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Editorial

A big step forward for PGT-M?

The recommendations for preimplantation genetic testing for monogenic conditions (PGT-M) are to use linked markers to allow a more confident determination of genetic status in preimplantation embryos (Harton et al., 2011; Thornhill et al., 2005). Historically, this was recommended as a means to prevent misdiagnosis due to allele dropout (ADO) and contamination (Pickering et al., 1994; Findlay et al., 1995; Rechitsky et al., 1999; Wilton et al., 2009). However, the requirement to create complex patient-specific tests incorporating multiple linked markers has increased the time and cost associated with the development of PGT-M testing.

Ideally, low-cost universal tests would be available, that can be applied to all patients, or to patients with specific indications, avoiding the need for the costly development of couple-specific tests. In this issue of *RBM Online*, Kubikova et al. (2018) describe a universal method for PGT-M for beta thalassemia and sickle cell anaemia, removing the need for individual development of tests for couples with mutations in the beta globin gene (HBB), at risk of having a child with beta thalassemia or sickle cell anaemia. The authors describe a method that they used to diagnose successfully 21 embryos from 3 couples with different beta thalassemia mutations, with 100% concordance with karyomapping.

This test uses next generation sequencing (NGS) to sequence the entire HBB gene alongside 17 very closely linked single nucleotide polymorphism (SNP) markers, providing a test that is suitable for the vast majority of HBB-affected couples. Unlike purely linkage-based methods, this test also allows the identification of almost all HBB mutations, reducing the need for accompanying family DNA samples which is one of the major disadvantages of linkage-based methods.

Additionally, panels such as this one, that have a relatively low number of total reads, enable a large number of samples to be analysed simultaneously, allowing the per-sample cost to be relatively low. In parts of the world where beta thalassemia and sickle cell anaemia are common, this could provide a way to access lower-cost universal preimplantation genetic diagnosis (PGD) for these diseases. In contrast, universal whole-genome linkage-only PGT-M methods, while providing a single method that can be used for multiple genes (Chen et al., 2016; Handyside et al., 2010; Natesan et al., 2014; Zamani Esteki et al., 2015), are relatively expensive per sample and may be cost-prohibitive for many. Additionally, purely linkage-based methods require supplementation with a direct mutation-detection method when family DNA samples are not available, or when mutations are *de novo* (Konstantinidis et al., 2015) and can also lead to difficulties in diagnosis when genetic recombination occurs close to the disease gene. In the method described here, the linked SNP markers were all located within 14 kb from the HBB gene, reducing considerably the risk of genetic recombination impacting on the diagnosis.

Of particular interest in this article, the authors reported 0% ADO from 141 heterozygous sites, including some blastomere samples that had high rates of ADO when measured by karyomapping. This appears to be due to the ~1000x read depth achieved with NGS, allowing alleles present in very low proportion to be visualised, whereas with less sensitive technologies (such as Sanger sequencing), the lower proportion allele would be obscured by the other allele that has been amplified preferentially, thus manifesting as ADO. This has also been described previously as an advantage of NGS (Yan et al., 2015), and is something that is worthy of further investigation. If the incidence of ADO proves much reduced using NGS, and as ADO was one of the main reasons for including linked markers in PGT-M, then the question arises, will there come a time when linked markers are no longer considered a necessary component of PGT-M? For the time being it would still seem prudent to continue to use them, and they also have a role in the detection of contamination, but there is definitely cause to revisit this in the future.

The test described in this article does not also provide a tandem solution for preimplantation genetic testing for aneuploidy (PGT-A), but this is not necessarily a disadvantage. The authors state that it is possible to perform PGT-A alongside, with a separate aliquot of the initial whole genome amplification being used with a suitable PGT-A method, obviously at an additional cost. It is important to mention also that there are currently several differing opinions regarding the routine use of PGT-A (Braude, 2018), and PGT-A is certainly not an essential component of a good PGT-M test.

In summary, the authors describe an excellent, low cost, universal PGT-M method for beta thalassemia and sickle cell anaemia. It provides a possible glimpse into the future, where panels for certain genetic diseases may be commonplace, with more expensive whole

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genome universal PGT-M methods, or custom methods, reserved for rare diseases for which it is less feasible to develop a gene-specific NGS panel.

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