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NEWS AND COMMENTARIES

Genetic Testing

From chromosomes to DNA, a revolution in prenatal diagnosis

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Chromosomal aberrations in the foetus have been detected for more than 30 years now by karyotyping cells from chorionic villi or from amniotic fluid. This is an elaborate and time-consuming process, requiring highly skilled technicians. The vast majority of aberrations detected are numerical, loss or excess of entire chromosomes.

Fluorescence *in situ* hybridisation (FISH) using DNA probes specific for the most frequently involved chromosomes is a faster alternative to karyotyping. However, FISH still requires considerable hands on time of trained technicians, in particular counting the signals on a sufficient number of nuclei, a process not easily automated in the diagnostic laboratory.

What we need is a rapid, easily automated technique, which will allow a prescreening of samples for the most common chromosomal aberrations, after which full karyotyping can be reserved for those cases in which there is substantially increased risk: the foetus showing developmental anomalies on ultrasonography, including enlarged nuchal translucency, and an abnormal DNA test.

Several variants of quantitative PCR have been shown to rapidly and accurately detect copy number changes characteristic for chromosomal aberrations.[1–3] The three approaches karyotyping, FISH, and quantitative PCR have been compared,[4] and cost-effectiveness of various testing regimes have been assessed.[5]

Why then, are so many cytogenetecists reluctant to abandon full karyotyping, and to have it replaced by a DNA test? There may be several reasons, one of which is the fact that the practical efficacy of a screening test in terms of success rate, positive, and negative predictive value, cost, etc. can only be assessed on very large numbers of samples. The report by Mann *et al*[6] fills part of this gap, since a very large number of samples have been tested in parallel using both quantitative PCR and karyotyping, showing the former to be a robust, reliable, and fast alternative to the latter.

The application of DNA techniques opens new possibilities that have as yet not been explored. So far only fragments of chromosome 13, 18, 21, X, and Y have been used. However, Rahil *et al*[2] used PCR fragments within genes, hinting already at the possibility to detect mutations at the gene level. In a multiplex PCR, a very large number of fragments can be amplified simultaneously. Various detection techniques are being developed for such large numbers of PCR products using microarrays or beads. Thus, subtelomeric regions, microdeletion syndromes, frequently occurring marker chromosomes, etc can easily be included. In addition, we could use for detection of the X chromosome exons of the Duchenne gene, which are most often deleted in patients. Moreover, we could include a fragment specific for the deltaF508 mutation, responsible for cystic fibrosis, exon 7 of the SMN1 gene, deleted in patients with spinal muscular atrophy, etc.

One may argue that prospective parents have not asked for this, and therefore we should stick to those conditions we used to be able to detect by karyotyping. But, do we really know what the parents want to know, and have tested ? It is not unreasonable to assume that the parents wish to have a test done which will detect any condition which will cause prolonged and sustained suffering to their child, irrespective of whether it is a chromosomal aberration or a gene mutation.

If however, we do include gene tests such as deltaF508 and exon 7 of SMN1 in our test, we will detect also heterozygous carriers in a relatively large number of pregnancies, which will cause much unrest and anxiety that could have been avoided, had the test been performed in the parents before conception.

Thus, the increased prenatal diagnostic capabilities dictate radical change in obstetric care. Preferably, the prospective parents should be counselled in advance to allow for reflection on which diagnostic possibilities they wish to use. Since the majority of pregnancies today are planned, such preconception advice should be incorporated in primary care.

If prenatal diagnosis not only includes the most frequent aneuploidies, but also a large number of other conditions, the question arises whether we should stick to serum screening, nuchal translucency, and maternal age to select high-risk pregnancies for invasive procedures. Harris *et al*[7] already belled the cat by contemplating eligibility to invasive prenatal testing of all pregnant women.

We can conclude that today, 30 years after its beginning, prenatal diagnosis is very much alive, and a great many questions on how to go about it are storming at us. In parallel with further developing our diagnostic capabilities we should pass on the information to prospective parents to allow for informed decision making and free choice. By much trial and little error, we should find a new standard of prenatal care which minimizes alarm and anxiety during pregnancy, and maximizes the benefits of improved diagnosis■

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Gene Therapy

The 'pro-sense' approach to Duchenne muscular dystrophy

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It seems that a clinically applicable gene therapy for Duchenne muscular dystrophy is within our grasp, now that two recent studies show that safe and efficient systemic delivery of antisense oligoribonucleotides (AONs) to induce exon-skipping in the dystrophin gene is possible.[1,2]

After years of struggle to develop a gene therapy for Duchenne muscular dystrophy (DMD), there now seems to be a tool with which we can utilize an escape route that nature had already hinted at. A deficiency of the membrane protein dystrophin causes the progressive deterioration of muscle fibres in DMD. Sometimes however, DMD patients have rare, dystrophin-positive fibres ('revertant fibres') that originate from exon skipping in the dystrophin gene, which generates a truncated transcript with a restored open reading frame.[3] Over the last 6 years, several laboratories have shown that we can actually enhance or induce this therapeutic exon skipping,[4] using small synthetic AONs.

The simplicity of the AON approach, as well as its specificity and efficacy, has been amazing. The important issue that remained, which the two new studies by Lu *et al*[1] and Goyenvalle *et al*[2] addressed, was how to develop a safe and efficient systemic delivery method that reaches all skeletal and cardiac muscles.

AON-induced exon skipping therapy is based on the reading frame rule.[5] This rule states that frame-shifting mutations in the DMD gene cause DMD, whereas frame-conserving ones mostly cause the milder Becker muscular dystrophy (BMD). Through the skipping of exons in DMD transcripts, AONs can restore the reading-frame, and convert DMD into BMD-like fibres. AONs vary in length between 16 and 22 nucleotides and are chemically modified to be resistant to intracellular nucleases and RNaseH. It is thought that they bind to specific sequences in the pre-mRNA, and thus disturb exon inclusion signals like splice sites, intronic branch point sequences, or exonic splicing enhancer elements. This, in turn, leads to the removal of the targeted exon from the processed mRNA.

These new studies[1,2] clearly show that the AON-induced skipping of exon 23 is therapeutic for the *mdx* mouse. This is an animal model that is dystrophin-deficient due to a nonsense mutation in the in-frame exon 23. Lu *et al* use an AON that targets the 5′ splice site of this exon, in combination with a 'drug carrier' called F127. This block copolymer belongs to the group of amphiphilic Pluronics that is extensively used in the pharmaceutical industry. F127 promotes the metabolic stability and circulation time of AONs in the blood circulation and their transport across cell membranes.

In a previous study, the same authors applied intramuscular injections and detected dystrophin expression that resulted from frame-restoring exon 23 skipping in up to 20% of muscle fibres.[6] This expression persisted for 2 months and significantly improved the strength of the treated muscles. In the recent study,[1] they injected 2 mg of the same AON with F127 through the tail vein in *mdx* mice. At 2 weeks after a single injection, significant numbers of dystrophin-positive fibres were detected in all muscle groups analysed, including the diaphragm. The distribution of dystrophin-positive fibres was highly variable: a pattern that the authors attributed to the cycles of degeneration and regeneration in individual *mdx* muscle fibres that led to differential uptake of the AONs. After repeated administration, dystrophin levels accumulated up to 1–5% of normal, while the variable dystrophin levels stabilized.

Other indications for a significant role of the regenerative process on the AON uptake were the absence of dystrophin expression in the heart (an organ without regenerative capacity), and the increased dystrophin induction in older *mdx* mice (6 weeks or 6 months) *versus* younger mice (3 weeks). This is a major advance for systemic AON administration. However, since regeneration in *mdx* mice is quite different to that in DMD patients, the clinical relevance of these results remains debatable. Last but certainly not least, while the role of F127 in the systemic uptake remains to be investigated, it