Bridging the gap between traditional Chinese medicine and systems biology: the connection of Cold Syndrome and NEI network

Tao Ma,†[a] Conge Tan,†[b] Hui Zhang,[b] Miqu Wang,[b] Weijun Ding[b] and Shao Li*[a]

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Systems biology is a general trend of contemporary scientific development. When coupling the classical traditional Chinese medicine (TCM) Cold Syndrome and methodology of systems biology, we conformed to the genome, transcriptome, proteome, and metabolome that are supposed to run through the overall macro behavior, and explored the macro and micro framework of systems biology of TCM Syndrome. We introduced a new way to probe into the implicit stratification of Cold Syndrome, after surveying 4575 cases of Cold Syndrome patients and examining gene expression information of a typical Cold Syndrome pedigree by microarray. We underlined the genetic background of the Cold Syndrome family based on the molecular foundation to understand Syndrome, one of our earlier discoveries in which genes and chemical compounds in neuro-endocrine-immune (NEI) system are scored as Cold or Hot (or both) property. Results indicate that Cold Syndrome related genes play an essential role in energy metabolism, which are tightly correlated with the genes of neurotransmitters, hormones and cytokines in the NEI interaction network. Therefore, NEI interaction not only opens out mechanism of classical TCM theory on Syndrome but also enriches current research on complex diseases as well as systems biology.

Microscopic "omics" information and macroscopic clinical symptoms need crashing

The fusion of "system" concept into many scientific fields, such as engineering, computer science, biology and medicine, is neither a stunt nor a coincidence, but a trend when we are trying to decipher the nature. Take medical science as an example: traditional Chinese medicine (TCM) has long been practised as an empirical system and retrieved tens of millions of lives from historically to currently, which happens to share the same concept with the coming systems medicine.[1] TCM can be considered as an ancient and classical paradigm of systems biology. In TCM, diagnosis and medication are based on "Syndrome" ("*ZHENG*" in Chinese Mandarin), which can be regarded as a profile of symptom combination, or clinical phenotypes, such as Cold or Hot Syndrome, and "Hot medication curing Cold Syndrome" is a standard therapeutic guide line. Cold or Hot Syndrome is a TCM based diagnostic perspective into disease. Diseases under Western Medicine's demarcation may also share some similar symptoms of Cold or Hot Syndrome. As a result, exploration of TCM Syndrome will benefit the research on complex diseases. This classical systems medicine at the macro level has been validated and developed by its repeated clinical practice for thousands of years. However, the intrinsic mechanism of phenotype-oriented diagnosis and therapy in TCM needs to be opened out by molecular biology at the micro level.

Nowadays, when celebrating our past triumphs over variolar, measles and so on through manipulation of antibiotics or vaccines, which are featured by a single pathogenic factor,we are also suffering from increasingly prevalent malignant tumors, metabolic syndromes, autoimmune diseases and highly infectious diseases. These diseases often originate from the complicated interaction between individual genetic background and environmental factors, and symptoms are presented in more than one organ or tissue and spread to the whole body.[2] For example, HLA-DRB1 genes are associated with the development of rheumatoid arthritis (RA),[3] and environmental factors, such as smoking,

[a] *MOE Key Laboratory of Bioinformatics and Bioinformatics Division, Tsinghua National Laboratory of Information Science and Technology/Department of Automation, Tsinghua University, Beijing 100084, China. E-mail: shaoli@mail.tsinghua.edu.cn; Fax: +86 10 62786911; Tel: +86 10 62797035*
[b] *Chengdu University of Traditional Chinese Medicine, Chengdu 610075, China*
† Contribute equally to this work.

Tao Ma is a PhD student in Professor Shao Li's research group.

Conge Tan is a graduate student in Professor Miqu Wang's research group.

diet and region of birth, contribute to the risk of RA.[4] Meanwhile, RA is featured by tormenting symptoms as synovitis,[5] subcutaneous rheumatoid nodule,[6] and constitutional symptoms[7] and so on. More symptoms of RA were also recorded by TCM literature "*Huang Di Nei Jing*" (The Yellow Emperor's Inner Classic, 475–221 B.C.) in the history, and were differentiated as Cold or Hot based on TCM diagnostic guideline.[8,9] According to American College of Rheumatology, RA is characterized by a series of clinical signs and symptoms.[10] This case suggests that phenotypic information still overrides molecular clues in some of today's clinical practices. In a similar manner, discovery of candidate molecular biomarkers for complex diseases seems to be at the very beginning, partially due to the multi-factorial property of these diseases. Could a single allele, RNA, or protein destine the disease roadmap? That sounds unrealistic.

This dilemma calls upon the debut of systems biology at the end of last century. Systems biology qualifies and quantifies constitution of the whole molecules (DNA, RNA and protein) and their interactions at a certain biological compartment or in a specific biological process, aiming at exploring complex structure, dynamics and function control at a systematic level. Its robustness lies on high throughput acquirement and analysis of myriad "omics" data with the rapid innovation of monitoring methods.[11] Indeed, concerning current systems biology, the following should also be addressed: Integration of "omics" information from different hierarchies, say genomics, transcriptomics, proteomics and metablomics, is still far away from the whole blue print of complex system; correlation between "omics" information at the micro level and body's overall presentation (means phenotype and function) at the macro level needs further interpretation in the long run. Currently, microscopic "omics" information and macroscopic clinical symptoms seemingly stand at the self-governed side, but look forward to mutual comprehension and promotion.

Mathematical Model-based symptom patterns of Cold Syndrome in traditional Chinese medicine

TCM is a holistic medical system which emphasizes harmonizing the integrity of body. Its methodology originated from ancient Chinese philosophy: "The myriad things have their backs to the Yin and face the Yang. Through the interaction of the Yin and Yang, a new harmony is created", said *Laozi* in his great work "*Dao De Jing*" (Taoism), a philosopher more than 2000 years ago. In TCM, disease is regarded as aberrancy from the balanced state of body and various pathogenic factors, including both endogenetic and exogenetic, are categorized as Cold, Hot or other typical patterns. If the Cold and Hot interact abnormally and depart from a steady state, the body will present a series of clinical symptoms, the combinatory profile of which is Cold or Hot Syndrome.[12] For example, Cold Syndrome refers that an individual has fixed parts of the cold, cold behaviors, and difficulty to adapt to the cold environment. TCM doctors carry out diagnosis and herbal therapy based on Syndrome differentiation, which can be regarded as a traditional phenotype-based medicine.

However, people often argue that there is a lack of objective diagnostic standards in clinical practice of the traditional methodology. Nevertheless, even in today's biological research, the experience of clinical doctors still adds valuable *a priori* knowledge. The popular NCBI OMIM (Online Mendelian Inheritance in Man) database is such a paradigm, and most of the works exploring genome and phenome relations rely on this database.[13] Moreover, Zhang and his colleagues developed a new clustering method in the form of latent tree model to quantify symptom profiles as "resultant clusters" in TCM kidney deficiency Syndrome differentiation.[14]

Here we standardized Cold Syndrome using the method of the latent tree model.[14] Firstly, we made Life Quality Scale of Cold Syndrome (scale of living quality in cold conditions), which is composed of 20 factors that are divided into three categories, namely, the 10 fixed cold body parts from head to feet, 6 obvious cold behaviors such as preferring hot drinks, 4 cold feelings such as cold susceptibility. Meanwhile, each cold factor is measured as 0, 1, 2 and 3, standing for none, mild, moderate and severe level, respectively. These 20 factors make the preparation of diagnostic rules. For example, a patient feeling slightly apathetic is diagnosed as mild, consciously feeling skin chills and apathetic from top to bottom as moderate, and consciously feeling icy in skin and a sense of clear cold as severe, when the patient's skin is pushed. Then 4575 cases were surveyed from China's Sichuan, Shanxi, Chongqing, Inner Mongolia and other provinces over the past decade.

For this study, the latent variables are three categories of elements derived by the latent tree model, namely cold adaptation, cold behaviors and cold areas, while manifest variables in this study are 20 elements which represent cold environment adaptation, behaviors in the cold, and human body's cold

Hui Zhang is a graduate student in Professor Miqu Wang's research group.

Professor Miqu Wang heads a Genetics Research Laboratory in Chengdu University of Traditional Chinese Medicine, working on genetic basis for traditional Chinese medicine Syndrome.

Weijun Ding is an associate professor in Miqu Wang's research group.

Professor Shao Li is the Vice Director of Bioinformatics Division, Tsinghua National Laboratory of Information Science and Technology, and heads a research group concentrating on traditional Chinese medicine Syndrome and combinatory drug therapy of complex diseases based on methodology of systems biology and bioinformatics.

areas. It was found that this classification has statistical significance and a typical cold pedigree with 16 family members was screened out in Pengzhou city, Sichuan province.

Cold Syndrome is a deviation from the homeostasis of NEI network

Current systems biology returns biology from local investigation, emphasizing structure and function of a specific molecule, such as DNA, RNA or protein, to global study, integrating molecular interaction into a whole system by today's cutting-edge "omics" technologies (genomics, transcriptomics, proteomics and metabolomics). This methodology still needs improving as the systematic interaction of different molecules at the micro level is not equivalent to the function of a cell or an organ, no mentioning the overall action of body at the macro level, although current systems biology did enlarge our vision of understanding complex biological systems as well as complex diseases.

Fundamental tasks of systems biology include enlightening the relationship between molecular interaction and body action and serving for the diagnosis and therapy of complex diseases. Under the biopsychosocial model,[15] complex diseases (or multi-factorial diseases) are often an outcome of abnormal genetic or environmental variation and evil consequence of their mutual interaction. The neuro-endocrine-immune (NEI) network is a paradigmatic system in Western medicine.[16] NEI system is also found to exhibit modularity features of the most significance in human phenotype–genotype association.[17] To maintain homeostasis between body and environment, NEI plays a pivotal role in information exchanges between these three systems and is important in lots of physiological and pathological processes.[18] Etiopathogenesis of many diseases, such as rheumatoid arthritis,[19,20] systemic lupus erythematosus,[21] ulcerative colitis[22] and aging,[23] is found from NEI abnormality.[24] A systematic perspective at both molecular and anatomical levels is required to understand complex diseases, for biological information of body flows through communications of neurotransmitters, hormones and cytokines produced by NEI system and ultimately presents as specific phenotypes of cells, organs or tissues. It is these messenger factors that ferry information of system and therefore make NEI from a conceptual system[16] into a molecule-based network.[25] Bridged by NEI system, "omics" progress at the micro level and TCM phenotype-based clinical practice at the macro level may not only promote each other, but also deepen our perception of complex diseases' mechanism.

Our previous study also evidenced that the long standing TCM diagnostic guideline "Syndrome" has its NEI background.[12] NEI system makes itself a united and coordinated whole by messenger molecules carrying communicating information from each of nervous, endocrine and immune system. Changes in neurotransmitters, hormones or cytokines at the micro level regulate body's response to the environmental stress and affect body's behavior, mood as well as disease phenotype at the macro level.[12] For instance, autonomic nervous system regulates and controls blood pressure, heart rate, perspiration, body temperature, gastrointestinal tract function, and so on. Disconcerted interaction between sympathetic and parasympathetic nerve systems will lead to a series of clinical symptoms, as constipation, irritability, rapid pulse, sweating or loss of appetite, decreased sweating and tight pulse, which TCM differentiates as Hot Syndrome or Cold Syndrome. There is also another example of NEI messengers' influence on disease phenotype: the endocrine axis rennin-angiotensin system (RAS) plays an important role in balancing fluid and electrolyte metabolism and blood pressure. Disorders in RAS will also result in TCM Cold or Hot Syndrome as facial flushing, clear urine or pallor.[26] Meanwhile, we discovered the microscopic molecular base to understand Syndrome by scoring the topological temperature of 187 genes and chemical compounds as Cold (topological temperature < 0), or Hot (topological temperature > 0), or both (topological temperature $= 0$) property, which measures the interactions among nodes in the network.[12] Following the paradigmatic approach of "The Connectivity Map" that connects small molecules, genes, and disease using gene-expression signatures, pioneered by Justin Lamb and his colleagues,[27] here we chose the TCM Cold Syndrome as a case to study NEI's role in bridging microscopic messenger molecules and macroscopic phenotypes.

As shown in Fig. 1, we firstly screened out a typical Cold Syndrome pedigree with 16 family members as mentioned above, and then gene expression levels were analyzed between 9 cases with Cold Syndrome (5 severe and 4 mild) and 5 normal people in this chosen family. Afterwards 25 differentially expressed (DE) genes ($P < 0.005$, by the two-sample T-test) are identified from 5062 gene expression values in the 14 experiments.[28] Human Protein Reference Database (HPRD, http://www.hprd.org/) is one of the most popular network resources for realizing protein–protein or gene–gene interactions,[29] especially the relationship between disease causal genes and drug targeted genes,[30,31] so we mapped 187 Cold or Hot NEI genes and 25 DE genes into HPRD network. 13 DE genes are identified in HPRD and 64 interactions between 43 NEI Cold or Hot genes and 13 DE genes are found after these DE genes are extended for 2 steps in this network. The relationships between DE genes and NEI genes through intermediate genes are listed in Table 1 and exhibited in Fig. 1(D). It is found that DE genes are much closer to Cold or Hot NEI gene group than any other randomly selected gene group ($P < 0.01$), measured by average shortest path as a network topological parameter. Moreover, many functional annotations in GO (Gene Ontology, http://www.geneontology.org/) related to inflammatory response, energy metabolism and environmental stress are significant ($P < 0.01$, by Fisher Exact test).[32] Then 12 pathways from KEGG (Kyoto Encyclopedia of Genes and Genomes, http://www.genome.jp/kegg/) are identified as metabolism or energy-related by using the GO comparison program in BRB ArrayTools.[28] There is considerable evidence to support the relationship between NEI system and energy metabolism.[33–36] Abnormity in energy metabolism will lead to Cold symptom profiles as chill, fear of cold, facial pallor and cold temperature of

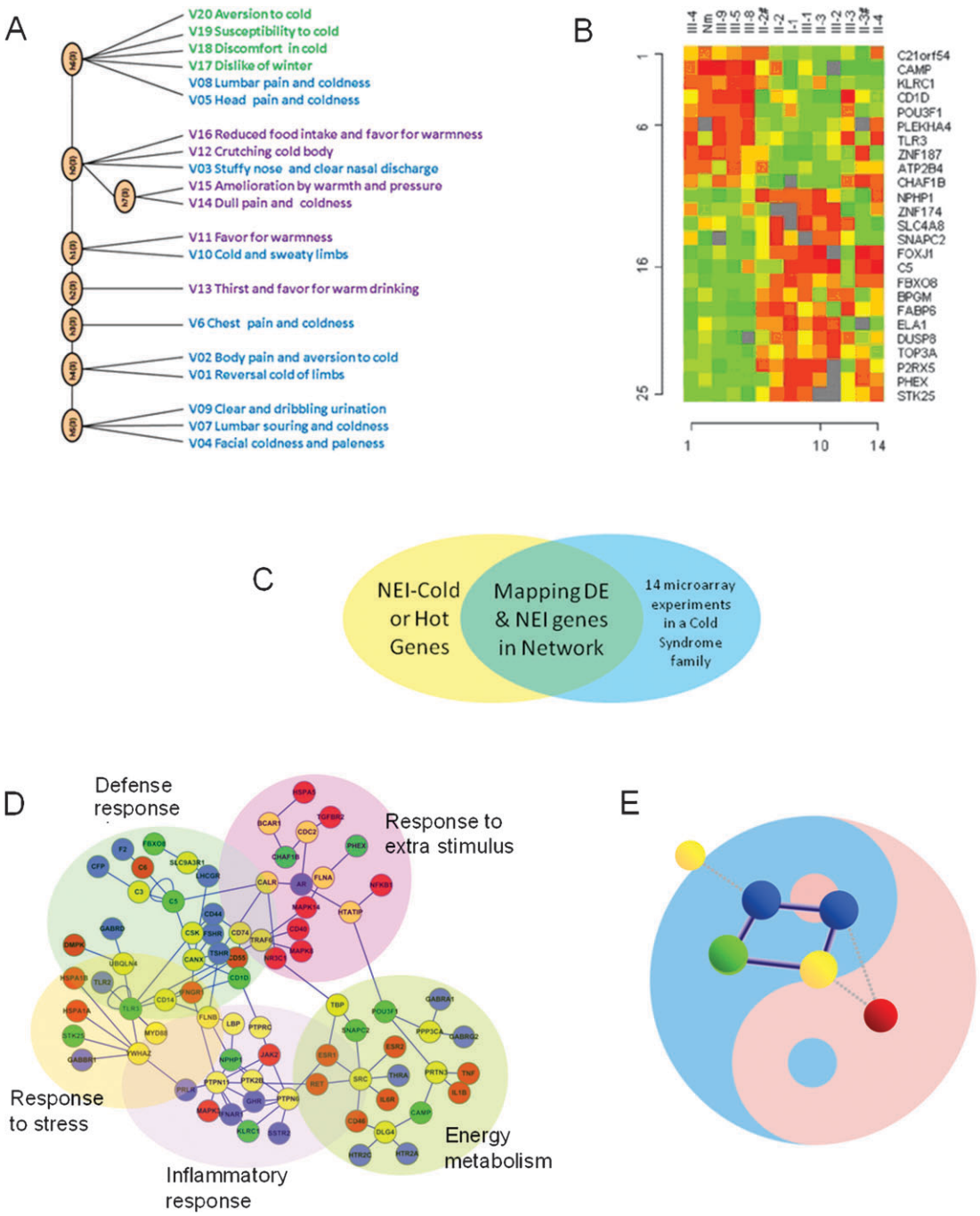

Fig. 1 Connection of TCM Cold Syndrome and NEI (neuro-endocrine-immune) system. (A) TCM Syndrome manifestations are clustered as clinical phenotypes (Green: Cold adaptation; Blue: Cold area; Purple: Cold behavior. h1–h8: Hidden variables in Latent Tree Model). (B) Expression level of genes from Cold Syndrome group.[28] (C) The strategy connecting NEI related Cold or Hot genes scored by "topological temperature"[12] and DE genes from microarray is depicted. (D) Differentially expressed (DE) genes interact with NEI genes in protein–protein interaction (PPI) network (from HPRD) and are backgrounded by typical significant Gene Ontology annotations. NEI related DE genes (Green), Hot genes (Red), Cold genes (Blue) and Intermediate genes (Yellow) interact with each other as functional modules in PPI network. (E) A putative network biomarker for Cold Syndrome is denoted as a functional module formed by predominant interactions between Cold genes and DE genes in the Cold background of the Cold–Hot (Yin–Yang) map measured by topological temperature.

body. These results not only validate our proposal that NEI system bridges transcriptomic information at the micro level and TCM phenotypic information at the macro level, but also reveal that the abnormal communication between NEI Cold and Hot gene groups[12] leads to TCM Cold Syndrome in all probability: symptoms of Cold Syndrome reflect deregulation of NEI system, which originates from loss of check and balance between the system's two properties: "Cold and Hot". As illustrated in Fig. 1(D), most of the DE genes are closer to Cold genes, and likewise genes

Table 1 Cold DE genes in microarray assay interact with NEI genes through intermediate genes in protein–protein interaction network[a]

DE	IM	NEI	GO Annotation (GO Number)
C5	C6	F2	
	C3	CFP	Acute inflammatory response (GO:0002526)
	C5	C6	Inflammatory response (GO:0006954)
		NR3C1	Complement activation (GO:0006956)
	CALR	AR	Response to wounding (GO:0009611)
		TSHR	Activation of immune response (GO:0002253)
	CANX	TSHR	
CAMP	DLG4	HTR2A	Behavior (GO:0007610)
		HTR2C	Response to stimulus (GO:0050896)
		CD46	Thermoregulation (GO:0001659)
	PRTN3	IL1B	
CD1D	CANX	IFNGR1	Immune system development (GO:0002520)
		FSHR	Immune system process (GO:0002376)
		LHCGR	T cell differentiation (GO:0030217)
	CD14	CD55	Lymphocyte mediated immunity (GO:0002449)
	PTPRC	JAK2	Adaptive immune response (GO:0002250)
	CD74	CD44	Defense response (GO:0006952)
CHAF1B	CDC2	TGFBR2	
		AR	N/A
	BCAR1	HSPA5	
FBXO8	SLC9A3R1	LHCGR	N/A
KLRC1	PTPN11	GHR	
	PTPN6	SSTR2	Cell surface receptor linked signal transduction (GO:0007166)
		GHR	
NPHP1	PTPN11	IFNAR1	JAK-STAT cascade (GO:0007259)
		PRLR	Phosphate metabolic process (GO:0006796)
		RET	Phosphorus metabolic process (GO:0006793)
		IFNGR1	Response to stimulus (GO:0050896)
		MAPK3	Response to stress (GO:0006950)
		JAK2	
	FLNB	TSHR	
	PTK2B	JAK2	
	LBP	CD14	
	PTPN6	IFNAR1	
		ESR1	
		JAK2	
PHEX	FLNA	AR	N/A
		MAPK14	
POU3F1	HTATIP	AR	
		NFKB1	Cell communication (GO:0007154)
	PRTN3	TNF	Gamma-aminobutyric acid signaling pathway (GO:0007214)
	PPP3CA	GABRA1	
		GABRG2	
SLC4A8	CA2	HSPD1	N/A
SNAPC2	SRC	CD46	Estrogen receptor signaling pathway (GO:0030520)
		ESR2	Regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic
		THRA	Process (GO:0019219)
		ESR1	Biopolymer metabolic process (GO:0043283)
		IL6R	
		RET	
	TBP	ESR1	
		NR3C1	
STK25	YWHAZ	PRLR	
		HSPA1B	Anti-apoptosis (GO:0006916)
		HSPA1A	Negative regulation of apoptosis (GO:0043066)
TLR3	TRAF6	CD40	Positive regulation of I-kappaB kinase/NF-kappaB cascade (GO:0043123)
		MAPK8	Inflammatory response (GO:0006954)
		MAPK14	Response to external stimulus (GO:0009605)
	MYD88	TLR2	Response to stress (GO:0006950)
	CSK	CD44	Defense response (GO:0006952)
	TLR3	CD14	Protein metabolic process (GO:0019538)
	UBQLN4	GABRD	Stress-activated protein kinase signaling pathway (GO:0031098)
	YWHAZ	GABBR1	Response to wounding (GO:0009611)
	UBQLN4	DMPK	

[a] DE: DE gene;[28] IM: Intermediate gene in protein–protein interaction network (from HPRD); NEI: Cold or Hot related NEI genes;[12] GO Annotation (GO Number): Examples of significant ($P < 0.01$, by Fisher Exact test) functional annotation of Cold or Hot related NEI genes, connected with DE genes by IM genes, and related to inflammatory response, energy metabolism and environmental stress in GO Biological Process.[32] All genes are listed as NCBI gene symbols. (DE: Differentially Expressed; NEI: Neuro-endocrine-immune; HPRD: Human Protein Reference Database; NCBI: National Center for Biotechnology Information; GO:Gene Ontology; N/A: No available significant GO annotation at $P < 0.01$ by Fisher Exact test).

in the Cold group are densely pooling around the DE genes in the network. Nevertheless, the detailed interactions of Cold and Hot gene groups still need extensively quantifying at the micro level in the future.

NEI network intermediates macro and micro systems medicine

The diagnosis and treatment of complex diseases rely much on the development of systems biology. An ideal system should not only answer why explicitly, but also show how effectively, bringing out the best between research on molecular mechanism and clinical experience based on phenotypes. NEI is a system which bridges information from microscopic "omics" to macroscopic TCM Syndrome. Current studies have transferred the conceptual NEI to the systematic NEI network.[25] Patterns of NEI genes' interaction in the network may provide an innovative biomarker for complex diseases as well as the so-called "network biomarker" (Fig. 1(E) for TCM Syndrome we defined previously.[12,37]

TCM Cold Syndrome has its rich theoretical foundation. As shown above, this phenotype-based diagnosis comprises information from body itself (Cold areas and Cold behaviors could be regarded as physical and psychological aspects, respectively) and environment outside (Cold Adaptation reflects interaction between body and environment), which nevertheless takes on the rudimentary form of systems medicine[37] that has been verified by its repeated clinical practice for thousands of years. As disease is a multi-factorial evil consequence, the corresponding TCM drug-combinational therapy deservedly takes its place. The progressive drug tolerance is becoming a nightmare in the treatment of complex diseases, bringing an increasing call for multi-targeted drug administration.[38–40] Here we not only tested the necessity for a multi-targeted regimen but also proposed a way to grasp this multiple information by tracing into NEI messengers' interaction in the network. Further, this method may also be promising in individualized medication, because of TCM's unique perspective of disease classification. There are always varied clinical symptoms that cannot be ignored, even within the same disease category. These tiny distinctions often give reliable evidence in individualized medication.[41–43] Rightly in this respect, TCM's "Syndrome differentiation based diagnosis and therapy" for each specific individual has long originated from this holistic but personalized methodology.

Complex diseases originate from intertwined body–environment interactions and present as miscellaneous phenotypes. The intermediate "multiple components of networks and pathways perturbed in the disease"[1] will deepen our understanding of the mechanism and corresponding medical intervention. However, information flows instantaneously and dynamically within the shifty network space and therefore makes itself a tricky fox, which is difficult to grapple even for the most cunning hunter. Nevertheless, computational and mathematical tools have armed us with weapons of mass advantage to excavate the interrelationship between microscopic and macroscopic information.[25,44–46] Hopefully, peeping into the NEI network will facilitate research on the ancient systematic medicine, TCM, as well as the systems medicine of predictability, preventability and personalization.[47,48]

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