Meeting report

Copy number variation goes clinical

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A report of the First Golden Helix Symposium 'Copy Number Variation (CNV) and Genomic Alterations in Health and Disease', Athens, Greece, 28-29 November 2008.

Since its development in the late 1990s, microarray-based comparative genomic hybridization (aCGH) has been widely used to screen for copy-number changes at high resolution across whole genomes. It has quickly become a reference method for the diagnosis of patients with severe developmental defects and has been instrumental in the discovery of a new abundant source of polymorphism in the human genome, called DNA copy number variation (CNV). Here we report some highlights of the First Golden Helix Symposium, focusing on the latest advances in the rapidly evolving field of research on CNV.

CNV is a major cause of chromosomal disorders in patients

Chromosomal imbalances are a major known cause of learning disability and developmental defects. A standard karyotype can only detect chromosomal imbalances (deletions and duplications) larger than 5 Mb, whereas aCGH is able to detect cryptic chromosomal imbalances that are not detectable by standard cytogenetic analysis. Since the recent implementation of aCGH in clinical diagnosis, large numbers of patients have been tested worldwide and genomic copy number alterations have been detected in around 10-15% of such patients with apparently normal karyotypes.

Pawel Stankiewicz (Baylor College of Medicine, Houston, USA) reported a large study in which more than 18,500 patients with mental retardation with or without anatomical malformations have been screened by aCGH. DNA samples from patients were analyzed using custom bacterial artificial chromosome (BAC) arrays until March 2007, when BACs were replaced by oligonucleotides. Overall, chromosomal imbalances with clinical significance were identified in 14% of the patients. This enormous dataset led to the discovery of several recurrent genomic disorders, in particular, microdeletions and microduplications on chromosome 1q21.1 in a series of individuals with developmental delay and neuropsychiatric abnormalities. The 1q21.1 microdeletions found in the Baylor study can be *de novo*, or be inherited from a mildly affected parent, or be inherited from an apparently unaffected parent. Patients with 1q21.1 microdeletions present with considerable variability in phenotype, including mild-to-moderate mental retardation, neuropsychiatric abnormalities, abnormal head size, dysmorphic features and congenital anomalies. The potential for reduced penetrance and variable expressivity in these syndromes raises difficult questions in the context of genetic counseling for newly diagnosed cases and particularly for prenatal diagnosis. aCGH was also used to determine the frequency of genomic imbalances in neonates with birth defects. Stankiewicz and colleagues screened 638 neonates and identified a clinically significant abnormality in 17.1% of them, showing that chromosomal microarray analysis is a valuable clinical diagnostic tool that allows precise and rapid identification of genomic imbalances and mosaic abnormalities as the cause of birth defects in neonates.

Joris Veltman (Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands) showed that instead of using aCGH arrays to screen patients with mental retardation, they have chosen single nucleotide polymorphism (SNP) arrays. In addition to detecting CNV, such SNP arrays enable the identification of uniparental isodisomies and the parental inheritance of imbalances. Veltman reported that since January 2008, the Nijmegen laboratory is using SNP arrays instead of karyotypes as the primary screening method for patients with mental retardation. If a chromosomal imbalance is identified using the SNP array, parental karyotyping and/or fluorescence *in situ* hybridization (FISH)

analyses are performed to identify whether it is a balanced chromosomal rearrangement, knowledge essential for accurate genetic counseling.

Currently, a number of different platforms are used to screen for CNV in patients with mental retardation. An initiative has been proposed to standardize diagnosis by aCGH. John Crolla (Wessex Regional Genetics Laboratory, Salisbury, UK) and his colleagues have designed a 44K oligoarray CGH to provide both genome-wide coverage at baseline resolution with the probes available and specific interrogation of known microdeletion and duplication syndromes. Crolla reported that together with David Ledbetter from Emory University in Atlanta, Georgia, his team will make publicly available a new 180K array design covering most of the known human microdeletion/duplication syndromes with a higher density, a reduced coverage of cancer genes and an even probe distribution across the whole genome.

Bert de Vries (Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands) presented his results on the 17q21.31 recurrent microdeletion syndrome. Patients with the 17q21.31 microdeletion present with a clearly recognizable clinical phenotype of mental retardation, hypotonia and a characteristic face. de Vries has found that the deletions encompass the *MAPT* (microtubule-associated protein tau) gene and are associated with a common inversion polymorphism predisposing to the deletion. Joris Vermeesch (Catholic University, Leuven, Belgium) demonstrated how CNV can be associated with Mendelian diseases. Gene deletions can not only be responsible for autosomal dominant pathologies (for example, by haploinsufficiency) but also cause autosomal recessive disorders in the presence of mutations inactivating the second allele. Duplications can be responsible for monogenic disease by dosage effect when the gene and its regulatory sequences are duplicated in their entirety, but intragenic duplications can also lead to haploinsufficiency and to recessive disorders. Vermeesch gave the example of partial duplications of the *ATRX* gene leading to an absence of ATRX mRNA and protein.

aCGH in prenatal and preimplantation diagnosis

To date, aCGH has been mostly used in postnatal diagnosis. Discussing its utility in prenatal diagnosis, one of us (CLC) presented a study by targeted BAC array of a series of 49 fetuses with multiple anatomical malformations and normal karyotype. Most of the known recurrent microdeletion syndromes, all 41 human subtelomeric chromosome regions, and 201 additional selected loci representing each chromosome arm were spotted on the array. All fetuses presented with at least three major malformations that led to a medically terminated pregnancy. In four cases, *de novo* genomic imbalances clearly underlying the pathological phenotype were identified. In one, the relationship between genotype and phenotype was unclear, as a subtelomeric 6q deletion was detected in a healthy mother and in her two fetuses bearing multiple malformations. The detection of causative chromosomal imbalances in 10% of these fetuses suggested the desirability of genome-wide screening by aCGH even when standard chromosome analysis is normal, and confirmed that aCGH will have a major impact on prenatal diagnosis.

However, aCGH results should be applied with extreme caution in prenatal diagnosis because of the possible detection of CNV with uncertain clinical significance, as indicated by recent work from elsewhere. Targeted arrays may be preferred over genome-wide arrays in prenatal diagnosis, to give better coverage of the targeted regions, to reduce the number of genes identified for which termination would be ethically questionable (for example, *BRCA*, *AZF*), and to reduce the number of regions of uncertain clinical significance.

Genome-wide CNV screening in single cells is of special importance for a variety of applications in basic research and clinical diagnostics. Prerequisites are technologies for capturing single cells, unbiased single-cell whole-genome amplification protocols, and an appropriate array platform for evaluating the amplification products. The maximal achievable resolution is a matter of debate. Jochen Geigl (Medical University, Graz, Austria) together with Michael Speicher has previously shown that when the amplification product is hybridized to a whole-genome tiling path BAC array, a resolution of 6-8 Mb is achievable. He described how high-resolution oligo-arrays and a new evaluation algorithm especially designed for the identification of small CNVs in noisy ratio profiles had led to improved resolution, with the identification of CNVs as small as 500 kb in cell pools (5 or 10 cells) and 2.6-3.0 Mb in single cells. Geigl described how the procedures are also suitable for polar body analyses in the context of prenatal genetic screening as they reliably allow the identification of aneuploidy patterns, the mode of chromosome segregation and segmental aneuploidies. The approach can also be applied to identify the amplification products of microdissected single chromosomes. Geigl reported that using this approach, they had narrowed down the breakpoint in a patient with a balanced *de novo* translocation involving chromosomes 7 and 13 to base-pair level.

Population genomics

Until recently, genome-wide interrogation of CNV was not easily applicable to population-based studies. One of us (RR) presented preliminary results from the construction of a new high-resolution CNV map by the Genome Structural Variation (GSV) consortium. GSV consortium members have carried out aCGH - using a set of 20 microarrays covering the whole human genome with 42 million oligonucleotides - on 40 individuals of African or European ancestry. They have discovered 11,700 CNVs of more than 500 bp in size

and have defined precisely CNV boundaries. Thus, it is now possible to accurately identify functional sequences that are variable in copy number, and provide new insights into mechanisms generating chromosomal rearrangements. New whole-genome CNV genotyping platforms based on this high-resolution map will become available within the next few months and allow more comprehensive case-control association testing with CNV.

Manolis Dermitzakis (Wellcome Trust Sanger Institute, Hinxton, UK) has worked for several years on defining the impact of nucleotide and copy number variation on gene-expression phenotypes. He gave an update on a new association survey currently in progress, comparing nucleotide variations and gene transcription levels in individuals from the HapMap project. By using cell-line samples from different sources, Dermitzakis and his colleagues have been able to accurately reproduce previously published data on the impact of nucleotide variants on gene transcription levels using SNP data from the phase 1 HapMap project. The new study will soon be extended to the high-resolution CNV map under construction by the GSV consortium and will provide new insights into the link between genetic variation and expression phenotypes.

In his keynote lecture, Stylianos Antonarakis (University of Geneva Medical School, Switzerland) reminded the audience that the concept of CNV was originally described more than 70 years ago in *Drosophila*. Reviewing his work on the identification of *cis*- and *trans*-regulatory elements in the human genome, focusing on chromosome 21, he showed how these data illustrate the new challenges faced by researchers in human genetics - to better understand the relationship between genetic variation and phenotypic diversity. The human genome can no longer be considered as a simple succession of linear DNA molecules carrying gene-coding information but should also be viewed as a three-dimensional regulatory network. Original and innovative strategies will be required in the future to decode all the genetic information that is currently released in extensive catalogs of single-nucleotide and copy number variation.

The participants at the first Golden Helix symposium enjoyed hearing about new and unpublished work and taking part in lively and informative discussions with the speakers. We look forward to the 2009 meeting, which will be on Stem Cell Biology.