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Review:

Whole genome amplification in preimplantation genetic diagnosis*

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Abstract: Preimplantation genetic diagnosis (PGD) refers to a procedure for genetically analyzing embryos prior to implantation, improving the chance of conception for patients at high risk of transmitting specific inherited disorders. This method has been widely used for a large number of genetic disorders since the first successful application in the early 1990s. Polymerase chain reaction (PCR) and fluorescent in situ hybridization (FISH) are the two main methods in PGD, but there are some inevitable shortcomings limiting the scope of genetic diagnosis. Fortunately, different whole genome amplification (WGA) techniques have been developed to overcome these problems. Sufficient DNA can be amplified and multiple tasks which need abundant DNA can be performed. Moreover, WGA products can be analyzed as a template for multi-loci and multi-gene during the subsequent DNA analysis. In this review, we will focus on the currently available WGA techniques and their applications, as well as the new technical trends from WGA products.

Key words: Whole genome amplification, Multiple displacement amplification, Primer extension preamplification, Degenerate oligonucleotide primed-polymerase chain reaction, Preimplantation genetic diagnosis

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1 Introduction

Preimplantation genetic diagnosis (PGD) is a technique that allows one to identify genetic defects in embryos created through in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI). It refers to a laboratory procedure to diagnostically analyze polar body, single blastomere, and blastocyst from biopsied embryos before the embryos are transferred, allowing the selection of normal embryos for transfer (Ouhibi *et al*., 2001; Harper *et al.*, 2009). PGD can be used for detecting at-risk couples who have already had a child with a genetic defect or whose family has a history of specific inherited disorders such as single gene defects and chromosomal abnormalities (Thornhill *et al*., 2005). The purpose of PGD is to improve the chance of conception for patients with genetic abnormalities, and to make it likely that their offspring will not suffer from the genetic defect carried by the family. In so doing, one avoids the moral or ethical dilemmas which arise from the termination of the pregnancy once it has begun. Handyside *et al*. (1989; 1990) were early pioneers in diagnosis of genetic diseases in human preimplantation embryos. Since then, PGD has been practiced widely throughout the world.

Polymerase chain reaction (PCR) and fluorescent in situ hybridization (FISH) are the two most commonly used methods in PGD for many IVF/ICSI laboratories (Thornhill and Snow, 2002). Although these methods have many advantages, there are still some limitations, such as providing very limited DNA fragments or genetic information among chromosome regions, only a few chromosomes can be detected simultaneously by FISH, and these cannot meet the needs of whole genome research (Wells, 2004; Wilton, 2005). The limitations have encouraged the development of more comprehensive techniques for

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genetic testing. Most researches in PGD are focused on new methods of single cell analysis, as well as improving current methods of PCR. For instance, nested PCR, multiplex PCR, and fluorescent PCR have been developed to reduce the disadvantages of conventional PCR (Findlay *et al*., 1995; Sermon, 2002; Malcov *et al*., 2007). However, the products from these methods are not yet available for whole genome research studies and the yields are limited.

Whole genome amplification (WGA) is one approach designed to overcome these problems (Handyside *et al*., 2004). This technique would amplify the entire genome from a cell up to microgram level, and consider the original sequence representation. Sufficient DNA can be amplified, allowing diagnosis of any known single gene defect that would have been impossible otherwise (Silander and Saarela, 2008), and multiple tasks which need abundant DNA can be performed from WGA products. Moreover, the WGA products can be analyzed as a template for multi-loci, multi-gene, and genome research during the subsequent DNA analysis.

An early approach for amplifying large fragments of a genome was to use primers directed at human Alu repeat element, providing a simple method for the isolation and analysis of specific chromosomal regions (Nelson *et al*., 1989). Because of the highly uneven distribution of Alu sequence in the human genome and the variable number of repetitions, this protocol cannot be used reliably in conjunction with molecular analysis of single cells (Wells *et al.*, 1999). Later, several other methods have been developed to amplify the whole genome. In 1992, Zhang *et al*. (1992) first used the primer extension preamplification (PEP) protocol for haplotyping of a single sperm cell. In the same year, degenerate oligonucleotide primed-PCR (DOP-PCR) was first described by Telenius *et al*. (1992) as a method for genome mapping studies. From then on, the two protocols have been often used as a part of WGA in PGD. Dean *et al*. (2002) used multiple displacement amplification (MDA) in PGD with great expectations. This is an ideal non-PCR-based method that may reduce the limitations of PCR-based WGA techniques, such as generation of relatively short DNA fragments and a high possibility of mutation introduction into the products (Peng *et al*., 2007). In this paper, we will review several currently available methods of WGA and their applications, and some new strategies from WGA products will be discussed as well.

2 Different methods of whole genome amplification

2.1 Primer extension preamplification

PEP involves PCR cycling and the use of *Taq* polymerase, a 15-base random oligonucleotide primer which is made up of 4^{15} (1×10^9) different sequences is included as well, and this facilitates the sequence-independent amplification of any sequence within the genome (Zhang *et al*., 1992). Unlike the conventional PCR, following denaturation, the primers are put at a low temperature (37 °C) to anneal to the DNA genomic template, and then the temperature increases slowly to 55 °C followed by a 4-min elongation step at 55 °C. At the low annealing and denaturing temperatures, the primers will nonspecifically combine with the DNA template, also including the products that are needed. Thus, these reproducible products will be easily parted during the following procedure. It is estimated that approximately 96% of the genome can be amplified at least 1000 times (Paunio *et al*., 1996). With this method, it is much more convenient to detect variance of different loci in PGD.

β-thalassemia is caused by any one of more than 200 point mutations or sometimes by deletions (Rund and Rachmilewitz, 2005). Such molecular heterogeneity requires a diagnostic strategy, not only to detect a range of mutations, but also to compound genotypes. Jiao *et al*. (2003) developed a strategy that involved PEP, followed by nested PCR and reverse dot blot (RDB) for simultaneous detection of more than one mutation in PEP products. This resulted in the thalassemia-free children being born (Jiao *et al*., 2003). PEP has also been clinically applied to the PGD of familial adenomatous polyposis coli (FAPC) (Ao *et al*., 1998), the approach was then followed by nested PCR, and it was used for amplifying two adenomatous polyposis coli (APC) fragments, i.e., an APC mutation site and an informative polymorphism. Both were detected by simultaneous single-strand conformation polymorphism and heteroduplex analysis. After transferring the unaffected embryo, no pregnancy resulted. Our research group had used single

cell PEP-PCR for dystrophin exons 8, 17, 19, 44, 45, and 48, and human testis-determining gene (*Sex-Determining Region Y, SRY*), and the results showed that this method could successfully cover the six dystrophin exons and determine the sex simultaneously with a high sensitivity (Xu *et al*., 2001).

In order to meet the clinical needs, a shorter PEP protocol has been developed, reducing the time required from >14 h to 5.5 h (Sermon *et al*., 1996), while the efficiency in amplifying different sequences in a single cell is retained. Furthermore, the PEP protocol has been modified and improved (improved-PEP, I-PEP), and has been shown to have an increased efficiency of amplification compared with PEP (Dietmaier *et al*., 1999). Cystic fibrosis (CF) is an autosomal recessive disorder caused by mutations in the CF transmembrane conductance regulator (*CFTR*) gene, leading to defective ion transport in the conducting pulmonary airways and exocrine glands (Mueller and Flotte, 2008). More than 1500 mutations have been described (Jonsdottir *et al*., 2008). Because of so many variant phenotypes in CF, it is important to detect as many of the mutations as possible during the single procedure in PGD. Sánchez-García *et al*. (2005) have developed an advanced method that can identify quite a few different mutations in single cells. A multiplex PCR and a fluorescent oligonucleotide ligation assay (OLA) were used for the identification of normal and mutant loci, and the results showed that 29 normal loci and the 31 most frequent CFTR mutations could be detected at the same time. In addition, I-PEP product appears to have higher specificity than MDA, particularly in the analysis of microsatellite loci (Sun *et al*., 2005).

However, the amplification bias, that varies from a low of 10^2 to a high of 10^4 (Paunio *et al*., 1996; Dean *et al*., 2002), and the existence of amplified fragments >1500 bp in length that cannot be demonstrated in PEP (Wells *et al*., 1999) have discouraged the broad implementation of this method in PGD.

2.2 Degenerate oligonucleotide primed-polymerase chain reaction

Another method for WGA is DOP-PCR which has been widely accepted, and it is a technically straightforward WGA method. Unlike PEP, DOP-PCR uses a partially degenerate primer which binds at many sites throughout the genome during several low-temperature annealing cycles. Then more specific priming at the fragments will be generated by increasing the annealing temperature. The starting template DNA of DOP-PCR can be as little as 15 pg or as much as 400 ng, and the quantity of DNA products is much greater than that of PEP (Wells *et al*., 1999; Peng *et al*., 2007).

The major advantage of this technique is that it can produce sufficient amplified products from single cells for use in comparative genomic hybridization (CGH) (Wells *et al*., 2002; Wilton, 2005), which requires 100 ng to 1 μg of test DNA, equivalent to more than 10000 cells (Harper and Wells, 1999; Wells and Levy, 2003). Voullaire *et al*. (1999) reported that it was possible to use CGH with DOP-PCR amplified DNA for the diagnosis of chromosomal aneuploidy in single cells. And later, the same research group successfully detected chromosome abnormality by using CGH in embryos in women with repeated implantation failure following IVF (Voullaire *et al*., 2002). However, the non-random DOP-PCR amplification had greatly influenced the accuracy of CGH, and we found that this phenomenon could be corrected by selecting the normal single cell DOP-PCR product as a reference (Jin *et al*., 2000).

DOP-PCR generates relatively short products, which is the same as PEP (Telenius *et al*., 1992). Luckily, new methods have been described to generate long amplification sequences. Products from LL-DOP-PCR (long products from low DNA quantities) protocol can range from 0.5 kb to >10 kb (Kittler *et al*., 2002). This was achieved by the 3′-5′ exonuclease proofreading activity of DNA polymerase Pwo and an increased annealing and extension time during DOP-PCR. In comparison with the conventional DOP-PCR method, it provides significantly better coverage for microsatellites and unique sequences.

Recently, a modification of the traditional DOP-PCR reaction (dcDOP-PCR), including the use of a more degenerate primer (10 nucleotides) and 12 nonspecific cycles, was developed by Bonnette *et al*. (2009). In this protocol, the longer amplification products were generated by additional proofreading enzymes; thus, increasing the genome coverage of the reaction. This gave an approximately 45% increase in the number of detected short tandem repeat (STR) alleles when compared with traditional DOP-PCR

(P=0.0003), and a nearly 34% increase when compared with traditional STR testing without WGA (P<0.0001). Further, allele dropout (ADO) was not observed in dcDOP-PCR products. However, there are not many studies in PGD with dcDOP-PCR, and more studies are needed.

2.3 Multiple displacement amplification

One of the most exciting developments in WGA methods is MDA, which has been the evolution of protocols designed for high performance. Depending on the use of MDA, the amount of the MDA product can reach the microgram level (1 000000 times more than that of single cell DNA) (Hellani *et al*., 2004). Since MDA appears to be an important technical breakthrough in the utilization of WGA methods, this method will be discussed in detail.

Principles of this technique are as follows: the random hexamers anneal to the single stranded target molecule. As the DNA polymerase elongates the primer, the upstream DNA strands are displaced, which can serve as templates for new priming events. These displaced DNA strands result in primer elongation in the opposite direction. The reaction of MDA continues, and new templates and a hyperbranched structure are produced by these new DNA strands, generating a great amount of copies of the target molecule (Lovmar and Syvänen, 2006). This reaction can be catalyzed by the φ29 DNA polymerase or by the large fragment of the *Bst* DNA polymerase. However, φ29 DNA polymerase is preferred over *Bst* DNA polymerases on account of the high efficiency and the low error rate (Spits *et al*., 2006). It was reported that the error rate of φ29 DNA polymerase was 100 times lower than that of *Taq* polymerase. φ29 DNA polymerase used for MDA has an error rate of 1 in 10^6–10^7 (Esteban *et al*., 1993) in contrast to ≈3 in 10^4 for PCR with *Taq* DNA polymerase (Eckert and Kunkel, 1991).

In clinical application, MDA has been shown to be highly efficient and accurate in many cases. Fragile X syndrome (FXS) is caused by a dynamic mutation in the fragile X mental retardation-1 (*FMR1*) gene. Normal individuals have <55 CGG repeats in the 5′ untranslated region, premutation carriers have 55–200 repeats, and full mutation ones have >200 repeats (Fu *et al*., 1991; Malcov *et al*., 2007). Conventional PCR amplification of the *FMR1* CGG triplet repeat-containing region is often difficult because of a "slippage effect". Full mutation and most high-repeat permutations could not be detected by the conventional PCR (Malcov *et al*., 2007). Burlet *et al*. (2006) used MDA products for fluorescent PCR analysis, which was performed to amplify the non-expanded CGG repeats with markers linked to *FMR1* and a sequence from the amelogenin gene. With the direct and indirect analyses of the *FMR1* gene, the embryonic gender can be determined simultaneously in preimplantation embryos. Lledó *et al*. (2008) developed an MDA-PGD protocol for X-linked retinoschisis. MDA products were used for PCR analyses of two polymorphic markers flanking the retinoschisis (*RS1*) gene and a new X/Y marker, X22, to sex embryos in an X-linked retinoschisis PGD program. One single pregnancy was achieved. This report shows that the MDA technique could allow the simultaneous amplification of different targets to perform diagnosis of any known gene abnormality and sexing determination in diseases that linked to gender.

When compared with PCR-based WGA methods, MDA appears to have several conspicuous advantages. The MDA products are of sufficient length and integrity, and the average product length is >10 kb (Dean *et al*., 2002), allowing accurate restriction fragment length polymorphism (RFLP) analysis. MDA products have been preferred over DOP-PCR products for array comparative genomic hybridization (array-CGH) (le Caignec *et al*. 2006), which requires approximately 300 ng of amplified DNA (Hellani *et al*., 2004; le Caignec *et al*., 2006). Array-CGH offers new possibilities for genetic analysis of aneuploidy screening and detection of unbalanced translocations in preimplantation embryos particularly (le Caignec *et al*., 2006).

Moreover, MDA offers a useful alternative to multiple locus-specific PCR preamplification for large single nucleotide polymorphism (SNP) genotyping scoring studies (Dean *et al*., 2002). In comparison with other WGA methods, Tzvetkov *et al*. (2005) found that MDA exhibits a significantly higher concordance of SNP genotypes following amplification, and suggested that MDA is the current WGA method which can be used in genome-wide SNP scans. The accuracy of genotyping varied from 89% to 100% (average 94%) for SNP from DNA amplified by MDA with φ29 DNA polymerase

(Kumar *et al*., 2008). Furthermore, when the MDA amplified product was used as reference, higher concordance for chromosome copy number from single cells could be achieved in comparison with genomic DNA (93.1% vs. 82.8%) (Ling *et al*., 2009), suggesting that MDA coupled with SNP mapping array may provide a reliable and accurate method for chromosome copy number analysis and the detection of single-gene disorders.

In addition, Jiang *et al*. (2005) have pointed out its great potential for producing high-coverage and high-yield WGA products from single sperm cells. They estimated that 76% of the loci can be amplified at least 2500-fold from single sperm DNA, and it can be further amplified >200-fold by a second-round MDA (Jiang *et al*., 2005). It may mark the beginning of a great new epoch in studies of local recombination rates.

2.4 OmniPlex whole genome amplification

Besides the MAD, OmniPlex is another useful method for obtaining sufficient DNA from a limited amount of samples for genetic diagnosis (Gribble *et al*., 2004; Uda *et al*., 2007). Fragmented genomic DNA can be converted randomly into inherently amplifiable DNA fragments of defined size, which formulates a library. With the help of a high-fidelity DNA polymerase, this library can be effectively amplified several thousand-fold. Then a final amplification of over a million-fold without degradation of representation can be achieved by reamplifying the library (Langmore, 2002; Barker *et al*., 2004).

Chen *et al*. (2008) reported the first successful application using the OmniPlex technology of WGA in PGD for single gene disorder in combination with human leucocyte antigen (HLA) typing. The examination of blastomeres using OmniPlex in PGD achieved an amplification efficiency of 90% and ADO rate of 6%–19%. The major advantages of this technology are that it permits the subsequent genotyping of multi-loci of a single blastomere in separate PCR reactions without mutual interference, as several PCR reactions using multiple primers in a tube might impede each other, and that it allows broadening of the test items and repeat confirmations for an ambiguous result. Bergen *et al*. (2005) demonstrated that OmniPlex WGA DNA exhibited a greater reduction in genotyping performance than MDA WGA DNA. The superiority between OmniPlex and MDA in PGD still needs further investigation.

3 Several problems of whole genome amplification

3.1 Preferential amplification and allele dropout

Both preferential amplification (PA) and ADO are the potential problems that can cause misdiagnosis, and they seem to be mainly generated during the WGA reaction (Wells *et al*., 1999). ADO is defined as the random amplification failure of one of the two heterozygous alleles whilst the other allele successfully amplifies, and PA means the failure of one allele to reach the threshold of detection (Findlay *et al*., 1995). The data on ADO and PA vary widely, and sometimes the figure can be considered unacceptably high. The PA rates in MDA could range from 6.9% to 60.7% (Spits *et al*., 2006), while the ADO rates in MDA could reach 10.0%–38.9% (Handyside *et al*., 2004; Hellani *et al*., 2004; Iwamoto *et al*., 2007; Ren *et al*., 2007; Renwick *et al*., 2007), which are comparable to those obtained by direct amplification of single cell loci (20%–40%) (Ray and Handyside, 1996; Rechitsky *et al*., 1998).

The following factors can account for PA (Walsh *et al*., 1992). First of all, differential denaturation because of the GC percentage differences between alleles was one important factor that could lead to PA. Moreover, the short allele product can be amplified preferentially, even when the target DNA has been sufficiently degraded. In addition, PA is closely associated to the initial number of genomes sampled. When it is very small, it can easily cause PA. Along with the factors mentioned above, less efficient priming of DNA synthesis of one allele is another factor that can result in PA of the other allele, because of mismatches between the primer and the specific allelic template.

As to ADO, it depends on the cell type analyzed, the genes tested, and the lysis conditions, as well as the PCR conditions (Sermon and de Rycke, 2007; Zeng *et al*., 2009). For autosomal recessive conditions when both partners are carrying the same mutation, ADO will not cause serious misdiagnosis, but the number of embryos available for transfer would decrease and this may result in a poor pregnancy rate.

For compound heterozygous or autosomal dominant conditions, the results are quite contrary to those autosomal recessive conditions, and the consequences of ADO could be catastrophic for transferring affected embryos (Thornhill and Snow, 2002): longer alleles, that is, with a larger number of repeats, either paternal or maternal. Frumkin *et al*. (2008) found that longer alleles had significantly higher dropout rates when compared with their corresponding shorter alleles. The small quantity of the WGA products that used in the following PCR-based analysis could also result in ADO.

These drawbacks seriously compromise the validity of PGD. Fortunately, in view of the disadvantages in MDA, methods to improve the accuracy of the techniques used will reduce the likelihood of these disadvantages. Several improvements have been suggested to overcome the ADO problem, such as the use of alkaline lysis buffer in order to break down the cell and make the DNA more accessible, and the use of lysis buffer containing proteinase and detergent as an effective way to help reduce ADO (Thornhill *et al*., 2001; Kim *et al*., 2009). Linker-adaptor amplification was also suggested to lower ADO rates (Renwick *et al*., 2007). Besides reducing ADO, the use of linked markers to increase the detection of ADO is another way to improve the accuracy of the techniques. With use of one or two linked markers, the undetected ADO would be reduced by approximately 50% and 75%, respectively, and with three linked markers ADO could be virtually entirely detected (Thornhill and Snow, 2002). General strategies to avoid PA have been discussed by Walsh *et al*. (1992), such as reaching a temperature capable of denaturing all alleles. One simple solution suggested by Spits and Sermon (2009) is to analyze more loci, where ADO rates generally are under 10% and PA is hardly an issue. However, new modified strategies are needed to improve the accuracy of PGD and they should be addressed in future studies.

3.2 Slippage of microsatellites

Microsatellites are short segments of DNA that have a repeated sequence, such as STRs or simple tandem repeats. The slippage of microsatellites is very common in PCR-based WGA methods, such as PEP and DOP-PCR. The amplification of repetitive DNA sequences, such as STRs, increased or decreased by a number of base pairs equivalent to one repeat length, presumably caused by slippage of the DNA chain during product generation (Wells *et al*., 1999), is probably due to the low annealing temperatures which are characteristic of PCR-WGA protocols. According to the report by Cheung and Nelson (1996), this problem mainly occurs when only a small quantity of samples are available; if providing sufficient amount of DNA, such problem maybe not exist. Moreover, the research using quantitative fluorescence multiplex PCR has shown that PA obviously exists in microsatellite heterozygote, and ADO occurs frequently as well. For these reasons, PCR-based WGA methods are infrequently used in aneuploidy diagnosis (Sherlock *et al*., 1998; Harper and Wells, 1999; Renwick *et al*., 2007). However, the slippage of microsatellites does not seem to occur among the MDA products (Lledó *et al*., 2006). Consequently, a misdiagnosis due to a discrepancy with genomic DNA could be substantially reduced by MDA, improving the outcome of PGD.

4 New strategies used in preimplantation genetic diagnosis following whole genome amplification

4.1 Comparative genomic hybridization and array-comparative genomic hybridization

CGH after WGA can permit visualization of all 46 interphase chromosomes. The entire test sample genome is amplified through the use of random primers and labeled with one color, and a normal reference sample is amplified and labeled with another color (Wilton, 2005). These samples are then used as probes to hybridize onto normal metaphase chromosomal plates. With image-processing software, duplication or deletion, and unbalanced structural differences between the normal reference and test specimens can be revealed. This technique has already been applied to PGD during the past few years, including chromosomal mosaicism, unbalanced structural chromosome aberrations, and chromosome breakage and aneuploidy (Malmgren *et al*., 2002; Shanske *et al*., 2004; Fragouli *et al*., 2006).

CGH does have limitations in that it is time-consuming and labor intensive. The long period required for hybridization (up to 72 h) has limited the

widespread use of this technique, as it is necessary to freeze the biopsied embryos, and the survival rate of the thawed embryos has been relatively poor (approximately 50% did not survive the thawing process) (Hu *et al*., 2004; Escribá *et al*., 2008). The development of a highly efficient cryopreservation technique is urgently needed to reduce fears concerning the impact of cryopreservation on embryo viability.

Recently, array-CGH has been developed, and it takes only 30 h to perform the hybridization step (Hu *et al*., 2004). Array-CGH is much easier to perform and more amenable to automation than CGH. It is also an effective tool for selection of embryos with a high implantation potential (Jones *et al*., 2008). It can be used for analyzing a wide variety of genetic mutations. The advantage of embryo fingerprinting and the potential for combined aneuploidy and single gene disorder diagnosis can be offered by accurate microarray platforms (Hu *et al*., 2004; 2007; Wells *et al*., 2008). This is a huge improvement over classical karyotyping, and it is considered to have great potential to replace much of the present diagnostic cytogenetics (Vermeesch *et al*., 2007).

However, several critical issues need to be addressed before array-CGH can be suitable for clinical PGD. First of all, the accuracy needs further evaluation. It was reported that array-CGH sometimes gave incorrect results for chromosomes 2, 4, 9, 11, 17, and 22 (Hu *et al*., 2004). In addition, the quality of WGA products might not be as perfect as we had expected (Paez *et al*., 2004), and the drawbacks of WGA that we mentioned above would reduce the efficiency for detection of chromosomal abnormality. More refinements will be required before general use in PGD. In addition, the present array-CGH protocol is expensive and does not seem to fit easily into all clinical PGD services. This requires us to find new ways to reduce costs and bring the advantages to more patients.

4.2 Haplotyping

Preimplantation genetic haplotyping (PGH) is a novel approach in PGD. The greatest advantage of this technique is that it can detect any mapped single-gene disorders even in the absence of an established mutation (Coskun and Alsmadi, 2007). However, it requires a careful study of the pedigree with at least one affected family member and finding several informative linked markers for the mutated genes before PGH can be applied, and these markers can be used for all carriers of the same monogenic disease.

Renwick *et al*. (2006) had successfully implemented PGH to CF and Duchenne muscular dystrophy (DMD) by MDA products, resulting in an on-going pregnancy. Moreover, the PGD of less common haematological mutations can be enabled by PGH (El-Toukhy *et al*., 2010), and the overall number of embryos suitable for transfer could be increased (Renwick and Ogilvie, 2007). PGH represents an alternative method in embryo diagnosis and the evolving technology of PGH following WGA may increase the diagnostic scope and availability of PGD in the future.

4.3 Single nucleotide polymorphism genotyping

SNPs account for most gene variants found in humans and may be associated with risks of developing common diseases. Therefore, many researches have been focused on SNPs as markers for genome-wide association studies in recent years (Carlson *et al*., 2003). These SNP-based association studies rely on the availability of abundant genomic DNA. WGA makes this possible.

WGA followed by SNP array analysis not only provides a reliable method to evaluate DNA products on genotype, but also can explore chromosomal aberrations with high accuracy and reproducibility (Iwamoto *et al*., 2007). Ling *et al*. (2009) reported that they had successfully detected chromosomal abnormalities by MDA coupled with SNP mapping array, such as trisomy and monosomy. Nevertheless, the study of SNP genotyping combined with WGA in PGD is in its initial stage, and the potential benefit from this method is not possible to estimate.

5 Conclusions

The WGA for genetic analysis in PGD is encouraging and the clinical uses of these techniques are on the rise. Currently, clinical applications of WGA provide very promising amplification efficiency. Especially, the advent of MDA opens the door to multiple tasks which need abundant DNA. It has been recognized to be the most effective WGA at present, and has become one of the main techniques in PGD. The new strategies used in PGD following WGA,

such as array-CGH, haplotyping, and SNP genotyping, may enable the detection of a wider spectrum of specific inherited disorders. In spite of different kinds of WGA which have already been developed and applied to clinical work, each method has its own merits and limitations. Further improvements of these protocols are required. We believe that more satisfactory methods will appear and play an important role in PGD in the immediate future.

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