Genes in concert

Maastricht University

Inaugural lecture

Prof. dr. Prof. dr. Theo M. de Kok

Faculty of Health, Medicine
and Life Sciences

Genes in concert : a toxicogenomics hymn towards disease prevention

Oratie Prof. dr. Theo M. de Kok

Rede uitgesproken ter gelegenheid van de aanvaarding van het ambt van bijzonder hoogleraar 'Population-based toxicogenomics' aan de Universiteit Maastricht, op vrijdag 22 april 2016 om 16.30 precies.

"Genes in Concert: a toxicogenomics hymn towards disease prevention"

Geachte mijnheer de pro-rector, zeer gewaardeerde toehoorders, beste vrienden en familieleden,
Dear guests and dear colleagues,

Van harte welkom bij mijn inaugurele rede waarin ik zal toelichten hoe het vakgebied van de Toxicogenomics kan bijdragen aan de het voorkomen van chronische ziekten als gevolg van blootstelling aan tal van omgevingsfactoren die aanwezig zijn in het milieu, voeding, medicatie en tal van andere consumentengoederen, en waarom er muziek zit in de ontwikkeling van preventieve strategieën die specifiek op het individu zijn gericht. Gezien de toenemende internationalisering van de wetenschap, de aanwezigheid van buitenlandse gasten voor het symposium eerder vandaag, en de buitenlandse collega's de werkzaam zijn binnen zowel de afdeling toxicogenomics als het instituut voor systembiologie MaCSBio waaraan ik eveneens verbonden ben zal ik deze rede uitspreken in het Engels.

Dear all, I just explained why my inaugural lecture will be in English and that I intend to explain why and how toxicogenomics research can contribute to the prevention of chronic diseases as a consequence of exposure to environmental chemicals and why there is music in the development of personalized prevention of disease.

What about Toxicology

[Slide 2: toxicants]

Human toxicological research aims to identify risk factors that may induce harmful effects as a result of exposure, which may result in acute illnesses, chronic disease or even instant death. These toxicants can be found in our food, in the air we breathe, in medication prescribed to us or in cosmetics products we use on a daily basis like shampoo and toothpaste. During evolution we have adapted ourselves and developed defence mechanisms to detoxify and eliminate a wide range of compounds. This relates to natural toxins found in poisonous plants and mushrooms as well as chemicals that are formed during burning of organic material, in for instance forest fires. The same type of combustion processes (such as the use of fossil fuels in car engines or industry) are a main source of environmental air and soil pollution nowadays. Additionally, over centuries mankind has learned from mistakes and registered this information in order to pass it on to later generations. This has made our daily life already a lot safer.

[Slide 3: Praegustator]

One of the most reliable approaches to guarantee the safety of foods and other consumer products, was probably already established by the roman emperors: the so called praegustator. This is a person that is supposed to test any food item before it is being consumed by the one to be protected. Although considered to be an honourable task, it is not without risk and several people have died performing such a job. It has been reported that even nowadays US presidents travel with pre-tasters to the Élysée palace in Paris or Buckingham palace London (which can be considered both the summit of security services and an insult to European cuisine and hospitality). Nevertheless, even this approach to food security has its limitations as it is not effective in establishing non-acute or low repeated dose effects.

[Slide 4 REACH]

It seems obvious that exposing people intentionally to chemicals has its limitations from both scientific and ethical perspectives. Therefore, chemical safety is nowadays predominantly established based on animal testing as an alternative to study toxic effects of environmental chemicals in humans. A wide range of animal models has been developed to test different types of toxicity, including tests for carcinogenicity, reproduction toxicity or immunotoxicity. New regulations of the European Union, called REACH, have been adopted to improve the protection of human health and the environment from the risks that can be posed by chemical exposures. One of the consequences of this regulation is an enormous increase in the number of laboratory animals needed to perform such safety evaluations.

This raises questions about the ethical acceptability of such tests, and this has resulted in a societal call for the development of animal free alternatives. Furthermore, animals are quite different from human beings and research on animals may not always be relevant for assuring human safety. The predictivity of some animal studies on carcinogenicity is for instance as low as 60%; hardly any better than flipping a coin.

[Slide 5: In Vitro alternatives]

These alternatives to animal test as an alternative to human studies are sought in use of human cell cultures. In the context of this inaugural lecture I will not explore further the exiting innovations in cell culturing techniques, using stem cells and 3D culturing systems for generating spheroids and micro-tissues that resemble much better normal human cell physiology, tissue structure and even disease development as compared to the traditional mono layer cultures of immortalized cell lines. Instead I want to move on to the transition of toxicology research into toxicogenomics, as it will guide us back to studying toxicity in human populations.

What makes toxicogenomics different from toxicology.

[Slide 6: Revolution]

Over the last 2 decades, a true revolution in biology has taken place, initiated by the human genome project in the US (1990 – 2003) in which the full human genetic code was determined by DNA sequencing techniques. The timeline on this slide shows how our understanding of genetics has started with the discovery of laws of genetics by Mendel in 1865 and resulted in the discovery of DNA as carrier of all genetic information by Watson and Crick in 1953. Thirty years later, in 1983, the first human disease was mapped to the genome (Huntington's disease).

[Slide 7: The cell]

In order to explain how this has impacted on toxicological research, I have to present some basic biological concepts.

Every cell in our body contains a complete set of our inherited genetic information: the DNA. DNA contains the code for all functional and structural properties of the human body and is located in the nucleus of the cell. In order to perform these functions, the information contained in the DNA has to be copied into a messenger molecule (mRNA) that can be transported from the nucleus into the cytoplasm where it provides the information for the formation of proteins. The proteins are the biologically active molecules that perform a wide range of biological functions. A part of the DNA that is coding for 1 specific protein is called a gene, and every human has approximately 20 to 25 thousand different genes.

Now let's assume a number of proteins is involved in the detoxification of a chemical. The chemical is usually converted into a slightly different molecule (called metabolite) which is more water soluble in order to be excreted in urine. Measuring metabolites gives therefore information on the outcome of the reactions that have taken place in the body.

[Slide 8: High through-put]

New 'high throughput' techniques have been developed to analyse DNA sequences in a few days and for a few hundred euro, whereas the sequencing of the first human genome took 13 years and several billion dollars. Nowadays, we can analyse whole genome gene expression responses using so called DNA-chip technology or microarrays, which allow to measure transcription of the DNA for all 25 thousand genes simultaneously. Slides carrying tiny spots of all different genes reflect the presence of high or low levels of each factor here shown by different intensities of green or red colours. Although most of the data in population studies until now have been generated with this type of technology, it is already being replaced by sequencing approaches as the next generation of techniques in biomedical research.

[Slide 9: AhReceptor]

How does this work in practice? Let's take an example from the work of Dr. Pim de Waard, one of the first PhDs, who was applying these gene expression techniques, in the context of the Human Nutrigenomics Centre in collaboration between our department, the department of Toxicology of the University of Wageningen and the RIKILT institute. His research focussed on compounds that were activating a specific receptor on the cell surface (Ah-Receptor) that needs to be activated in order stimulate the production of the proteins that can eliminate the exposure. This process is presented in the following overview:

If a compound like dioxin activates the receptor at the cell surface, it subsequently activates a cascade of signalling molecules in the cytoplasm. Next, the complex is transported to the nucleus where it binds to specific locations on the DNA to activate transcription of genes. This results in the formation of mRNAs that code for the production of enzymes that have the capacity to detoxify some of the compounds that activate the whole process.

One of the compounds that is known to act in this way is benzo[a]pyrene, a toxic compound that can be found in for instance barbecued meat, car exhaust and industrial emissions. These compounds are also known to have the capacity to induce modifications in DNA or induced DNA strand breaks, processes that are linked to the development of cancer or cardiovascular diseases as well as other chronic illnesses.
If the exposed cells are indeed damaged before the compound is eliminated, the cell may respond to the damage by activating repair mechanisms, again resulting in the formation of specific mRNA, the associated proteins (for instance enzymes that can fix broken DNA strands) and metabolites (e.g. removed DNA bases that were chemically damaged).
One can image that exposure to a specific compound leaves behind a trace of mRNA, proteins and metabolites that provide information on the type of exposure and the type of damage induced.

Molecular Barcode

[Slide 10: Molecular barcode]

If we look at the results from human colon cells that have been exposed to benzo[a]pyrene, we indeed see a number of genes being up and down regulated. In this graph, the strongest up regulated genes are found on the left whereas the strongest down regulated genes are on the right. Cytochrome P450 1A1, known to be involved in the detoxification of BaP, as well as other metabolizing enzymes were found to be activated. The graph also shows that other compounds that have a different chemical structure but are also known to activate the AhReceptor, such as TCDD, show a strong resemblance in their induced gene expression response.

This trace of genes that are activated or in activated, can be regarded as a barcode on a product in the supermarket: if you scan the code you get a list of information of the product: it is cheese, originating from France, and it costs 3 euro 95.

Here it means "this cell is exposed to something that activates the AhRecepter and may be toxic".

A remarkable finding in this study was that also natural compounds that can be found in healthy products are capable of activating the AhReceptor (in fact this was the main reason for setting up these studies).
This is for instance the case for indole-3 carbinol found in broccoli and Brussels sprouts or bergamottin found in grapefruit juice. Indeed, if we look at the curve for ICZ (which is indole-3 carbinol) we see indeed the same type of response. This may on the one hand imply that chronic intake at high concentrations of the compound may potentially be harmful (mind that the isolated compound can be bought in any grocery store and that the advised intake of I3C may be equivalent to consuming several kg of broccoli per day – days pass by that I don't do this); on the other hand it may explain why normal doses of foods containing these natural ingredients may actually be healthy. The induction of metabolizing enzymes may help the body to eliminate toxic compounds more effectively, without posing a DNA damaging effect itself. This implies that we should not only look at the similarities in the responses but also at the differences.

From this example we can learn that looking at gene expression profiles we may get insight in molecular responses that are relevant in health and disease risks, but also that we have to look how these expressed genes work in concert.

Genes in concert

[Slide 11: Genes in concert]

This brings me to the title of my inaugural lecture, where I compare biological processes to a piece of music, where the genes are the notes, the notes code form a message (either a symphony or a biological function), and where a combination or sequence of notes make a chord or a melody and a combination of genes reflects a biological function. In music, changing one note of a chord can change an A sharp to an A minor, which results in a completely different interpretation of the sound. In biology, changing a few genes in a gene expression pattern may reflect the induction of toxicity or disease.

In the rest of my lecture I will focus on the application of toxicogenomics markers in population studies, as this is of course the field of my professorship, and I will come back to the analogy between toxicology and music several times again.

Human population studies

[Slide 12: Human populations]

Let's go back to studies in humans. I started off with explaining the limitations of experimental toxicological research in humans, but having these new types of toxicogenomics markers creates new opportunities. Instead of looking at direct observable toxicity or disease, establishing molecular response profiles may be much more sensitive and thus allow detection of subtle changes at much lower doses of exposure. These markers may also allow earlier prediction of toxicity, as initial changes may be detected long before the onset of chronic disease.
Finally, these markers may help to establish causality, by providing information on the molecular mode of action. In population studies investigating relationships between for instance life style factors and disease risks, statistical associations may be found that are not necessarily causally related. Having information on relevant biological mechanisms that may

indeed explain a plausible relationship between exposure and effect adds to the likelihood that an association is indeed causal.

Newgeneris

[Slide 12: NewGeneris]

Over the last decade I was involved in a series of European projects looking at the relationship between environmental exposures and disease risks in larger populations. The first one was NewGeneris, which was coordinated by prof Jos Kleinjans head of our department, and focussing on parental exposures to environmental contaminants such as dioxin, PCBs and other persistent organic pollutants in relation to adverse health outcomes in children.

Particularly the incidence of childhood leukaemia, which is rising by about 1% a year since the 1950s is of concern, and could potentially be linked to increased exposure during pregnancy. In umbilical cord blood samples from more than 1000 new-born children from mother-child studies in various European countries, a significant association was found between chromosome damage and exposure to dioxins and PCBs.

Keven Hochstenbach, one of the PhDs working on this NewGeneris project, determined global gene expression levels in 120 Norwegian children and discovered important sex-specific differences in genomic responses. Specifically in boys, dioxin exposure was associated with activation of the proinflammatory factors whereas exposure to acrylamide (another compound known to damage DNA and occurring in specific foods such as French fries and coffee) was associated with activation of the Wnt pathway, which is associated with uncontrolled cell growth and the development of cancer.

This supports the hypothesis that childhood cancer, in particular leukaemia among boys, is causally related to the dietary intake of carcinogenic substances by their mothers during pregnancy.

Envirogenomarkers

[Slide 14: Envirogenomarkers]

Another large European project in which I was involved is Envirogenomarkers. I am very happy to have the coordinator of this project, prof. Soterios Kyrtopoulos from the National Hellenic Research Foundation (NHRF) in Athens, present in the audience.

Envirogenomarkers is built on the use of samples and exposure data from the European Prospective Investigation into Cancer and Nutrition (EPIC) study which is one of the largest cohort studies in the world, with more than half a million (521 000) participants recruited across 10 European countries and followed for almost 15 years. In the Envirogenomarkers project we specifically used the Italian and Swedish part of the EPIC study and focussed on participants that developed either breast cancer or non-Hodgkin lymphoma several years after inclusion in the study.

The conceptual basis of the project is the idea that exposure induces different responses at the level of gene expression and other omics responses, providing us with markers of exposures, whereas the disease endpoint itself would also be reflected in these markers, providing a molecular signature of the disease. In the overlap we would hope to find so called meet-in –the middle biomarkers that give information on both.

Having all technologies in place, we measured various genomics markers in the available samples from approximately 700 B-cell lymphoma cases and healthy controls. Our partners from the department of Epidemiology and Biostatistics at Imperial College London, headed by Prof. Paolo Vineis (also present in the audience) and particularly Dr. Marc Chadeau who was coordinating the complex data analyses, identified 745 genes that were related to the future risk of chronic lymphocytic leukaemia.

[Slide 16: Graph cases controls]
This graph shows the consistent over expression of the genes in cases (red) regardless of the location on the chromosomes. Looking at the biological processes in which these genes are involved, these were found to be highly relevant in the regulation of B-cell activities and the development of the disease. This finding demonstrates the clinical value of gene expression markers, predicting disease risk 10 years before clinical diagnosis, and allowing interventions at a very early stage that may improve the clinical cause of this disease.

This is truly a quite exciting outcome, but it does not give us any insight in the potential relationships between exposure and disease. For this I will highlight another example, but before I do this I have to go back to our genes in concert to explain just a little bit more complexity.

The role of the conductor: epigenetics
[Slide 17: The conductor]
Single musicians don't need a conductor as they can give their performance perfectly well on their own. However, the larger or orchestra gets, the more crucial the role of a conductor becomes in order to literally orchestrate the performance. Without tuning the instruments and regulation of the musicians (who plays when, how fast and how loud) the performance may end as a complete disaster; having played in a small band my younger years I have experienced and contributed to such disasters myself.
This need for regulation applies also to complex biological processes.

[Slide 18: Epigenetics regulation]
Going back to the series of events that make up a biological process, we can add regulatory mechanisms. At the level of the DNA, DNA-methylation and other modifications can switch gene on or off by changing the structure more open or condensed, thus making the DNA more or less available for transcription. At the RNA level, miRNAs may cause the rapid degradation of the mRNAs coding for the proteins and at protein levels, degradation processes may reduce the biological effect. As this does not change the actual genetic code these regulatory mechanisms are called epigenetic.

[Slide 18: Smokers]

Let's go back to the example I promised from the Envirogenomarkers project.
In order to have a strong proof of principle, we looked at gene expression, miRNAs and DNA methylation in smokers and non-smokers; not because we are interested in demonstrating the health risks of smoking (these are intensively studied and well know) but because differences in exposure are easy to establish and well validated information on health consequences is available, also at the molecular level.
The results show that more than 250 genes were expressed differently in smokers versus non-smokers, and that most of these genes were downregulated. At more than 1000 sites, DNA methylation was different between both groups, mostly showing loss of methylation in current smokers. Additionally, 34 miRNA were differently expressed.

[Slide 19: Hub genes]

Using a specific bioinformatics tool, called GORevenge, we tried to find out which are the potential master-regulatory factors (you could say what genes are playing the first violin). This identified 40 hub genes that have very strong links to multiple other biological functions in a network. These genes showed involvement in relevant molecular pathways in various diseases including cancer and heart disease.
This figure tries to visualize for instance the interactions between genes that have been identified based on different expression- or methylation levels and miRNAs, all related to lung cancer. These factors are represented by Diamonds, Rectangles and Triangles respectively.

Comparison of the smoking induced 'omic profiles with disease profiles reported in literature that were obtained from patients with either lung cancer or coronary heart disease, showed a remarkable overlap. This suggests that blood cells may respond to toxic exposures in a quite similar manner as found in the actual target tissue, e.g. the lung or the vascular system. It gives is a strong proof of principle that omic profiling in blood cells has the potential to identify at a very early stage disease-related responses caused by toxic exposures.

In the future we plan to generate new data sets on the 'omics responses to toxic compounds simultaneously in blood and target tissue. This is necessary to further validate to what extent the markers in blood reflect the actual toxicity in the crucial organs. Furthermore, such data are needed to construct mathematical models that can help us the predict toxicity and disease development more accurately.

Differences in genetic background

[Slide 19: Differences in genetic background]

Currently we are looking in these and other available data sets to discover how differences in genetic background may influence the effects of typical environmental exposures. Although we all have the same genes, different individuals in a population have different variants (or polymorphisms) of these genes. This determines for instance that we all have different colour of hair and eyes. Likewise, differences in genes coding for detoxifying enzymes, or one of the many other genes that are involved in toxic responses lead to differences in susceptibility to exposure. This explains why some people can develop lung cancer as a consequence of exposure to relatively low doses of environmental tobacco smoke whereas others can smoke cigars eternally without ever experiencing any serious health consequence.

Such gene-environment interactions are taken into consideration by Almudena Espin-Perez while analysing the Envirogenomarkers data. Previously she has shown in data from the Flemish Environmental Health Survey (FLEHS) that the combined exposure to multiple carcinogens (including heavy metals and organic pollutants), along with 28 genetic variants, resulted in the deregulation of cancer related molecular pathways. Subjects carrying a high number of risk alleles (gene variants that are theoretically related to increased risk) indeed appeared to be more responsive with respect to the induced expression of some cancer-related genes. This indeed suggests increased cancer risk as a consequence of environmental factors.

Prediction of toxicity: the Dutch national hymn

[Slide 20: Wilhelmus]

Apart from using genomics biomarkers for the identification of risk factors, these molecular barcodes can also be used for prediction of toxicity. If we know the key elements in the profile that are related to a specific toxic effect, let's say liver toxicity, we can use this information to predict potential liver toxicity induced by new compounds (such as drugs under development) that show the same signature. It is a bit like listening to the first three notes of a piece of music.

Based on these three notes you can easily predict the rest of the music simply because we carry the code in our own brain as data base for musical sequences. Having at least 15 nationalities in the audience, this means that some of you may not be able to predict the Dutch national Hymn 'Wilhelmus'. This emphasizes that we need to invest in having all relevant information in our data bases in order to be able to predict without mistakes.

[Slide 24 EU projects]

Fortunately, we have in Europe a very strong history in environmental health studies that allowed us to generate large databases and to build up expertise to extract the relevant information that can contribute to improved European public health. In addition to the projects already mentioned, other European projects are focussing on the environmental impact on children's health; these include the HELIX project, coordinated at CREAL, the Centre for Research in Environmental Epidemiology in Barcelona, directed by Manolis Kogevinas, and Environage, a project of Tim Nawrot at Hasselt University. The last projects I

want to mention in this context are HEALS and EXPOSOMICS. The EXPSOMICS project, coordinated by Paolo Vineis from Imperial college, has as a particular focus the advanced measurement of human exposure using for instance GPS based monitoring of individual exposure to air pollution. These last projects are all ongoing and in full progress.

Europe

Going through times were the added value of participating in the European Union is continuously being questioned, it should be emphasized that this level of scientific advancement and combined use of research expertise and infrastructure could never have been achieved at the level of individual member states or without a consistent EU support for scientific research on environmental health issues.
In order to profit optimally from investments already made, it is crucial to create an electronic infrastructure that is capable of safeguarding all collected data, and allowing harmonization between different project results. This is essential to allow analyses across all studies, identifying gaps in crucial information to be filled and serving as an indispensable source of data to further advance our analytical tools.
Creating new opportunities to do this, I consider as one of the top priorities on my research agenda.

Multidisciplinary work

[Slide 25: Multidisciplinary work and Macsbio]

It is a true challenge to get all relevant information out of the large and complex data sets, and in order to be successful this requires combined expertise from different disciplines including:

- Bioinformatics
- Molecular biology
- Epidemiology,
- Toxicology
- Mathematics and Statistics

Therefore it is good to have the Maastricht Centre for Systems Biology (MaCSBio) initiated by Maastricht University with support of the province of Limburg, now exactly one year ago. The institute brings together the expertise spread over the faculty of Health Medicine and Life Sciences, the Faculty of Humanities and Sciences, and the Faculty of Psychology and neurosciences.

Although different researchers have different definitions of what systems biology is, one of the crucial aspects is that it studies complete biological systems, which can be either an entire ecosystem, an intact human individual or a single cell. A second important characteristic is the close collaboration between researchers that generate the data, those that perform calculations and try to make mathematical models describing the system and those that perform the biological interpretation.

Together with prof. Ilja Arts I am directing one of the two research lines, called SYSTOPP which is focussing on the development of chronic diseases in people with obesity. We apply a top-down approach in which we use different type of 'omics data, life-style data and information on disease status coming from the type of human population studies that I described earlier.
We aim to develop new tools that help us to describe the obese system in a more accurate way and to map the 'omics markers we have from our population studies on the genome scale metabolic models that are available. This work is done in collaboration with the Luxembourg Centre of Systems Biomedicine.

The new tools that we aim to develop can also be applied in other fields of research, including systems toxicology. Eventually we aim to apply these approaches in the development of disease prevention strategies.

Disease prevention
[Slide 26: Prevention]
Prevention can be achieved in different ways. For instance:

- by identifying the most relevant hazards to be avoided;
- by identification of individuals that are more sensitive to exposures and may thus need specific prevention strategies; just think of the risk alleles in the Flemish study;
- or by trying to target crucial genes, like the hub genes in the smokers study, with preventive drugs or healthy food ingredients.

[Slide 27: IARC]
Any prevention strategy implies that we have to intervene somehow, which brings me to a different type of human population research: the intervention studies. I would like to explain this in the context of colon cancer risk. After a long journey studying potential dietary factors that may explain the relationship between typically Western diets and the risk of colorectal cancer, we eventually ended op looking into exposure to N-nitroso compounds which is associated with meat consumption. These compounds are not present in diet itself but can be formed in the gastrointestinal tract. This formation can be stimulated by nitrite which is added to processed meat such as cured ham, salami and other sausages, to prevent the growth of pathogenic bacteria. Also the presence of haem iron which is present only in red and not in white meat (such as chicken) stimulates the formation of nitroso compounds. This may explain why in epidemiological studies consumption of red but not white meat is associated with colon cancer risk.

[Slide 28: NOC signature]
In a range of experiments with cultured human colon cells, healthy populations, and a high meat intervention study in patients with inflammatory bowel disease, Dennie Hebels established a gene expression network that may explain the link between exposure to nitroso compounds and cancer development (once again a complex symphony of interacting genes and processes). In this network, the marked genes were suggested as a potential biomarker of exposure, which of course needs to be reproduced in an independent study. I will come back to that later. This was the first study in humans that actually confirmed at the molecular level the carcinogenicity of nitroso compounds observed in animal studies.

[Slide 29: Blueberry]

At the same time, Simone van Breda and Lonneke Wilms were studying the beneficial effects of the human diet. In contrast to meat intake, the WCRF identified bioactive compounds present in fruits and vegetables as most likely to reduce the risk of both lung and colon cancer. This was particularly based on the available mechanistic evidence and to a lesser extent on epidemiological studies.

In a dietary intervention study with blueberry juice rich in different classes of bioactive compounds including polyphenols, anthocyanins and vitamin C, the preventive effect on the induction of DNA damage by a challenge with hydrogen peroxide was established. It was clearly demonstrated that after 4 weeks of blueberry juice consumption, the white blood cells were much better protected against the same challenge. In this study also the impact of a number of different genetic variants in DNA repair genes and detoxifying enzymes was determined.

In the total population a DNA damage reduction of 20% was found, but this reduction was observed predominantly in individuals with specific genetic characteristics.

This demonstrates that looking at genetic differences between individuals is relevant and enables the identification of subgroups among the general population that may benefit more of protective effects of bioactive compounds. Such information provides the basis for more personalized approaches to disease prevention.

PHYTOME

[Slide 30 PHYTOME]

It is exactly at the intersection between the research lines on colorectal cancer risk factors and prevention that the PHYTOME project was formulated. This EU funded project that I coordinated myself, involved several partners from European research institutes as well as meat processing industry and aimed to replace nitrite (indicated as one of the culprits of colorectal cancer risk) by natural extracts of fruits and vegetables that are known to possess antimicrobial activity. This allows them to replace the function of nitrite in processed meat and at the same time to activate preventive gene expression profiles. These new meat products were again evaluated in a human dietary intervention study to establish the impact on exposure to nitroso compounds, measured in faecal material by dr Gunter Kuhnle at Reading University, who is also present in the audience.

[Slide 31: Reduction of ATNC]

The results show that after increased meat consumption the exposure significantly increases. After consumption of meat products enriched with natural extracts, the exposure level is reduced to background levels, even below the level found after consumption of white meat. The presence of the phytochemicals appeared to be more important than the reduction of added nitrite. Also DNA-adduct levels were higher after consumption of conventional products as compared to white meat, but not after consuming the PHYTOME meat products, again suggesting a phytochemical-induced preventive effect.

This suggests that meat consumption could actually be part of a well-balanced diet, without increasing cancer risk.

[Slide 32: Gene expression]

Gene expression analysis and DNA-methylation profiles, measured in colon biopsies from participants, taken by Ad van Bodegraven at the Zuyderland hospital in Sittard and by Ad Masclee at the academic hospital Maastricht, provided evidence which may mechanistically support such a preventive effects.

What I find particularly reassuring is that with the assistance of Diana Hendrickx and Almudena with regard to the bioinformatics, we now find some genes to be affected in the network show on this slide, that were also found in the earlier intervention study with increased red meat consumption.

This can be seen as the validation of previously identified biomarkers that we were looking for.

Overall these findings show that identifying risk factors based on toxicogenomics data and formulating food innovations using molecular understanding of preventive mechanisms may contribute to disease prevention.

Synergy

[Slide 33: Synergy and smood]

How to implement and valorise this; after all, putting science to work is one of the success criteria in modern academia. As a further continuation of this type of studies I am happy having found some enthusiastic partners in the province of Limburg who are aiming to bring a product to the market that has demonstrated beneficial health effects based on well-chosen combinations of bioactive ingredients in their original fruit and vegetable context.

New techniques available at a the company SMOOD in Horst, allow the extrusion of natural ingredients at low pressure and temperatures to keep the active ingredients in their original form and functionality. In the context of this collaboration we intent to set up a range of experiments to establish the synergistic effect of various ingredients by targeting multiple molecular processes at low dose.

Like in the blue berry intervention study, we intend to take genetic variability into consideration, aiming to develop a range of products that can be tailored to the specific needs of individuals.

Visualisation

[Slide 35: Mitochondrial damage]

One last aspect is the visualisation of all these complex outcomes. Composing a brilliant piece of music is one thing, but giving a stage performance is something else and requires different skills. Different performers can give a completely different interpretation of the same song. The classical Jazz performance of Nina Simone of 'Feeling good' dating back to 1965 has recently been transformed by Avicii using new tools to a completely modernized version and has brought it back to live again for a new generation. In systems biology we are facing a comparable challenge in finding new ways to presenting our data. The data we generate are typically boring and presented in tables and I have shown you already several representations of networks that try to visualize the biological interpretation.

Ideally we would like to see dynamic representations, as shown in this animation. It is an artist's impression (made by a young student in the Maastricht Science programme; Jip de Kok) of a dysfunctional mitochondrion, using gene expression data produced by Jian Jiang, in a study looking at mechanisms of drug induced liver injury in cells exposed to paracetamol. Although it looks dynamic, every interaction between chemicals and proteins is defined by the programmer. Further advancements in this field should generate tools that directly link our data to such visualisations in order to assist us in interpreting the biology hidden in our experimental results. Having artist's impressions adds yet another discipline to interact with in systems biology, again making the field more exiting. The fact that specific companies are emerging to provide services in this fields shows also here the potential for valorisation.

Education

[Slide 34: Education]

Over the years, we have shared the outcomes of our research programme on toxicological risks of chemical compounds, the rapid development of new genomics techniques and the risk-benefit analyses related to food research in a range of academic programmes of the faculty FHML. This includes Bachelor and Master programmes in Health Sciences, Biomedical Sciences, and European Public Health. This year, a new Master Systems Biology has been initiated and I think my first lecture and Journal club is planned in two weeks. I hope that with the increasing need for well-trained Systems Biologists in many fields of application, the programme will expand rapidly. I also think we should take the planned curriculum revision of Biomedical Sciences as an opportunity to strengthen the programme with regard to complex data analysis and bioinformatics; this also because we observe a decreasing number of applications for PhD positions coming from our own university.

Since the start of the master programme Health Food Innovation Management, offered at the UM campus in Venlo, I have been introducing the use of innovative biomarkers in food research. While expanding our research in this field, also involving the new Brightslabs in Venlo, it makes sense to involve students form this programme in the further development of the synergy-based SMOOD products.

Keep calm and acknowledge all musicians in the orchestra

[Slide 36 Acknowledgments]

Coming to my final slide. I would not be standing here without the work and support of many others. During my lecture I already mentioned several people that have contributed to the work I presented and I would like to extend my thanks and appreciation to all colleagues in the lab doing the practical work, the PhDs and postdocs I have been working with over the years, and the staff members both at the department of toxicogenomics and more recently also at MacBio. I would like to express my special thanks to all national and international partners in the numerous consortia for the fruitful collaboration and I hope that we will continue and expand our scientific exploration with even greater enthusiasm after today, aiming to compose some real masterpieces in the near future.

Two people I would like to highlight in my gratitude. First of all Jos Kleinjans, who convinced me after a few years at the Open University it was time to come back to Maastricht, and advance may career in toxicological research. You have always been a true source of inspiration and your incredible creativity in generating new project ideas, but also in finding opportunities for research funding by intensive networking and knowing your ways in Brussels, has learned me how to define of create my own research projects. Over the years you always remained the critical friend that I can come to for advice, and sometimes the advice even comes without asking. This is highly appreciated and has brought me to where I stand today!

Last and most importantly I want to acknowledge the support of Trudy who really understands the importance of all project board meetings abroad, even during weekends and holidays, and that interactions at international congresses and symposia are crucial sources of inspiration to generate new project ideas. I hardly ever invite you to travel along to such meetings, but on these very few occasions this has happened you blend in automatically as if you conducted the research yourself without hardly anybody noticing. This may not be all that surprising considering our diner table where Marloes brings in the evaluations of the medical curriculum at our faculty, Lieke reports on the Health Sciences Bachelor and the master Developmental Psychology, Ben (Lieke's boyfriend) discusses new insights obtained from the Biomedical Sciences master, and Jip (who is following the Maastricht Science Programme) shows the visualization of a dysfunctional mitochondrium. Just imagine conversation during Christmas dinner.
However, as this was apparently not sufficient Marloes attracted Joep to add a flavour of medical technology (directly flown in from Enschede – something that is considered to be a good habit in our University). Trudy I don't know what happened exactly but it seems a mini-FHML as grown around us…

For those of you who have counted well, there is still one vacancy at our table….
Jip, I suggest you come to MaCSBio Monday afternoon to discuss the algorithm needed to predict the crucial characteristics required to complete and survive our diner table.

I thank you all for your attention.

Ik heb gezegd.