Clinical implications of omics and systems medicine: focus on predictive and individualized treatment

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R1: The clinical implications of omics and systems medicine, focusing on predictive and individualised treatment

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Abstract

Many patients with common diseases do not respond to treatment. This is a key challenge to modern health care, which causes both suffering and enormous costs. One important reason is that common diseases are associated with altered interactions between thousands of genes, in combinations that differ between subgroups of patients that do or do not respond to a given treatment. **Such subgroups, or even distinct disease entities, have been recently described in common diseases like asthma, diabetes, autoimmune diseases and cancer. High-throughput techniques (omics) allow identification of such subgroups. This may have important clinical implications, such as identification of diagnostic markers for individualised medicine, as well as new therapeutic targets for patients who do not respond to existing drugs.** For example, whole genome sequencing may be applied to more accurately guide treatment of neurodevelopmental diseases, or to identify drugs specifically targeting mutated genes in cancer. A study published in 2015 showed that 28 % of hepatocellular carcinomas contained mutated genes that could potentially be targeted by FDA-approved drugs. Another translational study, which is described in detail, showed how combined omics, computational, functional and clinical studies could identify and validate a novel diagnostic and therapeutic candidate gene in allergy. Another implication is diagnostic markers and therapeutic targets for predictive and preventative medicine. By combing computational and experimental methods, early disease regulators may be identified, and potentially used to predict and treat disease before it becomes symptomatic. Systems medicine is an emerging discipline, which may contribute to such developments by combining omics with

computational, functional and clinical studies. The aims of this review are to give a brief introduction to systems medicine and how it may contribute to the clinical implementation of individualised treatment, as well as to give concrete examples of clinical relevance.

Introduction

What developments can today's medical students look back on when they retire in 40 years? If we instead look back 40 years, developments like ultrasound, computerised tomography and improved treatments come to mind. For example, only 30 years ago asthma patients were common in medical wards, while this is now rare because of inhaled glucocorticoids (GCs). In both cases, clinicians were and are not likely to consider limitations of state-of-the-art treatments, which may be substantial. For example, 10 to 20% of patients with asthma show limited or no response to inhaled GCs [1]. Because early disease manifestations generally do not differ from responders, variable disease courses and possible compliance problems, such patients might take a long time to diagnose. Variable treatment response is a general problem, which results not only in suffering, but also contributes to increasing costs for the society. The annual cost of ineffective drugs in the US alone is estimated at 350 billion dollars [2]. Variable efficacy is also adding to the huge costs associated with development of new drugs, which currently are estimated at 2.6 billion dollars/drug [3]. Such costs are a great challenge to financing of medical care. These problems all point to the same simple question: Why do patients that appear to have the same disease respond differently to the same treatment?

Asthma patients may give clues to answering this question. Asthma can be caused by infections, allergens or other environmental factors, all of which may give rise to different inflammatory responses, although the clinical phenotypes may be similar. Recent studies have led to characterisation of such responses, and potential diagnostic markers for individualised medicine, as well as novel drugs targeting different responses [1]. Although such developments are only in early clinical trial phases, they point to the advantages of understanding the reasons for variable treatment response. Returning to the initial question of what today's medical students may look back on when they retire in 40 years, individualised treatment of molecular subtypes of common diseases may be one of the major developments [4]. However, the example from asthma and several other diseases points toward such treatment coming much sooner (Table 1). Indeed, some have already reached the clinic, such as BRCA genotyping in breast cancer, CCR5 mutation status in HIV infection and new-born screening for metabolic defects [5]. Recently, optimisation of anticoagulant therapy based on genotyping of two genes was described [6]. However, these examples have one common and important limitation: they are based on only one or two variables.

3

Diseases are rarely caused by malfunction of one individual gene product, but instead depends on thousands of gene products that interact in a complex network [7]. Only a fraction of those gene products is likely to have large effects on the disease. Another problem is that although the remaining disease-associated genes individually have small effects, their combined effects may be large [8]. Thus, combined analyses of multiple large and small affect genes are likely to be required for diagnostic purposes. Is it at all possible to address this complexity in clinical settings? Systems medicine is an emerging discipline, which is based on combining high throughput (omics) and computational analysis with functional and clinical studies [1, 9-11]. The basic research aims are to gain systems-level understanding of the molecular changes underlying common diseases, and how they vary between subgroups of patients that appear to have the same disease. The clinical aims are to use this information for predictive and individualised medicine. Recent studies indicate that systems medicine may reach the clinic within the next five years, starting with omics-based diagnostics for individualised medicine in serious diseases that require expensive treatments [1]. For example, neurodevelopmental disorders (NDD) affect more than 3% of children and are attributable to single-gene mutations at more than 1000 loci. Traditional methods yield molecular diagnoses in less than one-half of children with NDD. A recent study showed that early whole-genome sequencing or whole-exome sequencing of such patients **could increase the number of children, for whom underlying molecular mechanisms and diagnosis could be defined**. **This resulted in improved treatment and could also be cost-effective** [12]. **Exome sequencing of more than 200 liver tumours resulted the identification of several novel driver mutations, of which 28 % were potentially targeted by drugs approved by the Federal Drug Administration [13]. An expression profiling study of colorectal cancers linked increased signaling of an anti-inflammatory cytokine, TGF-beta, to poor prognosis and showed that anti-TGF treatment had therapeutic potential** [14]**. Given the severity of the diseases, and the decreasing costs of omics the authors speculated that this type of analysis may reach the clinic in the near future.** Another study showed that a set of 10 lipids in peripheral blood could predict development of Alzheimer's disease with great accuracy [15]. These recent examples suggest the clinical potential of omics methods for predictive and individualised treatment. Clinical implementation may be accelerated by two economic factors: 1) Increasing costs of, for example, biological drugs, which may exceed €20,000 per

patient per year, and 2) the rapid decrease in costs for omics. Such drugs include biologicals targeting common diseases, including IgE for asthma, TNF for autoimmune disorders, and several As an example, the cost of whole genome sequencing has decreased 10,000 times in 13 years, and is expected to decrease 100,000 times within the next five years [16].

However, clinical implementation requires addressing significant challenges, like how to interpret and validate complex data, as well as how to make such data accessible in clinical settings. **A recent symposium devoted to systems medicine was organised by and described in this journal** [10]. The aim of this review is to provide an introduction to systems medicine for clinicians and translational researchers. The focus will be on integrated network-based analysis of omics and routine clinical data in order to gain a predictive, systems-level understanding of disease mechanisms, and how they vary between patients that appear to have the same disease. Such variations have important implications for diagnoses, treatments and drug development. However, it should be emphasised that methods other than network-based analysis can also be applied. For example, the same principles can also be applied to construct high-precision, mathematical models of how different variables relate to clinical outcomes such as treatment response [11, 17].

A brief introduction to Omics and its clinical implications

The word omics is derived from systems-level studies of all genes or gene products. For example genomics refers to all genetic information for an organism, including gene- and non-coding sequences. Other examples are transcriptomics (all coding and non-coding RNAs), metabolomics, lipidomics or proteomics. There are currently many different technologies for omics analyses. Since this review has an introductory aim, readers are referred to other reviews for detailed descriptions [18]. However, some of the most common forms should be mentioned here. Microarray technology is used for many different forms of omics analysis, of which the most common may be genomics and transcriptomics. In both cases known nucleic acid sequences are attached to a glass slide, and allowed to hybridise with complementary sequences in the samples that are analysed. If complementary hybridisation occurs this is indicated by fluorescence, whose intensity correlates with the amount of nucleic acid in the sample. Microarray technology is commonly used to analyse genetic variants in Genome-Wide Association Studies (GWAS). Such studies may include analysis of

millions of variants. In case of mRNA microarrays, expression of 60,000 genes and gene variants are commonly analysed. Limitations of the technology include the confounding effects of background noise and cross-hybridization. Recent advances suggest that genome-wide sequencing technologies may replace microarrays. Instead of hybridisation to predefined probes, short nucleotide sequences (such as mRNA) are sequenced in parallel. Advantages include a greater variety of transcripts, including isoforms of existing genes and novel non-coding variants, as well as a wider dynamic range. Third generation sequencing will extend to analyse thousands of base pairs. A disadvantage of sequence-based technologies compared to microarrays, is that the down-stream signal analyses are more complicated. Increasing analytical complexity is a natural consequence of increasing technological complexity and resolution. An important concept to address this problem is targeted omics, which refers to limiting the analysis based on other sources of information. For example, the results of mRNA microarray analyses may point to sequencing a part of the transcriptome that may be particularly relevant. As further discussed below, combinations of targeted omics may have important clinical implications, for example for high precision individualisation of treatment in case of serious diseases and expensive treatments.

It is also of note that the term omics is expanding to include other systems, such as all microbes (microbiome), environmental exposures (exposome), or even all diseases (diseasome). All these systems may be interrelated. Therefore an important challenge of systems medicine is to develop paradigms to integrate those systems. This challenge has already been partially addressed, using a generally applicable principle, namely network-based analysis. As an example, a landmark study showed that diseases could be organised in a network, which in turn, could be linked to another network of genes associated with those diseases [7] (figure 1). But how does this complexity relate to the clinic?

Systems medicine is a natural extension of clinical thinking

Clinicians rarely make therapeutic decisions based on individual variables. Instead such decisions generally involve balancing different symptoms and signs, as well as laboratory tests with environmental and social factors. Implicit in this decision process is that the variables are interdependent. Therefore, the same change in one variable may have different implications in two patients, because of different changes in other variables. For example, wheezing is a typical sign in asthma, which is

generally responsive to treatment with GCs. Such patients tend to have increased activity of inflammatory cells induced by Type 2 T-helper cells (Th2 high). By contrast, a subset of asthma patients that are Th2 low are less responsive to GCs, and may instead respond to treatment **specifically targeting Th17 cells** [19]. Diagnostic markers, such as sputum eosinophilia and exhaled Nitric Oxide are currently tried in clinical studies to distinguish between Th2 high and low patients [20]. However, as discussed above, the involvement of thousands of genes in common diseases like asthma, indicates that one or two biomarkers are unlikely to suffice for diagnostic purposes. Instead, a combination of clinical data and multiple omics-based markers may be needed. But can such complex combinations be organised and presented in such a way that clinicians can make informed diagnostic and therapeutic decisions? This would require some kind of clinical decision support system (CDS). Early examples of such systems, which are based on pharmacogenomics data, already exist and have been tested in the clinic [21]. In the next section, we describe how networks provide a template to organise clinical and omics variables in a way to understand disease mechanisms, as well as to compute diagnostic predictions. Network-based analysis of omics data plays a key role in systems medicine. As discussed below, such templates may be seen as a natural extension of current clinical decision-making processes, and have the potential to be integrated into CDS within the near future.

A brief introduction to networks

Networks provide a graphical and theoretical framework to describe and understand complex systems. In a landmark study in 1999, it was shown that networks depicting a wide range of technological, social, and biologic systems have common designs that are governed by simple and quantifiable organising principles [22]. Those principles are perhaps most obvious in social networks. Take for example a network that is constructed based on interactions between students in a school. Assume that each student is a node, which is connected to other students that she or he interacts most frequently with. It is unlikely that the connections, or links, will be randomly distributed. Instead, they are likely to form groups that correspond to the classes those students belong to. Moreover, groups of students belonging to similar educational programs are likely to be more connected than groups with unrelated programs. Another landmark study showed that the same organisation principles are also found in yeast cells [23]. Proteins with related functions formed groups, or modules. Similar

to school classes, a small number of proteins had very many links and formed "hubs". Those hubs had direct functional implications: systematic knockdown studies showed that silencing of hub proteins was more likely to affect cell survival than less connected proteins. One reason was that the hubs contributed to the small world property of the networks: even in very large networks, all nodes are generally connected by a limited number of links [7, 23]. Therefore, silencing of hub proteins is more likely to affect cell communications and survival. In the context of disease, the small world property increases the risk that a drug targeting a specific disease gene may have unexpected off-target effects [24]. Another important implication for medical research is that disease-associated genes identified by 'omics studies can be computationally mapped on models of the human protein-protein interaction (PPI) network, as described in figure 1. In other words, each disease-associated gene is mapped on its matching protein product. Generally, such mapping results in a portion of the genes co-localising in one or more modules, forming disease modules, while the remaining genes are dispersed in the network (Figure 2). As discussed below, disease modules are likely to contain the most disease-relevant genes [25]. Therefore, an important advantage of such modules is that they help to prioritise between the many disease-associated genes identified by high-throughput analyses.

The basic, clinical and pharmacological implications of disease modules

The general properties of modules described above support that genes in disease modules are more relevant for the disease than other genes. The two main reasons are that genes in a disease module have related functions and are highly interconnected. In other words, these two reasons imply that such genes can operate as an effective team, in contrast to other genes that are more dispersed in the network.

Several studies show that disease modules have important implications for basic and clinical research. Such modules may help to get an overview of disease-mechanisms by performing pathway analyses, as well as to identify novel disease genes, biomarkers or therapeutic targets. One of the first clinically oriented module-based studies showed how a disease-module derived from a mRNA microarray study of skin from allergic patients could be exploited to infer diagnostic protein markers that were validated by independent clinical studies [26]. Another, landmark study described a module relevant to breast cancer, and identified a novel candidate gene that was validated by functional and genetic studies [25]. Several module-based studies have

been performed in other diseases, including cancer [27-30], neurological [31-33], cardiovascular [34], and inflammatory diseases [35-37]. One of the studies showed how protein interaction modules could be used to predict outcome in breast cancer [25]. In a study of autoimmune diseases, mRNA modules were used to predict disease progression based on functional studies of underlying mechanisms [34]. In 2014, a module-based approach for drug discovery was described in rheumatoid arthritis based on a meta-analysis of GWAS of 100,000 subjects [35]. An mRNA microarray study of 1600 post-mortem brain samples from patients with Alzheimer's disease identified a disease module and a potential upstream regulator which was validated by functional studies [38]. General principles of networks may be exploited to analyse properties of genes in the disease modules. For example, the products of hub genes are likely to have larger effects than other genes in a disease module. This property may be helpful when prioritising between multiple genes in a disease module for functional and clinical studies, as well as when selecting genes as potential drug targets. Interestingly, genes targeted by drugs are more likely to be hubs [39, 40]. The network property that highly interconnected nodes are likely to be functionally related can be exploited to find novel diagnostic markers and therapeutic targets among the interactors of known disease genes [41, 42]. Taken together, the studies described above support that network-based analysis of omics data how many potential basic and clinical implications. It is, however, clear that the complexity of the analysis raises concerns about the feasibility of clinical translation. Recent studies may address such concerns. The translational feasibility of module-based analysis was shown in a study of seasonal allergic rhinitis [42]. **The study spanned from a genome-wide analysis of gene expression to high-throughput knockdowns of candidate genes, computational, functional and clinical studies. This resulted in the identification of a novel candidate gene in allergy, S100A4, which was validated by a knock-out mouse model of allergy, treatment of the mouse model and patient cells with an anti-S100A4 antibody, as well as diagnostic studies of patients** (figure 3). However, it was clear from this and many other studies mentioned above that individual genes are unlikely to suffice for diagnostic and therapeutic purposes. Although this complexity may seem daunting, preliminary studies of patients with seasonal allergic rhinitis and multiple sclerosis indicate that module subtypes may be exploited for individualised medicine [43]. An important limitation of the study was that it was based only on mRNA and protein expression. Studies of allergy and other

diseases have shown the importance of other genomic layers, such as genetic variants, DNA methylation and non-coding RNAs [44, 45].

These layers only partially correlate, and given the complexity of common diseases, it is likely that combinations of targeted omics analyses from different layers may be needed for diagnostic purposes, like individualised medicine. Indeed, some layers, like DNA methylation may be particularly suitable. A recent study showed that DNA methylation almost completely separated allergic patients from controls, and was highly correlated with disease severity [45]. However, can such complexity be addressed and translated for clinical purposes? This question will be discussed below, starting with a network-based strategy to integrate different sources of information of potential clinical relevance. After that, practical aspects of clinical implementation will be discussed.

Multilayer disease modules (MLDMs) to integrate multiple sources of omics and clinical data

Omics technologies allow systems-level analyses of different layers ranging from DNA to proteins, as well as metabolites and lipids [46, 47]. The human PPI network can be used to organise these layers into MLDMs. For example, one disease module can be formed by mapping disease-associated mRNAs on the human protein interaction network. Next, that module can be used to search for single nucleotide polymorphisms (SNPs) in GWAS of the same disease. If the SNPs map to proteins in the same module map, the two modules can be linked. This principle can be applied to all layers identified by omics, using statistical methods to test if the links are stronger than expected by chance. Another example is modules formed by genes and their regulators, such as a microRNA. The genes can be linked if they are regulated by the same microRNAs. A double-layer module can be formed by linking microRNAs that regulate the same gene [48] (figure 4).

An important implication of MLDMs is to study how genes, gene products and regulators interact with each other. Such studies can result in rejectable hypotheses. This is illustrated by the two examples above: Does a disease-associated SNP in a promoter region of a module gene change the expression of of that gene? Does a microRNA regulate its predicted target genes in a module? Such associations may explain disease mechanisms as well as how they vary between patients that appear to have the same disease. For example, the same gene may change differently between

two subgroups because its promoter region harbours a SNP or is differentially methylated. Such changes may have direct clinical implications. Both SNPs and methylation can be readily analysed in clinincal samples. Thus, MLDMs may provide a framework to identify optimal combinations of diagnostic markers from different layers, based on functional understanding of the pathogenic roles of those markers. Promising examples have already been reported in gliomas, where microRNAs and genetic variants may cause disease-associated variations in mRNA expression, which in turn can have relevance for predicting disease outcome [48, 49]. In allergy, mRNA modules were showen to be co-regulated by microRNAs. Functional and clinical studies showed that some of those microRNAS were hubs with potential diagnostic relevance. The same principles were shown to be applicable to other, highly diverse diseases [50].

The principle of linking molecular modules to MLDMs, can be extended to modules formed by other forms of clinical data, which can also be organised into networks. For example, diseases that show comorbidity can be linked and form disease networks. Similarly, disease modules that partially share the same genes can be linked and form disease module networks. Such multi-layer networks can be used to form rejectable hypotheses on diseasome- and genomewide scales: Do diseases that show comorbidity partially share disease modules? If not, is comorbidity due to socioeconomic or environmental causes? Moreover, the networks can be used to form hypotheses of increasing detail: Do genes in overlapping disease modules explain comorbidity? Can variations in expression or function of those genes explain why some patients only get one disease?

Such a change of scale highlights an old principle: To interactively switch between systems-level and detailed studies. It is perhaps best illustrated by a microscopic examination. You start in low magnification to get an overview and identify potentially important details, which can be analysed in further detail using higher magnification. The detailed analysis, may in turn lead to new questions for new analysis in low magnification. Multilayer networks provide a framework to extend that principle from diseasome/genome-wide scales to detailed functional or clinical studies.

It is also possible to expand the MLDM to include other sources of clinical information such as routine laboratory tests or medical imaging. For example, in a study of liver cancer, specific imaging traits could be linked to prognostic gene

expression changes [51]. Similarly, obesity traits could be linked to molecular changes [43, 52]. The principle of linking networks formed can also be extended to other types of variables that are relevant to disease. For example, social and environmental factors may each be modular and potentially possible to link to each other as well as molecular MLDMs. Thus, MLDMs could serve as templates to integrate and analyse multiple layers of disease-relevant information. As discussed in future implications below, user-friendly computational tools to describe MLDMs as graphical models already exist. Such models can potentially be used by researchers and clinicians alike. In principle, MLDMs, may not differ from how clinicians construct their own conceptual models of diseases based on different sources of information. In both cases, functional understanding plays a key role, but MLDMs allow integration of much more data and the option for computational predictions.

Problems and limitations

All systems medical studies involve significant methodological problems. In principle, these are either technical or related to the complexity of the underlying biomedical problem. Technical problems include accurate detection of signals when tens of thousands or more variables are measured, as well as methods to filter out the most relevant signals. Such problems are currently the focus of rapidly advancing research efforts, which have recently been discussed elsewhere, and led to strict guidelines for the use of high throughput studies in clinical contexts [53].

Another problem of a more principal nature is how to validate changes in thousands of genes, which individually may be small but in combination large. One important validation step is to search for genomic concordance. For example, is a transcriptional disease module enriched for polymorphisms identified by GWAS? MLDMs have already been shown to provide useful templates for such validation studies. Another principle is to combine silencing or up-regulation of individual genes with high throughput studies to study the network effects of the individual genes (figure 5).

If such genome-scale analyses support the findings, detailed functional and clinical studies can be performed, including disease models in mice [42].

Clinical implementation

To translate systems medicine into the clinic would require addressing the following challenges: 1) Clinical studies to determine which combinations of omics data are

needed for diagnostic purposes. Recent studies of asthma and seasonal allergic rhinitis support the feasibility of targeted transcriptomics to stratify patients for treatment response [54, 55]. However, given the complexity of common diseases it's likely that larger studies including other omics analyses are needed. 2) Development of laboratory technology for targeted omics that can be used in clinical settings. One currently available solution is that commercial providers of diagnostic omics kits perform and interpret the analysis. This is already possible in the diagnosis of breast cancer, where a targeted microarray measures expression of less than 100 genes in order to stratify patients. 3) Development of software for diagnostic classification based on the omics data. In the simplest case, patients may be stratified into high- and low risk groups, as in the breast cancer example. However, user-friendly software that may allow clinicians to make diagnostic classifications based on functional understanding of underlying mechanisms is already available. Such software can also include routine clinical data in the classification [56]. 4) Large-scale training of medical students and health professionals. Recommendations for such training are currently addressed by a multidisciplinary consortium initiated by the European Commission (https://www.casym.eu). 5) Analysis of the cost-effectiveness of targeted omics for individualized medicine should be initiated. Such analyses should balance the rising costs for drugs, drug development as well as ineffective medication vs. the costs of implementing omics-based diagnostics. A recent study indicated that targeted omics in breast cancer could be cost-effective at a treatment threshold of €20,000 [57]. This threshold is close to the costs of biological drugs, representing a significant challenge to modern health care. 6) Finally, clinical implementation would require multidisciplinary collaborations that include clinicians, representatives from patient organizations, experts in genomics and bioinformatics, participants from pharmaceutical and biotechnological industries, as well as healthcare and academic leaders. If successful, such collaborations could result in clinical implementation of stratification for treatment with, for example, biological drugs in specialized centres, within a five-year period. This could pave the way for similar projects for less costly drugs. Eventually, this could lead to a more general implementation, including in primary care. **The likelihood of clinical implementations reaching the clinic within the next five years is compounded by recent initiatives, such as president Obama's precision medicine initiative** [58, 59]**, and large-scale funding from the European Commission.**

Summary

The enormous complexity of common diseases, and the resulting problems, such as many patients not responding to treatment, increasing costs of drugs and drug development are strong motivations for new and complementary strategies for research and clinical practice. It is possible that omics and systems medicine will contribute to such strategies in the near future.

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Figure 1. Linking disease and gene networks to provide an overview of how diseases disease genes relate to each other. Disease-associated genes are mapped on the protein-interaction network. In general, genes belonging to the same or phenotypically similar diseases will co-localise in the network. Thus, the network can be used to identify or infer disease-associated genes.

Figure 2. A disease module. Conceptual model of how disease-associated genes (blue nodes) identified by high-throughput analysis tend to co-localize in the human protein-protein interaction network (white nodes), forming a module (blue oval). The genes in the module are assumed to be more important for the disease than extra-modular genes.

Figure 3. A strategy to identify and validate disease modules A) Identification of a disease module in allergy. We hypothesised that a known key gene in this disease, IL13, would be part of the module, and therefore co-regulated by the same transcription factors (TFs). Converging sources of biological information identified 25 putative regulators of IL13. B) siRNA knockdowns of the 25 TFs in Th2 polarised T lymphocytes from healthy controls showed that seven TFs had an effect on IL13. Repeated knockdowns of those seven, followed by microarrays, led to the identification of multiple putatively co-regulated genes. Those genes were computationally mapped on a network model of human protein interactions. A subset of the genes were highly interconnected and formed a module. The relevance of this module was validated by showing a significant overlap with a module derived from expression profiling of allergen challenged T lymphocytes from allergic patients, which was significantly enriched for biomarkers and therapeutic targets. This is an example of genome-scale validation. Another way is to search for concordance between different genomic layers, for example between disease associated changes in gene expression and enrichment of SNPs, as described by us [42] [38]

C and D) Extensive functional and clinical studies showed the diagnostic and therapeutic relevance of one of the genes in the module, S100A4. These studies included a mouse model of asthma and treating patient cells as well as a wild type mouse model with an S100A4 blocking antibody.

Figure 4. miRNA–mRNA regulatory networks in different human diseases. (A) Type 2 diabetes miRNA–mRNA network. (B) Chronic obstructive pulmonary disease miRNA–mRNA network. (C) Acute lymphoblastic leukaemia (B-lineage) miRNA–mRNA network. (D) Pancreatic cancer miRNA–mRNA network. (E) Renal cell carcinoma miRNA–mRNA network. (F) miRNA–mRNA regulatory networks in seasonal allergic rhinitis (allergomiR-target network). miRNAs are represented as red nodes, while their predicted mRNA targets are represented in blue. The links between the nodes represent regulation of the mRNAs by miRNAs.

Figure 5. Functional analysis of a candidate gene; a) the gene is knocked down using siRNA, b) the genes regulated by the candidate gene are identified by combined mRNA microarray- and pathway analysis after knockdown.
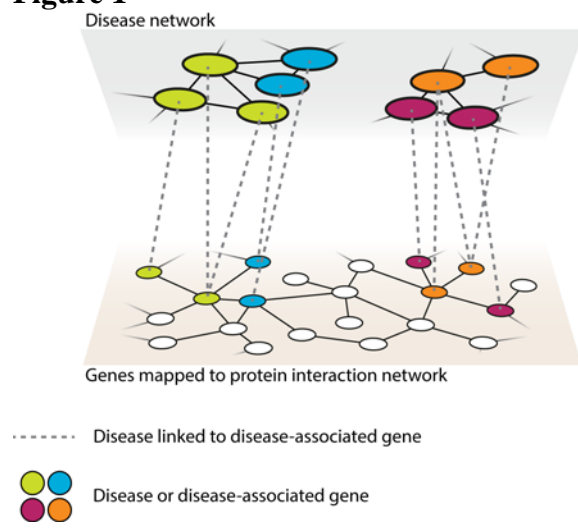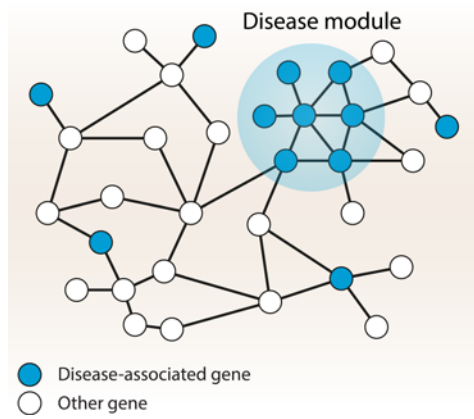
Figure 1

Disease network

Genes mapped to protein interaction network

- - - - - - - Disease linked to disease-associated gene

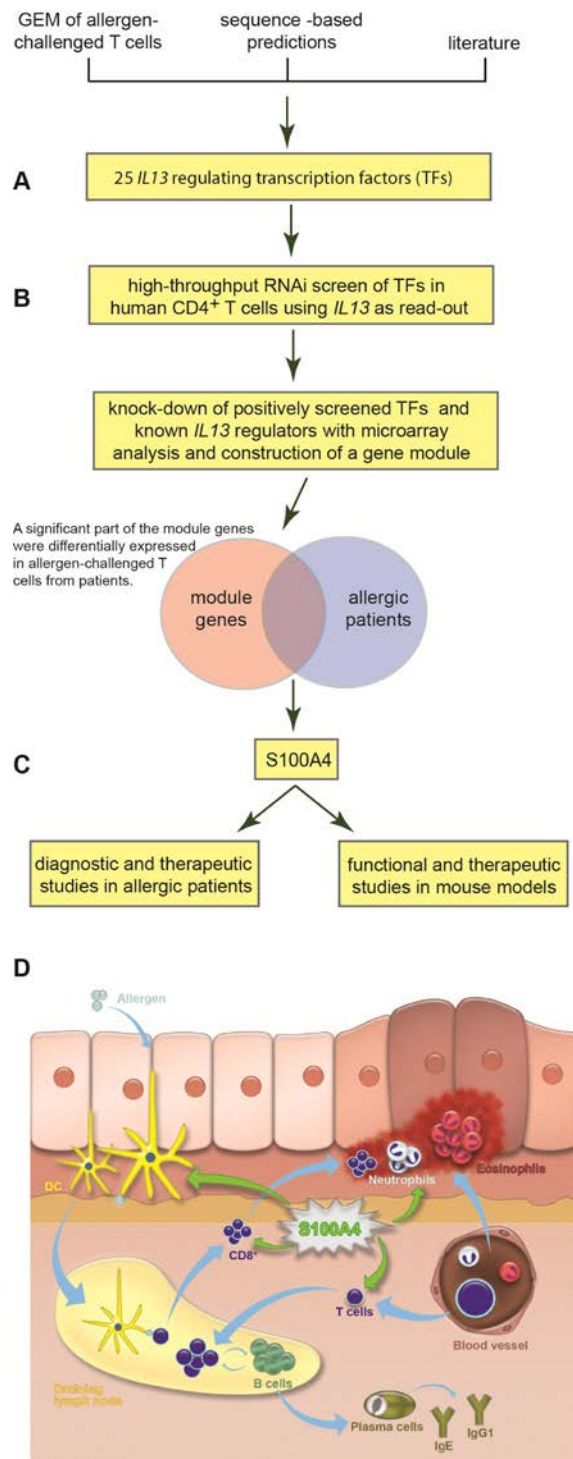Disease or disease-associated gene

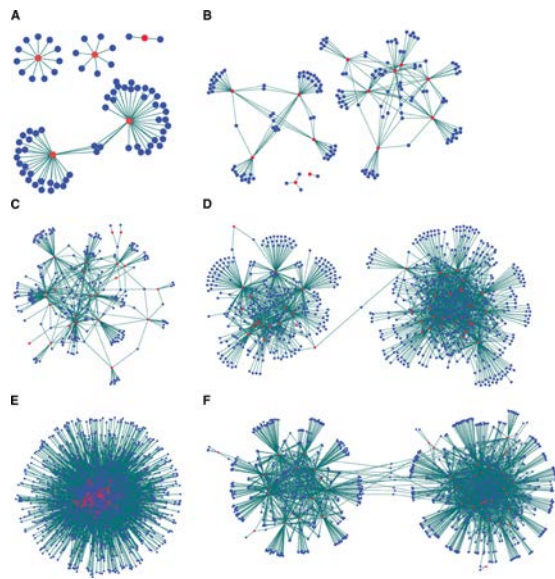**Figure 2.**

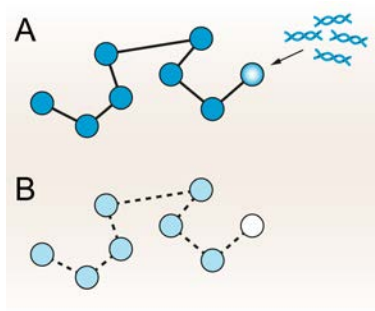**Figure 3**

Figure 4.

Figure 5

Table 1. Examples of potential or existing diagnostic or therapeutic options that are based on systems medical principles

Diseases	Method	Clinical potential	Clinical stage	Ref
Neurodevelopmental Disorders	Whole genome or exome sequencing	Earlier and improved treatment	Tried in the clinic	12
Breast cancer	expression profiling of less than 100 genes	Early classification of prognosis, Stratification for treatment	Available in the clinic	57
Hepatocellular carcinoma	exome sequencing	Identification of candidate genes that can potentially be targeted by FDA-approved drugs	Not tried in the clinic	13
Colorectal cancer	expression profiling identified increased TGFB signalling in patients with poor prognosis	Potential for early prediction and treatment targeting TGFB	Not tried in the clinic	14
Glioma	Expression profiling	Potential for early prediction of prognosis, and individualised medicine	Not tried in the clinic	48,49

Alzheimer's disease	Metabolomics	Potential for early prediction, Potential for preventative treatment	Clinical study	13 [15]
Allergy	Expression profiling identified several novel candidate genes, in particular S100A4	Potential biomarker for allergy Potential therapeutic candidate	Not tried in the clinic	40 [42]