Next Generation Sequencing in Cytogenetics

7.P1

NGS mapped breakpoints in balanced chromosomal rearrangements including the first large cohort of healthy carriers

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Next-generation sequencing (NGS) has revolutionised the mapping of balanced chromosomal rearrangements (BCRs), associating specific diseases to truncated genes and to regulatory landscapes associated with long range position effects (LRPE). The large majority of the published BCRs are from affected individuals, and very few BCRs from healthy carriers have been mapped by NGS.
We present the first data from The International Breakpoint Mapping Consortium (IBMC), a world-wide effort to NGS-map ultimately thousands of BCRs, including BCRs from healthy carriers. We mate-pair sequenced BCRs from 87 affected and 117 healthy carriers, doubling the number of mapped two-way breakpoints to >800. The proportion of truncated genes were similar in unaffected and affected carriers (44%-40%, respectively), but we observed a significant excess of truncated autosomal dominant (AD) disease genes ($p = 0.0024$) and loss-of-function (LOF)-constrained genes ($p = 0.0016$) in the affected carriers. However, truncation of known AD genes in apparently healthy carriers does occur, e.g. ABCC9, DCC, CACNB4 and TGFB2. Indeed, the breakpoint within ABCC9 orginates from a founder inv(12) with presently >120 known inversion carriers. Strikingly, 23% of the truncated genes in healthy carriers are LOF-constrained, at odds with exome sequencing data.
We also show that ~70% of the known LRPE-associated breakpoints/loci overlap with topological associating domains (TADs) that are highly enriched in evolutionary conserved non-exonic elements (CNEs). As the tip of the iceberg, we define ~400 CNE-TADs with this hallmark, covering ~16% of the genome, as high risk regions for LRPE. Breakpoints truncating CNE-TADs are more frequent among affected versus healthy BCR carriers ($p = 0.003$), and even more so are intergenic breakpoints within CNE-TADs ($p = 0.0015$).
Our study illustrates the importance of NGS-based mapping of BCRs irrespective of phenotype, and the potential of IBMC to confirm known disease genes, identify numerous novel candidate disease genes and dissect potentially hundreds of LRPE-regions. Mapping of healthy BCR carriers reveal a new resource for the study of why apparently normal individuals carry assumed deleterious mutations.

7.P2

CNV detection comparative study of array based methods and NGS

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Copy number variation (CNV) is one of the main forms of critical genetic variation. It is well known that CNV might directly or indirectly lead to some diseases, including cancer. During the past decade array-based methods were de-facto standard technologies for detecting CNV. Today Next-generation sequencing (NGS) based methodologies are occurring for identification of CNV.
Here we used 20 samples, for which CNVs were found using Affymetrix CytoScan HD Array (as standard approach) and Illumina sequencing with Agilent SureSelect Focused Exome capture in order to compare standard approach with NGS. For CNV detection we used CNVkit, method based on reads depth approach.
As a result for all 20 samples we detected 605 CNVs by array based method. For detected CNVs we used clinically stringent filtration: CNV should have length at least 25 kb or 50 kb and at least 25 or 50 probes for losses and gains respectively. For NGS data we found 28 CNVs. Then we intersected these CNVs between two sets of data in order to find precision/recall metrics for NGS based method. We found growth of recall metric with growth of CNV length, with recall up to 100% for CNV length > 3mb. The same tendency was observed for CNV divided by origin (losses and gains) and CNV that were detected in on-target regions. For off-target CNV we got only 7% recall for CNV length > 3mb. On the other hand precision is independent