

Understanding ZHENG in traditional Chinese medicine in the context of neuro-endocrine-immune network

S. Li, Z.Q. Zhang, L.J. Wu, X.G. Zhang, Y.D. Li and Y.Y. Wang

Abstract: Traditional Chinese medicine uses ZHENG as the key pathological principle to understand the human homeostasis and guide the applications of Chinese herbs. Here, a systems biology approach with the combination of computational analysis and animal experiment is used to investigate this complex issue, ZHENG, in the context of the neuro-endocrine-immune (NEI) system. By using the methods of literature mining, network analysis and topological comparison, it is found that hormones are predominant in the Cold ZHENG network, immune factors are predominant in the Hot ZHENG network, and these two networks are connected by neuro-transmitters. In addition, genes related to Hot ZHENG-related diseases are mainly present in the cytokine–cytokine receptor interaction pathway, whereas genes related to both the Cold-related and Hot-related diseases are linked to the neuroactive ligand-receptor interaction pathway. These computational findings were subsequently verified by experiments on a rat model of collagen-induced arthritis, which indicate that the Cold ZHENG-oriented herbs tend to affect the hub nodes in the Cold ZHENG network, and the Hot ZHENG-oriented herbs tend to affect the hub nodes in the Hot ZHENG network. These investigations demonstrate that the thousand-year-old concept of ZHENG may have a molecular basis with NEI as background.

1 Introduction

Traditional Chinese medicine (TCM) is a system with its own rich tradition and over 3000 years of continuous practice and refinement through observation, testing and critical thinking. TCM can be characterised as holistic with emphasis on regulating the integrity of the human body and the interaction between human individuals and their environments. TCM applies multiple natural therapeutic methods for patient management, with herbal formulas as typical treatments.

ZHENG is the basic unit and the key concept in TCM theory. All diagnostic and therapeutic methods in TCM are based on the differentiation of ZHENG, and this concept has been used for thousands of years in China [1]. ZHENG can be seen as the TCM theoretical abstraction of the symptom profiles of a disease, not simply an assemblage of disease symptoms. Also, ZHENG is used as a guideline in disease classification in TCM. For example, patients suffering from the same disease may be categorised into different ZHENGs, whereas different diseases may be categorised as the same ZHENG. The ‘Cold’ ZHENG (‘HAN ZHENG’ in Mandarin) and ‘Hot’ ZHENG (‘RE ZHENG’ in Mandarin) are the two key statuses of ZHENG, which therapeutically direct the use of Chinese

herbs in TCM. The terms of major symptom profiles for Cold and Hot ZHENGs are listed in Table 1 and a detailed description is given in Supplementary 1. Correspondingly, many Chinese herbs are categorised as either Hot-Cooling type or Cold-Warming type: Hot-Cooling herbs are used to remedy Hot ZHENG, and Cold-Warming herbs are used to remedy Cold ZHENG.

Owing to the importance of ZHENG in both the theory and the practice of TCM, much effort has been paid to investigate its biomedical fundamentals. Ou *et al.* [2] compared the antioxidation–oxidation balance in biochemical processes with the YIN–YANG balance, an ancient Chinese philosophy that underlies the concept of ZHENG in TCM [1]. Ko *et al.* [3] reported that the pharmacological basis of ‘YANG-invigoration’ in Chinese medicine might be primarily due to the enhancement of mitochondrial ATP generation. In spite of all these efforts, it is still far from a full understanding of the inherent mechanism of ZHENG. And as for the methodology, it is known that the scientific evaluation of TCM is very difficult using existing conventional methods [4, 5].

The objective of this work is to explore the molecular basis of ZHENG within the context of neuro-endocrine-immune (NEI) system. In modern Western medicine (WM), NEI system acts as a pivot in modulating host homeostasis and naturally optimising health through complex communications among chemical messengers (CMs), including hormones, cytokines and neuro-transmitters [6, 7]. If we consider CMs as the biochemical ingredients of the NEI system, then those genes that (directly or indirectly) encode these CMs can be considered as the genic ingredients of the NEI system.

Cold ZHENG and Hot ZHENG are widely applied in the diagnosis and the treatment of patients suffering from inflammation, infection, stress and autoimmune disorders

© The Institution of Engineering and Technology 2007

doi:10.1049/iet-syb:20060032

Paper first received 12th March and in revised form 26th July 2006

S. Li, Z.Q. Zhang, L.J. Wu, X.G. Zhang and Y.D. Li are with the Bioinformatics Division, TNLIST and Department of Automation, Tsinghua University, Beijing 100084, People’s Republic of China

Y.Y. Wang is with the China Academy of Chinese Medicine Sciences, Beijing 100700, People’s Republic of China

E-mail: shaoli@tsinghua.edu.cn

Table 1: NEI-related terms and major symptom profile terms of Cold ZHENG and Hot ZHENG

Subjects	Terms (keywords)
Cold-ZHENG-related symptom profile terms	Cold (chill, coldness); cold pain; tastelessness; clear abundant urine (clear urine in large amounts); loose stool; pale tongue; white fur (white moss); tight pulse (stringy pulse)
Hot-ZHENG-related symptom profile terms	Fever; heat (hot); diaphoresis; flushed face; burning pain; deep-coloured urine; red eyes; thirst; desire for drinking; constipation; red tongue; dry tongue; thin fur (thin moss); yellow fur (yellow moss); rapid pulse
NEI-related terms	Neuro-endocrine-immune; nerve-endocrine-immune; hypothalamic-pituitary; hypothalamus-pituitary; neuro-endocrinology; neuro-immunology; neuro-immunomodulation; immune-neuroendocrine; endocrine-immune; neuroimmunoendocrinology; psychoneuroimmunology

[8, 9]. According to WM, most of such disorders are related to the bi-directional communication between the neuro-endocrine and the immune systems [10]. And defects in NEI will result in various kinds of pathologies [11, 12]. Thus, two aspects make NEI an ideal context to explore the underlying molecular basis of ZHENG and to bridge the gap between the ancient TCM and the modern medicine. First, the NEI system acts as the host homeostasis mediator during the course of various body disorders. Second, there are some clues that patients with Cold ZHENG and Hot ZHENG, two representative and mutually controlled ZHENGs, present abnormal NEI functions [8, 9, 13]. For example, patients suffering from rheumatoid arthritis (RA) are found to experience an alteration of the NEI system [14, 15]. And in TCM, most RA patients can be categorised as either Cold-ZHENG-related RA or Hot-ZHENG-related RA, and treated by the Cold-Warming or Hot-Cooling TCM herbal formulas, respectively [9].

The vast amount of medical and biological literatures are a huge reservoir of human knowledge on complex life systems, but most of them exist in the form of pieces of isolated knowledge, for example the function of a single gene or the interaction of a small number of molecules. Literature mining techniques can help to build or to recover the whole picture buried in the isolated reports, for example building biological entity-relation networks from the literatures and then generating hypothesis [16, 17]. In this paper, we study the networks of both CMs and genes of NEI systems with regard to Cold ZHENG and Hot ZHENG, respectively, by using a co-occurrence literature mining approach [16–18]. We compare the networks built for Cold ZHENG and Hot ZHENG, and find interesting features of these two ZHENGs in the NEI system, showing a putative correspondence between ZHENGs and different aspects of the NEI system. Some key factors in the Cold and the Hot NEI networks are identified. Next, we selected two groups of diseases with their typical symptom profiles corresponding to Cold ZHENG and Hot ZHENG, respectively, in the view of TCM. Gene investigation about ZHENG-related diseases is performed to validate patterns derived from the literature mining approach. Finally, as RA has a close relationship with the NEI system and is widely studied in both WM and TCM [9, 14, 15], we choose RA as a disease model to explore ZHENG within the context of the NEI system. Unfortunately, the direct biological measurement of ZHENG is hardly available. Instead, we conducted experiments on the rat model of collagen-induced arthritis (CIA, the most widely used model for RA) [19, 20] to study the effects of the Cold-Warming and Hot-Cooling TCM herbal formulas (CWHF and HCHF, respectively) on the key CMs of the NEI network.

2 Computational and experimental methods

2.1 Building the NEI networks

We summarised the synonyms of NEI by querying Medline MeSH (medical subject headings) and excluding the irrelevant subheadings and postfixal terms. Eleven available keywords (Table 1) are used to search the PubMed database (<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi>). A total of 21 222 PubMed abstracts were downloaded, called the NEI PubMed pool. The 20 327 non-redundant gene symbols from the Human Genome Organisation (HUGO) glossary (<http://www.gene.ucl.ac.uk/nomenclature/>) are used to search for NEI-related genes and their relations. Moreover, a dictionary of NEI-related CMs, that is hormones, cytokines and neuro-transmitters, are manually collected from a total of 261 full-text review articles in English during the past 5 years (from September 30 2000 to September 30 2005) by using ‘hormone’, ‘cytokine’ and ‘neuro-transmitter’ as keywords, respectively, and ‘disease’ as the constrained condition to query the above NEI PubMed pool. This dictionary contains 109 CMs (corresponding to 242 terms, abbreviations and synonyms, see Supplementary 2). Using the HUGO genes and the NEI-related CMs, we build a gene network and a CM network for the NEI system via the co-occurrence literature mining approach, which assumed that when two biological entities are co-cited in the same text unit there should be a potential biological relationship between them [16–18]. The Neato program in the Graphviz software (AT&T; <http://www.research.att.com/sw/tools/graphviz/>) is adopted to visualise these networks.

2.2 Building the ZHENG networks

Keywords for Cold and Hot ZHENGs are collected according to the ZHENG symptom profiles defined by the authoritative and standard TCM terminology (Table 1) [21, 22]. We eliminated the homonymous terms that contain the word ‘hot’ or ‘heat’ but are unrelated to Hot ZHENG. For example, ‘Hot Shock Protein’ and ‘Heat Shock Protein’ are excluded. Using the Cold ZHENG and the Hot ZHENG keywords, 189 and 382 NEI-related PubMed abstracts are collected, respectively. Using the same method described in the NEI network construction, the gene/CM-based Cold ZHENG network and the Hot ZHENG network are built from their corresponding abstract pools. The function annotation of these genes and CMs are extracted from the NCBI database (<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?CMD=search&DB=gene>).

2.3 Topological analysis of the ZHENG networks

The topological characteristic of node i in a network is represented by a topology vector $\mathbf{v}_{i(W)} = [c_{i1}, c_{i2}, \dots, c_{in}]^T$, where n is the total number of unique nodes in both Cold and Hot ZHENG networks, $W = \{C, H\}$ refers to the Cold or Hot ZHENG network and c_{ij} is the indicator variable of the association between nodes i and j ($c_{ij} = 1$ if $i \neq j$ and there is an association; 0 otherwise). The topology distance between Cold ZHENG and Hot ZHENG networks is defined as $d_i = (\mathbf{v}_{i(H)} - \mathbf{v}_{i(C)})^T \cdot \mathbf{e}$, where \mathbf{v}_i is the topology vector of node i , and \mathbf{e} is the unit vector with all elements equal to 1. We normalise the topology distance to the range of $[-1, 1]$ as

$$\bar{d}_i = \frac{d_i}{(\mathbf{v}_{i(H)} + \mathbf{v}_{i(C)})^T \cdot \mathbf{e}} = \frac{(\mathbf{v}_{i(H)} - \mathbf{v}_{i(C)})^T \cdot \mathbf{e}}{(\mathbf{v}_{i(H)} + \mathbf{v}_{i(C)})^T \cdot \mathbf{e}}$$

As we classify a node i as a Hot ZHENG node if $\bar{d}_i > 0$, or as a Cold ZHENG node if $\bar{d}_i < 0$, we thus call \bar{d}_i the ‘topological temperature’ for the convenience of discussion. Then, we assess the observed Cold/Hot ZHENG proportion within each category of NEI using the cumulative binomial distribution.

$$P_{(c \leq c_0)} = \sum_{c=0}^{c_0} \binom{N}{c} \left(\frac{1}{2}\right)^N$$

where N is the total number of nodes in one category, excluding those with $\bar{d}_i = 0$; c_0 is the smaller number between the node number of the Cold ZHENG nodes and the node number of the Hot ZHENG nodes.

2.4 Construction of ZHENG-related disease data sets and ZHENG-related NEI pathways

From Clinical Medicine Database (<http://www.cintcm.com>) hosted by China Academy of Chinese Medicine Sciences, where information about more than 4000 diseases was described, we selected ZHENG-related diseases whose properties can be typically differentiated and categorised as either Cold ZHENG or Hot ZHENG. Genes associated with the obtained 21 Cold ZHENG-related diseases and

38 Hot ZHENG-related diseases (Table 2) were collected from the OMIM database (<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM>). The NEI-related pathways of these genes in KEGG (<http://www.genome.jp/kegg/>) were obtained via the DAVID database (<http://apps1.niaid.nih.gov/david/>). In the DAVID annotation system, Fisher’s exact test was adopted to measure the gene-enrichment in annotation terms (http://david.abcc.ncifcrf.gov/helps/functional_annotation.html#fisher). Here, Fisher’s exact test is used to measure whether the proportion of genes in a specific pathway for the query genes is significantly higher than that for the human genomic background genes.

2.5 Effects of ZHENG-oriented herbal treatments on hub nodes

Experiments are conducted on CIA rats to investigate the effects of the ZHENG-oriented herbal treatments, CWHF and HCHF, on the hub nodes in the Cold ZHENG and the Hot ZHENG networks. CWHF and HCHF (Table 3) have been clinically proved to be effective to RA [23], and these two formulas can also improve arthritis signs and joint damages in CIA rats [24, 25]. In our experiments, rats are randomly divided into four groups, namely normal, CIA, HCHF-treated and CWHF-treated. The variations of the concentration levels in the four groups, measured using radioimmunoassay at 6-h intervals from 0:00 (midnight) to 24:00, are recorded for a number of important hub CMs in the Hot and Cold ZHENG networks. Differences between the CIA rats and the normal rats, the CIA rats and each of the two treatment rats were analysed using the one-way ANOVA followed by Bonferroni *post hoc* test. $P < 0.05$ was considered significant. The experiments described were performed in accordance with the UK Animals (Scientific Procedures) Act of 1986. Details of the experiments are described in Supplementary 3.

3 Results and discussion

3.1 Computational NEI network

In the gene-based NEI network and the CM-based NEI network constructed in Section 2.1, the genes/CMs are the nodes in the networks, and an edge between two

Table 2: List of diseases related to Cold ZHENG and Hot ZHENG

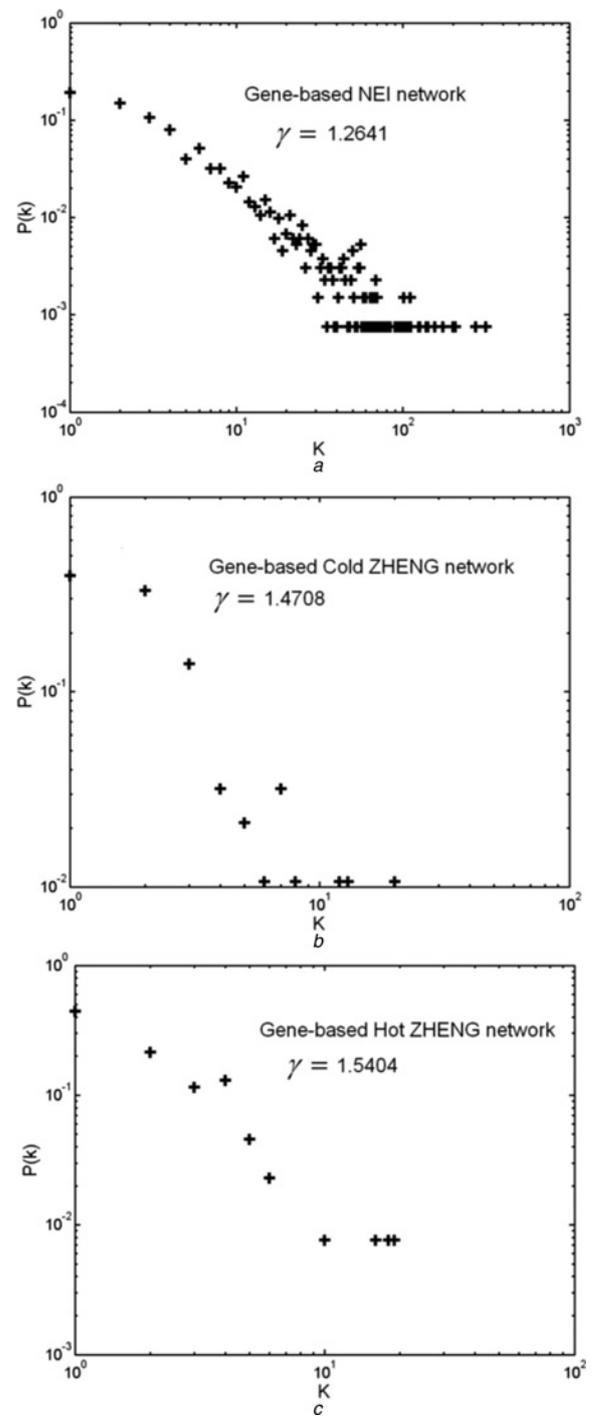
Disease data sets	Number	Diseases
Cold ZHENG-related diseases	21	Protein deficiency; hypothyroidism; child pituitary dwarfism; amenorrhoea–galactorrhoea syndrome; over weakness; deficiency and cold in large intestine; anaemic cardiopathy; adult anterior pituitary hypofunction; hypopituitarism; chronic pulmonary heart disease; occlusive arteriosclerosis; chronic renal failure; litchi disease; cretinism; neurosis; hyperprolactinaemia; empty sella syndrome; hypothalamus syndrome; psychonosema associated with hypothyroidism; impotence
Hot ZHENG-related diseases	38	Infectious mononucleosis; rheumatic fever; epidemic haemorrhagic fever; typhoid fever; paratyphoid; acute interstitial nephritis; acute pyelonephritis; acute enteritis; hyperthyroidism; acute appendicitis; acute pancreatitis; acute cholecystitis; suppurative infection; acute thyroiditis; acute peritonitis; trench fever; Australian tick-borne spotted fever; Q fever; Rocky mountain spotted fever; miliary fever; Kyasanur forest fever; Omsk haemorrhagic fever; Lassa fever; yellow fever; ratbite fever; child high-fever convulsion; kala-azar; epidemic haemorrhagic fever; relapsing fever; relapsing febrile non-suppurative panniculitis; septicemia; acute hepatitis gravis; epidemic cerebrospinal meningitis; epidemic encephalitis B; acute leukaemia; child acute pancreatitis; child viral myocarditis; acute febrile dermatosis of neutrophilic granulocytosis

Table 3: Composition of HCHF and CWHF

Formula	Herbs	Aims
HCHF	Ku-Shen (<i>Radix sophorae flaverscentis</i>)	Hot ZHENG
	Huang-Bai (<i>Cortex phellodendri</i>)	
	Qing-Feng-Teng (<i>Caulis sinomenii</i>)	
	Bi-Xie (<i>Rhizoma dioscoreae hypoglaucae</i>)	
CWHF	Fu-Zi (<i>Radix aconiti lateralis preparata</i>)	Cold ZHENG
	Bai-Zhu (<i>Rhizoma atractylodis macrocephalae</i>)	
	Gui-Zhi (<i>Ramulus cinnamomi</i>)	
	Juan-Bai (<i>Herba selaginellae</i>)	

nodes indicates that these two genes or CMs are related. The gene-based NEI network reflects a view of the whole structure of NEI at genic level, including 1585 genes and 8161 relation edges. The CM-based NEI network reveals the major functional component of NEI, which is composed of 108 CMs and 1607 relation edges. The number of edges a node has in a network is called the degree of that node [26–28], which indicates how many genes/CMs one gene/CM is related with. If the degree of a node is more than 2 fold of the median degree of all nodes in a network, such gene or CM is believed to play a critical role in the network structure, and we treat it as a hub gene or a hub CM [26–28]. As shown in Fig. 1a, the NEI network, has the property of a scale-free network, in which the connection degrees of the nodes follow a power law. Recent study [29] shows that the scale-free networks tend to contain centrally located, highly connected hub nodes that have dramatic influence on the way a network operates. Therefore it is believed that the hub genes in the network may play a key role in the NEI system. The CM-based NEI network is built on the specific CM list and therefore the power-law property is not observed.

Most of the hub nodes in the gene-based NEI network also appear in the CM-based NEI network. As shown in Table 4, these hub nodes include the tumour necrosis factor (TNF), proopiomelanocortin [POMC, other designations as adrenocorticotrophic hormone (ACTH) according to NCBI database], interleukin (IL)-6, prolactin (PRL), corticotropin releasing hormone (CRH) and so on. We also found that the hub nodes in the networks mainly belong to cytokines of the immune system as well as hypothalamic–pituitary (HP)–target organ axes such as HP–adrenal (HPA) and HP–thyroid (HPT) related hormones of the neuro-endocrine system. According to the schematic outline of the neuro-endocrine factors that regulate the secretion of the adrenal cortex [30], the hub nodes and their connections identified in our study are representatives of the NEI system. ACTH (79dCM: degree 79 in our CM-based network; 274dGene: degree 274 in our gene-based network) is released from the pituitary and is stimulated synergistically by CRH (75dCM and 142dGene) and arginine vasopressin (AVP, 58dCM and 102dGene). The secretion of ACTH is inhibited at the pituitary level by circulating glucocorticoids (cortisol in human, 41dCM, and corticosterone in rodents, 20dCM), which also regulate CRH and AVP through negative feedback control. CRH and AVP neurons are in turn subject to a wide range of influences – circulating cytokines and many neurotransmitters. For example, CRH and AVP are stimulated by acetylcholine (ACH, 32dCM and 8dGene), and are

**Fig. 1** Computational neuro-endocrine-immune and ZHENG systems

a Co-citation profiles in the gene-based NEI network

b Gene-based Cold ZHENG network

c Gene-based Hot ZHENG network

Shown on a log–log plot, the connectivity of the gene-based networks (NEI, Cold ZHENG and Hot ZHENG) follows a power law, $P(k) \simeq k^{-\gamma}$

CM-based networks identify approximately the same set of hub nodes as in their corresponding gene-based networks

inhibited by gamma-amino butyric acid (GABA, 41dCM). Moreover, each of the major inflammatory cytokines, interleukin (IL)-1 (73dCM and 25dGene), IL-2 (53dCM and 101dGene), IL-6 (74dCM and 201dGene) and TNF-alpha (146dCM and 315dGene), is capable of activating ACTH release [30]. The peripheral immune system provides a chemosensory system by which the presence of foreign molecules can be communicated to the brain and thus can induce an appropriate response [30].

Table 4: Hub nodes of the gene-based and the CM-based networks for NEI, Cold ZHENG and Hot ZHENG

NEI network (Number of co-citations)		Cold ZHENG network (Number of co-citations)		Hot ZHENG network (Number of co-citations)	
Gene-based (Median connectivity: 4)	CM-based (Median connectivity: 24)	Gene-based (Median connectivity: 2)	CM-based (Median connectivity: 3)	Gene-based (Median connectivity: 2)	CM-based (Median connectivity: 3)
TNF (315)	TNF (146)	POMC (20)	ACTH (18)	TNF (19)	TNF-alpha (13)
POMC (274)	ACTH (79)	TRH (12)	TRH (14)	IL6 (10)	IL-6 (9)
IL6 (201)	IL-6 (74)	CORT (8)	AVP (8)	IL1RAP (4)	IL-1 (7)
PRL (156)	IL-1 (73)	CRH (7)	CORT (6)		
CRH (142)	CRH (75)	AVP (5)	CRH (6)		
TRH (112)	PRL (69)				
AVP (102)	NF-kappaB (68)				
IL2 (101)	AVP (58)				
VIP (63)	IFN-gamma (57)				
NPY (70)	VIP (56)				
NA (69)	NA (54)				
NOS2A (44)	TRH (54)				
HTR1A (33)	IL-2 (53)				
IL1B (25)	NO (50)				
IGFBP7 (21)	IGF (49)				
IFNA (20)	NPY (49)				
NFKB (15)	5-HT (48)				

3.2 Computational Cold ZHENG and Hot ZHENG networks

Using the same method described earlier, we build a Cold ZHENG network and a Hot ZHENG network from the NEI-related PubMed abstracts with the standard Cold ZHENG and Hot ZHENG keywords (Table 1), respectively. For Cold ZHENG, the gene network is composed of 142 nodes with 120 edges, and the CM network is composed of 36 nodes with 69 edges. For Hot ZHENG, the gene network has 202 nodes and 169 edges, and the CM network has 43 nodes and 55 edges. Similar to the NEI network, the degree distribution in the gene-based ZHENG networks follows a power law (Figs. 1b and c), and most hub nodes in the gene-based ZHENG network are identified in the CM-based ZHENG network (Table 4). The hub genes/CMs in the Hot ZHENG network (TNF/TNF-alpha, -beta, IL6/IL-6, and IL1RAP/IL-1) belong to the category of immune cytokines, whereas the hub genes/CMs in the Cold ZHENG network are characterised as hypothalamus-pituitary hormones (POMC/ACTH, CRH/CRH, TRH/TRH and CORT/CORT) or neuro-transmitters (AVP/AVP).

Fig. 2a shows the combined CM network of Cold and Hot ZHENGs, where two networks are fused through the nodes and edges shared by both ZHENGs. The nodes appearing in Hot ZHENG network are shown in red, those in Cold ZHENG network are shown in blue and those appearing in both networks are shown in purple. A Yin-Yang map is drawn to discern the main bodies of two networks and indicate a mutual transformation of both may occur in given changes. It can be seen that in the Cold ZHENG network, HPA hormones such as cortisol, corticosterone, CRH and ACTH are closely interconnected; HPT hormones such as thyroid hormone (T) 3, T4, throtropin-releasing hormone (TRH) and thyroid-stimulating hormone (TSH) are also closely interconnected. In the Hot ZHENG network,

cytokines such as TNF-alpha, TNF-beta, IL-6 and IL-1 are closely interconnected.

Fig. 2b displays the ‘topological temperature’ of the CMs in the Hot and the Cold ZHENG networks. On the basis of cumulative binomial distribution, the CMs such as ACTH, TRH, TSH, GH and GIH secreted by hypothalamus or pituitary are mainly involved in the Cold ZHENG network ($P = 0.0059$), immune factors such as cytokines of TNF-alpha, IL-6, and IL-1 are mainly involved in the Hot ZHENG network ($P = 2.1 \times 10^{-6}$) and neuro-transmitters such as catecholamine are distributed in both ZHENG networks ($P = 0.254$). Fig. 2c shows the topological temperature of the genes presented in both ZHENG networks. It is observed that the genes in the gene-based Cold ZHENG and Hot ZHENG networks exhibit similar patterns as in the CM-based networks. Hormone-related genes are predominant in the Cold ZHENG network ($P = 0.046$), immune-related genes are predominant in the Hot ZHENG network ($P = 0.00047$) and neuro-transmitter-related genes are distributed in both networks ($P = 0.5$). These observations provide evidence that the ZHENG concept has its molecular base and different ZHENGs reflect their characteristics in different aspects of the complex NEI system.

3.3 ZHENG-related diseases and ZHENG-related NEI pathways

The typical symptoms of Cold ZHENG are chill without fever, whereas the typical symptoms of Hot ZHENG are fever without chill [21, 22]. We selected 59 diseases representing the major symptom features ZHENGs and divided them into two groups corresponding to either Cold ZHENG or Hot ZHENG. The ‘Hot’ group contains 38 diseases, whereas the ‘Cold’ group contains 21 diseases (Table 2). From the OMIM database, a total of 201 genes related to Cold ZHENG-related diseases and

Network structure of CM-based ZHENG network

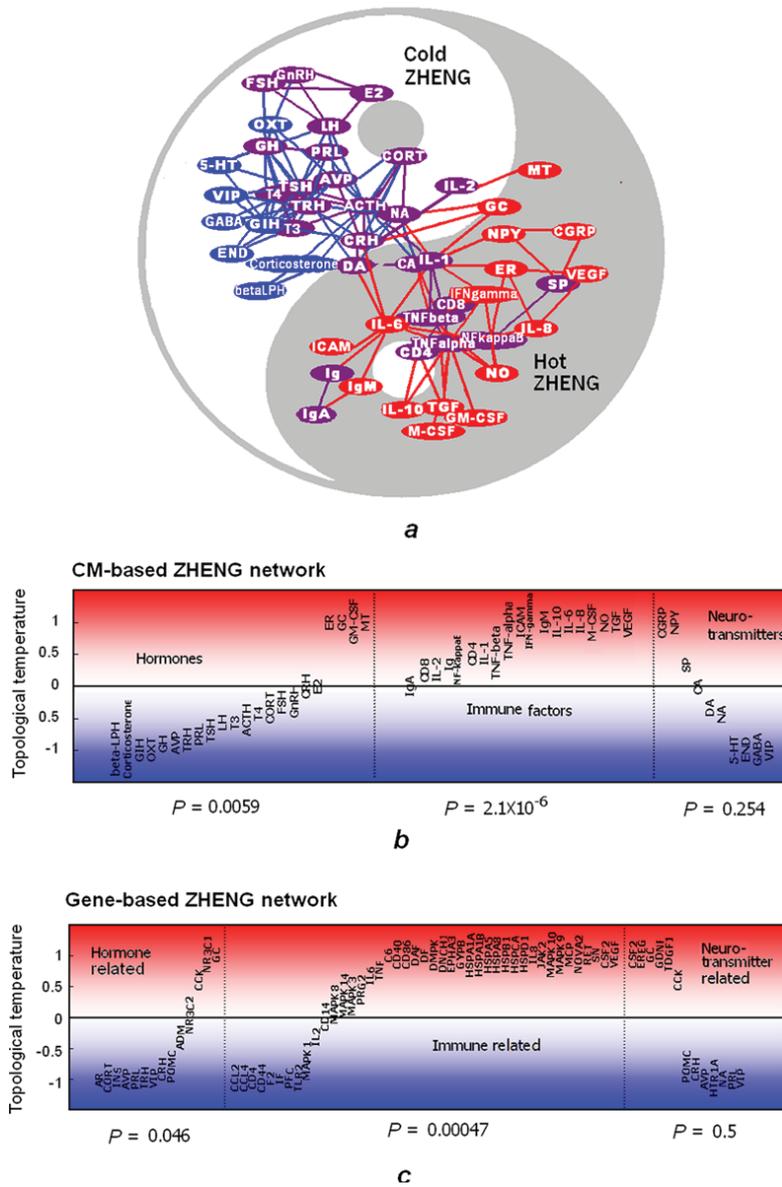


Fig. 2 Network patterns of ZHENG

a CM-based ZHENG network

Colour of a node signifies its ZHENG state (blue, Cold ZHENG; red, Hot ZHENG; purple, both Cold and Hot ZHENGs. The same to *b*)

Network can be divided into two major network clusters: Cold ZHENG network (composed of all Cold ZHENG-related nodes), and Hot ZHENG network (composed of all Hot ZHENG-related nodes)

The Yin-Yang background here is to indicate the possible relationship between Cold-Hot ZHENG networks and Yin-Yang theory of TCM, a mutual transformation of both may occur in given quantitative changes

b ‘Topological temperature’ (see methods) of each nodes in the CM-based Cold ZHENG (−1–0) and Hot ZHENG (0–1) networks

c Gene-based Cold ZHENG (−1–0) and Hot ZHENG (0–1) networks

Centre of the CM name or gene name corresponds to its topological temperature

603 genes related to Hot ZHENG-related diseases are collected, among which 60 genes are associated with both sets. By investigating the NEI-related KEGG pathways of these genes via the DAVID database, we found that in contrast to genes specific to ‘Cold’ diseases, genes specific to ‘Hot’ diseases are significantly enriched in the cytokine–cytokine receptor interaction pathway ($P = 5.9 \times 10^{-5}$ for ‘Hot’ diseases and $P = 0.225$ for ‘Cold’ diseases). The relationship between genes in ‘Cold’ diseases and hormones are not identified since KEGG contains fewer pathways for complex hormone regulations. These results are in agreement with the biological patterns we observed in the Cold and Hot ZHENG networks (see previous section). Moreover, the

results summarised in Table 5 show that genes shared by both ‘Hot’ and ‘Cold’ diseases are significantly enriched in the neuroactive ligand–receptor interaction pathway ($P = 2.21 \times 10^{-7}$). Together with the network structure of ZHENG in Fig. 2, we will speculate that the neuro-transmitters can be the common factors shared by both Cold ZHENG and Hot ZHENG.

3.4 Effects of ZHENG-oriented herbal treatments on the network hub nodes

Effects of the ZHENG-oriented herbal treatments on the hub nodes of ZHENG networks are evaluated using rat CIA, an experimental model of human RA [19, 20]. As shown in

Table 5: NEI-related KEGG pathways for genes in Cold ZHENG and Hot ZHENG-related diseases

Genes	Number of genes	NEI-related KEGG pathway (Homo sapiens)	P-values ^a
Genes related to Cold ZHENG-related diseases only	151	Neuroactive ligand–receptor interaction	0.180
		Cytokine–cytokine receptor interaction	0.225
Genes related to Hot ZHENG-related diseases only	543	Neuroactive ligand–receptor interaction	0.030
		Cytokine–cytokine receptor interaction	5.9×10^{-5}
Genes related to both diseases	60	Neuroactive ligand–receptor interaction	2.21×10^{-7}

^aP-values are calculated by Fisher exact test in DAVID. Fisher's exact test is used to measure whether the proportion of genes in a specific pathway for the query genes is significantly higher than that for the human genomic background genes

Table 3, we adopted a CWHF (which is used to remedy Cold ZHENG in TCM) to represent Cold ZHENG-oriented herbal treatment, and a HCHF (which is used to remedy Hot ZHENG in TCM) to represent Hot ZHENG-oriented herbal treatment in our experiment. Circulating concentrations of two hub nodes in the Hot ZHENG network, namely cytokines of TNF-alpha and IL-6, and two hub nodes in the Cold ZHENG network, namely HPA hormones of ACTH and corticosterone, are measured every 6 h during the 24-h period of the experiment.

Cytokines and HPA hormones are two key players in the classical neuro-endocrine feedback loop [11, 31, 32], where both TNF-alpha, a cytokine at the top of the inflammatory cascade [33], and IL-6, a key pathogenic element in inflammatory succeed, are potential activators of the HPA axis [34–36]. During inflammation, TNF-alpha is secreted first [37], IL-1beta and IL-6 successively [35, 36]. It is found that the cytokine system and the HPA axis are disorderly in many NEI-related diseases, including RA [32, 38]. In our study, as shown in Fig. 3, the secretion of serum cytokines increased significantly in rat CIA, for example TNF-alpha is increased at 24:00 ($P = 0.043$), IL-6 is increased at 6:00 ($P = 0.048$), 18:00 ($P = 0.021$) and 24:00 ($P = 0.002$) (ANOVA and Bonferroni test, similarly hereinafter). The over-production of TNF-alpha and IL-6 indicates the progression of arthritis in CIA rats 33 days after immunisation. Similar to the human RA patients, where normal ACTH secretion and abnormal corticosterone secretion are found [39], the secretion of corticosterone in the CIA rats evidently increases at 6:00 ($P = 0.039$) and decreases at 18:00 ($P = 0.037$), implying an adrenal disorder, which may be due to the stimulation of inflammatory cytokines such as TNF-alpha and IL-6 [32, 40]. Note that the normal behaviour of ACTH in CIA rats may be a result of the sparse sampling times. Some researchers consider that cytokines such as IL-6 also act directly on the adrenal cortex to stimulate corticosterone secretion [11, 41].

The production of both cytokines and hormones is known to exhibit diurnal rhythm in human RA and its rat models [10, 32, 42]. Peak production of the proinflammatory cytokines occurs at the time when plasma cortisol is at the lowest [43]. In early untreated RA patients, it is found that IL-6 precedes ACTH and cortisol by 1 and 2 h [40], respectively. In our study, CIA rats present the similar phenomena such that comparing with those of the normal rats, the lower level of plasma corticosterone and the higher level of serum IL-6 are observed at 18:00 simultaneously. The levels of both plasma corticosterone and serum IL-6 increase significantly at 6:00 clock in CIA rats, suggesting that the HPA axis of CIA apparently is insufficient to inhibit ongoing inflammation, although endogenous IL-6 may still stimulate the secretion of

corticosterone. Thus, TNF-alpha and IL-6 stimuli (24:00) are speculated to be responsible for the earlier peak of corticosterone (6:00) in CIA rats. The high levels of circulating IL-6 in the CIA group from 18:00 to 6:00 form a continual stimulation to the HPA axis, leading to adrenal insufficiency, a similar condition as in humans [32]. Such results demonstrate that CIA rats exhibit an impairment of the HPA-cytokine feedback loop in which inflammatory cytokines are overproduced and the HPA axis is hyporesponsive [31].

In our ZHENG-oriented herbal treatment, it shows that the HCHF-treatment can significantly suppress the hub nodes (e.g. TNF-alpha, IL-6) of the Hot ZHENG network in rat CIA (Figs. 3a and b). For example, in the HCHF-treatment group, significant suppression can be observed for IL-6 and TNF-alpha at 24:00 (IL-6, $P = 0.003$; TNF-alpha $P = 0.003$ against CIA rats). In addition, The HCHF treatment is observed to regulate the abnormal secretion of corticosterone, for example the high plasma corticosterone level of CIA at 6:00 ($P = 0.039$, CIA against normal rats) is reduced after the HCHF treatment ($P = 0.027$, against CIA rats). As described earlier, the inflammatory cytokines TNF-alpha and IL-6 are the potential activators of the HPA axis [34]. Thus, the improvement of corticosterone secretion at 6:00 caused by HCHF may be due to HCHF's ability to inhibit the production of TNF-alpha and IL-6 at 24:00, and then weaken the stimulation of both cytokines to the HPA axis, especially to the abnormal adrenal of CIA rats.

In contrast, the CWHF-treatment rats show suppression of the hub nodes (e.g. ACTH, corticosterone) in the Cold ZHENG network. For example, after CWHF treatment, plasma corticosterone is dramatically inhibited at 6:00 ($P = 0.032$) and 24:00 ($P = 0.046$), and ACTH is inhibited at 24:00 ($P = 0.002$). TNF-alpha, a cytokine shared by both ZHENG networks and highlighted in the Hot ZHENG network (positive topological temperature), is also inhibited by CWHF at 24:00 ($P = 0.002$). It is known that synthetic glucocorticoids, such as dexamethasone, can significantly suppress the rate of IL-6 gene transcription [44]. And the component extracted from Fu-Zi (*Radix aconiti lateralis preparata*), an herb in CWHF, is also found to affect the HPA axis directly [45]. Therefore CWHF may have an effect similar to exogenous hormones, leading to the suppression of endogenous hormones ACTH and corticosterone in CIA rats.

From the above results, we speculate that HCHF tends to affect the inflammatory cytokines (hub nodes in Hot ZHENG network) and then affect corticosterone through the cytokine-HPA pathway (grey line arrow in Fig. 3b), whereas CWHF tends to affect HPA axis hormones (hub nodes in Cold ZHENG network) and then affect some of cytokines through the HPA-cytokine feedback loop (black

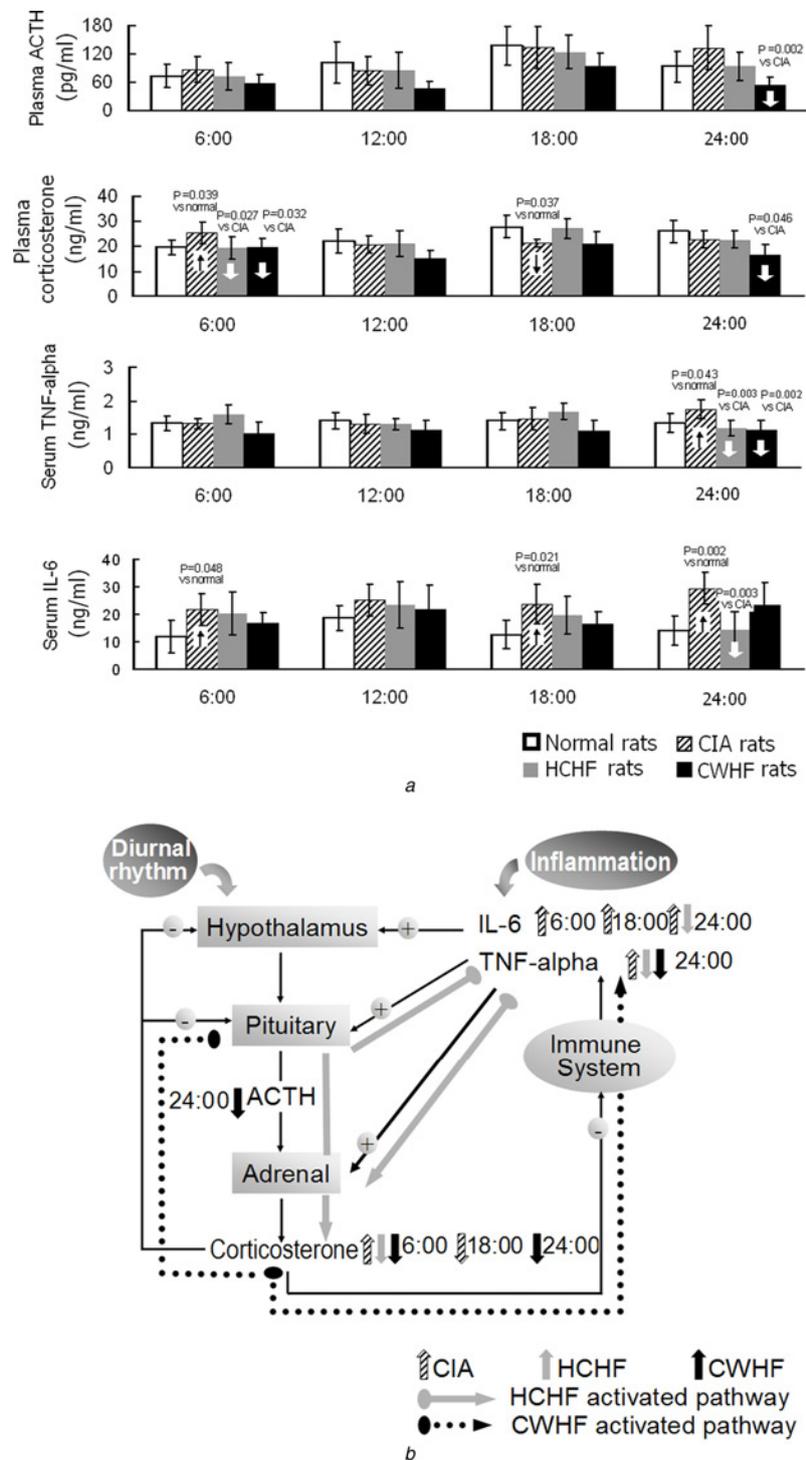


Fig. 3 Effect of herbal formula on the different hub nodes of the ZHENG Network

a Effects of the CWHF and the HCHF on the four hub nodes of the ZHENG network within 24 h using CIA rats

b HCHF tends to affect the inflammatory cytokines (hub nodes in Hot ZHENG network) then affect corticosterone through the cytokine-HPA pathway (grey-line arrow)

CWHF tends to affect HPA axis hormones (hub nodes in Cold ZHENG network) then affect some of cytokines through the HPA-cytokine feedback loop (black dot-line arrow)

dot-line arrow in Fig. 3b). Thus, with the combination of computational analysis and experiment, our investigation shows that the Cold ZHENG-oriented and Hot ZHENG-oriented Chinese herbs modulate the HPA-cytokine loop of CIA rats in different ways. CWHF tends to affect the HPA hormones, the hub nodes in the computational Cold ZHENG network, whereas HCHF tends to affect the immune cytokines, the hub nodes in the computational Hot ZHENG network. Such observations provide an interesting biological explanation for the traditional

ZHENG management philosophy of ‘Warming the Cold and Cooling the Hot’ [1] in TCM.

4 Conclusions

TCM diagnosis and treatment are characterised by the holistic view and the central concept of ZHENG. Here, we performed a co-occurrence text mining to learn which NEI-related genes/CMs participate in Cold ZHENG network and Hot ZHENG network, respectively. Some

relationships between Cold/Hot ZHENG and CMs/genes in the NEI system are found and hypotheses are generated by using this holistic method. Then, genes and corresponding NEI pathways from two groups of Cold ZHENG-related and Hot ZHENG-related diseases are statistically evaluated. The results validated the different biological patterns identified in Cold ZHENG and Hot ZHENG networks. We further experimentally confirmed that some key players distinguishing Cold/Hot ZHENG are affected by corresponding Cold/Hot ZHENG-oriented herbal treatments, respectively. As a result, this work highlights a possible molecular foundation for Cold ZHENG and Hot ZHENG with regard to the NEI system: hormones may be related to Cold ZHENG, immune factors may be related to Hot ZHENG and they may be interconnected by neurotransmitters. Thus, Cold ZHENG and Hot ZHENG reflect two typical conditions of the internal imbalances of NEI, and both of which should be taken into consideration during disease diagnosis and treatment. Moreover, from a methodological point of view, our work provides a new approach towards understanding and refining the theory and the principle of TCM within the framework of modern science, which, in turn, can provide an alternate perspective on the integrative NEI system. This preliminary study is the first step to understand ZHENG in viewing NEI network. And we believe that uncovering the molecular basis of ZHENG may help to design a tailored diagnosis and treatment for patients in the future by evaluating their ZHENG condition as well as the internal NEI imbalance, and ZHENG-related approach is very likely to play a role in the up-coming era of personalised medicine.

5 Acknowledgments

This work is supported by NSFC (30200365), the Key Project of MOE (104009), the FANEDD (200366) and the 973 Program (2003CB517106 and 2004CB518605) of the People's R. of China. We would like to express our great appreciation to Dr. B. Li (Boston University, USA), Dr. A. Lu (China Academy of TCM) and Mr. C. Zhang, B. Zhang in our laboratory for helpful discussions and comments.

6 Supplementary materials

Supplementary 1: explains the TCM terms about Cold ZHENG and Hot ZHENG.

Supplementary 2: gives the manually collected dictionary of NEI-related chemical messengers.

Supplementary 3: describes the experimental procedures for CIA rats under the Cold/Hot ZHENG-oriented herbal treatments.

7 References

- Gu, C.D. (Ed.): 'The inner classic of the yellow emperor, essential questions (Huangdi Neijing, Suwen)' (People's Medical Publishing House, Beijing, 1956), pp. 186–191
- Ou, B., Huang, D., Hampsch-Woodill, M., and Flanagan, J.A.: 'When east meets west: the relationship between yin-yang and antioxidation-oxidation', *FASEB J.*, 2003, **17**, pp. 127–129
- Ko, K.M., Mak, D.H., Chiu, P.Y., and Poon, M.K.: 'Pharmacological basis of "Yang-invigoration" in Chinese medicine', *Trends Pharmacol. Sci.*, 2004, **25**, pp. 3–6
- Xue, T., and Roy, R.: 'Studying traditional Chinese medicine', *Science*, 2003, **300**, pp. 70–71
- Wang, M., Lamers, R.J., Korthout, H.A., van Nesselrooij, J.H., Witkamp, R.F., van der Heijden, R., Voshol, P.J., Havekes, L.M., Verpoorte, R., and van der Greef, J.: 'Metabolomics in the context of systems biology: bridging traditional Chinese medicine and molecular pharmacology', *Phytother. Res.*, 2005, **19**, pp. 173–182
- Besedovsky, H.O., and Sorkin, E.: 'Network of immune-neuroendocrine interactions', *Clin. Exp. Immunol.*, 1977, **27**, pp. 1–12
- Roth, J., LeRoith, D., Shiloach, J., Rosenzweig, J.L., Lesniak, M.A., and Havrankova, J.: 'The evolutionary origins of hormones, neuro-transmitters, and other extracellular chemical messengers: implications for mammalian biology', *N. Engl. J. Med.*, 1982, **306**, pp. 523–527
- Hsu, C.H., Yu, M.C., Lee, C.H., Lee, T.C., and Yang, S.Y.: 'High eosinophil cationic protein level in asthmatic patients with "Heat", Zheng', *Am. J. Chin. Med.*, 2003, **31**, pp. 277–283
- Li, S.: 'Advances in TCM symptomatology of rheumatoid arthritis', *J. Tradit. Chin. Med.*, 2002, **22**, pp. 137–142
- Eskandari, F., Webster, J.I., and Sternberg, E.M.: 'Neural immune pathways and their connection to inflammatory diseases', *Arthritis Res. Ther.*, 2003, **5**, pp. 251–265
- Haddad, J.J., Saade, N.E., and Safieh-Garabedian, B.: 'Cytokines and neuro-immune-endocrine interactions: a role for the hypothalamic-pituitary-adrenal revolving axis', *J. Neuroimmunol.*, 2002, **133**, pp. 1–19
- Dorner, G.: 'Hormone-dependent brain development and new endocrine prophylaxis', *Exp. Clin. Endocrinol.*, 1989, **94**, pp. 4–22
- Zhang, X., Ji, B., Chen, B., and Xie, Z.: 'Relationship of cytokines and cold-heat syndrome differentiation in patients of duodenal ulcer', *Zhongguo Zhong Xi Yi Jie He Za Zhi*, 1999, **19**, pp. 267–269
- Wilder, R.L.: 'Neuroimmunoendocrinology of the rheumatic diseases, past, present, and future', *Ann. NY Acad. Sci.*, 2002, **966**, pp. 13–19
- Choy, E.H.S., and Panayi, G.S.: 'Cytokine pathways and joint inflammation in rheumatoid arthritis', *N. Engl. J. Med.*, 2001, **344**, pp. 907–916
- Jenssen, T.K., Lagreid, A., Komorowski, J., and Hovig, E.: 'A literature network of human genes for high-throughput analysis of gene expression', *Nat. Genet.*, 2001, **28**, pp. 21–28
- Zhang, C., and Li, S.: 'Modeling of neuro-endocrine-immune network via subject oriented literature mining'. Proc. 4th Int. Conf. on Bioinformatics of Genome Regulation and Structure, Novosibirsk, Russia, 2004, pp. 167–170
- Li, S., Wu, L.J., and Zhang, Z.Q.: 'Constructing biological networks through combined literature mining and microarray analysis: a LMMA approach', *Bioinformatics*, 2006, **22**, pp. 2143–2150
- Trentham, D.E., Townes, A.S., and Kang, A.H.: 'Autoimmunity to type II collagen an experimental model of arthritis', *J. Exp. Med.*, 1977, **146**, pp. 857–868
- Staines, N.A., and Wooley, P.H.: 'Collagen arthritis – what can it teach us?', *Br. J. Rheumatol.*, 1994, **33**, pp. 798–807
- State Bureau of Technical Supervision: 'The clinic terminology of traditional Chinese medical diagnosis and treatment – ZHENG' (Standards Press of China, Beijing, 1997), p. 52
- State Administration Bureau of TCM: 'The criteria of diagnosis and therapeutic effect of diseases and ZHENGs in TCM' (Nanjing University Press, Nanjing, 1994), p. 29
- Li, J.R., and Tong, X.L.: 'Discussion on the rheumatism (Bi Zheng Tong Lun)' (Anhui Science & Technology Publishing House, Hefei, 1987), p. 52
- Li, S., Lu, A.P., and Jia, H.W.: 'Therapeutic actions of the Chinese herbal formulae with cold and heat properties and their effects on ultrastructures of synoviocytes in rats of the collagen-induced arthritis', *J. Tradit. Chin. Med.*, 2002, **22**, pp. 290–296
- Li, S., Lu, A.P., Wang, Y.Y., and Li, Y.D.: 'Suppressive effects of a Chinese herbal medicine Qing-Luo-Yin extract on the angiogenesis of collagen induced arthritis in rats', *Am. J. Chin. Med.*, 2003, **31**, pp. 713–720
- Song, C., Havlin, S., and Makse, H.A.: 'Self-similarity of complex networks', *Nature*, 2005, **433**, pp. 392–395
- Jeong, H., Tombor, B., Albert, R., Oltvai, Z.N., and Barabasi, A.L.: 'The large-scale organization of metabolic networks', *Nature*, 2000, **407**, pp. 651–654
- Albert, R., Jeong, H., and Barabasi, A.L.: 'Error and attack tolerance of complex networks', *Nature*, 2000, **406**, pp. 378–382
- Han, J.D., Bertin, N., Hao, T., Goldberg, D.S., Berriz, G.F., Zhang, L.V., Dupuy, D., Walhout, A.J., Cusick, M.E., Roth, F.P., and Vidal, M.: 'Evidence for dynamically organized modularity in the yeast protein-protein interaction network', *Nature*, 2004, **430**, pp. 88–93
- Reichlin, S.: 'Neuroendocrine consequences of systemic inflammation' in (Committee on Military Nutrition Research (Ed.): 'Military strategies for sustainment of nutrition immune function in the field' (National Academy Press, Washington DC, 1999), p. 395
- Besedovsky, H.O., del Rey, A., Klusman, I., Furukawa, H., Monge Arditi, G., and Kabiersch, A.: 'Cytokines as modulators of the hypothalamus-pituitary-adrenal axis', *J. Steroid. Biochem. Mol. Biol.*, 1991, **40**, pp. 613–618

- 32 Cutolo, M., Sulli, A., Pizzorni, C., Cravioito, C., and Straub, R.H.: 'Hypothalamic-pituitary-adrenocortical and gonadal functions in rheumatoid arthritis', *Ann. NY Acad. Sci.*, 2003, **992** pp. 107-117
- 33 Pearce, G.J., and Chikanza, I.C.: 'Targeting tumour necrosis factor in the treatment of rheumatoid arthritis', *BioDrugs*, 2001, **15**, pp. 139-149
- 34 Imura, H., Fukata, J.U., and Mori, T.: 'Cytokines and endocrine function an interaction between the immune and neuroendocrine systems', *Clin. Endocrinol.*, 1991, **35**, pp. 107-115
- 35 De Simoni, M.G., Sironi, M., De Luigi, A., Manfredi, A., Mantovani, A., and Chezzi, P.: 'Intracerebroventricular injection of interleukin 1 induces high circulation levels of interleukin 6', *J. Exp. Med.*, 1990, **171**, pp. 1773-1778
- 36 Chrousos, G.P.: 'The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation', *N. Engl. J. Med.*, 1995, **332**, pp. 1351-1362
- 37 Perlstein, R.S., Whitnall, M.H., Abrams, J.S., Mougey, E.H., and Neta, R.: 'Synergistic roles of interleukin 6, interleukin 1, and tumor necrosis factor in the adrenocorticotropin response to bacterial lipopolysaccharide in vivo', *Endocrinology*, 1993, **132**, pp. 946-952
- 38 Wilder, R.L., Griffiths, M.M., Cannon, G.W., Caspi, R., and Remmers, E.F.: 'Susceptibility to autoimmune disease and drug addiction in inbred rats. Are there mechanistic factors in common related to abnormalities in hypothalamic-pituitary-adrenal axis and stress response function?', *Ann. NY Acad. Sci.*, 2000, **917**, pp. 784-796
- 39 Chikanza, I.C., Petrou, P., Kingsley, G., Chrousos, G., and Panayi, G.S.: 'Defective hypothalamic response to immune and inflammatory stimuli in patients with rheumatoid arthritis', *Arthritis Rheum.*, 1992, **35**, pp. 1281-1288
- 40 Mastorakos, G., and Ilias, I.: 'Relationship between interleukin-6 (IL-6) and hypothalamic-pituitary-adrenal axis hormones in rheumatoid arthritis', *Z. Rheumatol.*, 2000, **59**, pp. 75-79
- 41 Dunn, A.J.: 'Interleukin-6 access to the axis', *Endocrinology*, 2004, **145**, pp. 3578-3579
- 42 Li, S., Lu, A.P., Li, B., and Wang, Y.Y.: 'Circadian rhythms on hypothalamic-pituitary-adrenal axis hormones and cytokines of collagen induced arthritis in rats', *J. Autoimmun.*, 2004, **22**, pp. 277-285
- 43 Petrovsky, N., and Harrison, L.C.: 'The chronobiology of human cytokine production', *Int. Rev. Immunol.*, 1998, **16**, pp. 635-649
- 44 Miyazawa, K., Mori, A., and Okudaira, H.: 'Regulation of interleukin-1beta-induced interleukin-6 gene expression in human fibroblastlike synoviocytes by glucocorticoids', *J. Biochem. (Tokyo)*, 1998, **124**, pp. 1130-1137
- 45 Cai, D.F., Shen, Z.Y., and Chen, X.H.: 'Effect of aconitine on content of corticotropin-releasing hormone in hypothalamus of rats', *Zhongguo Zhong Xi Yi Jie He Za Zhi*, 1996, **16**, pp. 544-546