# From Arrays to NGS: How Chromosomal Genetics Evolved From Structure to Disease

Researchers from the MGZ Medical Genetics Center are using the Infinium® CytoSNP-850K BeadChip and the TruSight® Cancer Panel to analyze chromosomal abnormalities.

## Introduction

More than 100 years after chromosomes were first made visible under the light microscope, scientists made the discovery that humans possessed 46 chromosomes, rather than the 48 previously assumed.[1] Making up the programming core of the human body, the DNA these chromosomes contain is responsible for what makes each of us unique. However, the slightest chromosomal change can lead to harmful consequences. An extra chromosome can trigger congenital disorders such as Down syndrome. An unbalanced exchange of genetic material between chromosomes (eg translocation) can result in severe genetic disorders.

The myriad of cytogenetic alterations and their impact on human disease was intriguing to Udo Koehler, PhD, now Laboratory Director at MGZ Medical Genetics Center. Originally trained in primate evolution, he was fascinated by how small cytogenetic changes could have such profound consequences. It motivated him to shift his focus to human genetics studies and the development of cytogenetic diagnostics. Anna Benet-Pagès, PhD, also a Laboratory Director at MGZ found herself in a similar situation. Originally trained in biochemical genetics, she was drawn to the possibility of applying her expertise to human health.

Drs. Koehler and Benet-Pagès are part of a growing team performing cytogenetic analysis, next-generation sequencing (NGS), and research studies on human genetic disorders at MGZ. Founded in April 2000, MGZ has expanded from 1 human geneticist and 15 technicians to a team of 11 geneticists and more than 90 staff members, 20 of whom have PhD degrees. Over the course of their scientific careers, Drs. Koehler and Benet-Pagès have witnessed numerous technological advances, from early banding techniques to novel microarrays, and NGS.

### Leveraging Novel Technologies to Improve Resolution

Dr. Koehler began his studies in the prePCR and presequencing era of genetics. “We used conventional karyotyping by G-banding and fluorescence *in situ* hybridization (FISH) to study chromosomes,” Dr. Koehler said. Conventional karyotyping is the process of staining chromosomes with Giemsa stain (hence the name, G-banding) for visualization under light microscopy. FISH uses fluorescently labeled DNA probes to detect and localize the presence or absence of specific DNA sequences on chromosomes.

“We performed every FISH technique imaginable,” Dr Koehler stated. “Single-locus FISH, multiplex FISH, subtelomeric FISH, microdeletion FISH, and interphase FISH. We sampled blood, buccal smear, and urine to test for mosaics. It was a lot of microscope work.”

The introduction of the chromosomal microarrays in the early 2000s represented a paradigm shift in cytogenomics studies. Chromosomal arrays are used to scan the entire genome to identify small copy number variations (CNVs). At the start of the microarray era, arrays had a resolution of 1 megabase (1000 kilobases), which has since increased 100-fold or more. At a resolution of 10 kilobases or higher, scientists can feasibly detect copy number variants and alterations at the single gene, or even single exon level.

**Lab Directors Udo Koehler, PhD, and Anna Benet-Pagès, PhD, are part of an interdisciplinary team performing cytogenetic analysis, next-generation sequencing, and research studies at MGZ Medical Genetics Center in Munich, Germany.**

Using chromosomal arrays, researchers began discovering copy number variations rapidly, leading to the identification of new syndromes. “It was fascinating to take part in the evolution of microarray analysis,” Dr. Koehler said. “Arrays enabled me to identify the smallest genomic copy number variant, which wouldn’t have been detected by conventional karyotyping. It’s now a first tier approach for identifying developmental delay in children.” The straightforward and clear results from chromosomal microarray analysis “make the lab staff happy,” Dr. Koehler added. “They don’t have to look through a microscope anymore, which is time consuming and requires years of experience.”

### The Right Tool for the Job

Continuous growth has enabled MGZ to acquire technologies that support new cytogenomic approaches. It started using microarrays and later added an iScan® System. MGZ became the first German laboratory to use the Infinium CytoSNP-850K BeadChip after it was introduced in 2013. They use BlueFuse® Multi Software to analyze the data.

“The CytoSNP-850K BeadChip offers accurate cytogenomic testing,” Dr. Koehler said “I like that it is SNP-based, which means there are fewer false-positive copy number variants. With no labeling artifacts, the interpretation of results is much clearer, mosaics can be detected better, and I can clearly see the number of copies in the B allele chart.”

“When I look at the data using the BlueFuse Multi Software, there’s rarely any variant I can’t match with the databases,” Dr. Koehler added. “I like the links to the genome browsers and the zoom in and query capability to compare regions with other results. Creating batch files is easy, too. I can import the lab’s entire database to the BlueFuse Multi Software with just one click. I can analyze more patients in a shorter timeframe, which is very helpful for the lab workflow.”

About 90% of the samples MGZ analyzes with the CytoSNP-850K BeadChip are postnatal. These analyses help identify the causes of autism spectrum disorders, intellectual disability (ID), dysmorphic features, or neurological disorders such as epilepsy. MGZ also performs prenatal analysis with conventional karyotyping, chromosomal microarrays, and noninvasive prenatal testing (NIPT) for chromosomal aneuploidy (abnormal number of chromosomes).

### Assessing the Genetics of Cancer

The CytoSNP-850K BeadChip and traditional cytogenetic techniques aren’t the only genetic analysis tools used by MGZ. In 2012, Dr. Benet-Pagès brought NGS to the laboratory. She began using Illumina sequencing systems in 2007 during her postdoc, beginning with the Genome Analyzer and later moving to the HiSeq® System. At MGZ, she uses the MiSeq and NextSeq® 500 Systems to run a large number of custom panels to identify variants associated with rare hereditary diseases and the TruSight Cancer Sequencing Panel, which targets 94 genes with suspected associations to common and rare cancers. The sequencing panel also includes 284 SNPs suspected to correlate with cancer through genome-wide association studies (GWAS).

“The TruSight Cancer Sequencing Panel is a test method that anyone can adapt easily in their laboratory,” Dr. Benet-Pagès said. “It’s easy to implement in automated workflows and performs with high repeatability and reproducibility. The TruSight Cancer Panel enables us to analyze more genes in a single workflow than previous technologies. The advantage is that we can identify potential mutations in cancer associated genes not primarily tested.”

“The TruSight Cancer Sequencing Panel delivers results in 6–7 days,” Dr. Benet-Pagès added. “That includes the whole workflow process, from sample acceptance, DNA extraction, sample preparation, sequencing, and data interpretation, through to data reporting.”

### The Future of Cytogenetic Analysis

Drs. Koehler and Benet-Pagès would like their cytogenetics and NGS labs to work together more because the techniques are complementary. “Array-based technologies are useful in detecting CNVs such as large deletions and duplications, but not in detecting single nucleotide variants (SNV),” said Dr. Koehler. “NGS enables the detection of unexpected aberrations, such as SNVs, which aren’t well characterized. Pairing these 2 methods—dose analysis for CNVs and sequence analysis for SNVs—will determine the genomic basis for an increasing number of inherited disorders. This approach would benefit from a single software solution, perhaps BlueFuse Multi Software.”

## Reference

1. Genetic Timeline, National Genome Research Institute web site. www.genome.gov/Pages/Education/GeneticTimeline.pdf. Accessed May 12, 2015.

## Learn more about the Illumina products and systems mentioned in this article:

- Infinium CytoSNP-850K BeadChip, www.illumina.com/products/infinium-cytosnp-850k.html
- TruSight Cancer Panel, www.illumina.com/products/trusight_cancer.html
- BlueFuse Multi Software, www.illumina.com/clinical/clinical_informatics/bluefuse.html
- iScan System, www.illumina.com/systems/iscan.html
- HiSeq System, www.illumina.com/systems/hiseq_2500_1500.html
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