Meeting report

Copy number variation and genomic alterations in health and disease

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Published: 20 February 2009

Genome Medicine 2009, **1**:21 (doi:10.1186/gm21)

The electronic version of this article is the complete one and can be found online at http://genomemedicine.com/content/1/2/21

Abstract

A report of the 1st GOLDEN HELIX Symposium 'Copy number variation and genomic alterations in health and disease', Athens, Greece, 28-29 November 2008.

The 1st GOLDEN HELIX Symposium on 'Copy number variation and genomic alterations in health and disease' was recently held in Athens, Greece, where some 160 participants from 31 countries were updated on recent developments in the field of molecular cytogenetics, copy number variation and the direction in which the technology of array-based comparative genomic hybridization (array-CGH) is evolving. The meeting was supported by ten corporate entities, five of which presented their technologies for array-CGH and/or software tools for downstream analysis as company lectures embedded in the scientific program. Here, we report some highlights of this meeting from a genomic perspective.

GOLDEN HELIX Symposia: the concept

The GOLDEN HELIX Symposia (http://www.goldenhelixsymposia.org) are 2-3 day scientific meetings aiming to advance biomedical and life sciences by bringing together scientists within and across disciplines in the fields of human genomics and personalized medicine. This symposium series is named after the house of Francis Crick ('The Golden Helix'; 19/20 Portugal Place, Cambridge, UK) to emphasize their focus on human genomics and personalized medicine.

Conference venues are usually major cities or summer retreats in the Southern Mediterranean or Middle East regions. The symposia aim to maximize information exchange and promote collaborative relationships between regional research institutes and research centers of excellence in the United States and Europe. Such information exchange and collaboration ties are strengthened by interactions between participants and lecturers, the latter being internationally renowned scientists, recognized leaders in their field. Selected minireviews from the topics discussed in the symposia will be published in special issues of *Human Genomics and Proteomics* (http://www.sage-hindawi.com/journals/hgp).

Array-CGH technology

Molecular cytogenetics aims to bridge the gap between classical cytogenetics and modern molecular biology. In general, this involves the use of a series of techniques referred to as fluorescence *in situ* hybridization (FISH), either directly on metaphase chromosomes or interphase nuclei or indirectly using microarray-based comparative genomic hybridization (array-CGH) analysis to assess the entire genome for imbalanced chromosomal material. Michael Speicher (Medical University of Graz, Austria) provided a succinct overview of molecular cytogenetics.

Increases in resolution are achieved by advances that involve both the target and the probe, and microarray technologies provide a description of chromosome structure at a resolution that exceeds that of microscopic analysis. Array-CGH technology is an invaluable tool in oncology. Bauke Ylstra (Vrije Universiteit Medical Center, Amsterdam, the Netherlands) reported several applications of array-CGH in clinical oncology for tumor identification and stratification. Like Speicher, he stressed that integration of chromosomal copy number variation (CNV) with gene expression will probably identify new therapeutic targets that could not be identified by analysis of independent platforms alone.

Diagnostic genome profiling can also be performed using single nucleotide polymorphism (SNP) arrays. Joris Veltman (Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands) described the advantages of SNP arrays over bacterial artificial chromosome (BAC) arrays for genome profiling. In particular, with SNP arrays, intensity correlates with genomic copy number, one-color hybridization is performed and information on both genotype and copy number variation is provided. A SNP array platform was validated for the detection of submicroscopic deletions and duplications in patients with unexplained mental retardation, revealing rare *de novo* CNVs as explaining a significant proportion of mental retardation. Joris Veltman stressed, however, that CNV detection on commercial arrays is not yet perfect, and it is important to improve software tools and reference samples and to increase sensitivity. Philippos Patsalis (Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus) reported results from a custom-made X-chromosome-specific array for targeted locus copy number assessment and its application in understanding and characterizing the biological causes of X-linked mental retardation and its underlying mechanisms of X-chromosome disorders.

Chromosomal disorders and copy number variation

As previously mentioned, CNVs are often implicated as a cause of mental retardation and psychiatric disorders. Pawel Stankiewicz (Baylor College of Medicine, Houston, USA) reported the Baylor experience from a study of a large number of mentally retarded patients who were screened using molecular cytogenetic approaches. In particular, the study showed that large recurrent microdeletions (for example, on 1q21.1) and rare chromosomal deletions and duplications are associated with schizophrenia, that genomic alterations are also the cause of various cognitive disorders, behavioral traits and autism, and that CNV duplication can confer milder phenotypes than CNV deletion. Similarly, Shane McCarthy (Cold Spring Harbor Laboratory, Cold Spring Harbor, USA) described his research showing that, on the basis of their collective frequency in cases and controls, rare structural variants, both inherited and *de novo* CNVs, are associated with schizophrenia, and that frequent recurrent CNVs (e.g. on 1q21 and 15q13) are associated with schizophrenia in large cohorts. He also stressed that similar CNV risk factors may be shared between clinically distinct disorders (e.g. schizophrenia and autism spectrum disorders). Similar data were also presented by Corrado Romano (Oasi Institute for Research on Mental Retardation and Brain Aging, Troina, Italy), who reported the application of array-CGH in determining the underlying genetic cause of mental retardation and epilepsy.

Many visible chromosomal rearrangements occur *de novo*. Orsetta Zuffardi (University of Pavia, Italy) described the use of array-CGH to size unbalanced translocations and to reveal the mechanisms that lead to these rearrangements. Copy number alterations also occur frequently in human malignancies, and Sakari Knuutila (Haartman Institute and University of Helsinki, Finland) reported numerous examples of array-CGH revealing changes in the genomes of cancer patients, including cryptic gene copy number alterations in karyotypically normal cancers, uniparental disomy, deletions in presumed 'balanced translocations', duplication of fusion genes or detection of novel fusion genes, and episomal gene amplification.

CNVs are also the cause of numerous novel syndromes, with phenotypes varying from neoplasias and malformations to growth and mental retardation, and Bert de Vries (Radboud University Nijmegen Medical Centre) highlighted the use of array-CGH in the identification of these syndromes. Finally, gene dosage alterations can be often linked to disease phenotypes. Lisenka Vissers (Radboud University Nijmegen Medical Centre) highlighted two characteristic examples of disease-causing gene identification through deletion mapping strategies, for CHARGE syndrome (coloboma, heart malformation, atresia of choanae, retardation of growth and development, genital hypoplasia, ear abnormality including deafness) and 9q34 microdeletion syndrome.

Population genomics

CNV in DNA sequences probably has functional significance, but this has yet to be ascertained. Previous studies showed that approximately 12% of the human genome contains many CNV regions, including genes, disease loci, segmental duplications and other functional elements. These studies also revealed marked variation in copy number changes among populations and delineated linkage disequilibrium patterns for many CNVs. Richard Redon (Wellcome Trust Sanger Institute, Hinxton, UK) outlined his research showing that several CNVs are predisposing factors for several disorders, such as CNV in the *FCGR3* gene (encoding a low affinity IgG Fc receptor) that predisposes to glomerulonephritis, and low human β-defensin 2 gene copy number that predisposes to Crohn's disease. He summarized ongoing projects to understand the impact of CNVs on genes, characterize mutation mechanisms using sequence content at CNV breakpoints, genotype CNVs for all HapMap samples in order to characterize their population

properties, and initiate new association studies using the set of CNVs discovered in recent studies.

In some cases, CNVs have been shown to correlate with subtle phenotypes in healthy individuals. Joris Veltman (Radboud University Nijmegen Medical Centre) outlined preliminary results from an ongoing collaborative project in the Netherlands (the Brain Imaging Genetics project, BIG) to delineate the genetics of brain function and activity using brain endophenotypes, intermediate phenotypes that are heritable and genetically less complex, with the future goal of performing functional tests for significantly associated genes and loci and investigating possible links to brain disorders. Inherited, apparently benign CNVs can in some cases cause disease, depending on copy number, inheritance pattern or genetic and environmental background. Joris Vermeesch (Katholiek Universiteit Leuven, Belgium) described these so-called Mendelian CNVs, which cause autosomal recessive, autosomal dominant, X-linked and imprinted disorders. He provided examples, including autosomal dominant inherited microtia, which results from a 750 kilobase (kb) amplification on chromosome 4p, duplications in the X-linked genes *MECP2* and *ATR-X*, which result in various forms of severe mental retardation, and other examples of CNVs with variable expressivity and penetrance. He commented that the pace of Mendelian CNV discovery is currently in an exponential phase.

Genetic variation influences gene expression, and this variation in gene expression can be efficiently mapped to specific genomic regions. Moreover, variant gene expression is heritable and differs between populations. Emmanouil Dermitzakis (Wellcome Trust Sanger Institute) presented preliminary data from expression quantitative trait locus (QTL) analysis in eight human populations and three cell types. He concluded that *cis*-acting genetic variation (both SNPs and CNVs) influences gene expression and that differences between cell types are due to both alternative use of regulatory elements and differential levels of expression. This session was closed by the keynote lecture 'The renaissance of aneuploidy', delivered by Stylianos Antonarakis (University of Geneva School of Medicine, Switzerland), who commented on the past, present and future of cytogenetics and the impact of molecular cytogenetics in modern medical practice and presented some key examples from significant regulatory interactions in chromosome 21, using the 4C (chromosome conformation capture on chip) technology.

Prenatal diagnosis

Congenital malformations are a major cause of morbidity and mortality in infants. Chromosomal imbalances account for approximately 10-15% of the cases, but in up to half the patients the underlying genetic etiology of these malformations remains unknown. Array-CGH technology could, therefore, facilitate detection of genomic imbalances in these patients, and it could in the future be implemented for routine prenatal diagnosis. Cédric le Caignec (University Hospital, Nantes, France) presented results from a recent study to estimate the feasibility and the rate of detection of chromosomal abnormalities in fetuses with multiple malformations, a first step before the implementation of array-CGH in prenatal diagnosis. He concluded that array-CGH is feasible from amniotic fluid or chorionic villus samples and recommended a targeted array-CGH platform to be designed for prenatal diagnosis; this could be used for genome-wide screens for interstitial microdeletions and known genetic syndromes, including in subtelomeric and pericentromeric regions.

Comprehensive genome analysis of single cells is relevant not only for cancer genetics, to monitor small levels of residual disease after treatment and to assess heterogeneity within a primary tumor, but also for clinical diagnostics, breakpoint mapping, determination of mosaicism and forensic applications. In addition, genome analysis at the single cell level is important for non-invasive prenatal diagnosis, for example, analysis of fetal cells (for example, in maternal blood) and prenatal genetic screening (polar body analysis). Jochen Geigl (Medical University of Graz) outlined a representative whole-genome amplification protocol, coupled to a high resolution array-CGH method that is technically possible for single cells with the detection limit improved from 6-8 megabases (Mb) to less than 3 Mb (and even 500 kb for 5-10 cells). He also showed that the haploid genome from a polar body is suitable for array-CGH analysis for the identification of aneuploidy patterns and mode of chromosome segregation and for the detection of segmental aneuploidies as small as 10 Mb. Geigl reported an array painting method for rapid identification of disease-causing genes in cases with structural rearrangements. Molecular cytogenetic analysis is also feasible for ancient DNA samples isolated from extinct species (such as the woolly mammoth), Egyptian mummies, amber inclusions and coprolites. Holger Tönnies (University Hospital Schleswig-Holstein, Kiel, Germany) showed that conventional CGH analysis can be used for DNA isolated from ancient tissues to detect chromosomal aberrations, although the establishment of a reliable (unbiased) ancient DNA amplification protocol and a downstream array-CGH analysis for these purposes remains challenging.

Quality control

With so many commercial and customized array-CGH platforms currently available, there is a need for a consensus on a uniform and evidence-based platform for constitutional diagnostic oligonucleotide array-CGH, an array-CGH platform allowing researchers to survey the entire human genome for chromosomal abnormalities. John Crolla (National Genetics Reference Laboratory Wessex, Salisbury, UK) outlined the aims of an international consortium recently formed to design

and implement a uniform diagnostic array-CGH platform and to interpret array-CGH results. The consortium works towards reaching an agreement to create an evidence-based process for clinical array design, to set uniform guidelines for interpretation and reporting of array-CGH results, and to develop a centralized data repository for array abnormalities and benign variants. It has been agreed to cover all currently known microdeletion/microduplication and syndromic regions, of which there about 500 known so far, in order to achieve maximum backbone resolution with the probes available (which are about 30 kb long). Future challenges would be to define the minimum resolution for a diagnostic array-CGH and to host a 'cytogenetic' database of genomic variants in the DECIPHER database (database of chromosomal imbalance and phenotype in humans using ensembl resources, s) and/or other genome browsers [such as those at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) and the University of California at Santa Cruz (http://genome.ucsc.edu/)] for worldwide access.

Jacqueline Schoumans (Karolinska Institute, Stockholm, Sweden) reported a stepwise approach for validating the implementation of array-CGH platforms in the diagnostic setting. The ideal array-CGH platform should: (i) be cost-effective, widely used and with a probe design (share benign CNVs) for optimum resolution (at least down to 300 kb) to reliably detect CNVs; (ii) be commercially available; (iii) have a less laborious workflow than current platforms and include user-friendly software packages and certification of analysis; and (iv) make it possible to design custom arrays. Finally, Marcella Zollino (Università Cattolica del Sacro Cuore, Rome, Italy) concluded the meeting by presenting a checklist with criteria for selecting patients for array-CGH analysis and for validating subsequent results.

Conclusions

Overall, the 1st GOLDEN HELIX Symposium highlighted several main themes: the importance of CNV and genomic alterations in health and disease; the emerging array-CGH technology as a powerful and accurate tool for revealing existing and novel genomic alterations with high resolution in chromosomal and other disorders, such as schizophrenia, mental and growth retardation and malignancies; the occurrence of CNVs in healthy individuals and their relation to gene expression differences; the application of array-CGH in prenatal diagnosis and in the analysis of single cells; and the need for a uniform and evidence-based constitutional array-CGH platform. Gathering scientists from all these different disciplines allowed for cross-fertilization of ideas, thereby setting the horizon for new cutting edge research to be discussed at the next GOLDEN HELIX Symposium on CNVs, scheduled for 2010 in the Athenian Riviera.