



SEQUENCES IN ALL VARIATIONS

Systematic sequencing and comparative sequence analysis – those are two main tasks of the Systematic-Methodological Platform (SMP) DNA. "The sequencing data enable us to analyze phenotypes, genes and promoter elements including their modifications in detail," explains Professor Hans Lehrach, coordinator of the SMP DNA. "Moreover, we can gain insight into evolution by comparing the human genome with other genomes, for instance with the genome of chimpanzees." Besides this, the scientists of the SMP DNA are focusing on understanding the correlations between genotype and phenotype, by means of which they want to identify disease genes and elucidate disease processes.



Marie-Laure Yaspo

THE LITTLE DIFFERENCE

On the DNA sequence level the variation among people is only about 0.2 percent. However, in this seemingly so negligible variability lies the key to understanding complex diseases such as cancer, obesity or hypertension. Scientists worldwide are therefore working on recording the genetic variations within the human genome. Some scientists even describe the search for the variability of the human genome between individuals as the second act of the human genome project. To trace the differences, researchers are pursuing different approaches. In genotyping, for instance, the DNA sequences are systematically scanned for single nucleotide polymorphisms (SNPs). These deviations in single bases are the most common differences occurring in the human genome. Researchers estimate that there are about ten million SNPs in humans. Although many SNPs do not have any effect, some of them increase the risk for getting a specific disease or influence the effectiveness of a drug. Within the framework of the SMP DNA, six genotyping centers have

joined together to form the National Genotyping Platform (NGP): the GSF – National Research Center for Health and Environment in Munich under the direction of Professor Thomas Meitinger, the University of Kiel (Professor Stefan Schreiber), the Berlin research groups at the Max Delbrück Center (Professor Norbert Hübner) in Berlin-Buch and at the Max Planck Institute for Molecular Genetics in Berlin (Professor Hans Lehrach, Dr. Sascha Sauer) and the universities of Cologne and Bonn under the coordination of Professor Peter Nürnberg. Together they can provide the NGFN scientists with the entire spectrum of presently known genotyping methods.

TRACKING DOWN DISEASE GENES

"Today, due to modern high-throughput procedures, we can analyze 500,000 SNPs at one time," says Thomas Meitinger of the GSF in Munich. With the aid of genotyping, the scientists in the NGFN have already succeeded in identifying a number of disease genes. For instance, they found sequence variants in the gene for the protein leucine-rich repeat kinase 2 (LRRK2), which are associated with different forms of Parkinson's Disease. Furthermore, they discovered that several SNPs in the coding sequence for the protein FKBP5 are linked with a fast response to antidepressant drugs and a frequent recurrence of depressive episodes. However, genetic variants that predispose for a specific disease are not evenly distributed throughout the world. On the contrary, there are differences evident depending on the population. For instance, a population genetic study in Iceland showed that there are variants both in the gene for the 5-lipoxygenase activating protein (ALOX5AP) and in the gene for phosphodiesterase 4D (PDE4D), which make people with these genetic variants more predisposed to suffer a stroke. A study of the Munich genotyping platform on stroke patients from central Europe showed that in them, too, variants of the gene for ALOX5AP are associated with an increased stroke risk, whereas variants of the gene for PDE4D had no significant influence on stroke risk.

NOTHING LEFT OUT

Dr. Richard Reinhardt of the Max Planck Institute for Molecular Genetics (MPIMG) in Berlin runs the resequencing platform in the NGFN, consisting of the two groups in Berlin and Jena. "In contrast to genotyping we do not focus on individual SNPs when investigating a gene, but sequence the whole gene, or at least all exons," Richard Reinhardt explains. The advantage over all other methods is its unsurpassed accuracy. Resequencing captures all genetic variations – all SNPs but also deletions and insertions. The Berlin research-

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ers already have several achievements to their credit, such as the identification of the molecular causes of kidney diseases and cilia defects that play a role in chronic respiratory diseases. The resequencing platform is continually being expanded and developed to reduce the great outlay of effort and the higher costs involved in comparison to genotyping. "If we develop this here further, one day it will be possible to determine the entire genome of a patient within a short time and with a reasonable amount of effort, so that the costs will also be justifiable," says Richard Reinhardt, describing the future of the resequencing platform.

CHIMP GENOME AS COMPARISON

Dr. Marie-Laure Yaspo is pursuing quite a different approach to decode the functions of the human genome. Within the framework of an international project (the SMP DNA cooperates with the groups from Berlin, Braunschweig and Jena), she is working on the high-resolution sequence of the chimpanzee genome. "Although the chimpanzee is the closest living relative of human beings, there are nevertheless immense differences: for instance with regard to linguistic or intellectual capabilities or the susceptibility for various diseases," the biologist explains, who also conducts research at the MPIMG in Berlin. Within the framework of a German-Asian consortium she was involved in the deciphering of the first chimpanzee chromosome, the chromosome 22. "We assume the comparative analysis of the chimpanzee genome and the human DNA sequence will contribute to discovering disease-relevant genes. But in particular, we will also learn a lot about the evolution of the human genome," she adds. For this the scientists need an unbroken, top-quality sequence of the chimpanzee genome, because it differs from the human genome by only 1.7 percent. Marie-Laure Yaspo is working on the generation of a high-quality sequence of 42 megabases. Most of the segments she has investigated lie on the X chromosome. Numerous hereditary diseases are located on this chromosome, including many forms of mental disability. This is why decoding it is particularly significant. In addition, genomic segments which have been identified as medically interesting by the Disease-oriented Genome Networks in the

NGFN are being analyzed in the chimpanzee genome once again by Dr. Yaspo and her team.

INSIGHT INTO THE TRANSCRIPTION MACHINERY

However, within the SMP DNA there are also groups that are not focusing immediately on the whole genome, but rather on its specific segments. For instance, the group of Dr. Michal Janitz at the MPIMG: He and his staff are comparing the activity of promoter sequences in different cell types in order to understand the mechanisms of gene expression and regulation. "We use cell arrays to be able to analyze the promoter regions on a large scale. Our objective is to investigate 3,000 human promoter regions," Michal Janitz explains. To accomplish this task, plasmids containing a reporter gene driven by a promoter of interest are printed in an array format and then transfected into a number of human cell lines. After the transfection the activities of the promoters can be measured by detecting the reporter gene expression level. Michal Janitz plans to evaluate his data together with the findings from other projects, for example the promoter analysis in mice, in order to trace the global regulation patterns in mammalian cells.

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