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Karyomapping: a single centre's experience from application of methodology to ongoing pregnancy and live-birth rates

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KEY MESSAGE
This study has shown that karyomapping can be applied for couples requiring preimplantation genetic diagnosis for monogenic disorders and/or chromosomal rearrangements. Karyomapping can supersede direct mutation testing because of its superior diagnostic accuracy, shorter work-up time and its aneuploidy screening, which can minimize the risk of implantation failure.

A B S T R A C T

This study aimed to determine whether karyomapping can be applied to couples requiring preimplantation genetic diagnosis (PGD) for single gene disorder (SGD) and/or chromosomal rearrangement. 75/82 (91.5%) and 6/82 (7.3%) couples were referred for autosomal SGD and X-linked disease, respectively. One couple (1.2%) was referred for SGD and chromosomal rearrangement. Of 608 embryos, 146 (24%, 95% CI 21–28) day-3 and 462 (76%, 95% CI 72–79) blastocyst biopsies were performed. A total of 81 embryo transfers were performed; 16/81 (20%) were following day-3 embryo biopsy, 65/81 (80%) were following blastocyst biopsy and cryopreserved embryo transfer. Of 81 embryo transfers with known pregnancy outcome, 51 (63%, 95% CI 52–73) were on-going pregnancies, 6/81 (7%, 95% CI 3–15) resulted in first trimester miscarriages and 24/81 (30%, 95% CI 21–40) were failed implantations. Of the 51 on-going pregnancies, 15 (29%, 95% CI 19–43) couples had a singleton live birth at the time of write up. There have been no reports of abnormal prenatal, genetic testing or diagnosis of phenotype at birth. Karyomapping is reliable, efficient and accurate for couples requiring PGD for SGD and/or chromosomal rearrangement. Additionally, it provides aneuploidy screening, minimising risks of miscarriage and implantation failure.

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Introduction

Preimplantation genetic diagnosis (PGD) is an early form of prenatal diagnosis for couples at risk of transmitting a genetic disorder to their children. PGD requires the production of embryos through IVF procedures even for fertile couples. The embryos will later be subjected to biopsy, with a small number of cells removed for genetic testing. Only unaffected embryos are subsequently transferred to the uterus. Consequently, the risks and the physical and emotional burden of prenatal diagnosis can be greatly reduced.

PCR has been an essential component of PGD for single gene disorders since the early 1990s. The DNA of biopsied cells is released and subsequently amplified to a detectable level, in order for it to be analysed for the presence and/or absence of a mutation. In the early days of PGD, DNA contamination and the phenomenon of allele dropout (ADO) posed significant challenges for the diagnosis of embryos (Handyside, 2015). To mitigate the risk associated with these problems, polymorphic markers were incorporated into multiplex PCR strategies, providing redundant diagnostic tests, resistant to the effects of ADO, and providing simple DNA fingerprints able to reveal the presence of contaminants. However, such strategies generally required a high level of customisation, which sometimes required several months of design, optimisation and validation before they were ready for clinical use.

Single nucleotide polymorphism (SNP) genotyping and karyomapping is a novel technique for diagnosing single gene disorders and/or chromosomal rearrangements, which was first clinically applied in 2014 (Natesan et al., 2014). Cells biopsied from embryos are lysed, subjected to whole genome amplification and then analysed using a microarray capable of interrogating a large number of SNP. Approximately 300,000 SNP, distributed throughout the genome, are genotyped in the parents and also a close relative of a known genetic status (e.g. an affected child of the couple). Analysis of the data produced reveals whether the unique combination of alleles on the parental chromosome(s) carrying the mutation(s) has (have) been inherited. Karyomapping has the potential to enable simultaneous diagnoses of more than one serious monogenic disorder, or of a monogenic disorder combined with a chromosomal rearrangement. Furthermore, karyomapping has a much faster work-up time compared with conventional methods (in some cases just days rather than months). Natesan et al. (2014) compared the accuracy of karyomapping with direct mutation detection in 218 embryo samples from 44 PGD cycles. Karyomapping was concordant with direct mutation testing in 213/218 (97.7%) and the few non-concordant samples were all seen in consanguineous families. Karyomapping also enables the detection of most forms of aneuploidy resulting from errors occurring during meiosis as well as some associated with mitotic abnormality. This may potentially improve embryo selection, enhancing the likelihood that a transferred embryo will form a viable pregnancy by avoiding the transfer of those harbouring lethal aneuploidies. However, this remains to be conclusively proven.

This study presents a retrospective case series of PGD cycles from a single IVF-PGD centre, which was an early adopter of karyomapping technology. In total, pregnancy rates of 81 embryo transfers were available at the time of write up.

Materials and methods

All couples referred to the IVF Centre PGD from February 2014 and December 2015, where the work up by karyomapping was feasible were included in the study. Couples either self-referred or were referred to our lead PGD nurse (KD) from an National Health Service regional genetic centre by a clinical geneticist or genetic counsellor. The genetic nurse (KD) took a genetic history and discussed whether karyomapping was possible. Karyomapping was feasible if the DNA from a close relative of known genetic status (including son or daughter) or from an affected fetus (e.g. from a prenatal sample taken during a previous pregnancy) was available. The list of single gene disorders and/or chromosomal arrangements tested by karyomapping during the course of this study is shown in **Table 1**.

Table 1 – Single gene disorders and chromosomal rearrangements tested by karyomapping.

Single gene disorders
Anaemia adrenoleukodystrophy
Adult polycystic kidney disease
Anirida
Argininosuccinic aciduria
Betathalassaemia
Haemoglobinopathy E
BRCA1
BRCA2
Cardiomyopathy
Catecholaminergic polymorphic ventricular tachycardia
Charcot-Marie-Tooth 1a
Cystic fibrosis
DMD
Ectodermal dysplasia (hypohidrotic)
Familial adenomatous polyposis coli
Fascioscapulohumeral dystrophy
Fragile-X
Emery-Dreifuss type 2
Frax-E
Hereditary diffuse stomach cancer
Hereditary non polyposis colorectal cancer
Hypophosphatasia
Huntington disease
Incontinentia pigmenta
Leber congen amaurosis
Marfan syndrome
Meckel Gruber syndrome type 3
Neurofibromatosis type 1
Noonan syndrome
Norrie syndrome
Paraganglioma
Pendred syndrome
Pompe disease
Ponotcerebellar hypoplasia type 2
Retinoblastoma
Retinoschisis
Sickle cell disease
Spinal muscular atrophy
Spinocerebellar ataxia
Spinocerebellar ataxia 14
Spondylepiphyseal dysplasia congenital
Stuve Wiedeman syndrome
Usher syndrome
Vanishing white matter syndrome
Von Hippel-Lindau syndrome
Waardenburg type 2 syndrome
X-linked hypophosphatemic rickets
X-linked lissencephaly
X-linked optiz syndrome
Chromosomal rearrangements
Insertion (2;3)(q13;p24.3p25.3)

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