MEETING REPORT

Mutations, Structural Variations, and Genome-Wide Resequencing: Where to From Here in Our Understanding of Disease and Evolution?

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The 9th International Symposium on Mutations in the Genome, *Mutation Detection 2007*, was held on 23–27 September 2007 in Xiamen, China. Meeting participants reported on a broad range of advances in mutation detection technologies and their applications, including developments in SNP genotyping systems applicable to point-of-care diagnostic testing; and emerging views on structural variation, high-throughput sequencing and the importance of bioinformatic tools to support the growing amounts of genome variation data. This meeting report summaries the major themes presented at the meeting. Hum Mutat 29(6), 886–890, 2008. © 2008 Wiley-Liss, Inc.

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INTRODUCTION

The 9th International Symposium on Mutations in the Genome, sponsored by the Human Genome Organisation (HUGO) and the Genomic Disorders Research Centre (GDRC), was held in the scenic coastal city of Xiamen in southeast China. Continuing the tradition of these biannual Mutation Detection meetings for being hosted in exotic locations around the world, delegates from 27 countries converged onto the seaside resort in September 2007 to the backdrop of the Mid-Autumn Festival. During this lively festival, complete with fireworks and moon cakes, the Chinese celebrate the woman on the moon, a tradition that challenges the Western myth of a man on the moon. Gathered here at the only forum dedicated to mutation detection technologies and their applications, it was timely that researchers, clinicians, and developers of technology, challenged by the next wave of genetics in the post-genome era, were able to discuss the way forward in applying our understanding of the human genome, to rapidly and cost-effectively detect the genetic variation that will see the realization of point-of-care diagnostic tests (POCT) and personalized medicine and a better understanding of evolution than has previously been possible.

Comparisons of Mutation Detection Methods: Everything Old Is New Again

Before delegates were treated to a tantalizing array of emerging technologies and novel approaches for genetic analysis, the meeting began with a critical evaluation of established mutation detection methods and their usefulness in clinical and research applications. Meeting participants took a new look at some "old" mutation detection methods with comparisons to current technologies in terms of sensitivity and reproducibility of detection, as well as ease of use and cost, which are all important factors to consider when choosing a mutation detection platform for a particular application. It can be common practice to simply use the latest methods, developed for use with superior technologies such as fluorescent dyes and capillary electrophoresis (CE), without much consideration for older methods and their potential adaptation to modern platforms for improved assay sensitivity or throughput. It was evident from the findings presented by Vanessa Hayes (Sydney, Australia) that denaturing gradient gel electrophoresis (DGGE) remains an important technique for the detection of sequence variants. DGGE was reported to be the method of choice, outperforming high-resolution melting curve analysis (hrMCA), for the detection of multiple sequence variants within a single DNA fragment as well as defining *cis* and *trans* mutation orientation. Both methods reported similar sensitivities, detecting a 10% mutant allele fraction. The impressive adaptation of an older method to CE was presented by Annette Torgunrud Kristensen (Montebello, Norway), who reported the detection of remnant rectal cancer cells with a sensitivity of 0.4% mutation dilution using denaturant CE (DCE). The advantage of older methods when adapted to modern platforms was further validated by Paula Hedley (Stellenbosch, South Africa) who presented data on the detection of variants using PAGE-SSCP, multiplex CE-SSCP, and hrMCA. Traditional PAGE-SSCP has suffered from low sensitivity of mutation detection. Adapting the method to a CE platform with superior temperature control and using fluorescent primers has increased the sensitivity and usefulness of this method, detecting mutations in the *KCNQ1* gene of patients with Long QT Syndrome (LQTS; Romano-Ward Syndrome) that were not detected using the latest hrMCA approach. The utility of another

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old favorite DHPLC was demonstrated for prenatal screening and postnatal diagnosis of β-thalassemia mutations and was the optimal method for the detection of four previously unreported mutations, which were not detected in a blinded Sanger sequencing analysis (Wangwei Cai, Guangzhou, China).

Application and Evolution of Existing Mutation Detection Technologies

With the completion of sequencing the human genome 4 years behind us, we are now entering into the post-genome era. Our knowledge of genomes and their complex genetic and environmental interactions is gradually unraveling and pushing the boundaries of DNA technologies. It is becoming more evident that no one method will be appropriate for all mutation detection applications and more importantly that no single method will be able to detect all the complex variations that results in a disease or alternative phenotypic state. However, there still remains a need for more sensitive methods with a rapid time to result. A timely presentation to highlight this gap in technology described a study to detect mutations that inactivate the retinoblastoma tumor suppressor gene, which can be exonic and splice mutations as well as aberrant gene methylation, all of which can give rise to retinoblastoma. Using a combination of DNA sequencing to detect exonic and splice mutations, quantitative multiplex PCR to detect copy number variations (CNVs), and methyl-specific PCR to detect promoter hypermethylation, the approach reported a 92% sensitivity in unilateral retinoblastoma mutations (E. Zhang, Toronto, Ontario, Canada). Nancy Carson (Ottawa, Ontario, Canada) demonstrated the potential for combining two methods in order to achieve maximal mutation detection sensitivity for clinical diagnosis. When used independently, DHPLC and MLPA were more sensitive methods than the protein truncation test (PTT) for the detection of BRAC1 and BRCA2 mutations; however, neither method was 100% sensitive. The main limitation of DHPLC is that it is not suited to the detection of large genomic rearrangements, so when used in combination with MLPA, the combined methods were the most sensitive screening approach for detecting BRCA1 and BRCA2 mutations.

A simple approach to improve the sensitivity of mutation detection using hrMCA incorporated a preenrichment PCR step of the target DNA region before subsequent RT-PCR and hrMCA was performed using nested primers (Zhongyi Li, Canberra, Australia). It was reassuring to know that our needs are being addressed, as shown by a presentation from Roche (Christian Weilke, Penzberg, Germany) describing the new LightCycler 480 ResoLight dye. This dsDNA intercalating dye promises high signal intensity with low background fluorescence for greater sensitivity of mutation detection and has superior photostability, reducing temperature shifts from dye degradation as well as allowing more robust analysis using robotic reaction setup. Of particular note was the preeminence of real time PCR-based mutation detection technology in China stimulated by the severe acute respiratory syndrome (SARS) crisis. We anticipate and look forward to the development of many more mutation detection methods based on this simple and sensitive technology.

As Eileen Lim (Singapore) highlighted, the discovery of a mutation in an affected proband that is also detected in an unaffected parent may suggest the presence of other unknown mutations and/or environmental influences that have yet to be associated with the disease. This finding emphasizes the importance of mutation databases (see below) to complement the continual development of DNA technologies, as the increasing sensitivity of mutation detection methods will only be as useful as our knowledge of the disease-causing mutation. Jie-Yu Huang (Seattle, WA) described an interesting case of autosomal recessive Gaucher disease in which a common mutation (c.1226A>G) was identified in exon 9 of the glucocerebrosidase (GBA) gene in the proband. However, an informative follow-up screening of the parents only identified the c.1226A>G mutation in the father. Southern hybridization revealed a 1.5-kb deletion spanning intron 7 to intron 9, resulting in the complete deletion of exon 9 in the mother. This observation provides further support for a multi-analysis mutation detection approach and raises a new question: Should parents also be tested when possible to minimize misattribution of genotypes in autosomal recessive disorders?

The identification of hundreds of candidate genes for disease from genome-wide association studies has prompted the demand for more high-throughput and cost-effective DNA technologies to reduce the amount of sequencing performed on a segment of DNA that may or may not contain a variant. A promising new approach was described using thousands of padlock probes synthesized on microarrays to capture subsets of genomes containing SNPs. As Jin Billy Li (Boston, MA) described, mutations are then rapidly characterized by high-throughput sequencing rather than the conventional microarray analysis. Building upon his successful development of the padlock probes, Mats Nilsson (Uppsala, Sweden) presented a series of approaches utilizing these probes that allowed single-molecule detection, multiplexed targeted genome analysis, multiplexed targeted genotyping, and in situ genotyping [Dahl et al., 2007; Isaksson et al., 2007; Salmon Hillbertz et al., 2007]. Aglaia Athanassiadou (Patras, Greece) described a novel application for padlock probes in gene transfer studies to detect the presence of episomal vectors in the cell nucleus that could be applied both in solution and in situ without loss of cell viability.

A novel approach utilizing the conductivity of DNA to detect SNPs was described by electronically reading the chemical and biological information of a single DNA molecule wired to two electrodes [Chen et al., 2007]. In this technique described by Nongjian Tao (Tempe, AZ), the presence of a single-base mismatch is detected by the change in the conductance of the DNA molecule. Another method allowing single-molecule analysis was described that used quantum dot (QD) fluorescence resonance energy transfer (FRET) nanosensors for high-sensitivity mutation detection and quantitation that could be adapted to microfluidics for high-throughput and low-cost assay automation (Jeff Wang, Baltimore, MD). The QD FRET–based technology overcomes the disadvantages of molecular-based FRET systems by eliminating spectral cross-talk and background fluorescence.

Sequencing Beyond the Next Generation

The technology of Sanger sequencing is now referred to as the "first generation," as a second generation of sequencing technologies is entering the market that is useful for identifying rare mutations where whole genome association studies, biased toward SNPs with a high minor allele frequency, are limiting. However, as Ivo Gut (Evry, France) explains, once disease-causing mutations are identified, lower-cost sequencing technologies are needed that are developed for routine genotyping in diagnostic and research laboratories. Presenting a third generation of sequencing technology, he described an elegant technique using mass spectrometry combined with a modified PCR approach that utilizes ribonucleotides instead of deoxynucleotides, and a DNA polymerase that was engineered by Roche to specifically incorporate these nucleotides.

The products of "riboPCR" are incubated with sodium hydroxide to cleave amplicons at the site of the incorporated ribonucleotide, generating fingerprints for analysis by mass spectrometry to determine the genetic sequence. A modified manual Sanger sequencing approach was described that enabled the detection of sequence variants in complex polyploid genomes as well as mixed DNA pools, providing a tool for genetic analysis with both agricultural and medical application (Tania Tabone, Adelaide, Australia). Stephan Schuster (University Park, PA) compared the sequencing of genomes using the standard Sanger sequencing approach against the sequencing-by-synthesis techniques on a GS FLX (Roche) [Gilbert et al., 2007]. The demonstrated accuracy and superior throughput of second-generation sequencing technologies has opened the door of possibilities for sequencing many genomes once thought impossible, including degraded ancient and archival tissue, pathogenic microorganisms, and many economically important animal and crop species.

Point-of-Care Diagnostics

As high-throughput sequencing technologies become more attainable, perhaps the next major challenge to genetics is the development of POCT. Rapid, single-step, and affordable tests at the point of care that give a clear answer are needed that have similar accuracy as pregnancy and glucose tests. The plenary address, delivered by Yusuke Tuskahara (Tokyo, Japan) on behalf of Yusuke Nakamura emphasized the importance of pharmacogenomics to personalized medicine and the need for simple, rapid, and high-throughput SNP genotyping platforms to facilitate POCT. Addressing the goal of pharmacogenomics to identify biomarkers related to drug efficacy and toxicity for therapeutic use, he described the coordination of 12 institutes, 66 hospitals, and 15 universities in Japan to establish the Japan BioBank. The BioBank has set an enormous task to screen 2,000 individuals with over 40 diseases as part of a personalized medicine project initiated to understand the underlying disease mechanisms and identify individual genetic variation. To implement outcomes of this project, a SNP genotyping instrument was developed with a disposable chip enabling automated SNP typing from a single drop of blood in less than 80 minutes. The convenience and attractiveness of POCT is in the design of the plastic chip. All necessary reagents are included on the chip consisting of three parts: sample preparation to extract DNA directly from the patient's blood, multiplex PCR, and an Invader reaction for SNP detection. Using the anticoagulant Warfarin as the target drug, the technology is already in use in several hospitals around Japan to type SNPs in the two major response genes, *VKORC1* and *CYP2C9*, which lead to dose variation.

An innovative device that could revolutionize POCT was described by Michel Bergeron (Quebec City, Quebec, Canada). He described a microfluidic-microarray–based POCT on a compact disc that extracts DNA in a fully automated process in 5–12 minutes. Complementing this activity was the development of a microfluidic PCR thermoelectric (TE) unit incorporating a plastic disposable PCR card containing 12 individual PCR chambers. The design of the PCR card allows more efficient and rapid heat transfer, performing thermal cycling in half the time of standard PCR machines.

A major challenge to POC diagnostics and prenatal testing is early detection and minimal invasion to the fetus, without compromising sensitivity and accuracy of mutation detection. A new finding that may overcome this limitation was the discovery of fetal and maternal differences in DNA methylation patterns as well as placenta RNA circulating in maternal plasma. Nancy Tsui (Hong Kong, China) described how these two forms of fetal nucleic acids were used to detect chromosomal aneuploidies, such as chromosome 21 [Lo et al., 2007] and allows the potential development of noninvasive tests for other fetal chromosomal aneuploidies.

How Do We Detect the Somatic Variation?

It is a well-accepted limitation of traditional Sanger sequencing that a mutant allele present as a 20 to 25% dilution of wild-type alleles will be at the limit of detection, while many other mutation detection techniques report limitations between 5 and 10%. Hence there is a need for more sensitive DNA technologies that are suited to the detection of somatic changes which lead to cancer, aging, and many mitochondrial disorders. Qingge Li (Xiamen, China) reported an approach to increase the sensitivity of somatic mutation detection using a modified pyrophosphorolysis-activated polymorphism (PAP) technique that incorporates real-time PCR detection, to replace the gel-based electrophoresis approach. Using quantitative PAP (termed qPAP), he reported the detection of mutations in a 1/20,000 dilution of wild-type alleles, without the need to enrich or separate tumor cells from blood samples for rapid detection of circulating somatic mutations in cancer patients. Somasekar Seshagiri (South San Francisco, CA) reported the application of the *E. coli* mismatch repair detection (MRD) to detect somatic mutations with a specificity of 96% compared to standard Sanger sequencing with 55 to 83% and with a comparable sensitivity [Peters et al., 2007]. The MRD process, as he explained, enriches and sorts mutations for analysis on a tag array that can be conveniently coupled to a resequencing microarray for rapid mutation characterization. A simpler solution to enrich the mutant allele fraction combined DHPLC analysis and Sanger sequencing. In this approach, the DNA from heterozygous peaks detected by DHPLC analysis was collected and reamplified, allowing sequencing to characterize the mutation with an increased mutant allele to wild-type ratio. DHPLC analysis is a sensitive method, detecting a 5% mutant allele fraction, but still relying on sequencing to characterize a mutation. Therefore DHPLC-enrichment prior to sequencing is an effective method to increase the sensitivity and accuracy of detecting and characterizing somatic mutations (Bing Ya, Sydney, Australia). Perhaps the most simple and rapid approach available today for detecting somatic mosaicism was described by Idaho Technology (Jason McKinney, Salt Lake City, UT). Using unlabelled ssDNA (LUNA) probes and hrMCA, mutations present at a 5% dilution were readily detected.

Detecting CNV

Structural variants, including CNV are common and ubiquitous in the human genome, covering more nucleotides than SNPs. The association with cancers and many immunological and neurological disorders has led to a more intense and focused discovery and characterization of CNVs in recent years, increasing the number of known CNVs from 1,300 (June 2006) to 3,600 (March 2007). The genome-wide discovery and analysis of copy number and structural variants in "normal" individuals will lead to an understanding of how common structural polymorphisms may predispose to genetic disease [Eichler et al., 2007]. An already promising analysis of the structural variation within the human genome, as described by Evan Eichler (Seattle, WA), has identified previously undescribed microdeletion and microduplication syndromes associated with mental retardation, diabetes and renal disease. Existing DNA

technologies for CNV detection utilize hybridization (Southern blot, fluorescent in situ hybridization [FISH]) arrays, RT-PCR, and MLPA with variable sensitivity. Xiangning Chen (Richmond, VA) described the detection of CNVs in schizophrenia using TaqMan probes and pyrosequencing technologies by coamplifying and genotyping a reference and target loci [Chen et al., 2006]. The addition of an allele-specific probe allows the potential association between a CNV and a specific allele to be tested. Guohua Zhou (Nanjing, China) modified the MLPA method by incorporating pyrosequencing to simplify the probe design process and simultaneously quantify 19 target sequences containing CNVs and SNPs. The association of CNV with important disease highlights the need for improved assays that are more rapid and affordable than the current approaches. Using the popular EvaGreen DNA binding dye and two sets of primers, Xing Xin (Hayward, CA) described a simple hrMCA approach that utilizes one pair of primers to detect a reference locus and a second pair of primers to interrogate a target locus. The relative peak height and area ratio of the two melt peaks correlates with the relative gene dosage to determine the CNV of the DNA samples.

Databases and Informatics

The continual advancement in DNA technologies and their applications to identify genetic causes of disease will only be as powerful as the interpretation and analytical criteria for ascertaining pathogenic vs. nonpathogenic variants. DNA variant databases will become a more powerful tool to integrate genotype–phenotype data, which will require cooperation from the entire scientific community for the diligent reporting of variants. A solution offered by Johan den Dunnen (Leiden, Netherlands) was the "LSDB-in-a-box" to store sequence variation and patient data. The software is freely available online (www.LOVD.nl) and incorporates the MUTALYZER software to allow researchers to describe a sequence change and check the nomenclature of sequence variants. The INFEVERS database (http://fmf.igh.cnrs.fr/infevers), presented by the editor-in-chief Isabelle Touitou (Montpellier, France), provided a good example of a repository dedicated to mutations responsible for autoinflammatory diseases. Established in 2002, the database currently contains over 500 sequence variants in eight genes and has incorporated many new "user-friendly" modifications to assist users with the submission of new data.

A powerful use of genetic information and bioinformatic analysis was presented by Anthony Frudakis (DNAPrint Genomics, Sarasota, FL). As he described, only 0.1% of our genome differs between individuals and with some of this variation due to ancestry permits the identification of ancestry informative genetic markers (AIMs). Using a panel of AIMs, individual genomic ancestry admixture (IGAA) can be inferred and has been successfully used in forensic applications for the ethnic profiling of criminals, as a covariant to identify genetic determinants of complex traits such as myalgia [Frudakis et al., 2007b], and to elucidate the DNA markers for iris color, a complex and quantitative phenotype [Frudakis et al., 2007a]. This is also a timely application that has the potential to unravel the biological basis of individual variation in drug response, allowing a more rapid advance to phase III drug trials [Kishi et al., 2007].

Human Variome Project

A special presentation was given by the Chair of the Mutation Detection scientific organizing committee and leader of the Human Variome Project (HVP), Richard Cotton (Melbourne, Australia). The HVP was initiated in June 2006 in Melbourne Australia and represents the cooperation of 25 organizations, 14 journals, and 12 databases from 19 countries to collect and distribute human variation and its phenotypic effect [Cotton et al., 2007; Ring et al., 2006]. Speaking on behalf of the many collaborators involved in this project, Prof. Cotton described the vision of the HVP to produce a public catalog of curated variation in each of the 20,000 genes and associated phenotypes. Once completed, the HVP will support genetic healthcare and research outcomes by providing up-to-date and accurate lists of mutations and variations in genes that affect human health. At the conclusion of his presentation, Prof. Cotton opened the floor to the delegates of the Mutation Detection meeting to discuss current problems and possible solutions to the submission of genetic variation and associated phenotype information to online databases. It was a great opportunity for scientists in diagnostic and research laboratories to freely contribute ideas and experiences with data curation that will be fundamental to the successful completion and implementation of the HVP for improving diagnosis and treatment of disease.

CONCLUSION

The multidisciplinary nature of the Mutation Detection symposium was reflected in the range of topics, from detecting somatic variation for early detection of cancer and identifying genome-wide structural variation to developing new technologies for point of care diagnosis. DNA technologies are rapidly evolving to meet our ever-increasing understanding of the human genome and the genetic basis of disease. The reality of personalized medicine as a viable healthcare option is now upon us and DNA technologies to enable rapid POCT will be in demand. There is little doubt that future meetings will feature significant advancements in POCT, more sensitive technologies to detect mosaic mutations, and technologies specifically developed to detect and characterize structural variations. The improvement in the rate of sequencing has increased the rate of accumulation of sequence variants. With many of these variants still of unknown significance, future meetings will witness the discovery of many disease-causing mutations and a third generation of DNA sequencing technologies will offer low-cost approaches for rapid genotyping for routine diagnostic and research applications. Underpinning these advancements in technology and knowledge will be databases and bioinformatic solutions to support the integration of genetic information to provide early diagnosis and treatment for improved healthcare and quality of life.

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