Abstract Details

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Abstract title:

A novel long-range DNA sequencing approach improves the design of new protocols for preimplantation genetic testing of monogenic disease (PGT-M).

Biography

I am a final year DPhil (PhD) student at the University of Oxford, with research focused on the development of novel and less invasive methods for preimplantation genetic testing. Prior to my DPhil, I achieved a BSc (Hons) in Reproductive Biology from the University of Edinburgh and an MSc in Clinical Embryology from the University of Oxford.

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Study question:

Can long-range next-generation sequencing assist in the work-up of PGT-M cases, with potential to increase patient access, improve accuracy and reduce costs?

Summary answer:
Long-range DNA sequencing provides a powerful, low-cost method that reveals the informative SNPs closest to gene mutations and eliminates the need for additional family samples.

What is known already:
To avoid misdiagnoses caused by allele dropout (failure to amplify one of the two alleles in an heterozygous cell), PGT-M strategies usually involve parallel analysis of several diagnostically relevant sites (e.g. mutations+linked polymorphisms). Polymorphisms need to be as close as possible to mutation sites because of the possibility of recombination. To determine which polymorphic alleles are associated with the disease, DNA from additional family members is usually analysed. However, relatives are not always available, as patients may carry *de novo*mutations, relatives may be untested or deceased, or patients may not wish to disclose their PGT-M treatment to others.

Study design, size, duration:
A novel work-up method for PGT-M was evaluated. DNA was obtained from 13 couples undergoing PGT for different monogenic disorders. For each mutation, two primer sets (A and B) were designed, allowing amplification of the mutation plus an additional ~10kb upstream (A) or downstream (B). Amplicons were sequenced as single contiguous reads using the MinION (Oxford Nanopore). This identified informative single nucleotide polymorphisms (SNPs) and revealed which alleles exist on the same chromosome as mutations.

Participants/materials, setting, methods:
10kb regions flanking (and encompassing) mutation sites were sequenced from the 26 patients. Traditionally, candidate linked polymorphisms are identified from databases, but many turn out to be uninformative. Alternatively, parental samples can be analysed by microarray, simultaneously assessing many SNPs, but only evaluating a fraction of the variations in the genome. The SNPs found to be informative are sometimes relatively distant from the affected gene, increasing the chances of

recombination between the SNP and mutation.

Main results and the role of chance:
All 18 mutations in the 13 couples were successfully detected using long-range sequencing. Additionally, between 2 and 83 (average 18) informative SNPs were found in the 10kb flanking regions. The average distance from mutation sites to the nearest informative SNPs was 2529bp.This compares to an average distance of 32,179bp when microarrays were used to identify suitable SNPs. The extremely close proximity of polymorphisms identified by long-range sequencing means that diagnostic challenges due to separation of SNPs from the disease-causing mutation by meiotic recombination can be virtually ruled out. In 28% of patients the closest informative polymorphism had a minor allele frequency <0.1. Such SNPs are rarely heterozygous and are therefore unlikely to be included in SNP-microarrays and are also unlikely to be chosen from databases as a candidate marker for PGT-M work-up.Additionally, novel informative intragenic SNPs, not present in any database, were identified in three couples. Because mutations and SNP alleles were contained within the same sequencing ‘read’, phasing was successfully accomplished in all cases, without any need for samples from additional family members. This will be of great value for couples who have no relatives suitable for phasing of polymorphisms (approximately one quarter of all PGT-M couples).

Limitations, reasons for caution:
Unlike generic PGT-M methods (e.g. haplarithmisis/karyomapping), this strategy requires a customised protocol for each couple. The use of direct mutation testing combined with analysis of the closest possible SNPs flanking the mutation site is an approach that is unsurpassed in accuracy, but requires primer design and therefore slightly more work-up.

Wider implications of the findings:
By providing a simple, inexpensive, rapid method of identifying the closest informative polymorphisms to parental mutations, long-range sequencing potentially improves PGT-M accuracy, reduces costs of customised protocols and accelerates test development. Furthermore, this approach removes the need to obtain DNA from any family members other than the couple undergoing PGT.

Keywords:
PGT-M
DNA
Sequencing
Nanopore
SNPs