1. Introduction

Western medicine is based on a wide variety of disease management strategies that are employed with great success. For instance, the mortality of heart disease, stroke and cancer patients has dropped dramatically (Jemal 2009, Eheman 2012). Still, there is much room for improvement in various areas of medicine and care. Recently, within the American medical system medical interventions have become the leading cause of death with over 750.000 iatrogenic deaths each year (Null 2003). Alan Roses, past vice president of GlaxoSmithKline, reported in 2003 that 90% of the drugs work in only 30-50% of the patients. This means that large numbers of people receive medication that doesn't contribute to their health, leading to further hospitalizations due to adverse drug reactions and side effects.

Current Western drug development is based on the golden standard of randomized placebo controlled double-blind clinical trials. This type of study design is only suitable to discover average drug effects over large, homogenous groups of patients. However, when a drug enters the market it is largely unknown whether the drug is effective or toxic in the individual patient. On the other hand, drug candidates that might be very beneficial to a certain individual, will not reach the market when the average effect does not rise above a placebo effect in a large group of patients. In reality, drugs are developed for a statistically average patient which in reality doesn't exist, and not for the individual patient.

Personalized medicine

Personalized medicine has appeared on the horizon as a new drug development strategy, which aims to get the right drug, to the right patient, at the right time, in the right dosage, etc (van der Greef 2006) (Figure 1). The development of this type of medicine requires an entirely new way of conducting clinical trials and evaluating efficacy and effectiveness (Witt 2009). The effectiveness of drugs can be improved enormously when they are targeted to the right patients, and especially when they are developed for a specific sub-group of patients. Personalized medicine can only be developed in combination with proper diagnostic tools that are essential for matching individuals with a specific drug combination or treatment. This topic is extensively discussed in Chapter 2.

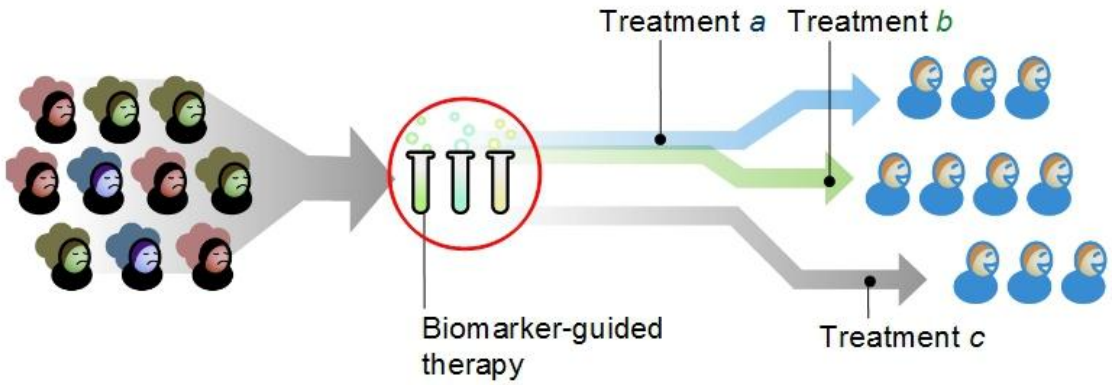

Figure 1. The concept of personalized medicine. Extensive diagnosis is the key for recognizing sub-groups of patients (differently colored unhappy characters on the left side of the figure). Medication is optimized for specific patient groups and eventually for the individual patient.

Better diagnostic tools can lead to better disease prevention strategies, and eventually to health promotion strategies. Health promotion is a largely unexplored area of science that for instance aims at improving the resilience of the system, and has the potential to offer enormous opportunities for improving quality of life and preventing diseases (Lindström 2005). Health promotion contrasts with current disease management strategies, which are mostly aimed at suppressing certain symptoms such as lowering cholesterol levels, reducing inflammation by suppressing the immune system, using insulin to control glucose levels, etc.

An intriguing challenge for scientists is how to measure health and health promotion. One step in this direction is the use of challenge tests such as the oral glucose tolerance test, exercise, or a high fat diet in healthy volunteers to measure the resilience of the system (Wopereis 2009). Not surprisingly, health is a very personal condition, each of us having to do with certain genetic predispositions, a constitution with certain strong and weak points, and more or less effective acquired behavioral patterns. Health can be promoted by certain life style changes that are generally good for everyone such as having a healthy diet and having a minimum of daily exercise. However, major improvements will be made possible by a personal targeted set of life style behaviors closely matched with the personal condition. Consequently, detailed diagnosis of the system is essential for the development of both personalized medicine as well as health promotion. Diagnosis is the topic of this thesis.

Systems thinking, systems biology & systems diagnosis

In the currently popular disease management mode of the health care system, people mostly wait to seek help until some serious symptoms are observed. For instance, when the pancreas function has already dropped dramatically, elevated blood glucose levels are detected, and diabetes type 2 can be diagnosed. This underlines that the body is quiet good at keeping processes within certain well-controlled boundaries without generating feelings of illness. This is called homeostasis. However, it might well be the case that other parts of the body are compensating for loss of function, while no changes in blood parameters are measured yet. In a disease management mode, it will be very hard to detect and prevent movements of the system towards unhealthy states.

It becomes increasingly clear that many chronic diseases are characterized by a combination of disturbances in various systems, such as the immune system, the autonomic nervous system, the gut microbiome and mental processes (Irwin 2011, Sterling 2011). For instance, deregulation of the cortisol circadian rhythm due to shift work or sleep disturbance has been found to increase cortisol levels in certain tissues, which can lead to immune system disturbances and diseases such as metabolic syndrome, cardiometabolic diseases and stroke (Cutolo 2012). In rheumatoid arthritis the role of gut microbiota in relation to joint problems 'the gut-joint axis' is becoming more and more important (Scher 2011). Studies such as these show that systems thinking, considering the interactions between various systems in the body, is essential for understanding complex diseases.

Systems thinking requires a shift in focus from objects to relationships. The quality of the organization of the system is more important than the quantity of the separate objects. At higher levels of the organization new properties emerge that cannot be observed at a lower level. For instance, cells working together in an organization called a liver show specific liver properties, which cannot be observed in the liver cells individually.

Current prevention strategies are generally based on treatment of populations at risk. For instance, the tendency is to prescribe statins for individuals with a 10 percent increased risk for cardiovascular diseases. This means that 90 percent of those individuals are receiving medication but will never develop the disease. However, these individuals might experience side effects. On top of that, statins work in only 25 percent of the patients and compliance is usually not very high, resulting in only one or two out of a hundred patients who would benefit from this prevention strategy. This leads to a medicalization of the society. Systems thinking is needed to design strategies for detecting early stages of disturbances of the body,

and finding the people who can really benefit from the drug. Changes in the relationships between processes are much more likely to be predictive of unhealthy conditions than single biomarker concentrations.

Systems biology is a biology-based interdisciplinary field that studies complex interactions in biological systems. Systems biology is based on the comprehensive measurement of processes for which a large variety of measurement techniques have been developed, ranging from genomics, proteomics and metabolomics to physiological measurements such as heart rate variability, blood pressure, ultra-weak photon emission and clinical chemistry parameters. Following the measurements, the data needs to be processed and analyzed, which requires specific statistical tools and approaches that deal with large numbers of features or variables compared to samples (Bijlsma 2006). Principal component analysis (PCA) is a key tool that is employed to search for the main sources of variation in datasets in which the variables are linearly related. Because most processes in nature are nonlinear, it is often more suitable to employ nonlinear principal component analysis techniques (Meulman 2003). Often unsupervised methods such as PCA are followed by supervised analysis methods. Classification and prediction of groups can be accomplished by applying for example partial least squares discriminant analysis (PLS-DA) (Barker 2003). Prediction based on large numbers of variables is susceptible to over-fitting (Westerhuis 2008), which requires extensive validation strategies such as double cross-validation and permutation testing (Hendriks 2007). The final step in the systems biology pipeline consists of the interpretation of the data analysis results. This non-trivial step usually involves extensive literature search, functional enrichment tools for -omics data and network analysis (Calvano 2005, Cline 2007, Bouwman 2012). The history, developments, and future perspectives in the field of metabolomics are discussed in Chapter 3.

The complex interactions in living systems, and between such systems and the environment are at the core of Chinese medicine. Chinese medicine evolved as a systems science with an emphasis on describing instead of explaining, which is the emphasis of the Western sciences. The following quotation from the Neijijng Suwen, the oldest classical Chinese medical text written around 3000 years BC, beautifully captures Chinese medical thinking:

'Huang Di stated, “People and nature are inseparable. In nature the cyclical movement of the heavenly bodies produces atmospheric influences that exert control over the rhythms of the seasons and is responsible for change to the myriad living and nonliving things. These cycles are repeated endlessly with patterns of predictability, and yet simultaneously with a tendency

towards chaos. It is this chaos in the macrocosm that upsets the balance of the delicate ecology within people that produces disease." (Ni 1995)

The Chinese descriptive approach is actually another systems biology approach in which patterns of complex organization are described and treated. For instance, in Chinese medicine a headache by itself is not treated, because it is a single symptom without context. Only when headache appears in a combination with other symptoms such as irritability, dry eyes, painful menstruation and an oppressed feeling in the chest, the pattern acquires a meaning and can be treated. More or less stable patterns of symptoms are recognized. In fact Chinese diagnosis can be termed a systems diagnosis approach. Treatment of these patterns or syndromes is aiming for a change or shift towards another more favorable or healthy state of the system. This concept of health closely relates to the attractor and bifurcation concepts from complexity theory (see introduction texts by Steven Strogatz (Strogatz 2001) and James Bassingthwaighte (Bassingthwaighte 1994)).

Further development of personalized and preventive medicine will require both Western and Chinese system thinking approaches. The key issue is how to communicate between the two sciences and how to translate and integrate the advantages of both sciences into one new future systems science. This is a major issue explored in this thesis. Two symbols of systems biology are shown in Figure 2 to illustrate this challenge. On the left side is a network model of the interactions between genes, proteins and metabolites measured in an atherosclerosis rat model using modern -omics techniques. On the right side a Chinese functional description of the body is presented in which the persons and the relationships between them represent organ functions and the relationships between the organs. Both sides of the picture show regulatory systems and the organization of regulation. The descriptive approach is therefore a nice addition to the more detailed explanatory approach.

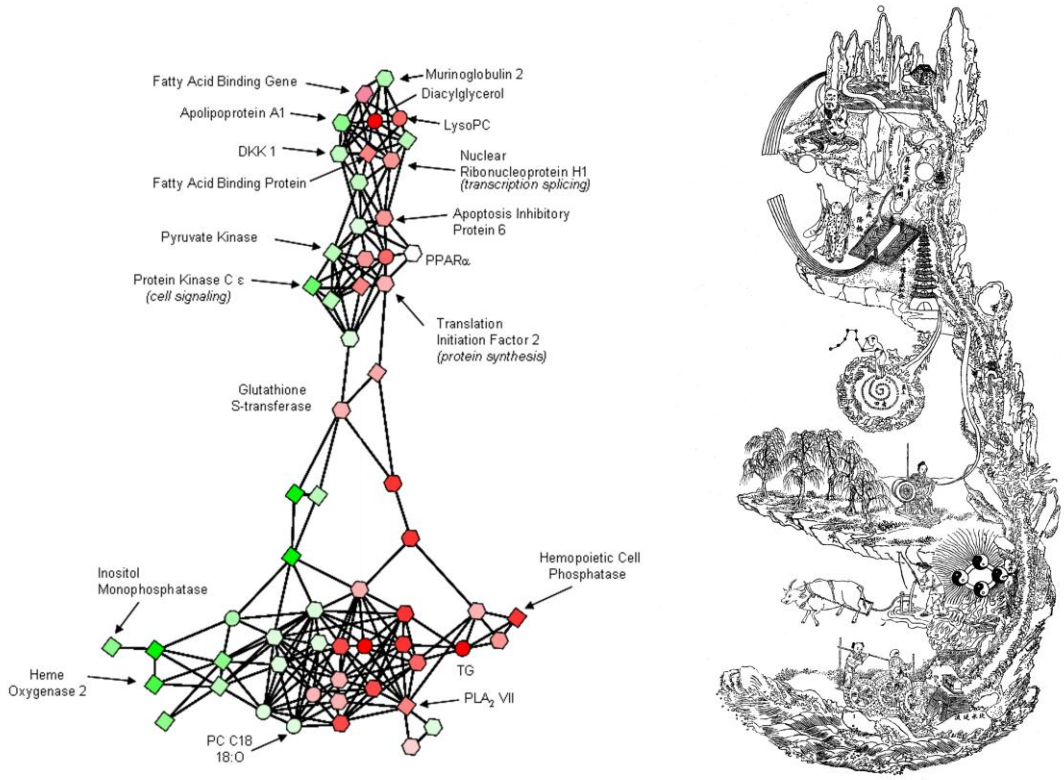

Figure 2. Illustration of systems thinking in Western science and the Chinese Taoist traditions. The left panel shows a network of correlations between metabolites, genes and proteins revealing the complex interactions related to the onset of atherosclerosis. The right panel shows the Neijing Tu, a chart of the inner landscape of the body from the White Clouds Taoist temple in Beijing. The Taoist school of Highest Clarity envisions the body as a complete world onto itself that is also a reflection of the world.

Sub-phenotyping approach for targeted care

In this thesis a step is taken in the direction of personalized medicine by integrating Chinese diagnosis with systems biology. The main focus of the research was to find relevant sub-types of rheumatoid arthritis (RA) patients. The patients in the studies were diagnosed according to the official American College of Rheumatology classification criteria for rheumatoid arthritis. On top of that a Chinese diagnosis was obtained for each patient and used for assigning patients to sub-groups. Systems biology, especially metabolomics and transcriptomics measurements were used to find biological differences between these sub-groups of patients.

The next step was to design new studies to test the clinical relevance of the possibly newly discovered sub-groups of RA patients.

Several sub-types of RA patients have been identified based on particular clinical and molecular features (van Baarsen 2010, van der Pouw Kraan 2007). Factors such as disease duration and age have been identified that predict response to treatment (Anderson 2000, Wolfe 1991). Although some molecular markers have been found to predict functional and structural outcomes, these markers rarely find their way into clinical practice. One reason is the difficulty to translate markers found in trial populations to routinely measurable and cost-effective predictors for individuals (Conaghan 2011). Another reason is that the discovery of novel relevant sub-types of RA patients is hampered by the unsupervised nature of the currently employed research strategies. Without prior knowledge it becomes very hard to sift through large amounts of gene expression, proteomics and metabolite information and come up with useful models and interpretations. This indicates that there is a need to develop new robust and reliable clinically applicable tools to identify sub-types of patients for rheumatoid arthritis.

Outline of the thesis

Chapter 2 gives an in-depth exploration of how personalized medicine can be envisioned and developed. Diagnosis is discussed as a key concept in this endeavor. A further integration of systems thinking into the life sciences and medical sciences is proposed aiming to further develop a systems science. Additionally, the value of Chinese systems thinking for personalized medicine is extensively discussed and methods for integration with Western science are described.

In Chapter 3 metabolomics as a key systems biology technology is reviewed in the light of 30 years of metabolomics at TNO. The chapter shows how metabolomics started out from a technology push that turned into a biology pull around the year 2000. A large variety of metabolomics applications that have been developed over 30 years illustrates this. The review is concluded with future perspectives for the metabolomics field and how the field can contribute to changing views on health care and healing.

Chapter 4 provides deeper insights in metabolic processes that are related to rheumatoid arthritis. This literature overview attempts to provide a comprehensive view of these processes that have been described over the last several decades. Subsequently functional

enrichment tools are used to integrate the information and find the dominant biological processes.

In Chapter 5 the first clinical study is described in which two sub-types of rheumatoid arthritis patients are found based on a Chinese diagnosis. The two sub-types of patients are characterized by gene expression profiles measured in CD4+ T-cells and by a GC-MS metabolomics analysis of plasma. This chapter provides the first evidence for biological differences between two sub-types of RA patients based on a combination of Western and Chinese diagnosis.

Chapter 6 describes an important step following the study presented in the previous chapter, namely the standardization of the Chinese diagnosis used to sub-type the patients. This step involves the development and application of a systems diagnosis questionnaire that allows the collection of extensive symptom patterns of arthritis patients. These symptom patterns are explored for 49 patients who filled in the questionnaire.

In Chapter 7 a more extended clinical study is described in which the same two sub-types of rheumatoid arthritis patients are explored. In this study the patients are classified by a Chinese medicine expert, but in addition the systems diagnosis questionnaire was completed by the patients as well. Extensive untargeted LC-MS metabolomics measurements were performed in addition to standard clinical chemistry measurements to characterize the sub-types of patients.

Finally, in Chapter 8 the contribution of this work towards personalized medicine is discussed. In addition ideas for future studies that could follow from this work are presented.

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