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Review

Karyomapping and how is it improving preimplantation genetics?

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Abstract

Introduction: Preimplantation genetic diagnosis and screening (PGD/PGS) has been applied clinically for >25 years however inherent drawbacks include the necessity to tailor each case to the trait in question, and that technology to detect monogenic and chromosomal disorders respectively is fundamentally different.

Areas Covered: The area of preimplantation genetics has evolved over the last 25 years, adapting to changes in technology and the need for more efficient, streamlined diagnoses. Karyomapping allows the determination of inheritance from the (grand)parental haplobocks through assembly of inherited chromosomal segments. The output displays homologous chromosomes, crossovers and the genetic status of the embryos by linkage comparison, as well as chromosomal disorders. It also allows for determination of heterozygous SNP calls, avoiding the risks of allele dropout, a common problem with other PGD techniques. Manuscripts documenting the evolution of preimplantation genetics, especially those investigating technologies that would simultaneously detect monogenic and chromosomal disorders, were selected for review.

Expert Commentary: Karyomapping is currently available for detection of single gene disorders; ~1000 clinics worldwide offer it (via ~20 diagnostic laboratories) and ~2500 cases have been performed. Due an inability to detect post-zygotic trisomy

reliably however and confounding problems of embryo mosaicism, karyomapping has yet to be applied clinically for detection of chromosome disorders.

Keywords: PGT, Karyomapping, PGD, PGS, IVF

1. Introduction

1.1 The need for karyomapping

Over the last 25 years or more, one of the main advances in assisted reproduction technology (ART) has been the development of preimplantation genetics. Preimplantation genetics refers to the genetic profiling of oocytes or embryos before transfer into the uterus, through use of cytogenetic and/or molecular biology techniques [1]. These technologies collectively allow for the diagnosis of monogenic defects, chromosome copy number abnormalities and/or unbalanced segmental chromosomal rearrangements in a bid to eliminate or at least reduce the risk of affected live-born individuals, implantation failure and pregnancy loss [2-4]. The technique was first introduced in the late 1960s as proof of principle allowing the successful birth of selectively sexed rabbits [5]. However, it wasn't until 1990 that this technology was successfully applied clinically with the use of sex selection for two couples at risk of transmitting X-linked disorders Adrenoleukodystrophy (ALD) and X-linked mental retardation [6]. At the same time, Verlinsky and colleagues described a protocol for polar body testing for patients at risk of transmitting PI type ZZ alpha-1-antitrypsin deficiency (AATD). Although no pregnancies were established, this study showed that proof of principle for polar body testing for monogenic disease [7]. Later Handyside and colleagues reported the first live birth following PGD for a single gene disorder, by screening for the deltaF508 mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene [8].

Typically, clinical use of preimplantation genetics is divided into preimplantation screening (PGS) and preimplantation genetic diagnosis (PGD). PGS (or PGD-A) refers to non-targeted technologies that detect chromosome abnormalities (principally aneuploidy), whereas PGD generally pertains to the detection of single gene disorders, translocations and Human Leukocyte Antigen (HLA) matching for immunological compatibility testing [9-11]. This technology was applied clinically for

the first time for the treatment of Fanconi anaemia (FA) by HLA matching [12] and has since been shown in many clinical studies that PGD in combination with HLA typing is an effective therapeutic tool for treatment of an affected sibling [13-16]. PGD is theoretically applicable to any monogenic disorder, but the most common are cystic fibrosis, beta-thalassemia, myotonic dystrophy, Huntington's disease and fragile X syndrome [11, 17]. Several studies have reported the combining the detection of single gene disorders and chromosomal abnormalities in IVF samples [18-21]. Rechitsky and colleagues first described the simultaneous detection of chromosome copy number and cystic fibrosis [22], however the ultimate goal of *a single test that would simultaneously detect monogenic and chromosomal disorders* had been a key aim of preimplantation genetics for many years [23, 24]. Karyomapping has provided this opportunity.

1.2 Technical issues associated with PGD/PGS

The development of polymerase chain reaction (PCR) and the increase in its sensitivity to be applicable to single cells paved the way for interrogation of target genomic sequences that code for known genetic defects [25, 26]. DNA is released from a lysed cell that has been collected following embryo biopsy on day three or day five of development and the relevant locus is amplified to a detectable level. Traditionally, the amplified DNA is then analysed for the presence or absence of the mutation [27]. This process has a series of technical challenges as the small amount of DNA (5–10pg) found in a single cell increases the risk of DNA contamination, amplification failure [25]. A major drawback that imposed limitations on single cell PGD is allele dropout (ADO), which can have a significant impact on diagnostic accuracy. ADO is the failure to amplify one of two alleles at a heterozygous locus, thereby making what should be a heterozygous call appear homozygous. The issue of ADO was also highlighted by Rechitsky et al. who investigated the incidence of ADO in polar body and blastomere testing. This study emphasised the importance of determining ADO frequency for all loci to avoid cases of misdiagnosis [28].

ADO is a particular problem single cell PCR due to the low amount of starting DNA [27]. In the original clinical PGD study [6] PCR was used to perform sex determination through the amplification of repetitive Y-specific sequences that

provided a larger original target sequence on which to work. This technique was however found to be susceptible to amplification failure and contamination, which can lead to misdiagnosis. Even with newer approaches to detecting sex chromosome sequences (e.g. [29-32]) similar problems of misdiagnosis remained. Greater specificity was achieved through nested PCR [8], which, in part, allowed for the detection of sequence-specific changes. Early examples included detecting the causative mutations associated with cystic fibrosis and Alpha-1 Antitrypsin (A1AT) deficiency [8]. This heralded an era of mutation detection in PGD (e.g. [33-36]).

1.3 The utility of polymorphic linked markers to increase diagnostic accuracy

Data gleaned from the sequencing of the human genome [37-40] identified polymorphic markers across the genome that could be used in a multiplex PCR protocol for single cell diagnosis [24]. Targeted haplotyping of the embryo through multiplex PCR of short tandem repeat (STR) markers provided increased accuracy of testing and minimized potential errors caused by undetected allele dropout (ADO) or contamination [24, 41]. This is due to the fact that the markers close to the affected gene, when compared through linkage analysis by establishing the variant present in the affected parental DNA, provided verification of the results received from the direct mutation detection [24]. Such analyses were however limited to the number of PCR experiments that could reasonably be performed and genome-wide analyses e.g. with SNP microarrays (see later) greatly increased the utility of linkage-based analyses for preimplantation genetics.

Furthermore, technical advances in the amplification of whole genomic DNA (see later), as well as the development of fluorescent PCR, allowed an increase in the number of additional informative linked markers, which subsequently increased the accuracy of the test [17, 42, 43]. Harper and colleagues reported a misdiagnosis rate of 10/3727 (0.27%) between 1997 and 2007 after embryo transfer that was then determined to be from contamination or allele dropout [27, 44]. One of the main uses of multiplex strategies in part using polymorphic markers was in order to provide analysis of the Human Leukocyte Antigen (HLA) region in order to match embryos to affected children [20, 45]. Thus, the method of using closely linked STR markers flanking the gene of interest became established as the gold standard method for

PGD at the turn of the century in comparison to other technologies [23, 46]. An underlying problem of the above approach is that the development of a robust, accurate multiplex PCR test that is patient, disease or locus specific, is labour intensive and time consuming. Therefore, the couple typically had to wait for a significant amount of time, sometimes several months, before a treatment cycle could take place. This delay can cause much stress to them and possibly a reduction in fertility potential, especially couples with advanced maternal age, as they wait for test completion. Further to this, this targeted approach provided little information about chromosome copy number, which is known to be a major contributing factor of implantation failure, recurrent miscarriage and mental retardation [24, 47-50]. Karyomapping was developed to circumvent these problems.

1.4 Detection of chromosome copy number

The first recorded case of PGS in non-humans was in fact a chromosomal diagnosis [5], by detecting the Barr Body in rabbit blastocysts. In a clinical setting, following the ultimately unsuccessful attempt to sex biopsy samples reliably using Y specific PCR [6], the group of Joy Delhanty introduced fluorescent *in-situ* hybridisation (FISH) for the sex chromosomes in preimplantation embryos using X and Y chromosome specific probes [51]. This was followed in 1992 by simultaneous detection of these chromosomes for the application of sexing human preimplantation embryonic nuclei [52-54] and between 1992 and 1994, twenty-seven treatment cycles were completed using this technique that resulted in nine pregnancies and five female live births [53-55]. FISH was subsequently applied to aneuploidy screening and mosaicism detection in human preimplantation embryos, chiefly by Munné and colleagues [49, 56-58] and this method for PGS was the most popular approach for the following 15 years. It was applied for patients with indications of advanced maternal age, recurrent miscarriage and recurrent implantation failure. The technique (Figure 1) used a non-targeted approach, initially screening for e.g. chromosomes 13, 16, 18, 21, 22 (X and Y) [49, 56-58]. However, this technology was the subject of controversy in the field [59], with retrospective analysis suggesting benefits in some clinics but randomized trial data suggesting that there was no demonstrable clinical benefit to performing chromosomal screening. Most controversially, a study

suggested that there was a detrimental effect of performing PGS [60], with others showing at least no beneficial effect and, when results were backed up by larger trials [61-68], confidence in the technology was lost [69]. Approaches to screening for chromosome changes across the whole genome thus became a priority.

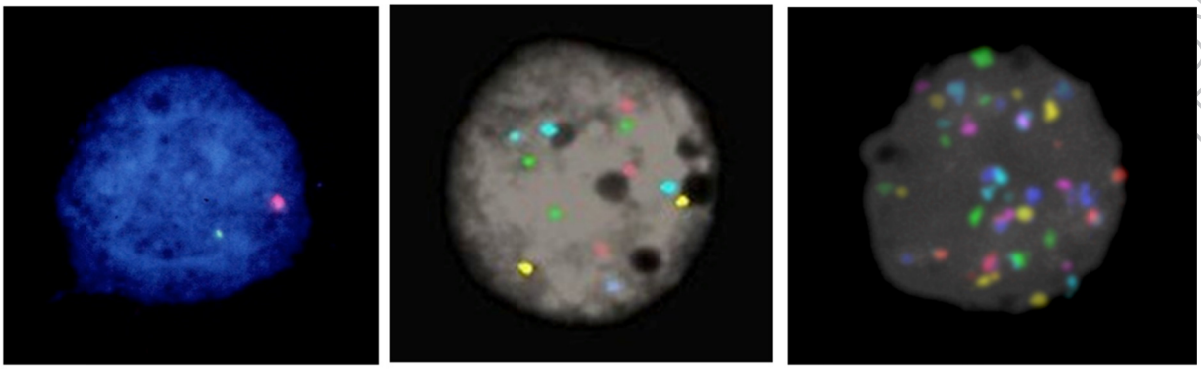

Figure 1. Three views of FISH on human embryos. Left - sexing with X and Y specific probes; middle classic approach to screening for chromosome disorders: 5 or more colour FISH; right – 24 chromosome FISH with 4 consecutive layers of 6 colour probes embryos [70, 71].

Although 24 chromosome FISH has been demonstrated in human embryos [70, 71] problems with overlapping signals, failed probes, complex imaging systems etc. means that FISH should now largely be considered a research tool for e.g. assessing levels of mosaicism and nuclear organization. Although it is nonetheless still performed in a few clinics around the world for PGS, genome-wide analysis by aCGH and ultimately genome sequencing (see below) is now the state of the art.

In order to perform such a genome-wide analysis then amplification of the whole genome from a single or small number of cells was an essential pre-requisite. The development of techniques such as multiple displacement amplification (MDA) and PCR library whole genome amplification for the amplification of minute quantities of DNA from single cells revolutionised not only PGS but PGD also [26, 72], opening the door for the opportunity to investigate the use of genome-wide methods for mutation and/or chromosome copy number analysis [36].

Comparative genomic hybridisation (CGH) is a cytogenetic technique that took the principles of FISH one step further, by using differentially labelled test and control

(normal) DNA, that are competitively hybridized to metaphase chromosomes [73]. The signal intensity of the test DNA is then measured against that of the reference for each chromosome using computer software, thereby permitting the identification of copy number abnormalities [74]. This technology was initially used in 1992 to detect copy number changes in solid tumours and was at the forefront of cancer genetics research [75, 76]. Unlike other traditional techniques such as FISH, CGH allowed for the more rapid assessment of chromosome copy number in the entire genome [77]. However, one of the main limiting factors of this technology was the resolution, which was is limited to approximately 5-10Mb in most clinical applications [74, 78].

Array comparative genomic hybridisation (aCGH) is a means of aneuploidy screening across the whole karyotype and involves WGA of biopsied cells followed by fluorescent labelling of both a test DNA sample (green) and a reference (normal) DNA sample (red) [79]. These samples are then allowed to hybridize to a tiling path microarray and the colour ratio is determined in order to identify whole or segmental chromosome copy number differences within the test sample. Therefore, aCGH allows for aneuploidy screening as well as identification of deletions and duplications of specific chromosomal regions [80-83]. In parallel to the development of aCGH, a general shift in preference for the timing of biopsy from the cleavage to the trophectoderm stage (and less commonly to the polar body stage) has largely brought about a renaissance in PGS, however the technology cannot detect a loss or gain of an entire set of chromosomes (e.g. triploidy). A study by Munné and colleagues determined that around 1.8% of embryos (n=91) were homogenously polyploid with no other detectable abnormalities [84-86]. Furthermore, the problem of chromosomal mosaicism (where embryos have populations of normal and abnormal cells) remains. Given the complexities of mosaicism however it is beyond the scope of this review to discuss in detail.

An alternative method for detecting chromosome copy number was developed and validated by Treff and colleagues using real-time quantitative PCR (RT-qPCR) [87-89]. RT-qPCR entails a pre-amplification step, followed by a high-order multiplex PCR reaction to amplify two regions on each arm of all the chromosomes. Rapid quantification of each product using RT-qPCR then allows for the evaluation of copy number over the whole genome [36]. One unique feature of this technology is that

the PCR is performed directly on the sample, without any whole genome amplification (WGA) step first unlike other technologies such as aCGH and SNP microarrays (see below); minimising the risk of misdiagnosis through artefacts known to be introduced by WGA technology [90]. However, it is important to note that due to this, RT-qPCR can only be used on trophectoderm samples, meaning that sufficient blastocyst embryos need to be available [36, 89].

The availability of benchtop sequencing technology allowed for the development of next generation sequencing (NGS) technology for chromosome screening. This technique involves fragmentation of the whole genome amplified DNA into small pieces (100–200 base pairs). These fragments are then sequenced using fluorescent signals to indicate the relevant sample, an approach that can be achieved at very low cost. This occurs until a sufficient sequencing depth has been achieved [91]. The sequence data across the genome are compared with a reference genome and then counted with the use of specialist software [91]. The number of sequences from a specific chromosome is proportional to chromosome copy number, therefore trisomy or monosomy will result in greater or lower numbers of reads, respectively [90, 92]. This allows for both whole chromosome aneuploidy and segmental imbalances to be detected [93], has a greater dynamic range than aCGH (Figure 2) and is the technology currently utilised in most modern PGS cycles.

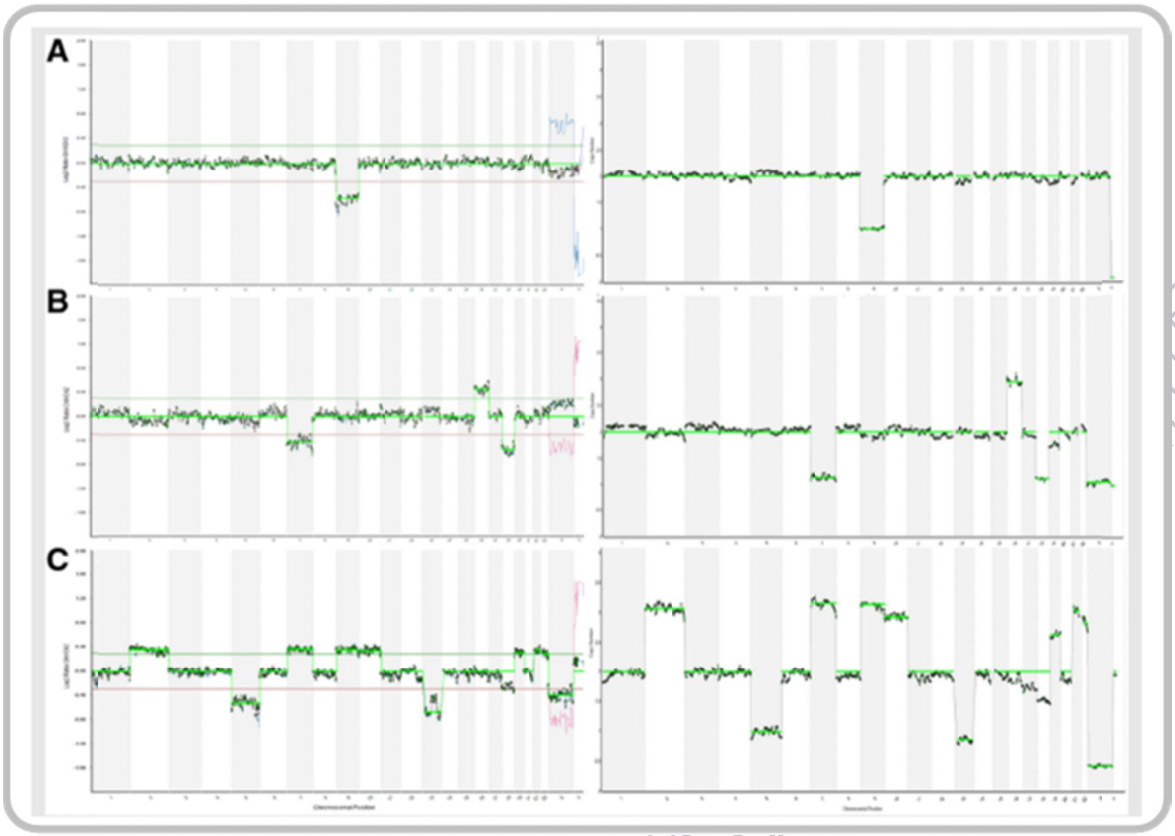

Figure 2. Comparison of the same diagnosis with array comparative genomic hybridisation (left) and sequencing (right). The greater dynamic range of sequencing is apparent.

1.5 SNPs and SNP chips

The use of microarray technology was further developed as a means of determining the genotype of an embryo by detecting thousands of single nucleotide polymorphisms (SNPs) distributed across the human genome [23]. First developed for genome wide association studies, SNP arrays take advantage of positions at which there are two distinct alleles, each at a similar frequency, and these are reasonably evenly spread across the genome [94, 95]. As a result of interrogation such SNP arrays (or “SNP chips”) each allele is differentially fluorescently labelled after hybridisation with amplified embryonic DNA. The total fluorescence and the fluorescence ratio of the two different fluorophores allow analysis of homozygosity and heterozygosity as well as, to some degree, identification of duplications or deletions [95] and aneuploidy. Genotypes are classified as AA, BB (homozygous) or

AB (heterozygous) and the raw data from these provide the basis of karyomapping analysis.

Almost 40 million SNPs have been validated that reside mostly in non-coding regions, with arrays generally detecting 660,000 to 2 million SNPs across the length of all chromosomes [36]. Due to the biallelic nature of SNPs, it is suggested that, on their own, they are less informative than STR markers [23]. However, by determining the genotype of the parents, and a relative of known disease status, four distinct sets of markers can be identified across each of the parental chromosomes [23]. A study by Rabinowitz *et al*., using SNP array technology, showed a 79% chemical pregnancy rate after screening for single gene defects [96]. Treff and colleagues applied this technology for PGD of unbalanced inheritance of rearranged chromosomes and aneuploidy screening of 12 patients, with a 75% birth rate [97] showing that SNP arrays may be particularly suited to PGD for monogenic disorders or translocation chromosome imbalance combined with comprehensive detection of aneuploidy [36]. Karyomapping however combines all these advantages of SNP arrays to create a single, widely applicable approach to preimplantation genetics.

2. What is Karyomapping?

Karyomapping [98] allows the determination of inheritance from the parental (or grandparental) genetic material through the assembly of haploblocks (inherited chromosomal segments). The approach involves genome-wide SNP analysis of parental DNA, amplified embryo DNA and an appropriate ‘reference’ such as a close relative (e.g. older child affected by the disorder).

The first step is the identification of ‘informative’ loci for each of the parental haplotypes [99] at which one parent is homozygous and another heterozygous. All other loci are then disregarded as uninformative. These then need to be compared to the reference individual of known disease status in order to establish phase (i.e. assign a reference “affected” haplotype). At this stage, the genotype (SNP chip output) of each embryo within the cohort needs to be compared to the reference genotype to determine similarity at each informative locus. The resulting output (Figure 3- 4) creates a karyomap showing homologous chromosomes and

crossovers. Comparison of the SNP markers present on the parental chromosomes at the chromosomal position of the gene(s) of interest with the reference genome against those present in the cells taken from the embryo allows the determination of the presence or absence of the mutant allele(s) by linkage [23] rather than direct mutation detection. Karyomapping can further be used for the diagnosis of aneuploidy (monosomy and meiotic trisomy (Figure 5)) triploidy, parthenogenetic activation and uniparental heterodisomy (which can lead to imprinting disorders such as Prader–Willi or Angelman syndromes), as well as abnormal patterns of genome duplication seen with, for example, molar pregnancies [98-100]. Specifically, karyomapping identifies monosomies and deletions by the absence of either haplotype from that parent and trisomies of *meiotic origin* only through the presence of both haplotypes from one parent in one or more sections of the chromosome [98]. One crucial advantage of karyomapping is that it allows for determination of heterozygous SNP calls, referred to as “key SNPs” which allows the risks associated with allele dropout, a common issue with other PGD techniques, to be avoided (see above).

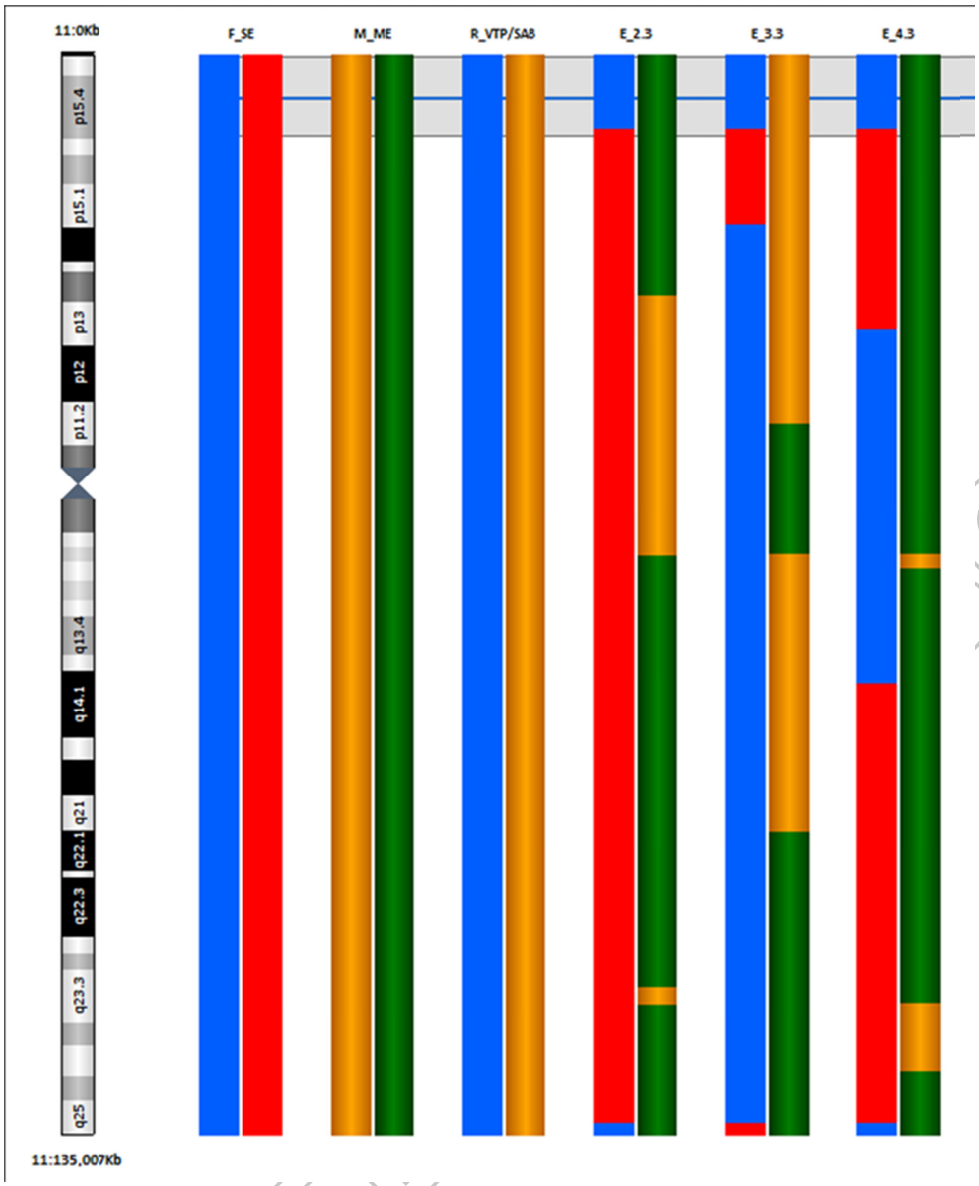

Figure 3. Example karyomapping output on chromosome 11. From left to right, the ideogram of chromosome 11, the paternal chromosomes (red and blue); the maternal chromosomes (yellow and green); haplotype of an affected child (blue and yellow parental chromosomes) and three embryo biopsy samples compared to the parents and affected child.

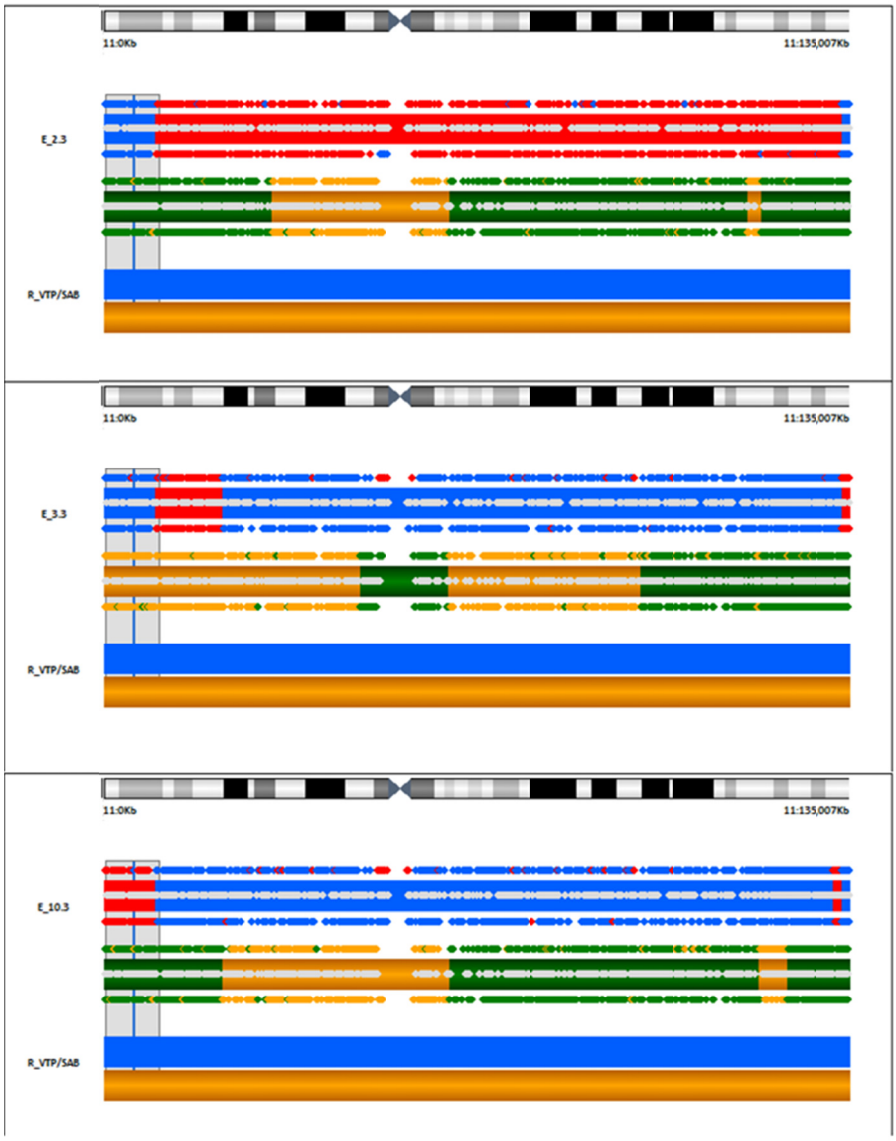

Figure 4. More detailed view of the three embryos above compared to the affected child.

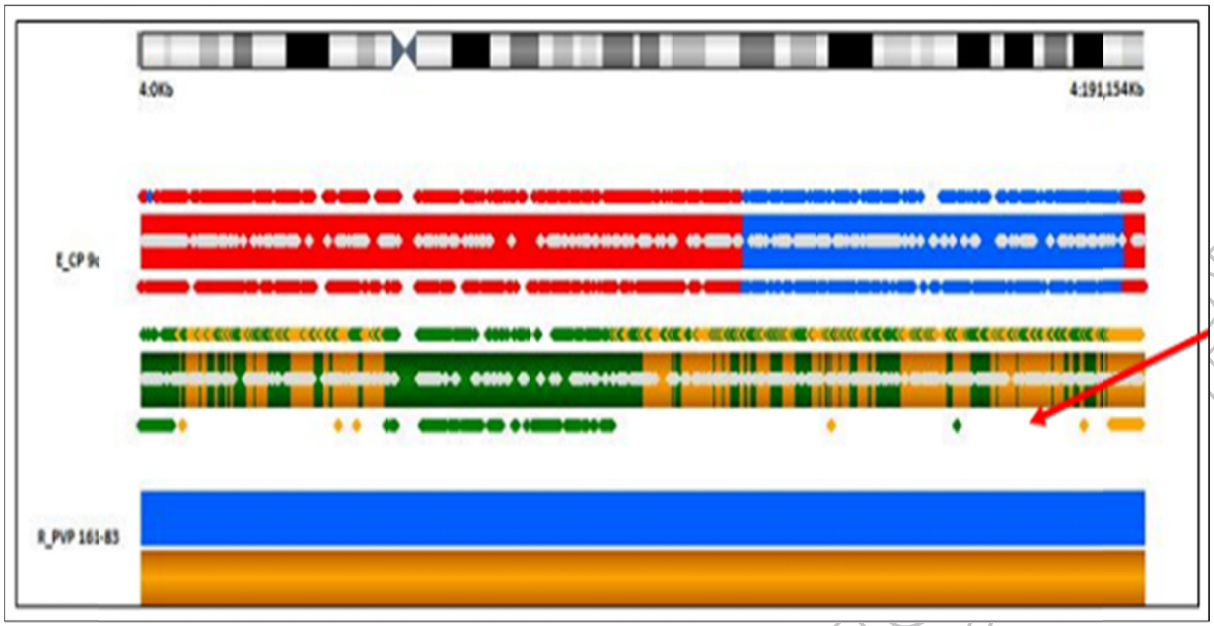

Figure 5. Detection of meiotic trisomy by karyomapping (alternative yellow and green stripes).

Karyomapping is thus a novel tool for the simultaneous detection of single-gene disorders and chromosome copy number imbalance in PGD embryos in a single assay, with test development taking between one and two weeks to complete to ensure appropriate level of SNP coverage at the site of interest [27, 36, 98]. In practice, karyomapping has a dedicated Illumina SNP array designed to interrogate around 300,000 SNPs and dedicated software to analyse the results (see Figures 3-5). The approach has been validated by blinded retrospective analysis and comparison with the 'gold standard' STR marker and mutation detection approach. This was preceded by two case reports in which karyomapping was undertaken in parallel with conventional analysis have been completed as validation of the technology [24, 99].

The first case report on clinical validation of karyomapping was published for Smith–Lemli–Opitz (SLO) syndrome, an autosomal recessive condition leading to multiple congenital abnormalities and mental retardation [99]. Retrospective analysis with karyomapping determined full concordance with the clinical analysis of the samples by STR PCR and for aneuploidy testing [99, 100]. The second case study was conducted for the detection of Marfan Syndrome, an autosomal dominant condition

that affects the connective tissue to varying degrees [24]. This study determined that both methods for PGD differentiated between affected and unaffected embryos with high efficiency and accuracy (after cleavage stage biopsy). However, karyomapping was much more time efficient process due to the shortened pre-test work up requirement [24]. In a subsequent study the analysis compared karyomapping with direct mutation detection in 218 embryo samples from 44 clinical cycles. The study determined that karyomapping was concordant with direct mutation testing in 213/218 (97.7%) cycles. Furthermore, the non-concordant samples were all in consanguineous regions [99, 100]. Giménez *et al*. looked at the use of karyomapping for a *de novo* deletion in the TSC2 gene, which is responsible for tuberous sclerosis [101]. An attempt at conventional PGD was completed; assessing a total of 26 SNP within the deleted region, the protocol developed was still insufficient for the conclusive diagnosis of all potential embryos produced by the couple. However, it was determined that karyomapping was able to detect the mutation. The patients underwent a PGD cycle using karyomapping which resulted in a healthy live-born child [101]. This study therefore confirmed that karyomapping is a powerful and versatile new approach for mutation detection in preimplantation embryos, some of which may be not be possible through conventional PGD methods.

Furthermore, karyomapping is platform independent (though to date we believe only used with Illumina chips) as the output is binary [99], this allows any platform, not just SNP chips, to be used including whole-genome sequencing [101]. It is suggested that a whole genome sequence (a basic interrogation so that it takes a shorter time to generate) followed by karyomapping would allow an accurate, but rapid diagnosis [99]. However, it is important to note that even with the major developments in whole genome amplification; gaps would inevitably arise in the assembly. It is suggested that karyomapping could combat this by adaptations to the algorithm showing that the technology can evolve [101]. Karyomapping, therefore, is seen to have inherent ‘future-proofing’ and thus has the potential to form the foundation for most PGD worldwide [99]. Furthermore, it has been shown that karyomap analysis can be extended to include allele-specific intensity data, which allows sequence-identical chromosome duplications to be detected [102, 103]. It has also been demonstrated that adaptations of karyomapping can be used to assess post-zygotic copy number in embryos allowing the origin of trisomies to be

differentiated [101]. This is an important advantage as mosaic trisomies of meiotic origin invariably lead to clinical problems however those of post-zygotic origin can, in certain circumstances, proceed uneventfully to term. The issue of PGS for chromosomal abnormalities remains controversial, however mounting data have provided evidence that it can be used to reduce the risk of miscarriage and disorders such as Down Syndrome [101, 104-107]. From a patient care perspective, the additional information relating to parental origin of meiotic errors provided by karyomapping (but not other PGD technologies) can help couples to determine which treatment option to try next, such as donor gametes [24].

It is important to note that significant savings can be made in labour as karyomapping does not require the in-depth workup required for customised tests as when performing multiplexed STR analysis. With this in mind, the per-sample cost for karyomapping is comparable to or less than the cost of traditional PGD technologies, depending on the complexity of the analysis [99, 100].

2.1 Simultaneous detection of monogenic disorders and chromosome copy number: Alternatives to karyomapping

There are other techniques that allow for the simultaneous detection of monogenic disorders and chromosome copy number in IVF derived human embryos. Haplarithmisis [103, 108] is one such method, which allows B allele frequencies to be called as well as the standard AA, BB or AB alleles we expect from SNP data. Zamani *et al*. argue that the process of whole-genome amplification is in itself problematic due to artefacts and thus other haplotyping methods suffer from error-prone SNP genotypes (AA, AB, BB) and the relatively subjective nature of discriminating chromosome copy number changes from these artefacts [103, 108]. They suggest that Haplarithmisis could be used to diagnose specific disease causing alleles throughout the genome, as well as indicating the presence of numerical and structural chromosomal abnormalities in the embryos. Furthermore, it has been shown that using this technique, meiotic segregation errors can be distinguished from mitotic ones [108].

Treff et al. developed the use of RT-qPCR, demonstrating a targeted NGS strategy and a multiplex PCR reaction that included the chromosome-specific target sequences along with the mutation site [88]. This strategy reduced the necessary read depth for accurate sequencing of the mutation site as well as parallel RT-qPCR for chromosome copy number, which allows for a reduction in per sample cost as well as the time required to run the test [36]. Zimmerman and colleagues determined that this strategy was more reliable than other techniques [109] with 303/304 (99.7%) embryos getting a definitive diagnosis and 1/304 (0.3%) recorded as inconclusive due to a recombination event. This study also demonstrated an 82% (27/33) pregnancy rate [109].

Another interesting method is the use of NGS technology with linkage analysis. Yan and colleagues describe a technique called “mutated allele revealed by sequencing with aneuploidy and linkage analyses” (MARSALA) [110]. This method involves multiple annealing and looping-based amplification cycles (MALBAC) for whole-genome amplification and subsequently, aneuploidy is determined by CNVs, whereas SNVs associated with the monogenic diseases are detected by PCR amplification of the MALBAC product. Aneuploidy is then detected by copy number variations (CNVs) and then detection of single-nucleotide variations (SNVs) in the PCR amplified MALBAC product determines the disease status of the sample. The false-positive and false-negative SNVs are avoided by an NGS based linkage analysis [110]. Furthermore, the study demonstrated that by using this method two viable and healthy live births were achieved [110].

2.2 Limitations of karyomapping

It is important however to note that karyomapping also has a number of limitations. The need for DNA from a close relative of known disease status can limit the use of karyomapping, especially in cases where the disorder leads to shortened life expectancy. However, this is a limitation of the premise of PGD for single gene disorders in which linkage analysis is involved, not specific to the karyomapping technology itself. Secondly, if a recombination event in either parent, reference individual or embryo is next to the position of interest this may make the data difficult interpret and thus a diagnosis inconclusive. Regarding *de novo* mutations, it may not

be possible with karyomapping to establish which parental chromosome is linked to the defect therefore, mutation testing is essential in these cases. As with all PGD technology therefore, karyomapping does not *a-priori* detect new mutations [111]. However, karyomapping can still be used to identify the affected parental chromosome in single sperm or embryo samples [23]. One other area of development for this technology, as is the same with all PGD technologies based on linkage analysis, is in cycles dealing with consanguinity. In these cases, the pattern of key and non-key SNPs identifies regions in which the parents and possibly the close relative share one or more sequence-identical chromosome regions. It is suggested that as these regions are less informative combined karyomapping and mutation detection would be the most appropriate course of action [23, 99]. Another issue with karyomapping, already alluded to, is the fact that it cannot easily detect trisomies of post-zygotic origin unless combined with quantitative approaches.

From a practical standpoint, it is important to note is that there are cost implications regarding the implementation of karyomapping. If the lab follows the published Illumina protocol, karyomapping requires 4 products: SureMDA™, DNA Analysis Kit, the iScan® System or NextSeq® 550 System, and BlueFuse® Multi analysis software [112]. The scanning system required to read the BeadChips are different for those required for NGS (VeriSeq®), and further to this karyomapping requires MDA to amplify the DNA instead of WGA commonly used in aCGH and NGS. Due to these requirements, there is also a need for dedicated workrooms for each stage of sample preparation that adds to the logistical costs of running a karyomapping assay.

Although patient work up for karyomapping is acknowledged to be shorter than that of other methods for mutation detection, in the case of disorders that have not been mapped by classical PGD techniques, STR marker tests need to be developed before karyomapping can be performed. This then means that the work up time for karyomapping is the same other technologies.

3. Expert Commentary

Karyomapping was first commercialized by Illumina in 2013 and is currently a routine procedure for PGD detection of single gene disorders. At time of writing (November 2016) around 1000 clinics worldwide offer karyomapping, with detection largely serviced by 20 diagnostic laboratories. Approximately 2500 cases have been performed, a figure almost certainly out of date by the time this article is being read [113]. Because of issues of inability to detect post-zygotic trisomy reliably however and the confounding problems of embryo mosaicism, karyomapping has yet to be applied clinically in a widespread manner for the detection of chromosome disorders. When this occurs then it will be able to reach its full potential as a method to simultaneously detect chromosomal abnormalities and monogenic disorders.

4. Five Year View

Over the next five years we predict that the use of karyomapping will increase. With its current widespread use for monogenic disorders this seems inevitable, and thus a range of manuscripts associated with its validation is very likely. Given its potential for chromosomal detection, validation for cytogenetic diagnoses is likely to follow, however the issue of detection of post-zygotic chromosome imbalance (which cannot be achieved by karyomapping alone) needs to be addressed. This will be achieved by combining karyomapping with quantitative SNP detection and this has already been applied to some degree in the Haplarithmisis algortihm [102]). For a test to become truly widespread it also need to be affordable and again it seems likely that the overall cost of the test will reduce as economies of scale become apparent. Given that NGS technologies are also becoming widespread for aneuploidy detection it would be refreshing to see the karyomapping algorithms adapted to use sequencing data rather than SNP chips.

Key issues

- Preimplantation genetic diagnosis and screening (PGD/PGS) has been applied clinically for >25 years however inherent drawbacks include the necessity to tailor each case to the trait in question

- Technologies to detect monogenic and chromosomal disorders respectively are fundamentally different to one another

- Adapting to changes in technology has been a challenge over the last 25 years and there is constantly a need for more efficient, streamlined diagnoses.

- Karyomapping allows the determination of inheritance from the (grand)parental haplobocks through assembly of inherited chromosomal segments. The output displays homologous chromosomes, crossovers and the genetic status of the embryos by linkage comparison, as well as chromosomal disorders.

- Karyomapping also allows for determination of heterozygous SNP calls, avoiding the risks of allele dropout, a common problem with other PGD techniques.

- Manuscripts documenting the evolution of preimplantation genetics, especially those investigating technologies that simultaneously detect monogenic and chromosomal disorders, are reviewed here.

- Karyomapping is currently available for detection of single gene disorders; ~1000 clinics worldwide offer it (via ~20 diagnostic laboratories) and ~2500 cases have been performed.

- Due an inability to detect post-zygotic trisomy reliably however and confounding problems of embryo mosaicism, karyomapping has yet to be applied clinically for detection of chromosome disorders.

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Declaration of Interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Reference annotations

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*** Of considerable interest*

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