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Cystic Fibrosis Research News

Title:

Single–cell High Resolution Melting Analysis: A novel, generic, pre-implantation genetic diagnosis (PGD) method applied to Cystic Fibrosis (HRMA CF-PGD).

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What was your research question?

For a variety of genetic conditions which are known to be inherited, pre-implantation genetic diagnosis (PGD), where IVF embryos are tested for inherited conditions like cystic fibrosis (CF) before being implanted into the mother's womb, represents a safe, established alternative to prenatal diagnosis where the tests are carried out on the baby in the womb. We aimed to develop a flexible, low-cost and reliable method of identifying a person's genetic profile, to allow the analysis of any combination of CF genes inherited from the parents.

Why is this important?

Currently, most laboratories offering PGD for CF test for the presence of the most common mutation (Phe508del) coupled with chromosome markers which are closely linked to the CFTR gene in a process, called "haplotyping". By looking at chromosome markers, laboratories can detect chromosomes inherited from the parents and identify which affected or unaffected CFTR genes are transmitted to the embryo. To haplotype embryos, samples from relatives (such as healthy or affected siblings or grandparents) must be analysed at the same time. This is often challenging, as it is not always possible to obtain samples from relatives for a variety of reasons i.e. deceased grandparents or unavailable sibling.

What did you do?

This new approach allows us to detect a wide range of CFTR mutations at the same time and haplotype single-cells without designing new tests for new mutation combinations. The method we developed overcomes the need to test relatives for haplotyping by making the best use of the widely used High-Resolution Melting Analysis (HRMA) technology to analyse single cells which are appropriate for testing during PGD. In the absence of samples from

relatives, haplotyping is achieved by building up the single cell's genetic profile through the combination of its status (carrier or normal or affected) with its haplotype markers. This single cell's profile is used as a "seed" to "type" its sibling embryos.

What did you find?

We developed a method that for the first time can use the HRMA technology in single cells. We successfully applied this approach in 15 cycles of clinical PGD in 15 carrier couples representing 14 distinct combinations of different genetic profiles. CF genetic profiles were identified in 88 out of 93 samples using HRMA and were confirmed using a different method at the same time. 57 embryos were suitable to be implanted into the womb, which was done in 14 out of 15 couples. Six procedures resulted in pregnancy (2 sets of twins and 4 single babies) and the PGD genetic profiles were confirmed using conventional prenatal diagnosis.

What does this mean and reasons for caution?

Couples who are carriers of CFTR genetic mutations have benefited greatly from PGD targeted at selecting embryos not at risk of having CF. Given the genetic differences in CF, especially in Mediterranean populations, it would be expensive, difficult and time-consuming to develop and make best use of an individually tailored PGD method to diagnose specific combinations of mutations in the CFTR gene. HRMA is a simple and reliable method which is easily adapted for the analysis of virtually any CFTR mutation. It allows the highly sensitive and specific detection of DNA sequence variants and this study shows it is a valuable tool in identifying CF genetic profiles from single cells.

What's next?

The HRMA approach using single cells which we described above can be used to detect mutations in other types of PGD for any disease that is inherited when a single defective gene is passed on from each parent. It can also be used in other fields besides PGD, wherever single cell genetic profiling is required.

Original manuscript citation in PubMed

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