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MEETING REPORT

Spell-checking our genes: report from the symposium *Mutation Detection in Large Genes*, 14 May 1999, Vicoforte, Italy

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Detection of DNA sequence differences, both misspellings in our genes and normal sequence variants, has become increasingly important in research and clinical application. One particular recurring problem, that of searching for any sequence alteration in a large gene suspected of being mutated, was the subject of a recent symposium, arranged as a special session during the Fifth International Mutation Detection Workshop (Vicoforte, Italy, 13–16 May 1999). Such mutation scanning is common during the 'end game' of disease gene mapping, when the culprit gene has been confined to a limited region through linkage or association studies. Likewise, many large genes can be suspected to be altered in specific diseases and require mutation scanning in a clinical context. Examples are the breast cancer genes, and in Duchenne muscular dystrophy the dystrophin gene, whose genomic size approaches that of the total *E. coli* genome.

The importance of developing and sharing expertise in this area prompted an initiative by the European Union to bring about and support this symposium through a contract to the Human Genome Organization (HUGO). The Mutation Detection workshop was the ideal setting for such an event, because it brought together inventors and major users of mutation detection methodology. The meeting was attended by 120 registrants from 22 nations, all of whom either gave a poster or an oral presentation. The aim of the large genes session during this workshop was to illustrate the present state of the art in mutation detection in large DNA fragments or in genes with large numbers of exons. The focus was both on presenting the most recent methods and ones still in progress, as well as methods more widely used in the routine clinical laboratory. Apart from invited lectures, a special poster session was organised with contributions from participants.

Besides the gold standard Sanger DNA sequencing technique, a large number of methods are used to screen large genes for mutations with the aim of increasing throughput and reducing cost.

Recent developments in the very popular method Single Strand Conformation Polymorphism (SSCP) analysis were presented by Drs Steve Sommer and Kenshi Hayashi. The application of five running conditions (Dr Sommer) or fluorescence capillary electrophoresis (Dr Hayashi) allows detection of all or almost all mutations. Dr Sommer applied the technique to clinical mutation detection, whilst the purpose of Dr Hayashi's work was to identify single-nucleotide polymorphisms (SNPs), for their use as genetic markers.

Denaturing gradient gel electrophoresis (DGGE) is suitable for laboratories that perform numerous analyses of the same gene, as the main effort seems to be in setting up the conditions. Multiplexing in gel lanes is used in clinical laboratories (Dr Robert Hofstra). A simple automated 2D instrument has been developed, allowing initial size separation followed by DGGE separation without manual interference. This approach allows better discrimination of multiplex products and

enables complete visualisation of an entire gene in one gel, immediately revealing each exon and variants thereof (Dr Nathalie van Orsow).

The chemical cleavage of mismatch (CCM) technique is well known for its suitability for scanning long genes. New advances have rendered the method increasingly suitable for high throughput applications. In particular, a new protocol using potassium permanganate instead of the toxic osmium tetroxide, and automation of the procedure using a non-specific solid phase are important advances that were reported (Dr Richard Cotton).

The enzymatic cleavage of mismatch (ECM) method was presented by one of the inventors, Dr Rima Youil, who applied the method to mutation detection in the large fibrillin gene, and by Dr Mats Inganas, Amersham Pharmacia Biotech, who reported on the company's proprietary ECM system 'Passport'. It appears that in experienced hands ECM may reveal more mutations than have previously been detected by other methods, such as SSCP and DGGE. However, some concern was raised by the audience about the reproducibility of the method.

The protein truncation test (PTT) is another established method widely used in mutation scanning, despite its limited sensitivity. This method specifically detects mutations that interrupt reading frames, thereby altering the length of protein products translated from amplified DNA molecules. It is particularly suited to scanning cancer genes where mutations leading to abnormal or generally shorter mRNAs may be more frequent than missense mutations. PTT was presented by its inventor, Dr Johan den Dunnen, who highlighted both advantages and shortcomings of the method. He suggested the use of 3' tags to reduce the background of translation products, and to balance the signal strengths of full-sized products compared with truncated ones. He also discussed a multiplex approach for scanning the dystrophin gene where as much as 20 million bp could be scanned per gel.

The denaturing HPLC technique (dHPLC) was described by one of its developers, Dr Peter Underhill, who illustrated the speed and efficiency with which mutations can be found in this automated procedure. A very high sensitivity of mutation detection in many studies was reported. The method has been often applied for efficient scanning of large DNA segments in the search for sequence variants to be used as genetic markers: the case of the more than 100 Y-chromosome haplotypes characterised by dHPLC provided an elegant application of this technique to the study of human evolution.

One important trend in genetic analyses involves the use of DNA microarrays, as exemplified during the symposium by the use of high density oligonucleotide arrays to scan large genomic regions for the identification of single-nucleotide polymorphisms (Dr Janet Warrington). This approach (GeneChip probe array technology from Affymetrix) combined with laboratory automation enables a throughput of 10 Mb per week, corresponding to 100 genes across 40 individuals.

Several examples were presented of approaches currently in use for specific long genes, such as the tuberous sclerosis (TSC) genes (Dr Nicola Migone), inherited colorectal cancer genes (Dr Riccardo Fodde), and the dystrophin gene (Dr Gian Antonio Danieli). Posters on cancer genes such as RET, BRCA 1 and 2, APC, and on other diseases due to changes in large genes, such as Alport disease, were also presented.

The role of personal preferences in selecting the methodology was apparent in presentations dealing with the same genes. Moreover, even within the same laboratory often two or more procedures are used to analyse different portions of the same gene. This was illustrated by Dr S Pages *et al* who set up a screening protocol for the entire *BRCA2* gene by combining the advantages of several scanning methods. They used DGGE to scan 9 short exons and fluorescence-assisted chemical cleavage of mismatch (FAMA) to scan larger segments of the gene, ie 15 amplicons larger than 1 Kb. Dr Migone presented his experience on a three-step search for mutations in the *TSC* genes: first Southern blotting to detect large mutations, followed by PTT, and as a third step by FAMA to detect point mutations; recently, however, PTT and FAMA have been substituted by dHPLC which, according to him, appears more reliable and more sensitive, though quite laborious to set up. Dr Danieli reported that diagnostic laboratories dealing with the dystrophin gene commonly perform a preliminary run of PCR amplification to detect intragenic deletions, then a second run to detect intragenic duplications. An SSCP screening for point mutations is reserved for those samples that did not exhibit either deletions or duplications. To avoid this multistep approach he has tested double-stranded conformational analysis (DSCA) to screen the 79 exons of the dystrophin gene and found it useful, despite an estimated detection rate of only 70–75%.

It is generally considered useful to confirm the presence of a mutation, and to further characterise it by

using a different method, most often by direct sequencing. This should probably be taken as an indication that the 'perfect' method for scanning large genes for mutations is not available yet. The convenience of a push-button spell-check for our genes is nowhere in sight so far, and future development will have to address both efficiency of detection and the economy of these increasingly common investigations.

Abstracts for the presentations at the meeting can be read on the HUGO Website: www.gene.ucl.ac.uk/hugo/.