



CHALLENGE

Medical researchers seek fast and robust validation of results as they investigate the link between genetic factors and polycystic kidney disease (PKD).

SOLUTION

JMP® Genomics from SAS

RESULTS

Using JMP Genomics, researchers at the University of Heidelberg have identified a gene suspected to play a role in the progression of PKD in rats. This discovery opens the door to powerful new avenues of research for the development of therapies that could ultimately benefit human PKD sufferers worldwide.

MORE INFORMATION

Discover more about JMP Genomics:
www.jmp.com/genomics

Learn more about SAS:
www.sas.com

German researchers find clue in fight against kidney disease

JMP® Genomics aids study of PKD, which can lead to end-stage renal failure.

The discovery of a spontaneous mutation in a single amino acid has led German researchers to a finding that may help slow the progression of a debilitating kidney disease that afflicts millions of people around the world.

Professor Norbert Gretz is Director of the Medical Research Center (Zentrum für Medizinische Forschung, or ZMF) in Mannheim, Germany. The Medical Faculty Mannheim provides clinical training to medical students at Ruprecht Karls University of Heidelberg and is also the faculties' central provider of research services.

For more than 20 years, Gretz has directed a team of ZMF researchers examining the molecular basis of polycystic kidney disease (PKD) – a condition characterized by the growth of multiple cysts in both kidneys. PKD is genetically transmitted and afflicts 1 in 1,000 people in the general population. Left untreated, it progresses to end-stage renal failure in 50 percent of patients afflicted by the most severe forms of the disease. As the disease progresses, patients today have limited treatment options, the most common being either dialysis or kidney transplant.

The researchers have examined dozens of generations of offspring from a single pair of rats with the same gene mutation that causes PKD in humans. Gretz's breakthrough came when his team, using JMP Genomics, discovered a different gene expression profile that caused the disease to progress more slowly in one of two substrains of the rats.

"This difference tells us that there is at least one gene that is a modifier for the disease," he explained. After identifying a "gene of interest," he sought to prove that it was the culprit. So he administered a drug to inhibit the function of a single protein. "Renal function did not deteriorate as fast as it had," Gretz says. "This presented functional proof of the modifier and showed this inhibitory drug could be used for delaying the progression of the disease."

The long-term implications of this discovery are potentially momentous. "If this [drug] works similarly in humans with PKD, the progression rate could potentially be halved," says Gretz, who is documenting his findings in an article for publication in a scientific journal.

**STATISTICAL
DISCOVERY.™
FROM SAS.**

“With JMP Genomics we can quickly advance investigations from raw microarray data to analysis results. Formerly, the validation of results took several weeks to achieve, but today we get the results in just a few hours. We can very easily implement additional analysis processes, giving us a decisive advantage in our daily research.”

Professor Norbert Gretz, MD, PhD
Director, Medical Research Center, Medical Faculty Mannheim, University of Heidelberg

A ‘complete workflow’

Gretz began using JMP Genomics for microarray analysis in 2007, soon after it was introduced to the market. In the first 18 months, ZMF scientists used JMP Genomics from SAS to process and analyze nearly 5 terabytes of raw data. Adoption of JMP Genomics “was a logical evolution,” Gretz says. For nearly 30 years, Gretz had used SAS® software to analyze the vast volumes of data associated with genomic research. JMP Genomics combines the high-powered analytics of SAS with the point-and-click ease and interactive data visualization capabilities of JMP, a desktop product from SAS. It’s a combination that works well for Gretz.

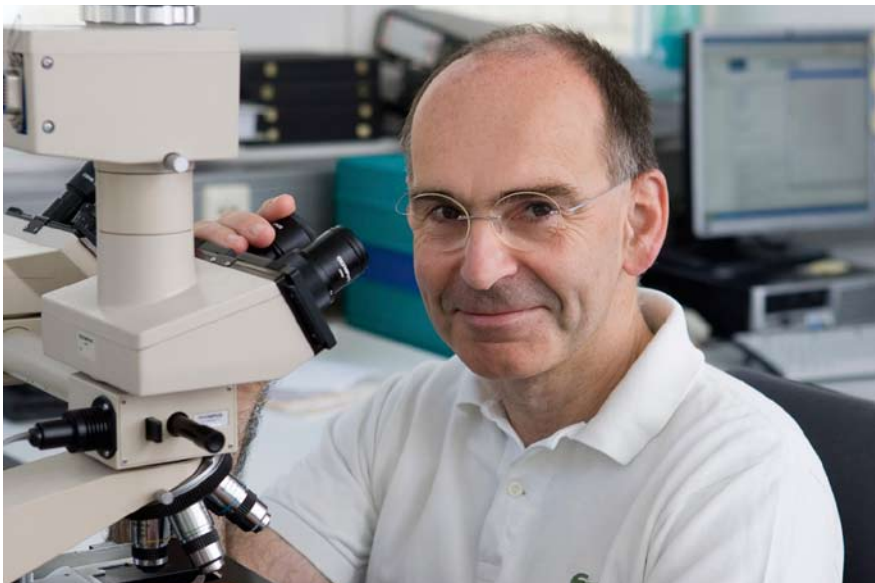
“The output is easier to handle, the graphs are more convenient, and you still have the functionality of SAS,” he says. “It’s really a complete workflow.”

For Gretz’s team, JMP Genomics helps in five steps of the research process:

- Verification of microarray data quality.
- Incorporation of updated probe set annotation to keep up with advances in the understanding of genome structure and gene organization.
- Assessment of the statistical significance of detected differences using flexible mixed models.
- Principal component analysis and hierarchical clustering of samples and genes.
- Pathway analysis – a “crucial step” that assesses whether certain gene sets clustered in functional pathways show more expression changes than would be expected by chance.

“With JMP Genomics we can quickly advance investigations from raw microarray data to analysis results,” Gretz says. “Formerly, the validation of results took several weeks to achieve, but today we get the results in just a few hours. We can very easily implement additional analysis processes, giving us a decisive advantage in our daily research.”

For the kidney disease study, Gretz’s team used a rat model system containing the same gene mutation that causes PKD in humans. mRNA from heart and kidney tissue was processed, and the resulting cDNA hybridized to 24 Affymetrix rat DNA GeneChips containing 15,924 probe sets. After scanning and preprocessing by Affymetrix software, raw data from Affymetrix CEL files were imported and evaluated using JMP Genomics. Researchers applied cross-array correlation analysis during the initial data quality assessment to identify statistical outliers, which were excluded from further analysis. Once analysts were satisfied with the results of normalization and quality control tests, they applied analysis of variance (ANOVA) to determine whether observed deviations in transcriptional patterns were statistically significant.



Dr. Norbert Gretz has spent two decades investigating the molecular basis of polycystic kidney disease.

RNA and microRNA – on to new horizons

Despite its substantial investment in array technology platforms, the ZMF team had hit an analysis bottleneck before the adoption of JMP Genomics. Working primarily with expression data from Affymetrix arrays, ZMF analysts evaluated two spreadsheet-based systems, but these did not meet their analytic needs. “The results of the evaluation systems were not robust because their statistical capabilities were limited,” explained Gretz. They turned instead to the trusted SAS and JMP analytics underlying JMP Genomics, and report that thorough microarray analyses can now be completed within a few hours and results delivered quickly to researchers’ desktops.

In addition to robust statistics, the ZMF team also sought a platform flexible enough to accommodate diverse data sets from emerging technologies. In recent years, scientists have identified and isolated remarkably small RNA components of the genome, commonly known as microRNAs, which play an important role in regulation of protein production. ZMF now also provides researchers with the technology to generate rich information about microRNA patterns and uses JMP Genomics to analyze the resulting large data sets. Researchers receive reliable statistical information about observed patterns, which can then be placed into the context of gene pathways to identify groupings of genes that signal the outbreak of a particular disease. Many scientists



Members of the research team, from left, Dr. Li Li, Dr. Gretz, Maria Saile, Dr. Carsten Sticht.

believe that understanding differences in microRNA patterns between diseased and healthy organs will assist in the development of future diagnostic tools and more effective medications.

Encouraging collaboration and customization

Microarray analysts at ZMF have provided important feedback to the JMP Genomics team, resulting in the addition of new quality-control functions in the software. In addition, JMP Genomics developers added a new annotation process to link gene identifiers to global bioinformatics databases like UniGene, Gene Ontology and Entrez Gene. These tools make it possible for scientists to verify the relevance of research results and to formulate new hypotheses. ZMF analysts may access a wide variety of analysis options in JMP Genomics, and may

even develop their own custom processes to call SAS through point-and-click dialogs.

“Through targeted progress and the possibility of analyzing large data sets quickly, we learn more and more about the gene mutation which is responsible for the diseased kidney,” says Gretz. “JMP Genomics from SAS is the right tool for our purposes.”



SAS Institute Inc. World Headquarters +1 919 677 8000 To contact your local JMP office, please visit www.jmp.com

SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc. in the USA and other countries. ® indicates USA registration. Other brand and product names are trademarks of their respective companies. Copyright © 2009, SAS Institute Inc. All rights reserved. 103835.529908US.0209