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TECHNOLOGICAL INNOVATIONS

Familial haplotyping and embryo analysis for Preimplantation Genetic Diagnosis (PGD) using DNA microarrays: a proof of principle study

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Abstract
Purpose Development of PGD assays for molecular disorders is based on analysis of a familial mutation together with linked polymorphic STR markers; a process which is lengthy and requires the identification of multiple informative markers prior to PGD analysis. On the other hand, whole genome amplification (WGA), in conjunction with microarray platforms, allows the use of a universal assay for the analysis of a very large number of SNP markers at once. The aim of this study was to test high throughput pre-PGD familial haplotyping for in-case blastomere analysis in order to eliminate time-consuming pre-case preparations for each family.
Methods A PGD cycle was performed for a couple with paternal Charcot Marie Tooth 1A (CMT1A) using a classic multiplex nested PCR approach. Mutant embryos from the case were blindly reanalyzed, as single or multi-cell biopsies, using a multiple displacement amplification-based WGA protocol and microarray SNP analysis. In parallel, relevant genomic DNA samples from the family were also analyzed by SNP microarray.
Results After applying a 'unique informative allele' selection algorithm to the data, this array-based assay reconfirmed the initial diagnosis in all samples.
Conclusions We describe a PGD method that is both accurate and feasible during the time-frame required for embryo transfer. This strategy greatly reduces the time for pre-case haplotype preparation.

Keywords Preimplantation genetic diagnosis · SNP microarray · Single cell · Haplotyping · Whole genome amplification

Capsule The classic STR-based method for PGD of monogenic disorders requires time consuming pre-case preparations. In this proof of principle investigation, we describe a SNP microarray-based methodology that accomplishes pre-PGD case preparations in a fraction of the time without compromising on diagnostic accuracy.

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Introduction

Preimplantation genetic diagnosis (PGD) was developed two decades ago for couples at high genetic risk of having affected children. The technique can be performed by blastomere, polar body, or blastocyst biopsy for Mendelian and chromosomal disorders [6]. Since only unaffected embryos are transferred to the uterus, PGD provides an alternative to current post conception diagnostic procedures (amniocentesis or chorionic villus sampling), which can be followed by pregnancy termination. The main causes of misdiagnosis in PGD are occurrence of undetected recombination events and allele dropout (ADO) in single cell analysis, due to unequal allelic amplification. Therefore, molecular PGD relies upon the use of multiple linked polymorphic markers (short tandem repeats [STRs] or single nucleotide polymorphisms [SNPs]) in combination with the specific mutation for diagnosis [17]. The addition of informative polymorphic markers to mutation analysis has been shown to decrease misdiagnosis rates from 3–4 % to 0.3–0.5 % [19].

The PGD process begins by selecting informative markers, in the vicinity of the mutation, based on analysis

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of genomic DNA from the couple together with relevant affected and unaffected family members. Subsequently, a haplotype is built to identify alleles that are linked to the mutation. The use of at least three polymorphic markers surrounding the gene has been shown to decrease the ADO misdetection rate from almost 27 % in blastomeres, if only the mutation is analyzed, to almost 0 % if at least three markers are analyzed [16]. Moreover, by selecting polymorphic markers in the vicinity of the analyzed mutation the chance of misdiagnosis, resulting from an undetected recombination event, is very much reduced [10]. However, for some families, identification of even three informative mutation-proximal polymorphic markers might be challenging, because the gene of interest is located in a less variable genomic region. Furthermore, for couples in inbred populations (such as Ashkenazi Jews, and Bedouins), informative marker selection is further complicated by common allele sharing. Thus, given that informative markers must be tailored not only to the disease but also for each individual family, the family workup process before the PGD cycle can be time consuming and labor intensive.

Not long ago, SNP and comparative genomic hybridization (CGH) arrays were developed for post and prenatal diagnoses, including single cell PGD analysis for Mendelian and chromosomal disorders (reviewed in [15]). These methods are universal and save the necessity for informative marker selection prior to a PGD case. However, array-based technologies require a large amount of starting DNA for analysis, and, as a result, whole genome amplification (WGA) must be performed on single cells as a prerequisite.

Recently, Handyside et al. [5] demonstrated that multiple displacement amplification (MDA) WGA products provide DNA of sufficient quality and quantity for accurate single cell haplotyping analysis by SNP microarray. However, this group did not test this new method on the type of biopsy most frequently used for PGD, namely single blastomeres. Moreover, the feasibility of the technique was assayed only for the diagnosis of an autosomal recessive disorder without further evaluation in the diagnosis of an autosomal dominant disorder. This latter issue, in particular, should be addressed in single cell haplotyping protocols because fewer informative markers are available for autosomal dominant disease diagnosis. Regarding autosomal recessive disease (for which carrier embryo transfers are performed) diagnosticians may choose from two different parental wild type alleles when weighing embryo transfer. Therefore even if a misdiagnosis occurs in one of two wild type alleles, the embryo will be still be a healthy mutation carrier. This scenario must be avoided when diagnosing autosomal dominant disease because only one non-mutation-linked parental allele may be returned to prevent disease in the embryo.

In this study we further develop array-based haplotyping for molecular PGD by demonstrating that the method is, indeed, feasible for the accurate diagnosis of autosomal dominant diseases in single blastomeres. Moreover, we also describe a novel analytical methodology, termed 'unique allele identification,' by which contradictory calls resulting from ADO can be overcome. This, coupled with mutation and whole chromosome analysis, improves fine-mapping of recombination events, thereby facilitating the inclusion of a large number of genetic markers in haplotype construction.

Materials and methods

Patient workup

A patient with Charcot Marie Tooth 1A (CMT1A), due to a duplication on chr. 17p12, presented to our unit for PGD. The presence of the duplication was validated and further investigated by analysis of peripheral blood DNA from the patient, his healthy spouse, and his affected mother. Chromosome copy number variation (CNV) was evaluated by SNP microarray analysis using the Cytogenetics Whole-Genome 2.7M Array™ (Affymetrix, Santa Clara, CA, USA) according to the manufacturer's protocol. Subsequently, polymorphic microsatellite markers surrounding the diseased gene were identified and tested for informativity in the family. Based on informative markers a haplotype map was constructed for the family and a PGD cycle was performed. The panel of markers used for PGD analysis is shown in Fig. 1.

Whole genome amplification and SNP oligonucleotide genotyping

Single and 3–5 cell biopsies were performed on day 4 cleavage stage embryos that were diagnosed as non-transferable. Biopsies were placed in 2 μL of Ca^{2+}- and Mg^{2+}-free phosphate buffered saline and immediately subject to alkaline lysis and MDA, as previously described [12]. MDA samples, together with genomic DNA from the proband and family members described in Patient workup, were then subject to GeneChip 250K Nsp SNP microarray analysis (Affymetrix, Santa Clara, CA, USA). CNV analysis and SNP genotyping were performed using the Genotyping Console version 4 (Affymetrix, Santa Clara, CA, USA) and genotype data was exported to Microsoft Excel for interpretation.

Blastomere biopsy, ICSI and embryo cultures

Blastomere biopsy, ICSI and embryo cultures were performed as previously described [1].

Ethics

Since PGD is a clinical procedure, IRB approval was not required for the performance of a PGD cycle and for the

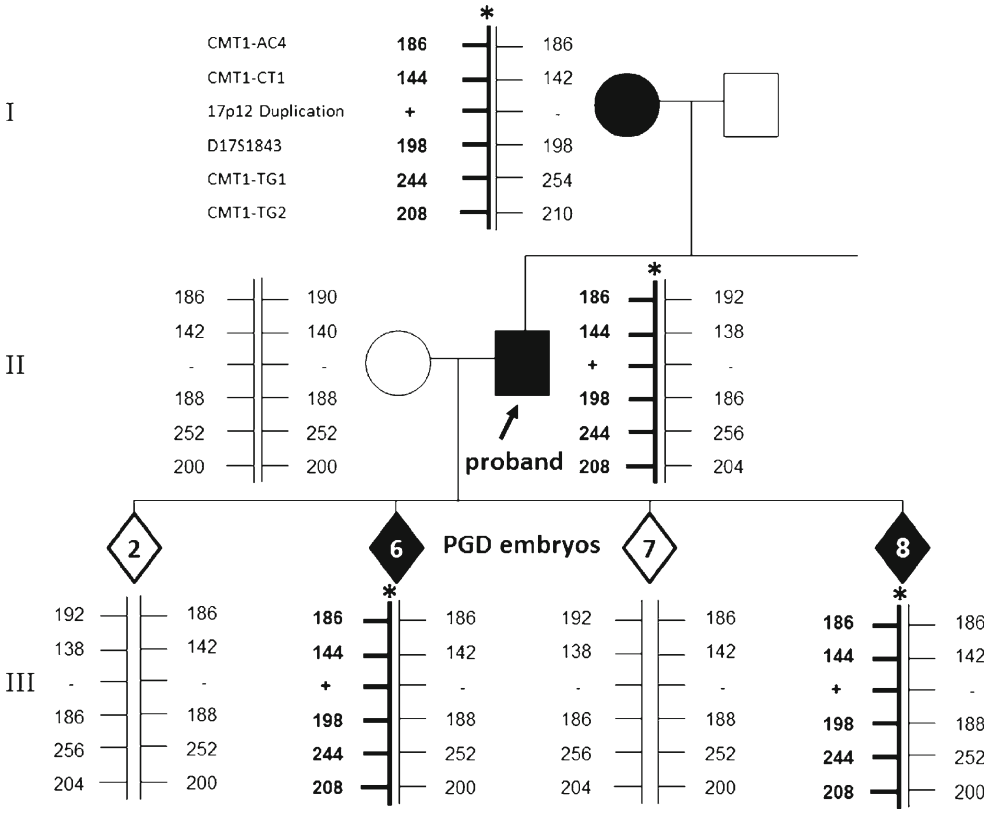

Fig. 1 Family haplotype map based on 5 polymorphic microsatellite markers surrounding the 17p12 duplication (as indicated by (+) when present, and by (−) when absent). The physical position of the duplication is indicated together with 5′ +1 positions of each flanking marker on chromosome 17 (genome build hg19). *Asterisks* (*) indicate the affected allele. Allele length in base pairs is indicated next to each marker for each family member. Generation III depicts four different embryos, as diagnosed by PGD. ADO was not detected at any of the tested loci. Embryos diagnosed with the proband duplication-linked haplotype are represented by shaded diamonds whereas embryos diagnosed with the proband wild type haplotype are not shaded

testing of PGD-related methods in this study. Nevertheless, the family signed a letter of informed consent for all materials described herein.

Results

A male suffering from distal leg muscle weakness, who also has a wheelchair bound brother suffering from the same disorder, presented to our unit with his spouse for PGD. The proband was diagnosed with a duplication on chromosome 17p12 encompassing a region that includes the CMT1A–implicated PMP22 gene. Five informative polymorphic microsatellite markers, surrounding the duplicated region on chr. 17 (within a +/− 2 Mb interval), were identified and used to create a haplotype map for the patient and his family. A classical PGD cycle was performed using duplication-linked microsatellite markers and two embryos were identified as Wild Type (WT) and transferred. Two additional embryos (embryo 6 and embryo 8) were diagnosed as mutant and were used in this study after receiving the family's consent (Fig. 1).

On day 4 post-fertilization, single and multi-cell biopsies were performed on mutant embryos, and these cells were used as a substrate for multiple displacement amplification WGA followed by SNP microarray analysis. The SNP genotype data provided sufficient quality for haplotyping of WGA samples after applying the algorithm described below.

DNA samples from the CMT1A patient, his spouse, and affected mother were subjected to genotyping microarray analysis along with the WGA embryo biopsies. The laboratory personnel performing the embryo genotyping analysis were blinded to our previous results obtained during the PGD cycle. Preliminary qualitative analysis of the SNP array datasets identified high call rates in single cell WGAs (approaching 90 %) that increased, as expected, in multi-cell WGAs (to 92 % and 96 % in embryo 8 and embryo 6, respectively; Table 1). ADO was assessed in embryo biopsies by analyzing SNP markers for which the proband and his spouse were homozygous for opposite alleles requiring obligate heterozygote SNPs in the embryo. Thus, homozygote calls on the same loci were indicative of ADO. Using this qualitative analysis we found that single cell biopsies presented with ~30 % ADO while the multi-cell biopsies presented with much lower ADO rates (3 % and 18 % in embryo 8 and embryo 6, respectively; Table 1). These levels of ADO, however, did not interfere with genotyping analysis due to the relatively high number of informative SNPs that distributed rather evenly around the locus of interest (see below). In contrast, CNV analysis of these samples was inconclusive due to noisiness resulting from numerous

Table 1 Qualitative analysis of SNP microarray datasets

Sample	Affected Father (Proband)[a]	Mother[a]	Affected Mother of Father[a]	Embryo 6-single[b]	Embryo 6-multi[b]	Embryo 8-single[b]	Embryo 8-multi[b]
Number of Calls (n, %) (Total number of probe sets=262,264)	259,274 (98.86)	254,160 (96.91)	258,277 (98.48)	235,486 (89.79)	253,215 (96.55)	235,932 (89.96)	242,515 (92.47)
SNPs used for ADO analysis (n, %) [parents homozygous for opposite allele]	14,204	14,204		11,246 (79.17)	13,042 (91.82)	11,379 (80.11)	11,810 (83.15)
ADO (n, %)				3,731 (33.18)	398 (3.05)	3,141 (27.06)	2,116 (17.92)

[a] peripheral blood genomic DNA

[b] blastomere/s biopsy after WGA

preferential amplification and ADO events in the SNP data. This challenge of CNV analysis with MDA-based single cell WGA samples has been addressed at length in previous studies [5,7,9,11,14].

In order to address the genetic status of the embryo biopsies, a mutant haplotype was first assembled on chr. 17 by comparing heterozygote SNPs on the proband's dataset with homozygote SNPs, on the same loci, from his affected mother's dataset. Figure 2 illustrates this selection process for informative paternal mutant SNP marker identification. Subsequently, the patient's spouse was used to construct haplotypes on each of the biopsies as shown in Fig. 3. Paternal informative SNPs, for which the spouse was homozygotic at the same locus, were used for haplotyping the embryo biopsies. However, owing to the possibility of ADO in the embryo genotype, some calls could not be used to construct the embryo haplotypes. Since all informative SNPs were, by definition, heterozygote in the affected father, and homozygote in the healthy spouse, loci in which the embryo's genotype appeared to be homozygous similar to

Fig. 2 Identification of disease/paternal-informative SNPs. A representative list of SNP genotypes on a random 19 SNP region of chr. 17 from the proband and mother-of-proband microarray datasets. Both the proband and his mother are affected with CMT1A stemming from a duplication on chromosome 17p12. Accordingly, a duplication-linked paternal/proband haplotype was constructed by identifying heterozygous proband loci that were homozygous in the mother-of-proband at the same loci (*red circles*)

the healthy mother could not be definitively called. This is because it is not possible to differentiate between a true homozygote genotype at this locus in the embryo or a false homozygote owing to ADO of the father's allele ("*" haplotype calls in Fig. 3). These possible ADO genotypes were eliminated from our analysis. Nevertheless, there were other obvious instances of ADO that did not interfere with accurate haplotyping, and were not excluded from the analysis. Accordingly, for SNPs in which the embryo genotype was called as a homozygote for the unique paternal allele, it was obvious that ADO of the mother's allele had occurred. In these situations, the father's allele was still useful for constructing the paternal inherited haplotype (which, in this case, was the haplotype of interest; see italicized "*A*" haplotype call in Fig. 3). This novel analytical methodology, which allows allele determination despite some cases of ADO, is termed hereafter as 'unique allele identification.'

Using unique allele identification genotyping analysis on all of chromosome 17, paternal haplotypes were constructed for each embryo biopsy. These entire chromosome haplotypes consisted of approximately 100 SNPs per biopsy out of a total of 4,844 chr17 SNP probes on the array. Noticeably, unique alleles from the proband mutant haplotype were predominantly represented in all embryo biopsies (Fig. 4). The average distance between these haplotype informative markers ranged from 0.75 to 0.81 Mb in single blastomere biopsies and from 0.58 to 0.75 Mb in multi-cell biopsies, substantially reducing the risk of recombination events that could potentially distort the analysis. Importantly, the same analysis also identified 10–16 mutation linked SNPs per biopsy (out of 17 mutation informative SNP probes) flanking the disease-causing mutation (+/− 2 Mb). None of the 26 mutation-proximal WT haplotype informative SNPs were detected in any of the biopsies.

Two distant recombination events were identified in all samples at +13.88 Mb and −54.94 Mb from the CMT1A disease-associated gene. This region included 71–88 mutation informative SNPs between recombination sites. Moreover, the proband's mutant haplotype was clearly identified in the non-recombined regions surrounding the locus of interest in all embryo biopsies confirming the previous PGD case results (Fig. 5). Overall, this SNP-array based diagnostic procedure was completed in 56 net hours.

Discussion

This proof of principle study shows that unique allele identification, in SNP array-based whole chromosome embryo haplotyping, may effectively replace classical STR-based approaches for the diagnosis of CMT1A. The user-friendly methodology we describe is universal and should be fit for implementation in PGD of other monogenic disorders as well. Future application of our technique in multiple clinical

Mother (spouse of proband) genotype	Affected Father/Proband genotype	Embryo 6-single genotype	Embryo 6-single paternal inherited haplotype	Embryo 6-multi genotype	Embryo 6-multi paternal inherited haplotype
BB	AB	BB	*	AB	A
AA	AB	AB	B	AB	B
BB	AB	No Call		AB	A
AA	AB	AB	B	AB	B
BB	AB	AA	*A*	No Call	
BB	AB	BB	*	BB	*
BB	AB	BB	*	BB	*
BB	AB	BB	*	BB	*
BB	AB	BB	*	BB	*
AA	AB	AA	*	AB	B
AA	AB	AA	*	AB	B
BB	AB	BB	*	BB	*

Fig. 3 Identification of paternal/proband transmitted haplotypes in PGD embryos by SNP microarray analysis. A representative list of microarray dataset SNP genotypes on chr. 17 are shown for the proband (father), his spouse (mother), and embryo no. 6 (see Fig. 1) single blastomere (embryo 6-single) and multi-blastomere (embryo 6-multi) biopsies. Paternal/proband inherited haplotypes were derived for both single and multi-cell biopsies. A single, *non-italicized letter* indicates that the paternal/proband transmitted allele was identified and ADO was not detected at the locus. A single, *italicized* letter indicates that maternal ADO was detected at the locus, but the paternal/proband transmitted allele was identified nonetheless. An *asterisk* (*) indicates that paternal allele transmission could not be resolved due to the possibility that ADO had occurred at the locus

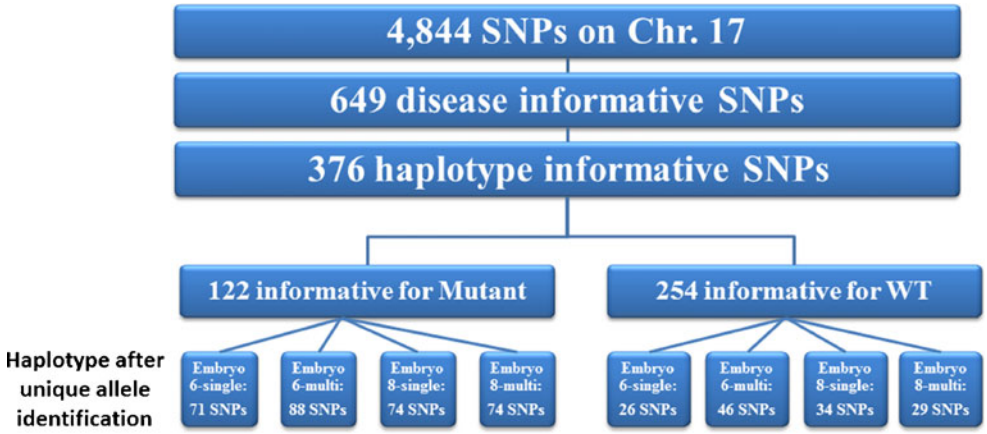

Fig. 4 Genetic marker selection for whole chromosome haplotyping. The microarrays used in this study provided 4,844 SNPs for analysis of the entirety of chr. 17. Approximately 100 of these SNPs were used to correctly haplotype blastomere biopsies. The selection process for the identification of these SNPs was as follows. First, disease informative SNPs, for which the proband (or embryo father) was heterozygous and the CMT1A-affected mother of proband was homozygous (see Fig. 2), were selected. Next, haplotype informative SNPs, for which the healthy spouse of proband (mother) was homozygous at the same loci as the disease informative SNPs, were identified. These SNPs were then subdivided into mutation-linked and WT-allele linked groupings. Technically, all of the haplotype informative SNPs could be used to generate a paternal haplotype in the descendents of the proband and his spouse. However, due to the possibility that ADO may lead to misleading genotypes in single cell embryo biopsies, 'unique allele identification' was performed as in Fig. 3 in order to select fully informative genetic markers for haplotyping of cleavage stage embryos. The numbers of SNPs in these unique allele identified haplotypes are indicated, for each biopsy

cases will be required to validate this hypothesis going forward.

In theory, array-based whole chromosome haplotyping should facilitate the diagnosis of multiple genomic loci (for HLA matching) and multiple monogenic disorders in parallel, without compromising on accuracy. Indeed, some of the major advantages of this method are speed and enhanced diagnostic confidence. These advantages arise from the ability of SNP arrays to provide a large sampling of informative genetic markers per embryo in high throughput. Thus, the time for pre-case family workup is much reduced for cleavage stage embryo diagnosis because accurate family haplotype maps can be constructed in one assay prior to the commencement of a PGD cycle. This array-based pre-case genetic workup saves time in comparison with traditional molecular PGD protocols because it does not demand new assay design, calibration, and testing for rare disorder diagnosis. On the other hand, at the present time the monetary cost of this method, per embryo, is relatively high due to marked up microarray prices. This drawback makes it difficult for researchers to conduct large-scale studies using array-based PGD. However, we do anticipate that the cost, per array, will decrease in the future, at which time our analytical methodology will be readily available for implementation on a larger scale.

The SNP arrays used in this study analyzed ~260,000 different loci across the entire genome with thousands of genetic markers dispersed throughout each chromosome (except for chromosome Y). In classical STR-based PGD, 3–8 genetic markers are chosen flanking the mutation (usually within 2 Mb) due to the possibility that meiotic recombination will confound genotyping analysis [2]. However, SNP arrays provide more than sufficient SNP markers surrounding any relevant mutation (typically 400–500 SNP probes per gene locus +/− 2 Mb), and in addition overcome the problem of recombination by mapping recombination events across entire chromosomes due to sufficiently high resolution coverage of the genome [3]. In some cases, a single recombination event will occur in close proximity of the gene of interest [2], but rarely does this occur twice in the same region. In order to account for this possibility, the average interval between SNP loci should be under 1 Mb so as to ensure that at least 4 markers will be available for haplotyping within +/− 2 Mb of a relevant gene. If this resolution is not obtained with 260,000 markers on a typical genotyping array, then embryos do not need to be re-biopsied for diagnosis. Rather, theoretically familial genomic DNA and embryo WGA samples may be further probed with higher resolution commercially available SNP arrays for subsequent embryo transfer in later IVF cycles.

A previous study [5] described a whole chromosome haplotyping technique similar to that of the current report, with a few notable differences. Handyside et al. utilized their technique, termed 'karyomapping,' to diagnose blastocyst biopsies and whole cleavage stage embryos for the autosomal recessive disorder, cystic fibrosis. In preliminary experiments on single lymphoblastoid cells, they reported higher resolution coverage of informative SNPs on chromosome 1 (0.18 Mb avg. SNP interval) than that on chromosome 17 (0.75–0.81 Mb SNP interval in blastomeres) in this report.

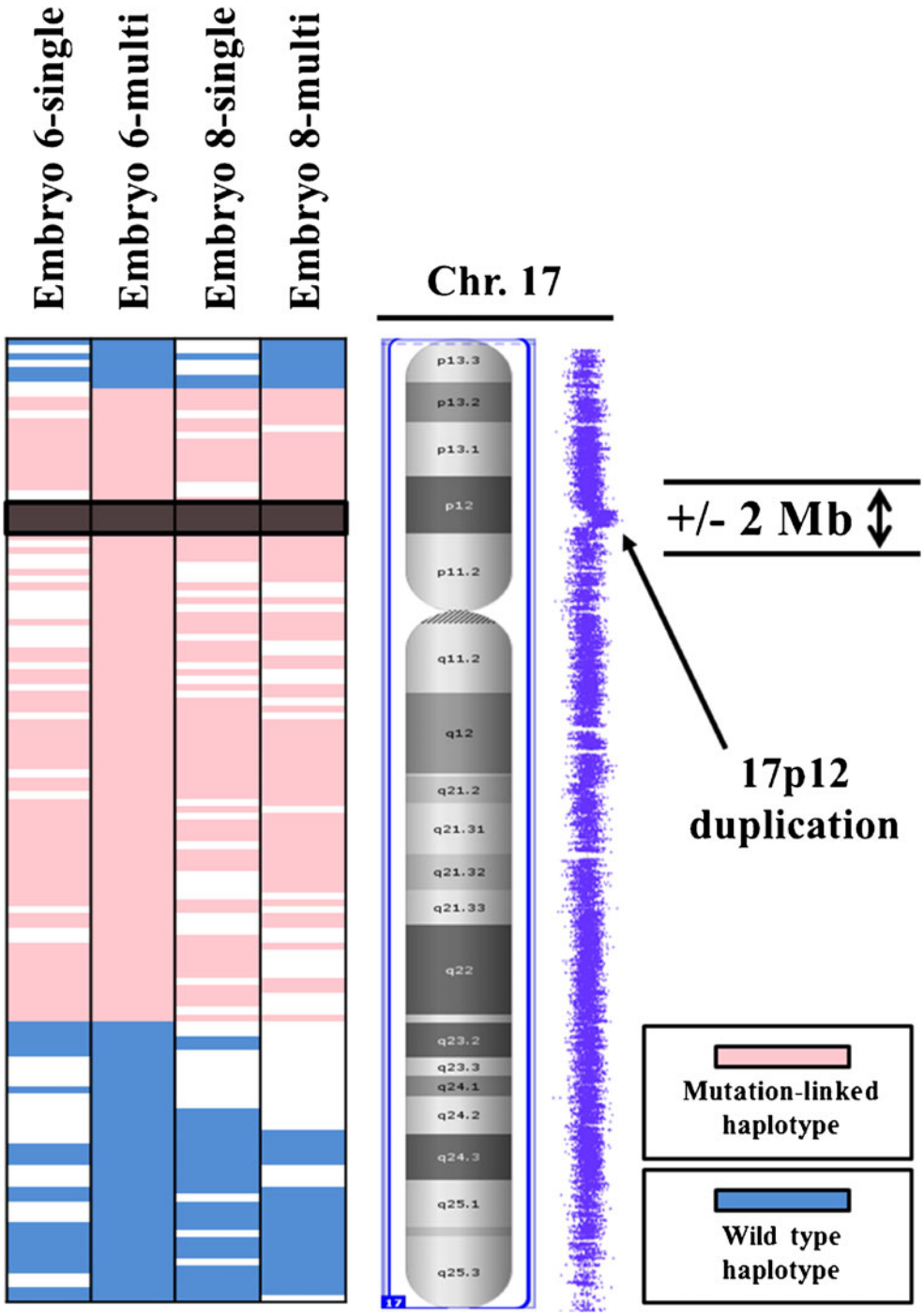

Fig. 5 Graphical representation of array-based whole chromosome haplotyping analysis of embryo biopsies. The proband mutant and wild type haplotypes are shown for each embryo biopsy, as indicated. Blank regions were not haplotyped due to ADO or failed genotype calling. An ideogram of chr. 17 and CNV analysis of proband genomic DNA are juxtaposed to the whole chromosome haplotyping results for reference

While this difference may be attributed to differing cell types analyzed (lymphoblast vs. blastomere), microarray platforms utilized (Illumina vs. Affymetrix), and differing array densities tested (~310,000 SNPs vs. ~260,000 SNPs) in the two studies, the most significant factor effecting the large observed discrepancy was the assayed mode of inheritance. Handyside et al. tested the inheritance from both parents, as appropriate for diagnosis of autosomal recessive disease (providing haplotyped SNPs from both parents); and this report tested solely paternal/proband inheritance, as appropriate for autosomal dominant disease diagnosis. Indeed, once both parents were analyzed in the current report dataset, the informative SNP density (0.12 Mb avg. SNP interval on chromosome 17; data not shown) was comparable to that of Handyside et al. and even slightly higher. This slight improvement in SNP coverage can then be explained by the different SNP selection methodologies that were employed by both groups. Handyside et al. used a stringent algorithm

to account for ADO-related errors by incorporating only heterozygote embryo SNP calls into haplotyping analysis, whereas the current study demonstrates that both heterozygote and homozygote calls (indicating ADO that is not relevant to disease diagnosis; for example, see italicized "*A*" haplotype call in Fig. 3) can be incorporated into the analysis without compromising diagnostic accuracy. Another important lesson gleaned from this study is that whole chromosomal haplotyping can be effective on blastomere biopsies. This notable difference from the report of Handyside et al. (which did not assess single blastomeres) suggests that embryo diagnosis can be performed within a short enough window to allow for fresh embryo transfer during a PGD case (see below).

Altogether, the workflow for cleavage stage embryo haplotyping was performed over a 72 h time period in this report (56 net hours). Thus, a PGD case could theoretically begin with a single blastomere biopsy on day 3 and end with diagnosis and transfer of a blastocyst stage embryo on day 6 (72 h). However, the current workflow may also be adjusted to allow completion in less than 48 h as well. For example, WGA was performed with a conservative 16 h 30 °C incubation step in this study, but we and others [13] have found that this can be replaced by a 4 h incubation that is equally effective. Hence, this rapid WGA protocol would allow the entire process to complete in only 44 h thereby enabling diagnosis and transfer of day 5 cleavage stage embryos. In addition, some of the incubation steps in the SNP array sample preparation workflow can also be shortened so as to further narrow the window of diagnosis.

Interestingly, recent studies have shown that fresh embryo transfer does not necessarily lead to higher implantation and pregnancy rates when compared with blastocyst transfer of warmed vitrified embryos [8,20] With this obstetrical background in mind, it may be even more advantageous to biopsy blastocysts, ahead of vitrification, for array-based diagnosis. For one, multi-cell biopsies such as blastocysts are more likely to present with higher quality SNP-array data because they feature lower amplification failure and ADO rates than single cell biopsies. Secondly, if embryo vitrification is decided upon at the outset of a PGD case, the pressure of achieving a genetic diagnosis in time for fresh embryo transfer is eliminated. Thus, multiple options exist to facilitate the practical incorporation of array-based haplotyping in PGD analysis.

It should be noted that the array-based protocol, described here, can provide CNV information together with single gene data. Although the CNV analysis in the current study was noisy, various protocols for performing 24 chromosome preimplantation genetic screening (PGS) on single cell MDA samples have recently been developed [4,9,11,15,18] and these protocols are all amenable to the single gene array-based PGD methodology described here. Thus, it appears that the means for full and accurate genetic analysis of cleavage stage embryos are now in place, and soon it will be possible to transfer comprehensively diagnosed embryos during a combined molecular PGD and PGS cycle.

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Conflicts of interest The authors declare that they have no conflict of interest.

References

1. 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. Hum Reprod. 2006;21(8):2047–51. doi:10.1093/humrep/del064.
2. Altarescu G, Eldar Geva T, Brooks B, Margalioth E, Levy-Lahad E, Renbaum P. PGD on a recombinant allele: crossover between the TSC2 gene and 'linked' markers impairs accurate diagnosis. Prenat Diagn. 2008;28(10):929–33. doi:10.1002/pd.2070.
3. Chowdhury R, Bois PR, Feingold E, Sherman SL, Cheung VG. Genetic analysis of variation in human meiotic recombination. PLoS Genet. 2009;5(9):e1000648. doi:10.1371/journal.pgen.1000648.
4. Fragouli E, Wells D. Aneuploidy screening for embryo selection. Sem Reprod Med. 2012;30(4):289–301. doi:10.1055/s-0032-1313908.
5. 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. doi:10.1136/jmg.2009.069971.
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. doi:10.1038/344768a0.
7. Iwamoto K, Bundo M, Ueda J, Nakano Y, Ukai W, Hashimoto E, et al. Detection of chromosomal structural alterations in single cells by SNP arrays: a systematic survey of amplification bias and optimized workflow. PLoS One. 2007;2(12):e1306. doi:10.1371/journal.pone.0001306.
8. Jin R, Tong X, Wu L, Luo L, Luan H, Zhou G, et al. Extended culture of vitrified-warmed embryos in day-3 embryo transfer cycles: a randomized controlled pilot study. Reprod Biomed Online. 2013;26(4):384–92. doi:10.1016/j.rbmo.2012.12.003.
9. Johnson DS, Gemelos G, Baner J, Ryan A, Cinnioglu C, Banjevic M, et al. Preclinical validation of a microarray method for full molecular karyotyping of blastomeres in a 24-h protocol. Hum Reprod. 2010;25(4):1066–75. doi:10.1093/humrep/dep452.
10. Kakourou G, Dhanjal S, Daphnis D, Doshi A, Nuttall S, Gotts S, et al. Preimplantation genetic diagnosis for myotonic dystrophy type 1: detection of crossover between the gene and the linked marker APOC2. Prenat Diagn. 2007;27(2):111–6. doi:10.1002/pd.1611.
11. Konings P, Vanneste E, Jackmaert S, Ampe M, Verbeke G, Moreau Y, et al. Microarray analysis of copy number variation in single cells. Nat Protoc. 2012;7(2):281–310. doi:10.1038/nprot.2011.426.
12. Renwick PJ, Trussler J, Ostad-Saffari E, Fassihi H, Black C, Braude P, et al. Proof of principle and first cases using preimplantation

genetic haplotyping–a paradigm shift for embryo diagnosis. Reprod Biomed Online. 2006;13(1):110–9.
13. Spits C, Le Caignec C, De Rycke M, Van Haute L, Van Steirteghem A, Liebaers I, et al. Optimization and evaluation of single-cell whole-genome multiple displacement amplification. Hum Mutat. 2006;27(5):496–503. doi:10.1002/humu.20324.
14. Treff NR, Su J, Tao X, Northrop LE, Scott Jr RT. Single-cell whole-genome amplification technique impacts the accuracy of SNP microarray-based genotyping and copy number analyses. Mol Hum Reprod. 2011;17(6):335–43. doi:10.1093/molehr/gaq103.
15. Vanneste E, Bittman L, Van der Aa N, Voet T, Vermeesch JR. New array approaches to explore single cells genomes. Front Genet. 2012;3:44. doi:10.3389/fgene.2012.00044.
16. Verlinsky O, Kuliev A. Atlas of preimplantation genetic diagnosis. 2nd ed. London: Taylor & Francis; 2004.
17. Verlinsky Y, Cohen J, Munne S, Gianaroli L, Simpson JL, Ferraretti AP, et al. Over a decade of experience with preimplantation genetic diagnosis. Fertil Steril. 2004;82(2):302–3. doi:10.1016/j.fertnstert.2004.02.108.
18. Voet T, Vanneste E, Van der Aa N, Melotte C, Jackmaert S, Vandendael T, et al. Breakage-fusion-bridge cycles leading to inv dup del occur in human cleavage stage embryos. Hum Mutat. 2011;32(7):783–93. doi:10.1002/humu.21502.
19. Wilton L, Thornhill A, Traeger-Synodinos J, Sermon KD, Harper JC. The causes of misdiagnosis and adverse outcomes in PGD. Hum Reprod. 2009;24(5):1221–8. doi:10.1093/humrep/den488.
20. Zhu D, Zhang J, Cao S, Zhang J, Heng BC, Huang M, et al. Vitrified-warmed blastocyst transfer cycles yield higher pregnancy and implantation rates compared with fresh blastocyst transfer cycles-time for a new embryo transfer strategy? Fertil Steril. 2011;95(5):1691–5. doi:10.1016/j.fertnstert.2011.01.022.