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ARTICLE

Karyomapping allows preimplantation genetic diagnosis of a de-novo deletion undetectable using conventional PGD technology

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Carles Giménez obtained his Bachelor's degree in Biology in 1989 at Universitat Autònoma de Barcelona (UAB, Catalonia). He started his research work focused on the development of PGD techniques, and gained his PhD in Biological Sciences from UAB in 1995. He is also a Senior Embryologist (ESHRE, 2008), Genetic Counsellor (Pompeu Fabra University, 2009) and accredited geneticist (AEGH, 2015). He is one of the PGD/PGS pioneers, and was involved in the teams that achieved the first pregnancies to term after PGD in Spain. In 2003 he cofounded Reprogenetics in Barcelona. Since then, he has been the molecular laboratory director and the scientific co-director.

Abstract Preimplantation genetic diagnosis (PGD) was carried out for a couple carrying a de-novo deletion in the *TSC2* gene, responsible for tuberous sclerosis. Karyomapping, a method employing genome-wide analysis of single nucleotide polymorphisms (SNP), was used as PGD protocol. Analysis of DNA from the affected parent using karyomapping confirmed the region covered by the deletion and revealed more than 30 SNP located within the affected region. These SNP were subsequently used for embryo diagnosis (deletion revealed by hemizygosity and/or reduced probe intensity). Seven blastocyst embryos underwent trophectoderm biopsy followed by vitrification. Biopsied cells were subjected to comprehensive aneuploidy screening using microarray comparative genomic hybridization (aCGH), with karyomapping for the detection of embryos carrying the mutant *TSC2* gene carried out in tandem. Two embryo transfers were performed, the second of which resulted in the birth of a child. This study highlights that karyomapping may be applicable to a subset of de-novo mutations undetectable using standard PGD strategies. Additionally, karyomapping results were in complete concordance with aCGH, both methods revealing the same aneuploidies in the embryos tested. It was concluded that karyomapping may represent a valuable advance in cases of PGD for monogenic diseases.

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Introduction

Tuberous sclerosis (TSC) (OMIM: 191100) is a multi-system disorder with a dominant mode of inheritance, characterized by hamartomas in the brain, skin, eyes, heart, lungs and kidneys (Yates, 2006). These tumours are usually benign, but they are associated with significant morbidity and mortality. Of most clinical consequence are central nervous system tumours and renal disease. The disease is caused by mutations in two tumour-suppressor genes: *TSC1* (9q34, OMIM 605284) and *TSC2* (16p13, OMIM 191092).

The diagnosis of TSC is usually based on clinical and radiological findings. Mutations can be identified in around 85% of individuals who meet diagnostic criteria for TSC, with the majority (69%) found in *TSC2*. Approximately two-thirds of affected individuals have TSC as the result of a de-novo mutation (http://www.ncbi.nlm.nih.gov/books/NBK1220/).

Preimplantation genetic diagnosis (PGD) is an alternative to prenatal testing that enables people with a specific inherited condition in their family to avoid passing it on to their children. It involves the creation of several embryos using assisted reproductive technology, followed by the removal of one or more cells and detection of the mutant gene. Embryos found to be unaffected can be transferred to the mother's uterus whereas those with the mutation are discarded. Using PGD, high-risk patients can avoid issues of pregnancy termination or the birth of an affected child.

The detection of a single gene disorder (SGD), such as TSC, in cells biopsied from preimplantation embryos remains challenging because sensitive multiplex-polymerase chain reaction (PCR) methodologies are required in order to amplify specific DNA fragments (e.g. the mutation site and/or linked polymorphisms) to detectable concentrations. Problems such as allele drop out (ADO – the failure to amplify one of the two parental alleles in the biopsied cell) and contamination with extraneous DNA are important sources of misdiagnosis. To reduce the impact of these difficulties, current standard practice guidelines recommend combining amplification and analysis of several closely linked polymorphisms, along with (when possible) direct mutation detection (Harton et al., 2011; The Preimplantation Genetic Diagnosis International Society, 2008). Identification of informative polymorphisms and design and optimization of a multiplex-PCR capable of amplifying all of the necessary loci from a single cell, requires a significant amount of laboratory work. This usually leads to a delay in treatment while the protocol is created and validated and adds considerably to the cost of PGD.

Recently, karyomapping has been proposed as an alternative to conventional PCR-based protocols (Handyside et al., 2010; Natesan et al., 2014). Essentially, karyomapping involves genome-wide linkage analysis, in which several hundred thousand single nucleotide polymorphisms (SNP) scattered throughout the genome are genotyped in the two parents and their embryos. Each chromosomal region has a unique SNP fingerprint, allowing the inheritance of chromosomal segments (and the genes they contain) to be tracked from one generation to the next. By comparing SNP results obtained from the parents to those obtained from other family members of known genetic status (e.g. another relative carrying the same mutation as one of the parents) the combination of SNP alleles associated with a chromosome carrying a mutant gene can be identified. Transfer of embryos carrying this chromosome (or SNP pattern) can then be avoided.

The main advantage of karyomapping is that it can be used for diagnosis of any familial SGD without the need to develop a patient-specific test, greatly reducing the time required for work-up prior to PGD. Moreover, the detection of individual parental chromosomes using SNP allows trisomies of meiotic origin and monosomies to be revealed. Testing for aneuploidy in the embryos of patients undergoing IVF treatment, with transfer of those found to be chromosomally normal, has been shown to yield an improvement in ongoing pregnancy rates and is also anticipated to reduce risks of miscarriage and Down syndrome (Forman et al., 2013; Scott et al., 2013; Yang et al., 2012). Thus, the combination of SGD diagnosis together with aneuploidy screening may improve the clinical outcome of PGD cases.

Materials and methods

Patient information – genetic and clinical

A 32-year-old woman diagnosed as affected with tuberous sclerosis type 2, with renal symptoms and one year of secondary infertility, was referred to us to undergo an IVF–PGD cycle. Hysterosalpingography showed a non-permeable Fallopian tube. Her 33-year-old husband presented normal spermiogram and fluorescence in-situ hybridization (FISH) sperm test. Both had normal karyotypes.

Using multiplex ligation-dependent probe amplification (MLPA) the woman was shown to be a carrier of a de-novo deletion encompassing exons 1 to 15 and also the 5′ region of the *TSC2* gene.

Preliminary work-up

The patients received appropriate counselling and informed consent was obtained for all procedures undertaken. The clinics and laboratories involved in providing treatment hold all licences required to permit IVF and PGD to be offered. Since PGD is considered to be a standard treatment, which requires patient-specific optimization and the development of novel protocols as a matter of routine, no further ethical approval was sought on this occasion. Traditional PGD work-ups for patients at risk of transmitting a single gene disorder require a high degree of customization, comprising the design and optimization of PCR primers for amplification of the mutation site and several linked short tandem repeat (STR) polymorphisms. However, in this case, a de-novo mutation was responsible for the disorder. Since no other family members carried the mutation, linkage analysis was not possible. Direct