Expanding and improving the service for testing single embryonic cells by preimplantation genetic haplotyping

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Abstract

The problem of having offspring with inherited diseases can be resolved in some cases through preimplantation genetic diagnosis (PGD). Spinal Muscular Atrophy (SMA) is one of these diseases. In my short term visit to Guy's Hospital in London, I set up a panel of markers which can be used for preimplantion genetic haplotyping in affected families with this pathology.

Introduction

Preimplantation genetic diagnosis and preimplantation genetic haplotyping

Preimplantation genetic diagnosis (PGD) is an option for couples at risk of transmitting inherited diseases to their offspring who wish to avoid an affected child, recurrent miscarriages, termination of pregnancy or having to adopt children (1-3). In this technique, embryos are generated by *in vitro* fertilization (IVF) and the diagnosis in them permits the transfer of only unaffected embryos.

PGD is now routinely applied for three different groups of inherited genetic disorders: X-linked disorders, monogenic diseases (single gene defects) (4-6) and chromosomal abnormalities (6-11). PGD can be considered a very early stage of prenatal diagnosis with testing being performed either immediately following fertilization, or at day 3 or day 5 post fertilization. Almost all PGD centres perform cleavage stage biopsy at day 3 post fertilization (when the embryo has 6-8 cells).

The application of PGD for single gene defects is limited by the need to develop family-specific single-cell PCR mutation test. The very small amounts of DNA targeted and analyzed by these procedures means that not only are they extremely vulnerable to contamination by extraneous DNA, but also to allele dropout (ADO; where one or both of the 2 alleles at any locus fails to amplify) which can compromise the accuracy of the diagnoses (12-14).

In order to provide large quantities of DNA from only one cell, efficient whole genome amplification can be performed by Multiple displacement amplification (MDA) which uses bacteriophage Φ29 polymerase (15-17). DNA from a single biopsied blastomere undergoes approximately 10exp6 fold amplification by MDA, allowing multiple PCR tests to be performed on the amplification products. The ADO rates when you use PCR on MDA products, are between 5 and 31% and these results are not acceptable for direct mutation testing (18,19), nevertheless MDA has been applied clinically in PGD of some diseases (20).

To overcome the problem of a high rate of ADO, multiple DNA markers form within and around a disease gene can be used to provide indirect testing by haplotype analysis (a kind of DNA fingerprinting), following genotyping of appropriate family members. The amount of DNA provided through MDA from a single cell makes testing with multiple markers straightforward. So, even with high ADO you can determine the haplotypes if you use sufficient markers. This technique has been termed preimplantation genetic haplotyping

(PGH) and has been used clinically for Duchenne muscular dystrophy and Cystic Fibrosis (21). In addition, the use of fluorescent PCR allows the development of sophisticated and sensitive assays, so extreme preferential amplification can be distinguished from true ADO (22). Fluorescent PCR permits multiplexing of different primer pairs with the use of semi-automated analysis through a capillary electrophoresis system (23).

Haplotype analysis depends upon the testing of linked markers within, or in close proximity to, the gene of interest. A minimum of two alleles is required for confident assignment of a haplotype. If you use sufficient markers you will overcome the difficulties associated with particular markers being uninformative for a given family, problems of ADO and potential problems that might result from undetected recombination between a mutation and a linked markers, since the large number of loci tested will increase the likelihood of identifying such recombinants and identify the location of recombination with great precision. Family members must be tested to deduce the high-risk haplotype(s) that have been inherited by the affected individual(s) and the low-risk haplotypes. Once the high and low risk haplotypes within a family have been established they can be used to determine the genetic status of embryos for that family (21,23).

Aim of study

To expand the PGH (preimplantation genetic haplotyping) service by establishing new panels of linked markers for further monogenic disorders as required by the Preimplantation Genetic Diagnosis Centre.

Patients and Methods

Using on-line genome databases and published literature to select a panel of polymorphic markers, designing primers and multiplex PCR assays. Assessment of the suitability of the chosen markers by looking at their results in a panel of 30 individuals (60 chromosomes), several families and MDA products from a panel of single blastomeres.

Results

The main disease studied was Spinal Muscular Atrophy (SMA). The spinal muscular atrophies are a clinically and genetically heterogeneous group of neuromuscular disorder caused by degeneration of anterior horn cells. This disease has a birth prevalence of 1/10.000, and a carrier frequency of 1/40-1/60; SMA is among the most frequent autosomal recessive hereditary disorders.

The Survival of Motor Neuron (SMN) gene exists as two homologues, in unaffected individuals each chromosome 5 has at least one telomeric gene (SMN1) and in the vast majority of chromosomes at least one centromeric gene (SMN2), although chromosomes exist with an intact SMN1 gene that lack SMN2. Deletions of SMN1 gene appear to be directly involved in SMA, since exon(s)7 (and 8) of SMN1 are undetectable in over 95% of patients, irrespective of their clinical type, either as a result of homozygous deletions, or because of conversion of sequences of SMN1 into those of the SMN2 gene (30).

PGD for SMA is currently offered at Guy's Hospital by testing directly for the SMN1 deletion by fluorescent PCR restriction digest and the use of a single linked marker D5S610. The test has been transferred across to the new approach of PGH.

A panel of linked polymorphic markers for SMA has been designed. Some of them had been used diagnostically before were found in the European Best Practice Guidelines for

Molecular Analysis in Spinal Muscular Atrophy, others were found using the Santa Cruz genome browser. Fluorescent Multiplex PCR assays were performed to determine the het value (heterozygosity value) of each marker, to know the level of ADO in blastomere panel, and to know the suitability of these markers in families affected by SMA. In particular, the use of a marker has been assessed, known to be present in multiple copies on chromosome 5 for inclusion in the marker panel as it is located in the promoter region of the SMN1 gene.

Also designed a panel of markers for chromosome 21 has been designed.

Discussion and Conclusions

The assay developed to test for SMA by PGH was completed and used in a clinical PGD case and an unaffected embryo was identified and replaced.

The panel of markers for chromosome 21, hopefully, will be assessed and introduced in the future as a test for trisomy 21 which will be offered to women undergoing PGD for single gene defects who are at increased risk of Down Syndrome due to raised maternal age.

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