100 births [8].

Conventional PGT for β-thalassemia combined with HLA typing is to detect *HBB* (hemoglobin subunit beta) gene mutations directly, and perform short tandem repeat (STR) analysis at the same time [9–11]. As second-generation linkage markers, STRs are limited in number and are unevenly distributed throughout the human genome, such that the available informative STRs are quite limited in the target region, as well as in its adjacent upstream and downstream regions, and can result in unavailable linkage markers. In addition, the distance between

Jing Wang and Bao-Min Lu contributed equally to the work.

✉ Yan-Wen Xu
xuyanwen@live.cn

[1] Reproductive Medicine Center, The First Affiliated Hospital, Sun Yat-sen University, Guangdong, China

[2] Guangdong Provincial Key Laboratory of Reproductive Medicine, No.58, Zhongshan II Road, Guangzhou 510080, China

markers will lead to the inaccurate location of gene recombination, resulting in reduced feasibility and accuracy of haplotype analysis. With the development of high-throughput genome-wide technology, a technique termed Karyomapping has emerged, which is a genome-wide haplotype analysis of the parents based on single nucleotide polymorphisms (SNPs). The SNP array contains 300,000 SNPs covering the whole genome. Considering the coverage of the whole genome, theoretically, all known genes or chromosome segments, and even the entire chromosome states, can be obtained. By comparing the SNP markers present on the parental chromosomes at the position of the single gene defect in the child with those present in the embryo biopsy, the presence or absence of the affected gene(s) can be diagnosed indirectly using linkage analysis [12]. The emergence of Karyomapping has provided a general linkage analysis-based approach for PGT, which can detect any monogenic disease, together with HLA typing [13, 14]. Therefore, a single test could not only obtain data for the whole family (including embryos) concerning the target gene but also can determine the recombination of each chromosome, as well as the aneuploidy status of the chromosomes [13]. This could potentially improve embryo selection, enhancing the likelihood that a transferred embryo will form a viable pregnancy by avoiding the transfer of those harboring lethal aneuploidies. However, this remains to be proven conclusively [15]. Karyomapping is not specifically designed or recommended by the supplier for comprehensive chromosome screening (CCS). The first clinical application of Karyomapping was reported by Natesan *et al*. A total of 218 embryo samples from 44 cycles were detected by this technique blindly, with a concordance rate of 97.7% compared with that detected by polymerase chain reaction-short tandem repeat (PCR-STR). The inconsistent result was speculated to be caused by factors such as insufficient STRs in the original results or the uncertainty of the original judgment caused by semi-informative STRs [16]. Subsequently, a number of papers have summarized and reported the clinical application of this technique in PGT-M [12, 15, 17–19]. However, the application of Karyomapping in HLA matching has rarely been reported. In the present study, we retrospectively analyzed the diagnostic data and clinical outcomes of PGT for β-thalassemia and HLA matching using PCR-STR and Karyomapping.

The study aimed to evaluate the advantages of Karyomapping compared with conventional PCR-STR and to analyze the possible problems associated with the clinical application of Karyomapping.

Materials and Methods

Patient population

Our PGT laboratory has established single-cell PCR with reverse dot blotting (RDB) to diagnose β-thalassemia and HLA matching since 2009. In the last 4 years, we have adopted the Karyomapping technique. In the present study, we recruited 128 PGT cycles of 102 couples for β-thalassemia combined with HLA matching from 2014 to 2017, when both techniques were applied in our laboratory. Among the 128 cycles, Karyomapping was used in 88 cycles (case group), and the PCR-STR method was adopted in 40 cycles (control group). A total of 1205 embryos were biopsied. Of the 102 couples, 83 had only one biopsy cycle, 13 had two, and the remaining six couples had three or more. In addition, five couples were subjected to both techniques in different cycles. Clinical pregnancy was confirmed by a gestational sac with a fetal heartbeat approximately 5 weeks after frozen embryo transfer. Spontaneous miscarriage was defined as pregnancy loss in the first trimester.

All couples were given genetic counseling before treatment and were informed of the treatment protocol and procedure, the misdiagnosis rate of PGT, and the possible failure to obtain HLA-matched embryos. All patients were informed about relevant risks and provided written informed consent.

This was a retrospective analysis, and no private information was involved; therefore, the Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University exempted the study from ethical approval.

In-vitro fertilization (IVF)–embryo transfer cycle, blastocyst biopsy, and whole genome amplification

The conventional luteal-phase long protocol was used for controlled ovarian stimulation with intracytoplasmic sperm injection (ICSI) for insemination; fertilization was observed at 16–18 h after insemination. Embryos that were fertilized normally and developed to six cells or more were submitted for blastocyst culture on day 3. Six to eight trophectoderm cells were biopsied in the blastocyst stage. All blastocysts were vitrified after biopsy, while the cells for biopsy were placed in 3.5 μl of phosphate-buffered saline (PBS). A REPLI-g Single Cell Kit (QIAGEN, Hilden, Germany) was used for whole-genome amplification (WGA).

Direct detection of HBB gene mutation and haplotype analysis of HBB and HLA gene

Direct detection of HBB gene mutation was performed using PCR with RDB. According to previous reports [20, 21], STRs in *HLA* genes and their flanking regions were detected by multiplex fluorescent PCR to establish the haplotype for *HLA* genotyping. In addition, linkage analysis of the *HBB* gene and its 1-Mbp flanking regions were performed to reduce the risk of misdiagnosis caused by Allele-drop out (ADO). According to the 2011 European Society of Human Reproduction and Embryology (ESHRE) PGD consortium [8], each family had at least one heterozygous STR in the adjacent upstream and downstream regions of *HLA-A*, *HLA-*

B, *HLA-DRA*, and *HLA-DQB*1, respectively. Table 1 describes the specific *HBB* and *HLA* gene-related STRs.

Karyomapping analysis and comprehensive chromosome screening

The peripheral blood DNA of parents and reference samples (children with β-thalassemia major), together with the embryo samples from the WGA, were analyzed using HumanKaryomap-12 BeadChips (Illumina, San Diego, CA, USA) according to the manufacturer's protocol. The scanning results were analyzed using BlueFuse Multi V4.3 software provided by Illumina. HLA typing was performed in the *HLA-A*, *HLA-B*, *HLA-DRA*, and *HLA-DQB*1 regions as well as 2 Mb upstream and downstream of each gene. At the same time, the B allele and Log R Ratio data were obtained from the scanning results produced by Illumina Genome studio 2.0 software for aneuploidy screening.

Statistical analysis

SPSS 19.0 software (IBM Corp., Armonk, NY, USA) was used for statistical analysis in this study. The mean of two groups was compared using a *t* test. The Mann-Whitney *U* test was applied for data with a non-normal distribution. The comparison of ratios used the χ^2 test. $P < 0.05$ indicated that the difference was statistically significant.

Results

There were no differences with regard to the average maternal age (31.59 ± 3.60 *vs.* 31.88 ± 4.61) and the mean number of biopsied blastocysts (9.67 ± 3.94 *vs.* 8.85 ± 3.66) between the case and control groups. The clinical pregnancy rate per transfer cycle was 65.08% in the case group, which was significantly higher than the 41.86% in the control group (P = 0.018). In addition, the spontaneous miscarriage rate was 2.44% in the case group, which was significantly lower than the 22.22% in the control group (P = 0.045). In addition, the fetuses of two couples in the control group were selectively terminated because of the detection of Down syndrome. Detailed information is shown in Table 2.

There was no discrepancy in the diagnosis of β-thalassemia between direct mutation detection and STR analysis in the control group. Furthermore, no statistical difference

Table 1 Overview of STR markers in the HBB gene and HLA complex

	STR locus	Heterozygosity	Approximate location		Distance from the target gene (Mbp)
			Starting bp	Ending bp	
Tel	D11S988	0.88	4,518,621	4,518,740	0.7
	D11S4181	0.86	4,746,612	4,746,974	0.4
	D11S2362	0.81	4,790,917	4,991,191	0.2
	HBB		5,225,466	5,227,071	
	D11S4891	0.73	5,229,694	5,229,798	< 0.1
	D11S1760	0.88	5,263,107	5,463,378	< 0.1
Cen	D11S1338	0.74	5,966,682	5,967,038	0.7
Tel	D6S276	0.79	24,185,574	24,185,941	
	D6S105	0.81	27,803,496	27,803,624	
	D6S248	0.82	28,728,418	28,728,688	
	HLA-A		29,909,037	29,913,661	
	HLAC-CA	0.72	88.4 kb telomeric of HLA-C[10]		
	D6S265	0.76	115 kb centromeric of HLA-A[10]		
	HLA-C		31,236,526	31,239,907	
	HLABC-CA	0.78	39.4 kb centromeric of HLA-C[10]		
	HLA-B		31,321,649	31,324,965	
	MIB	0.82	24.9 kb centromeric of HLA-B[10]		
	82–1	0.81	Between BAT5 and LTB[10]		
	D6S273	0.78	96 kb telomeric of HSP70[10]		
	HLA-DRA		32,407,619	32,412,823	
	*HLA-DQB*1		32,627,244	32,636,160	
	D6S291	0.72	36,297,737	36,297,945	
Cen	D6S426	0.85	40,674,444	40,674,713	

Table 2 Overall clinical results of β-thalassemia combined with HLA matching

Result	Control group (PCR-STR)	Case group (Karyomapping)	*P* value
No. of patients/cycles	32/40	75/88	
Maternal age (years, mean ± SD, min–max)	31.88 ± 4.609(23-40)	31.59 ± 3.601(22-39)	NS
Paternal age (years, mean ± SD, min–max)	35.18 ± 4.601(26-42)	34.31 ± 5.625(23-47)	NS[a]
No. of oocytes retrieved	13.52 ± 7.423	17.24 ± 7.085	$P < 0.05$[a]
No. of biopsied embryos	8.85 ± 3.655	9.67 ± 3.941	NS[a]
Clinical pregnancy rate per transfer cycle (%)	41.86	65.08	$P < 0.05$
Spontaneous miscarriage rate (%)	22.22	2.44	$P < 0.05$
No. biochemical pregnancies	3	1	–
No. of babies born	12	42[b]	–

[a] Statistical analysis was performed according to Mann–Whitney *U* test

[b] By the time of the article was written

was found between the case and the control group in the diagnosis of β-thalassemia. With regard to the inconclusive diagnosis by haplotype analysis, there was a statistical difference between the two groups (0.85% vs 2.69%, $P = 0.014$ in *HBB*; 0.85% vs 2.40%, $P = 0.034$ in *HLA*). The inconclusive results of PCR-STR in the control group were mainly attributed to the occurrence of ADO at the STR locus and the poor heterozygosity of the locus itself. For Karyomapping in the case group, the inconclusive result might be related to the aneuploidy of the chromosome containing the target gene or copy-neutral loss of heterozygosity (LOH) in the gene region.

There was a significant difference in the HLA matching rate between the case and control groups (21.34% *vs.* 14.37%, $P = 0.007$). Meanwhile, the rates of normal and carriers of β-thalassemia with matched HLA were 15.72% and 14.07%, respectively, with a slight difference between the groups ($P = 0.048$). Subsequently, CCS was conducted in the case group, and 33.37% were found to have aneuploidy. Finally, the percentage of HLA-matched embryos decreased the transfer rate to 9.79%, because of the exclusion of aneuploid embryos. The detailed results are presented in Table 3.

Among 820 embryos subjected to SNP haplotype analysis for the *HBB* gene in the case group, recombination in the *HBB* gene and its adjacent 2 Mb upstream and downstream was found in 94 embryos (11.46%), with 54 recombination events on the paternal allele and 44 on the maternal allele. In addition, three embryos indicated recombination on both alleles, and the reference samples indicated recombination in seven families, with six on the paternal allele and one on the maternal allele. Additionally, recombination in the *HLA* gene and its adjacent 2 Mb upstream and downstream was found in 46 embryos (5.61%), with 18 on the paternal allele and 28 on the maternal allele. Meanwhile, the reference samples indicated recombination in six families, with three on paternal alleles and three on maternal alleles. The location of the recombination was mostly found between *HLA-A* and *HLA-B* and downstream of *HLA-DQB*1 (as shown in Table 3 and Fig. 1).

In the case group, there was one case in which the mother had a copy-neutral LOH at 6p21.31-p22.3 (6:24,946,167–34,166,599) of about 10 Mb, which just covered the entire HLA region. The lack of effective informative SNPs in the HLA region meant that HLA matching could not be determined in two embryos. Subsequently, PCR-STR was used for HLA matching in this family. The results showed that 4 out of 11 STR loci were informative. Finally, one embryo was determined as HLA-matched and a successful pregnancy was achieved after transfer. The amniotic cell genetic test result was in accordance with the result predicted by PGT-HLA (as shown in Fig. 2).

Discussion

Verlinsky *et al.* first reported preimplantation genetic diagnosis (PGD) for Fanconi anemia combined with HLA matching in 2001 [22]. In 2010, Handyside *et al.* put forward the concept of Karyomapping [13]. Several studies have confirmed the feasibility and accuracy of this technique to detect monogenic diseases before embryo implantation [13, 15–17]; however, few studies have reported HLA matching.

The present study reported the clinical application of Karyomapping combined with HLA matching in patients with β-thalassemia. In the diagnosis of β-thalassemia, Karyomapping was similar to conventional PCR-STR in terms of the normal homozygote rate, the heterozygote rate, and the affected rate. The inconclusive diagnosis in the control group was speculated to be related to insufficient STRs or the semi-informative STRs and ADO, which led to failure to determine the haplotype. There are a limited number of STRs in the genome, and STRs may be far apart from each other, with an average spacing of 0.7 cM [23–25]. Consequently, the informative STRs available for the target gene and its flanking regions are quite limited. At the same time, ADO is inherent in minimal DNA amplification, which further reduced the

Table 3 The laboratory data of preimplantation genetic testing for β-thalassemia and HLA matching using PCR-STR and Karyomapping

Result	Control group (PCR-STR)			Case group (Karyomapping)		*P* value
	HBB-RDB	HBB-STR	HLA-STR	HBB	HLA	
No. of failure amplification	20	–	–	24	–	
Diagnosis rate	334/354(94.35%)	325/354(91.81%)	326/354(92.09%)	820/851(96.36%)	820/851(96.36%)	
Normal rate of β-thalassemia	78/334(23.35%)[a]	73/325(22.46%)[a]	–	207/820(25.24%)[a]	–	
Heterozygosity rate of β-thalassemia	179/334(53.59%)[a]	172/325(52.92%)[a]	–	407/820(49.63%)[a]	–	
Abnormal rate of β-thalassemia	77/334(23.05%)[a]	80/325(24.62%)[a]	–	206/820(25.12%)[a]	–	
Incomplete/inconclusive diagnosis by haplotype analysis	–	9/334(2.69%)	8/334(2.40%)	7/827(0.85%)	7/827(0.85%)	$P < 0.05$
HLA matching rate	–	–	48/334(14.37%)	–	175/820(21.34%)	$P < 0.05$
Normal/carriers and matched HLA	47/334(14.07%)			130/827(15.72%)		$P < 0.05$
HLA-matched allowed for transfer	47/334(14.07%)			81/827(9.79%)		$P < 0.05$
Aneuploidy	–	–	–	276/827(33.37%)	–	–
Haploidy	–	–	–	2/827(0.24%)	–	–
Triploid	–	–	–	1/827(0.12%)	–	–
The recombination rate of embryos				94/820(11.46%)	46/820(5.61%)[b]	–

[a] There was no statistical difference between the two

[b] No informative SNPs of maternal in HLA region were included

number of STRs available in the embryo samples. For example, in our study, there were six STRs in *HBB* and its flanking regions. In a family, the available STRs (informative and semi-informative STRs) must be less than or equal to 6. Then, in embryo testing, the number of available STRs may be further reduced because of ADO. In addition, these available STRs may be located only in the upstream or downstream regions of *HBB*. Meanwhile, in the case group, the inconclusive diagnoses were mainly caused by aneuploidy of the target chromosome and copy-neutral LOH in the target region. The large number of SNPs greatly weakened the impact of ADO. SNPs cover all chromosomes; therefore, the copy number of the whole chromosome can be evaluated accordingly, thereby promoting aneuploidy screening to prevent spontaneous miscarriage and the misdiagnosis of target genes caused by chromosomal abnormalities.

SNPs are distributed more evenly with less space between them in the human genome compared with STRs; therefore, they are more accurate to determine the parental origin of target genes, especially chromosome recombination and its location. A recombination rate of 5.61% within the *HLA* region was observed in our study. The recombination location of *HLA* observed was mostly located between *HLA-A* and *HLA-B* and in the downstream region of *HLA-DQB*1. The results reported by Kakourou *et al.* were different from our results. In their study, the rate of recombination of *HLA* was 3.5%, which occurred mostly in the upstream region of *HLA-A* and downstream of *HLA-DQB*1. [26]. However, before that, Little *et al.* and Rechitsky *et al.* reported over 4% recombination within the *HLA* region [27, 28]. Our recombination rate was closer to these two reports. The STR analysis was applied in a previous study. Thus, further study will be needed to estimate the

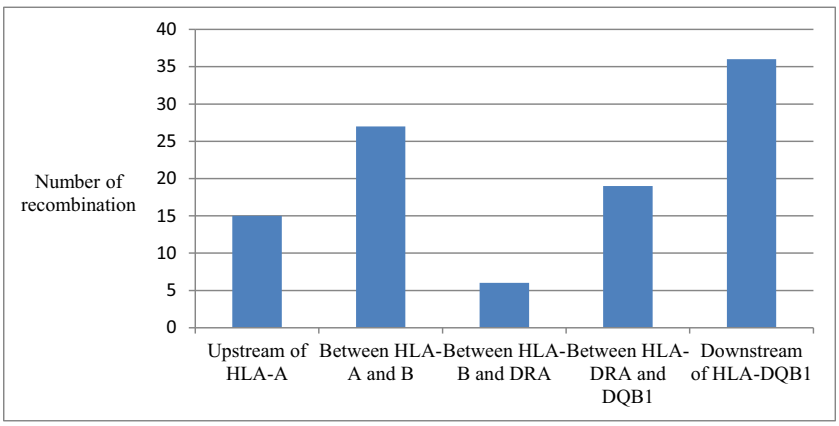

Fig. 1 Distribution of recombination in HLA region

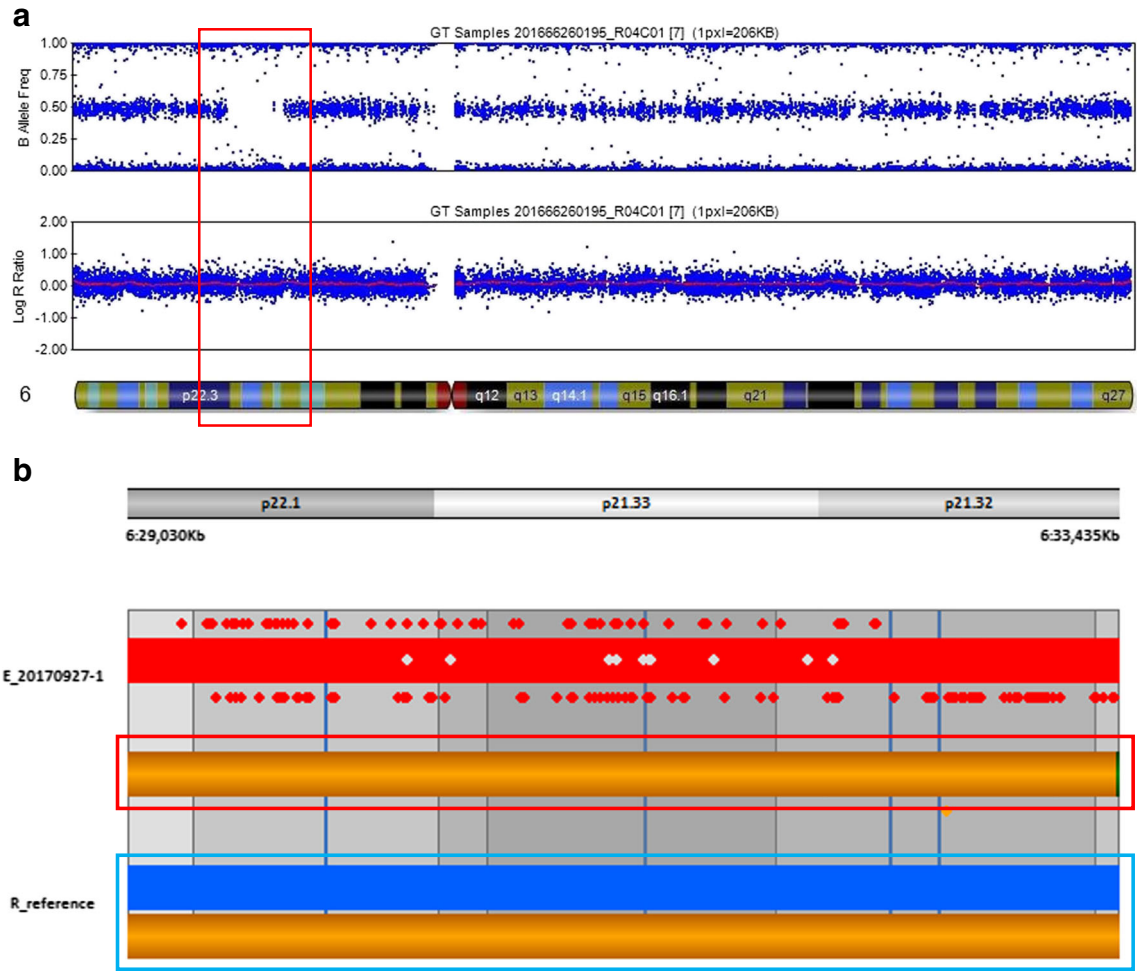

Fig. 2 Preimplantation genetic testing of β-thalassemia combined with HLA matching. There was one case in which the woman had a copy-neutral LOH at 6p21.31-p22.3. **a** B allele and Log R Ratio result of chromosome 6 in the mother. The region within the red frame contains the HLA region. All SNPs are homozygous AA or BB; meanwhile, the baseline value of the Log R Ratio is not offset. **b** SNP haplotypes analysis of the HLA region. The region within the red frame shows the maternal haplotypes of an embryo; there are no informative SNPs. The region within the blue frame shows the haplotypes of the reference sample. **c** Schematic diagram of the distribution of four STR loci available for the HLA region. **d** STR haplotypes analysis of the HLA region, F father, M mother, E embryo, tel telomere, cen centromere. The black bold number is the peak of the reference sample. Embryo E2 was confirmed to be incompatible with the reference sample because of an unmatched paternal chromosome. Karyomapping was unable to determine the HLA type in E3 and E6. E3 was determined to be an HLA-matched embryo by PCR-STR. Successful pregnancy was achieved after transfer. The amniotic cell results were in accordance with the results predicted by PGT-HLA

recombination rate of the *HLA* region by other methodologies, such as Karyomapping [26]. Karyomapping might be able to provide a more accurate location of chromosomal recombination, thereby improving the accuracy of haplotype analysis. Theoretically, the HLA matching rate is 25%. Our results further confirmed the accuracy of Karyomapping, with a rate of HLA matching (21.34%) being closer to the theoretical value.

In addition, a whole-genome SNP array can be used for aneuploidy screening. Especially for HLA-matched families, it is important to select the most suitable embryos (unaffected HLA-matched euploid blastocysts) for transfer, thereby increasing the time and opportunities for hematopoietic stem cell transplantation treatment. Recent reports support this view, suggesting that PGT-HLA should include aneuploidy screening as standard [26, 29–31]. In our study, the aneuploidy rate was 33.37% after CCS. The average maternal age was 31.59 ± 3.60 years. These results were similar to previous reports. Vanneste *et al*. revealed that women aged < 35 years had an aneuploidy rate of 30–40% at the blastocyst stage. Hou *et al*. reported that the rate of chromosomal abnormalities was 33.59% in young women (< 35 years) [32, 33]. Although the rate of matched transferable embryos fell from 15.72% to less than 10%, the clinical pregnancy rate and spontaneous miscarriage rate were significantly improved in the case group compared with those in the control group. By contrast, two cases in the control group were selectively terminated because of the presence of Down syndrome.

Although improvements in technology provide more opportunities and possibilities for diagnosis, Karyomapping

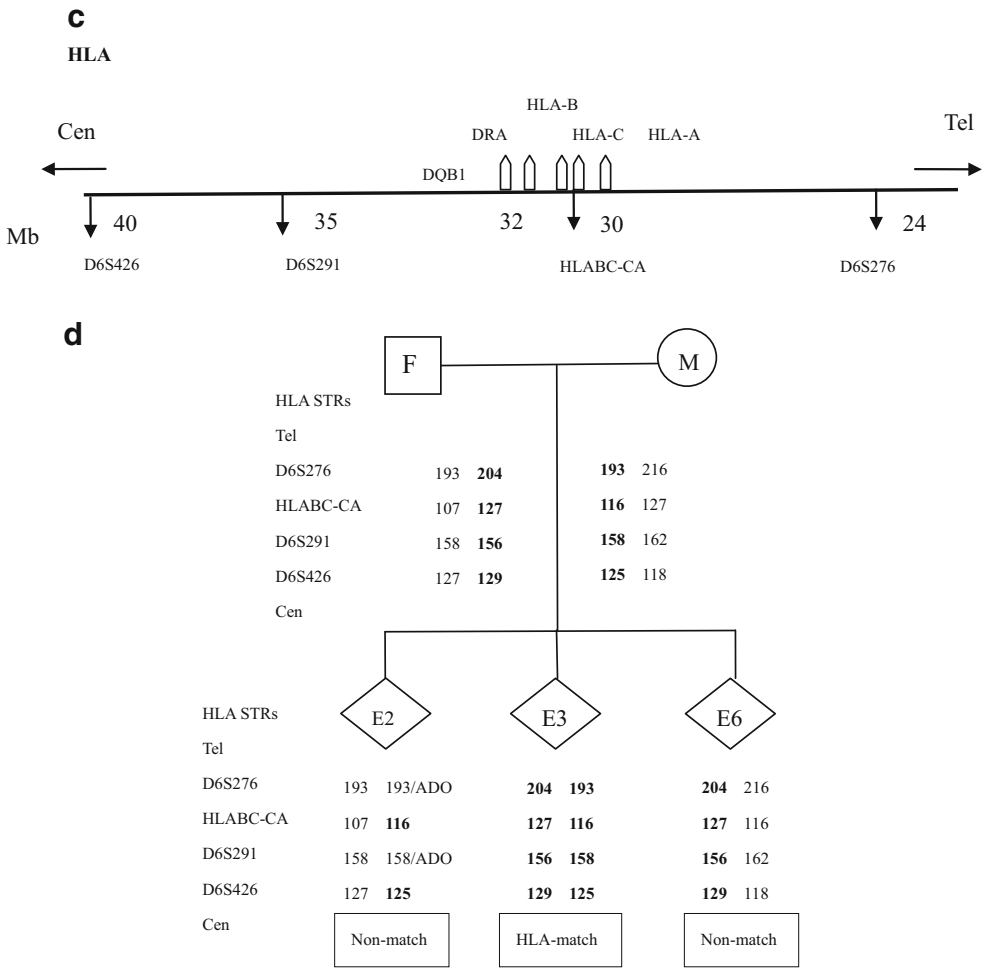

Fig. 2 (continued)

itself also has certain limitations. For example, the principle of Karyomapping is still a linkage analysis, and chromosome recombination remains the main cause of poor accuracy and mistakes in diagnosis. This might lead to difficulty in diagnosing all cohorts of embryos, especially if the reference sample itself has crossovers in the target region. In this study, this phenomenon occurred in seven families. All the embryos in the family showed a recombination in the same location adjacent to the *HBB* gene region. A similar recombination occurred in *HLA* of all embryos in six families. According to the report by Ottolini *et al.*, such recombination could be attributed to the crossover of the reference sample itself at this site, namely, a common crossover [34, 35]. In the case of the *HBB* genes, the risk of transferring an affected embryo is minimal if a chromosome of the embryo has been identified as coming from a normal parent chromosome. However, if the embryo has inherited one abnormal chromosome and a common crossover occurs in the other one, it may not be possible to diagnose the embryo. Direct mutation detection of the *HBB* gene may be considered in this situation; however, accuracy cannot be guaranteed because of the possibility of ADO, and it is extremely risky to transfer such embryos. In the case of HLA, it may not be possible to determine whether the embryo is compatible with the reference sample, which may result in patients undergoing several PGT-HLA cycles or not being able to obtain matched embryos.

In addition, a case of copy-neutral LOH was encountered in part of the short p arm of chromosome 6 in our study. The copy-neutral LOH covered the entire *HLA* gene region, making the diagnosis of HLA a challenge. We attempted to use STRs to analyze the results. There were four available informative STRs, and the *HLABC-CA* locus was found to be located between *HLA-B* and *HLA-C* (as shown in Fig. 2). A SNP is a biallelic polymorphism of double alleles, while an STR is a multiple allelic polymorphism. Each SNP locus usually contains only two alleles; there are thus only two variants in a single SNP, and the degree of variation is lower than that of microsatellite DNA. In other words, the amount of information provided by approximately 700–900 SNPs is equivalent to that provided by 300–400 microsatellites. In this regard, if

an SNP array is used for PGT-HLA, STR analysis can be considered in the case of copy-neutral LOH in the *HLA* gene region or where there are insufficient SNPs in the region itself. Konstantinidis *et al.* also mentioned that direct mutation detection of the target gene and STR analysis could be considered if the SNP coverage was extremely low in the target region [19].

In summary, Karyomapping is a simple and efficient method of PGT for β-thalassemia when combined with HLA matching, and with the advantage of CCS to exclude chromosomal abnormalities simultaneously. However, this technique still has its limitations. There is still room for conventional STR analysis in cases of insufficient SNPs for linkage analysis. Next-generation sequencing (NGS) technology can directly detect target genes while carrying out linkage analysis. However, there have been few publications of clinical applications in PGT for β-thalassemia combined with HLA matching by NGS. In the future, more relevant data will be needed to validate the methodologies.

Acknowledgments The authors would like to thank all the patients and the staff at the First Affiliated Hospital of Sun Yat-sen University who participated in this study. The authors also thank the native English-speaking scientists of Elixigen Company (Huntington Beach, California) for editing our manuscript.

Funding information This work was supported by the National Key Research and Development Program (Grant No.2018YFC1003102) and the National Natural Science Foundation of China (Grant No.81771588) and the Guangzhou Science and Technology Project (Grant No.201804020087), and the Guangdong Province Science and Technology Project (Grant No.2016A020218008).

References

1. Li CG, Li CF, Li Q, Li M. Thalassemia incidence and treatment in China with special reference to Shenzhen City and Guangdong province. Hemoglobin. 2009;33(5):296–303. https://doi.org/10.3109/03630260903211698.
2. Pan HF, Long GF, Li Q, Feng YN, Lei ZY, Wei HW, et al. Current status of thalassemia in minority populations in Guangxi, China. Clinical Genet. 2007;71(5):419–26. https://doi.org/10.1111/j.1399-0004.2007.00791.x.
3. Xiong F, Sun M, Zhang X, Cai R, Zhou Y, Lou J, et al. Molecular epidemiological survey of haemoglobinopathies in the Guangxi Zhuang Autonomous Region of southern China. Clin Genet. 2010;78(2):139–48. https://doi.org/10.1111/j.1399-0004.2010.01430.x.
4. Yin A, Li B, Luo M, Xu L, Wu L, Zhang L, et al. The prevalence and molecular spectrum of alpha- and beta-globin gene mutations in 14,332 families of Guangdong Province, China. PLoS One. 2014;9(2):e89855. https://doi.org/10.1371/journal.pone.0089855.
5. Higgs DR, Engel JD, Stamatoyannopoulos G. Thalassaemia. Lancet (London, England). 2012;379(9813):373–83. https://doi.org/10.1016/s0140-6736(11)60283-3.
6. Gaziev J, Lucarelli G. Hematopoietic stem cell transplantation for thalassemia. Curr Stem Cell Res Ther. 2011;6(2):162–9.
7. Fang JP, Xu LH. Hematopoietic stem cell transplantation for children with thalassemia major in China. Pediatr Blood Cancer. 2010;55(6):1062–5. https://doi.org/10.1002/pbc.22742.
8. De Rycke M, Belva F, Goossens V, Moutou C, SenGupta SB, Traeger-Synodinos J, et al. ESHRE PGD Consortium data collection XIII: cycles from January to December 2010 with pregnancy follow-up to October 2011. Hum Reprod (Oxford, England). 2015;30(8):1763–89. https://doi.org/10.1093/humrep/dev122.
9. Fernandez RM, Pecina A, Lozano-Arana MD, Sanchez B, Guardiola J, Garcia-Lozano JC, et al. Experience of preimplantation genetic diagnosis with HLA matching at the University Hospital Virgen del Rocio in Spain: technical and clinical overview. Biomed Res Int. 2014;2014:560160. https://doi.org/10.1155/2014/560160.
10. Fiorentino F, Biricik A, Karadayi H, Berkil H, Karlikaya G, Sertyel S, et al. Development and clinical application of a strategy for preimplantation genetic diagnosis of single gene disorders combined with HLA matching. Mol Hum Reprod. 2004;10(6):445–60. https://doi.org/10.1093/molehr/gah055.
11. Van de Velde H, Georgiou I, De Rycke M, Schots R, Sermon K, Lissens W, et al. Novel universal approach for preimplantation genetic diagnosis of beta-thalassaemia in combination with HLA matching of embryos. Hum Reprod (Oxford, England). 2004;19(3):700–8. https://doi.org/10.1093/humrep/deh153.
12. Handyside AH. Live births following karyomapping - a "key" milestone in the development of preimplantation genetic diagnosis. Reprod Biomed Online. 2015;31(3):307–8. https://doi.org/10.1016/j.rbmo.2015.07.003.
13. Handyside AH, Harton GL, Mariani B, Thornhill AR, Affara N, Shaw MA, et al. Karyomapping: a universal method for genome wide analysis of genetic disease based on mapping crossovers between parental haplotypes. J Med Genet. 2010;47(10):651–8. https://doi.org/10.1136/jmg.2009.069971.
14. 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. J Assist Reprod Genet. 2015;32(3):347–56. https://doi.org/10.1007/s10815-014-0405-y.
15. Ben-Nagi J, Wells D, Doye K, Loutradi K, Exeter H, Drew E, et al. Karyomapping: a single centre's experience from application of methodology to ongoing pregnancy and live-birth rates. Reprod Biomed Online. 2017;35(3):264–71. https://doi.org/10.1016/j.rbmo.2017.06.004.
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. Genet Med. 2014;16(11):838–45. https://doi.org/10.1038/gim.2014.45.
17. Gimenez C, Sarasa J, Arjona C, Vilamajo E, Martinez-Pasarell O, Wheeler K, et al. Karyomapping allows preimplantation genetic diagnosis of a de-novo deletion undetectable using conventional PGD technology. Reprod Biomed Online. 2015;31(6):770–5. https://doi.org/10.1016/j.rbmo.2015.08.017.
18. Gould RL, Griffin DK. Karyomapping and how is it improving preimplantation genetics? Expert Rev Mol Diagn. 2017;17(6):611–21. https://doi.org/10.1080/14737159.2017.1325736.
19. Konstantinidis M, Prates R, Goodall NN, Fischer J, Tecson V, Lemma T, et al. Live births following Karyomapping of human blastocysts: experience from clinical application of the method. Reprod Biomed Online. 2015;31(3):394–403. https://doi.org/10.1016/j.rbmo.2015.05.018.
20. Fiorentino F, Kahraman S, Karadayi H, Biricik A, Sertyel S, Karlikaya G, et al. Short tandem repeats haplotyping of the HLA region in preimplantation HLA matching. Eur J Hum Genet. 2005;13(8):953–8. https://doi.org/10.1038/sj.ejhg.5201435.

21. Hussey ND, Davis T, Hall JR, Barry MF, Draper R, Norman RJ, et al. Preimplantation genetic diagnosis for beta-thalassaemia using sequencing of single cell PCR products to detect mutations and polymorphic loci. Mol Hum Reprod. 2002;8(12):1136–43. https://doi.org/10.1093/molehr/8.12.1136.
22. Verlinsky Y, Rechitsky S, Schoolcraft W, Strom C, Kuliev A. Preimplantation diagnosis for Fanconi anemia combined with HLA matching. Jama. 2001;285(24):3130–3.
23. Edwards A, Hammond HA, Jin L, Caskey CT, Chakraborty R. Genetic variation at five trimeric and tetrameric tandem repeat loci in four human population groups. Genomics. 1992;12(2):241–53.
24. Marshall E. 'Playing chicken' over gene markers. Science. 1997;278(5346):2046–8. https://doi.org/10.1126/science.278.5346.2046.
25. Litt M, Luty JA. A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. Am J Hum Genet. 1989;44(3):397–401.
26. Kakourou G, Kahraman S, Ekmekci GC, Tac HA, Kourlaba G, Kourkouni E, et al. The clinical utility of PGD with HLA matching: a collaborative multi-centre ESHRE study. Hum Reprod (Oxford, England). 2018;33(3):520–30. https://doi.org/10.1093/humrep/dex384.
27. Little AM, Green A, Harvey J, Hemmatpour S, Latham K, Marsh SG, et al. BSHI Guideline: HLA matching and donor selection for haematopoietic progenitor cell transplantation. Int J Immunogenet. 2016;43(5):263–86. https://doi.org/10.1111/iji.12282.
28. Rechitsky S. Preimplantation genetic diagnosis with HLA matching. Reprod Biomed Online. 2004;9(2):210–21.
29. Goldman KN, Nazem T, Berkeley A, Palter S, Grifo JA. Preimplantation genetic diagnosis (PGD) for monogenic disorders: the value of concurrent aneuploidy screening. Journal of genetic counseling. 2016;25(6):1327–37. https://doi.org/10.1007/s10897-016-9975-4.
30. Rechitsky S, Pakhalchuk T, San Ramos G, Goodman A, Zlatopolsky Z, Kuliev A. First systematic experience of preimplantation genetic diagnosis for single-gene disorders, and/or preimplantation human leukocyte antigen typing, combined with 24-chromosome aneuploidy testing. Fertil Steril. 2015;103(2):503–12. https://doi.org/10.1016/j.fertnstert.2014.11.007.
31. Sermon K. Novel technologies emerging for preimplantation genetic diagnosis and preimplantation genetic testing for aneuploidy. Expert Review of Molecular Diagnostics. 2016;17(1):71–82. https://doi.org/10.1080/14737159.2017.1262261.
32. Hou W, Xu Y, Li R, Song J, Wang J, Zeng Y, et al. Role of aneuploidy screening in preimplantation genetic testing for monogenic diseases in young women. Fertil Steril. 2019;111(5):928–35. https://doi.org/10.1016/j.fertnstert.2019.01.017.
33. Vanneste E, Voet T, Le Caignec C, Ampe M, Konings P, Melotte C, et al. Chromosome instability is common in human cleavage-stage embryos. Nat Med. 2009;15(5):577–83. https://doi.org/10.1038/nm.1924.
34. Ottolini CS, Capalbo A, Newnham L, Cimadomo D, Natesan SA, Hoffmann ER, et al. Generation of meiomaps of genome-wide recombination and chromosome segregation in human oocytes. Nat Protoc. 2016;11(7):1229–43. https://doi.org/10.1038/nprot.2016.075.
35. Ottolini CS, Newnham L, Capalbo A, Natesan SA, Joshi HA, Cimadomo D, et al. Genome-wide maps of recombination and chromosome segregation in human oocytes and embryos show selection for maternal recombination rates. Nat Genet. 2015;47(7):727–35. https://doi.org/10.1038/ng.3306.