REVIEW ARTICLE

Genomic insights into ayurvedic and western approaches to personalized medicine

BHAVANA PRASHER[1]*, GREG GIBSON[3] and MITALI MUKERJI[1,2]*

[1]*CSIR Ayurgenomics Unit-TRISUTRA, CSIR-Institute of Genomics and Integrative Biology, New Delhi 110 020, India*
[2]*Genomics and Molecular Medicine, CSIR-Institute of Genomics and Integrative Biology, Mathura Road, New Delhi 110 020, India*
[3]*School of Biology and Center for Integrative Genomics, Georgia Institute of Technology, Atlanta, GA 30332, USA*

Abstract

Ayurveda, an ancient Indian system of medicine documented and practised since 1500 B.C., follows a systems approach that has interesting parallels with contemporary personalized genomic medicine approaches to the understanding and management of health and disease. It is based on the *trisutra*, which are the three aspects of causes, features and therapeutics that are interconnected through a common organizing principle termed '*tridosha*'. *Tridosha* comprise three ascertainable physiological entities; *vata* (kinetic), *pitta* (metabolic) and *kapha* (potential) that are pervasive across systems, work in conjunction with each other, respond to the external environment and maintain homeostasis. Each individual is born with a specific proportion of *tridosha* that are not only genetically determined but also influenced by the environment during foetal development. Jointly they determine a person's basic constitution, which is termed their '*prakriti*'. Development and progression of different diseases with their subtypes are thought to depend on the origin and mechanism of perturbation of the *doshas*, and the aim of therapeutic practice is to ensure that the *doshas* retain their homeostatic state. Similarly, western systems biology epitomized by translational P4 medicine envisages the integration of multiscalar genetic, cellular, physiological and environmental networks to predict phenotypic outcomes of perturbations. In this perspective article, we aim to outline the shape of a unifying scaffold that may allow the two intellectual traditions to enhance one another. Specifically, we illustrate how a unique integrative 'Ayurgenomics' approach can be used to integrate the *trisutra* concept of Ayurveda with genomics. We observe biochemical and molecular correlates of *prakriti* and show how these differ significantly in processes that are linked to intermediate patho-phenotypes, known to take different course in diseases. We also observe a significant enrichment of the highly connected hub genes which could explain differences in *prakriti*, focussing on *EGLN1*, a key oxygen sensor that differs between *prakriti* types and is linked to high altitude adaptation. Integrating our observation with the current literature, we demonstrate how *EGLN1* could qualify as a molecular equivalent of *tridosha* that can modulate different phenotypic outcomes, where hypoxia is a cause or a consequence both during health and diseased states. Our studies affirm that integration of the *trisutra* framework through Ayurgenomics can guide the identification of predisposed groups of individuals and enable discovery of actionable therapeutic points in an individualized manner.

[Prasher B., Gibson G. and Mukerji M. 2016 Genomic insights into ayurvedic and western approaches to personalized medicine. *J. Genet.* **95**, 209–228]

Introduction

Ayurveda is an Indian system of predictive, preventive, personalized and promotive medicine documented and practised since 1500 B.C. Its primary aim is maintenance of health in healthy people and alleviation of disorders in diseased people (Sharma 1981, 1999). In this sense it is no different from the contemporary efforts that use molecular diagnostics for the purposes of predictive health and personalized medicine. Ayurveda describes the subject matter into three major categories termed '*trisutra*', meaning the three interconnected aspects of causes (*hetu*), features (*linga*) and therapeutics (*aushadha*) both for healthy and diseased people (Sharma 1981). The question thus arises as to whether there are molecular and genomic correlates of *trisutra*.

Understanding the molecular mechanisms of a disease that can be rationalized to design effective drugs and improve human health care remains a fundamental goal of medical science. In medical terms, a disease is defined as a condition that demonstrates adverse effects on normal human physiology

*For correspondence. E-mail: Bhavana Prasher, bhavana.p@igib.res.in; Mitali Mukerji, mitali@igib.res.in.

Keywords. ayurgenomics; human individuality; system biology; *tridosha*; P4 medicine; *trisutra*; *prakriti.*

under the influence of various factors, which are mainly characterized as either genetic or environmental. Since a disease is characterized by its symptoms, the current principle of medical science seeks its cure in a symptom-guided manner, which often ignores the process of disease onset. Symptoms are the outcomes of an evolutionary process that a disease onset adopts spanning the genotype–phenotype space of a given individual. It therefore becomes imperative to comprehend such genotype–phenotype relations of a given human individual in the context of its environment. In the posthuman genome sequence era, our understanding of genotype–phenotype relation has improved significantly. Decades of investigations suggest that phenotypic manifestation is a consequence of an enormous amount of complexity due to the underlying orchestration between various biological components such as DNA, RNA, protein and metabolites involved in various cellular processes that are responsive to the environment. Effects can be seen across the range from single-cells to epidemiology. While the current systems-level framework is capable of deconvoluting the complexity of disease process in a discrete manner, it however remains challenging to comprehend such complex relations in a time-dependent manner. Network medicine is evolving as an emerging area of research as we begin to comprehend the knowledge of networks in biological systems and evolve the tools of systems biology (Oltvai and Barabási 2002; Loscalzo *et al.* 2007; Agustí *et al.* 2011; Barabási *et al.* 2011; Loscalzo and Barabási 2011; Fabbri *et al.* 2012).

We would argue that recognition of the underlying systems biology has been effective in the translation of network medicine into clinical practice of Ayurveda for thousands of years in India. Evidence from the textual references (Sharma 1981, 1999) suggest that this field has evolved following intensive observations spanning long period of time in large number of individuals which has lead to establishment of tenets that are still contemporary (figure 1). Today, this practice continues in India side-by-side with western medicine, with the ongoing training of ayurvedic doctors and, opening of clinics and hospitals as a prominent feature of Indian healthcare. The concepts and practice of Ayurveda resonate with the aims, observations and the promise of contemporary P4 medicine; it is predictive, preventive, personalized and participatory medicine (Hood and Friend 2011; Tian *et al.* 2012; Agustí 2013). Currently the science of network medicine is primarily observational, involving big data and large amounts of correlations (Barabási 2007; Park *et al.* 2009; Suthram *et al.* 2010; Agustí *et al.* 2011; Barabási *et al.* 2011; Vidal *et al.* 2011; Li *et al.* 2014). This review highlights the *Trisutra* aspects of Ayurveda, its relevance and contemporariness in systems medicine.

The aim of this study was to propose a theoretical framework of Ayurveda that can help systematize these observations to have a comprehensive understanding and a foundation for P4 medicine. We start by introducing the concepts of Ayurveda, using the Sanskrit terms as described in the classical and most widely cited ayurvedic literature, namely, the English translations of *Charaka samhita* (Sharma 1981), *Sushruta samhita* (Sharma 1999) and *Ashtanga sangraha* (Srikanthamurthy 1992). To specifically direct the reader to the relevant part of these books and to highlight the extent of documentation available in these ancient texts, we have followed the convention described in table 1. Subsequently, we discuss three potential modes of synthesis of the principles of Ayurveda with contemporary genomic medicine, namely: (i) stratification of heterogeneous populations to enhance the effectiveness of genetic association studies, (ii) gene expression profiling to find molecular correlates of *tridosha*, the physiological entities responsible for understanding human individuality, and (iii) discovery of genetic variants that may contribute to development of phenotypes that are key to ayurvedic classification.

Ayurveda: translational medicine with systems approach

The personalized approach of Ayurveda begins with the fundamental understanding of interindividual differences in baseline health states, starting with their development *vis-a-vis* determinants and contributing factors. Responsiveness to environment and robustness of the systems are also largely determined by baseline health states. This, further feeds on to the variability in disease susceptibility, course of clinical presentation and progression (1: *C.I.1*, *C.Vi.8*; *C.Su.10* & *C.Vi.6*). These baselines of health also guide the recommendations made by ayurvedic practitioners regarding therapeutic regimens both for maintenance and promotion of health as well as for alleviation of disease conditions. The latter include interventions regarding the line of treatment, choice of drugs, and their dosage and mode of delivery, all of which are tailored to disease subtype, severity and stage (1: *C.Vi.8*).

Ayurveda also considers the dynamicity of an individual's environment, both internal (age, basic constitution, metabolic capacity, mental state, etc.) and external (time, place, season) for assessment of the levels of perturbation from his/her basic homeostatic state, and in turn the selection of drugs and dietary regimen (1: *C.I.1* & *C.Sa.6*). This, explains the use of word 'healthy' and 'diseased' instead of 'health' and 'disease' in the descriptions of *trisutra* in Ayurveda (1: *C.Su.1* & *C.Su.30*; 2: *S.Su.1*).

Tridosha: common organizing principle shaping human individuality

The interrelatedness of internal and external environment is due to a common origin, *panchamahabhuta* (five fundamental elements) whose biological effects in humans are explained in terms of a unifying organizing principle called the *tridosha* (3: *A.S.Su.20*). *Tridosha* perform all major physiological functions of the body starting from fertilization through development, continuing later in life until ageing and death. *Tridosha* comprises three ascertainable physiological entities, namely, *vata* (kinetic), *pitta* (metabolic) and *kapha* (potential) that are pervasive across systems, work in conjunction with each other, and respond to external

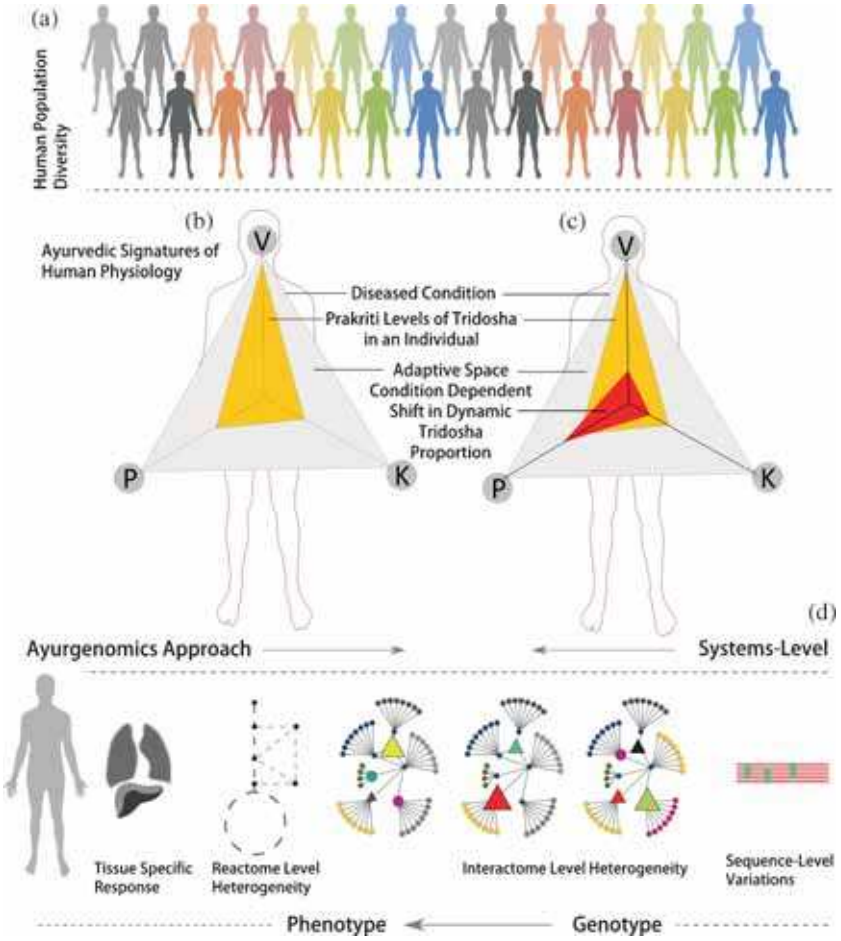

Figure 1. *Tridosha* as a common organizing principle in health and disease. Concept of personalized medicine of Ayurveda based on *prakriti* can allow integration of population genomic approaches (a) with system's understanding of physiological networks, (d) through the molecular analysis of common underlying principle of *tridosha* (b and c). Figures b and c represents the *tridosha* concept in human physiology. The three vertices of the triangle represent *vata*, *pitta* and *kapha* axes. Figure b represent the static state (*praakrit*) and c the dynamic (*vaikarik*) represented by yellow and red triangles respectively. The dynamic component can fluctuate along any of the three axes in response to intrinsic and extrinsic stimuli. Health state depicted as the adaptive space is constrained within the limit depicted by the grey triangle beyond which, is the diseased state. Disease onsets when the dynamic component crosses the threshold towards any of the vertices as depicted in b. Figure d depicts all levels of organization where *prakriti tridosha* proportions can bring about variability. An individual is more likely to cross the threshold of adaptive space in the direction of its own *prakriti*.

environmental conditions to maintain homeostasis (1: *C.Su.1* & *C.Su.18*; 2: *S.Su.21*; 3: *A.S.Su.19* & *A.S.Su.20*). Distinct properties and functions have been ascribed to each *dosha* (Prasher *et al.* 2008). For instance, *vata* (V) contributes to manifestation of shape, cell division, signalling, movement, cognition and excretion of wastes. It is also considered to be an initiator of the activities of *kapha* (K) and *pitta* (P) (1: *C.Su.12* & *C.Ci.28*). *Pitta* is primarily responsible for metabolism, thermoregulation, energy homeostasis, pigmentation, vision and host surveillance (1: *C.Su.12&18*; 3: *A.S.Su.19,20*). *Kapha* is responsible for anabolism, growth and maintenance of structure, storage and stability (1: *C.Su.12 & C.Su.18;* 3; *A.S.Su.1920*).

Each individual is born with a specific proportion of *tridosha* (V, P and K) that determines his/her basic constitution, which is termed their '*prakriti*' (1: *C.Vi.8*; 2: *S.Sa.4*; 3: *A.S.Sa.8*). The proportions of *tridosha* in the gametes of the parents at the time of fertilization contribute to the process of foetal development, and they shape and influence multisystemic phenotypic traits, including each person's responsiveness to extrinsic and intrinsic cues, thereby influencing their susceptibility to diseases (1: *C.Vi.8*; 2: *S.Sa.4*; 3: *A.S.Sa.8*; figure 2).

The proportions of VPK in a *prakriti* are not only genetically determined in the gametes (*shukra shonita*), but also influenced by diet, life style and age of the transmitting parents as well as maternal diet and environment during foetal development (*matur-ahar-vihara, kala garbhashaya, mahabhutavikara*). Genetic aspects are a cumulative effect of ethnicity (*jati-prasakta*), familial aspects (*kula-prasakta*) and geoclimatic (*desha-anupatini*) conditions including individual specific aspects (*pratyatmaniyata*). Geoclimatic conditions exert their effects during foetal development as well as on phenotypic manifestations in later life (figure 2).

Table 1. The convention followed for original textual references cited in the text.

Text name	Collective name of group of chapters (predominantly dealt subject)	Example references from all texts dealing with different subjects
Charaka samhita (C.)	*Sutra sthana* (*Su.*) (conceptual framework and abridged version of entire compendium)	*C.Su.1*, first chapter of *Charaka samhita sutra sthana*; *C.Vi.8*, eighth chapter of *Charaka samhita vimana sthana*
Sushruta samhita (S.)	*Sharira sthana* (*Sa.*) (anatomy, physiology and developmental biology) *Nidana sthana* (*Ni.*) (principles of etiopathogenesis including clinical features of diseases)	*S.Ni.1*, first chapter of *Susruta samhita, nidana sthana*
Ashtanga samgraha (A.S.)	*Vimana sthana* (*Vi.*) (principles of analysis for disease, drug and individuals for personalized management of health and disease)	*A.S.Sa.4*, fourth chapter of *ashtanga samgraha, sharira sthana*; *A.S.Su.20*, twentieth chapter of *ashtanga samgraha sutra sthana*
Chakrapani commentary (Ck)	*Indriya sthana* (*I.*) (clinical methods for assessment of prognosis and outcome of disease) *Chikitsa sthana* (*Ci.*) (principles of internal medicine including regenerative and reconstructive surgery)	*Ck on C.Su.7*= Chakrapani's commentary on seventh chapter of *Charaka samhita sutra sthana*
Dalhana commentary (Dl)	*Kalpa sthana* (*Ka.*) and *Siddhi sthana* (*Si.*) (pharmaceutical processing of medicinal formulations mainly for *panchkarma* (cleansing/regenerative) therapies)	Dl on *S.Sa.4*, Dalhana's commentary on fourth chapter *Susruta samhita sharira sthana*

These textual references are available in English translations in Sharma 1981, Sharma 1999 and Srikanthamurthy 1992 and have been referred with the number 1, 2 and 3 respectively in the text citations. For example '1: C.Vi.8' would be a citation available in reference '1' i.e. P.V. Sharma's English translation of Chapter 8 of *Charaka Samhita Viman Sthana.*

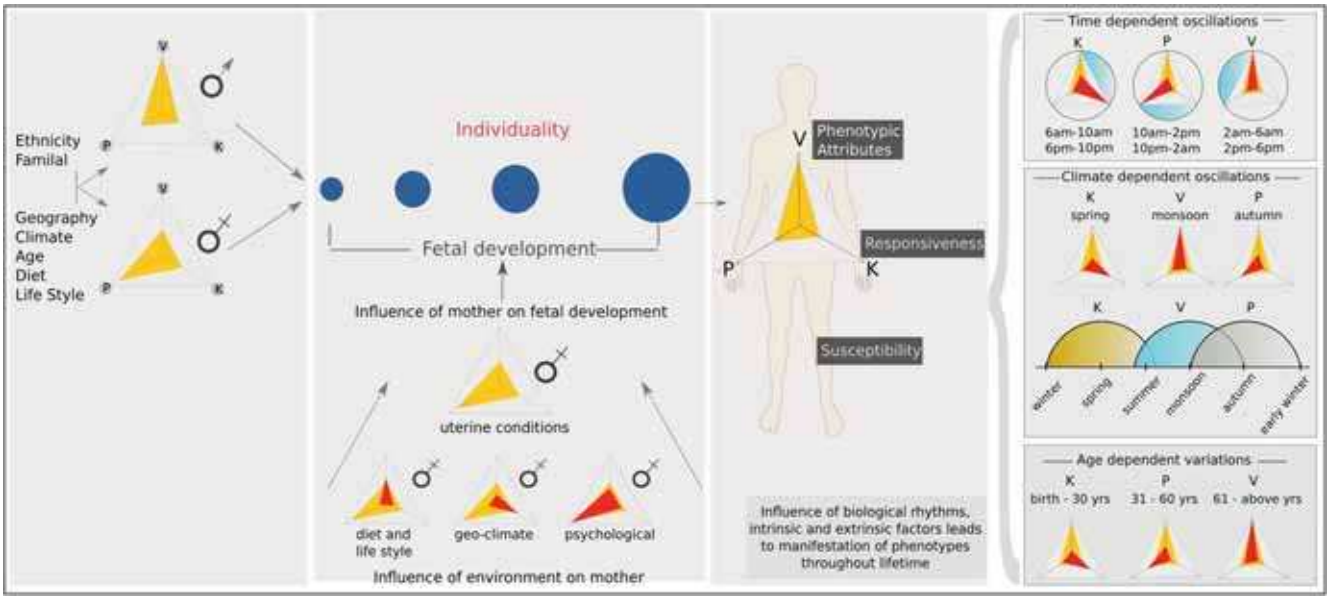

Figure 2. Mechanism of development and expression of *prakriti* in an individual with the factors governing them at different stages: prenatal, developmental and at later stages of life. The dynamicity of proportions of *tridosha* during day, age and season is depicted for a representative *vata prakriti.*

Thus, *prakriti* is a consequence of the cumulative effects of genetic and epigenetic factors, ethnicity, heritability, geo-climatic adaptations, age, time and season which have all been independently shown in modern times to affect human phenotypes and its responsiveness to external environment (1: *C.I.1* & *C.Vi.8*; 3: *A.S.Sa.8*).

If the VPK proportions exceed allowable limits of variability, it is possible that a viable foetus does not develop. Similarly, disproportions in localized systems/tissues may lead to malformations/developmental defects (*garbha vikriti*) of that system. The proportions of VPK that are physiologically viable only lead to development of foetus with corresponding *prakriti* (1: *C.Su.7* and Ck commentary ; 2: *S.Sa.4* and Dl commentary).

Tridosha at different levels of organization in development and health

Prakriti tridosha governs the entire process of development including building of the basic body tissues (seven *dhatus*). The *tridosha* and *dhatus* together build organs (*koshthanga*) and systems (*srotas*). Each organ system is developed with its own characteristic proportions of VPK and basic body tissues in accordance with their physiological functions (1: *C.Su.20* & *C.Ci.28*; 2: *S.Sa.4*; 3: *A.S.Su.19, 20*). The cumulative effect of differential proportions of *tridosha* (*prakriti*) would thus be reflected in multisystem attributes observable at various levels of anatomy, physiology and mental aptitude as well as in response to diet, environment, life style and stress. Thus variable states of health (*prakriti*) are an outcome of the different proportions of the *tridosha* during development, and these remain invariant in an individual. The observation that each organ system has its own proportions of VPK will likely complicate efforts to study the molecular correlates of *prakriti*, since it would presumably be necessary to perform genomic profiling on each tissue.

Phenotypic diversity, according to Ayurveda, is a consequence of a continuum of the relative proportions of VPK, resulting in seven possible constitutional types namely *vata* (V), *pitta* (P), *kapha* (K), *vata-pitta* (VP), *pitta-kapha* (PK), *vata-kapha* (VK) and *vata-pitta-kapha* (VPK). The first three are considered as phenotypic extremes, exhibiting readily recognizable phenotypes (table 2 and 1: *C.Vi.8*; 2: *S.Sa.4*; 3: *A.S.Sa.8*). It is worthwhile to emphasize that each of these states are healthy, as they are systems that have developed and tuned to adapt to the person's basal levels of VPK. However, perturbation from these levels predisposes people to specific diseases (2: *S.Sa.4*; 3: *A.S.Sa.8*; figures 1 and 3). For example, *vata prakriti* is likely to cross the thresholds of *vata* more readily than any other *prakriti* types (1: *C.Vi.6*). *Prakriti* assessment in an individual is carried out with the phenotypes described in Ayurveda and incorporated in questionnaire (Prasher *et al.* 2008). It takes into account the anatomy, physiology, metabolism, response to diet and environment, physical activities and movements, higher functions of brain and psychosocial behaviour etc. The comprehensive extent to which each of these attributes is examined is provided in table 3. This phenotype scaffold keeps in context the ancestry, genetic background and geoclimatic conditions as well as the age of the individual during assessment.

Dynamic component of tridosha: links between health and disease, in response to internal and external environment

In an individual, the VPK comprises both static and dynamic components. The proportions of VPK that govern the developmental process and shape the *prakriti* of an individual are fixed at the time of birth and are called *praakrit* (static) *doshas* (3; *A.S.Sa.8*) (figure 2). These levels of VPK are replenished through nutrition with specific diets that are suitable for maintenance of *prakriti* proportions of VPK (1: *C.Su.28*). The *doshas* continue to perform their physiological function throughout a person's lifetime and maintain homeostasis in harmony with the external environment (1: *C.Sa.4* & *C.Su.18*, *20;* 2: *S.Su.15*; 3: *A.S.Su.19,20*). VPK also oscillate with cues from sunlight (*surya*), moon (*soma*) and wind (*anil*), which are expressed as rhythmic/cyclical changes of day, night and seasons (*aho ratri ritu*) (1: *C.Su.12* & *C.Su.6*; 3: *A.S.Su.1* & *A.S.Su.4*; figure 3). Their levels are also influenced by internal rhythms such as sleep, stages of digestion and metabolism (*nidra, avasthapaka, nishthapaka*) as well as ageing (*vaya*) and other lifestyle practices (*Vihara*) (1: *C.Ci.15;* 3: *A.S.Su.1*). This dynamic component of the *dosha* is called *vaikarika*, and its patterns are determined by an individual's *prakriti tridosha* (*praakritaa vikrutanam beejbhoota*) (Srikanthamurthy 1992). This in turn is manifested as rhythmic and temporal expression of *prakriti* phenotypes apportioned to the time (*kalanupatini*) as well as age (*vayonupatini*) components (figure 3). These temporal *doshic* proportions also contribute to interindividual differences in the overall physical and mental strength, tolerance for exercise, dietary habits, metabolism and excretion tendencies (table 2). For instance, as an effect of old age, *vata* increases in every individual. However, a *vata prakriti* person would exhibit the effects of elevated *vata* such as erratic metabolism and excretion, lack of sleep etc., more intensively and the onset could also be earlier. Six basic tastes (sweet, sour, salty, bitter, pungent and astringent) have been ascribed to food and drugs wherein, a group of three tastes are described to increase each *dosha* and the remaining three decrease them, thereby making them suitable or unsuitable for corresponding *prakriti*. For instance bitter, astringent and sweet keep *pitta* in check whereas sour, salty and pungent items are not suitable for *pitta* and accordingly ayurvedic practitioners make dietary recommendations with these tastes in mind (1: *C.Su.1*, *C.Vi.1*, *C.Su.7* 18: *A.S.Sa.8*). Also the suitability of season, geography, food and climate vary amongst *prakriti* types and the peak activity hours also differ between *prakriti*. Just like the circadian rhythms, the proportions of *tridosha* vary temporally throughout the day with different peak times of *vata*, *pitta* and *kapha* in a 12 h cycle and also with the seasons (figure 3). Importantly, the *tridosha* are restrained within normal limits in health while perturbations beyond an individual's threshold (*praakrit* plus allowable *vaikarik* component) lead to diseased (*vikriti*) states (3: *A.S.Sa.8*). The robustness or fragility of the system depends upon whether a person has balanced or extreme *dosha* proportions in the basic constitution respectively. This

Table 2. Distinguishing features of predominant *prakriti* types: phenotype scaffolds (1: *C.Vi.8*; 2: *S.Sa.4*; 3: *A.S.Sa.8*).

Features	*Vata*	*Pitta*	*Kapha*
Anatomical			
Body frame	Thin	Medium	Broad
Body build and musculature	Weakly developed	Moderate	Well developed
Skin	Dry and cracked	Soft, thin, with tendency for moles, acne and freckles	Smooth and firm, clear complexion
Hair	Dry, thin, prone to breaks	Thin, oily, early graying	Thick, smooth and firm
Physiological			
Appetite for food and digestive capacity	Frequent, variable and irregular food habit	Higher digestive capacity for food and water consumption	Regular and stable food habits with low amount; lower digestive capacity
Taste preference	Sweet, sour, salty	Sweet, astringent, bitter	Sweet, bitter, pungent and astringent
Thirst and perspiration	Variable	High	Low
Body odour	Mild	Strong	Very less
Body temperature	Low or variable	High	Low
Weight gain	Difficulty in gaining	Gain and loose easily	Tendency to obesity
Bowel habit tendency	Irregular towards constipation	Loose motion	None
Sleep (amount and quality)	Less and shallow	Moderate/sound	High and deep
Physical activities and movements	Excessive and brisk	Moderate/spontaneous	Less mobile
Tolerance for seasonal weather	Cold intolerant	Heat intolerant	Endurance for both
Metabolism of toxic substances	Moderate	Quick	Poor
Ageing	Fast	Moderate	Slow
Higher mental functions			
Communication	Talkative	Sharp, incisive communication with analytical abilities	Less vocal with good communication skills
Initiation capabilities	Quick, responsive and enthusiastic	Moderate, upon conviction and understanding	Slow to initiate new things
Memory	Quick at grasping and poor retention	Moderate grasping and retention	Slow grasping and good at retention
Disease susceptibility, resistance and healing power			
Disease predisposition/poor prognosis	Developmental, neurological, dementia, movement and speech disorders, arrhythmias	Ulcers, bleeding disorders, skin diseases	Obesity, diabetes, atherosclerotic conditions

Distinguishing features of predominant *Prakriti* types: phenotype scaffolds (1: *C.Vi*.8; 2: *S.Sa*.4; 3: *A.S.Sa*.8). The numbers 1, 2 and 3 refer to the English translations of Sharma 1981, Sharma 1999 and Srikanthamurthy 1992, respectively.

resonates with the fundamental strategies of physiological adaptation in living organisms (Baffy and Loscalzo 2014). The three most contrasting types (*pitta*, *vata* and *kapha*) are the most vulnerable as they are likely to cross the thresholds of normal limits more readily (1: *C. Su.7* & *C.Vi.6*). It can be seen that the assignment of an individual's *prakriti* represents predictive health in contemporary terminology.

Tridosha in disease development: systems understanding

Baseline *prakriti tridosha* (homeostatic state) in an individual is a prelude for dynamic states of health in response to extrinsic as well as intrinsic factors and evolution of diseased states (perturbed state of *doshas*). Correspondingly, the development and progression of different diseases depends upon the origin (*Hetu*) and mechanism of perturbation

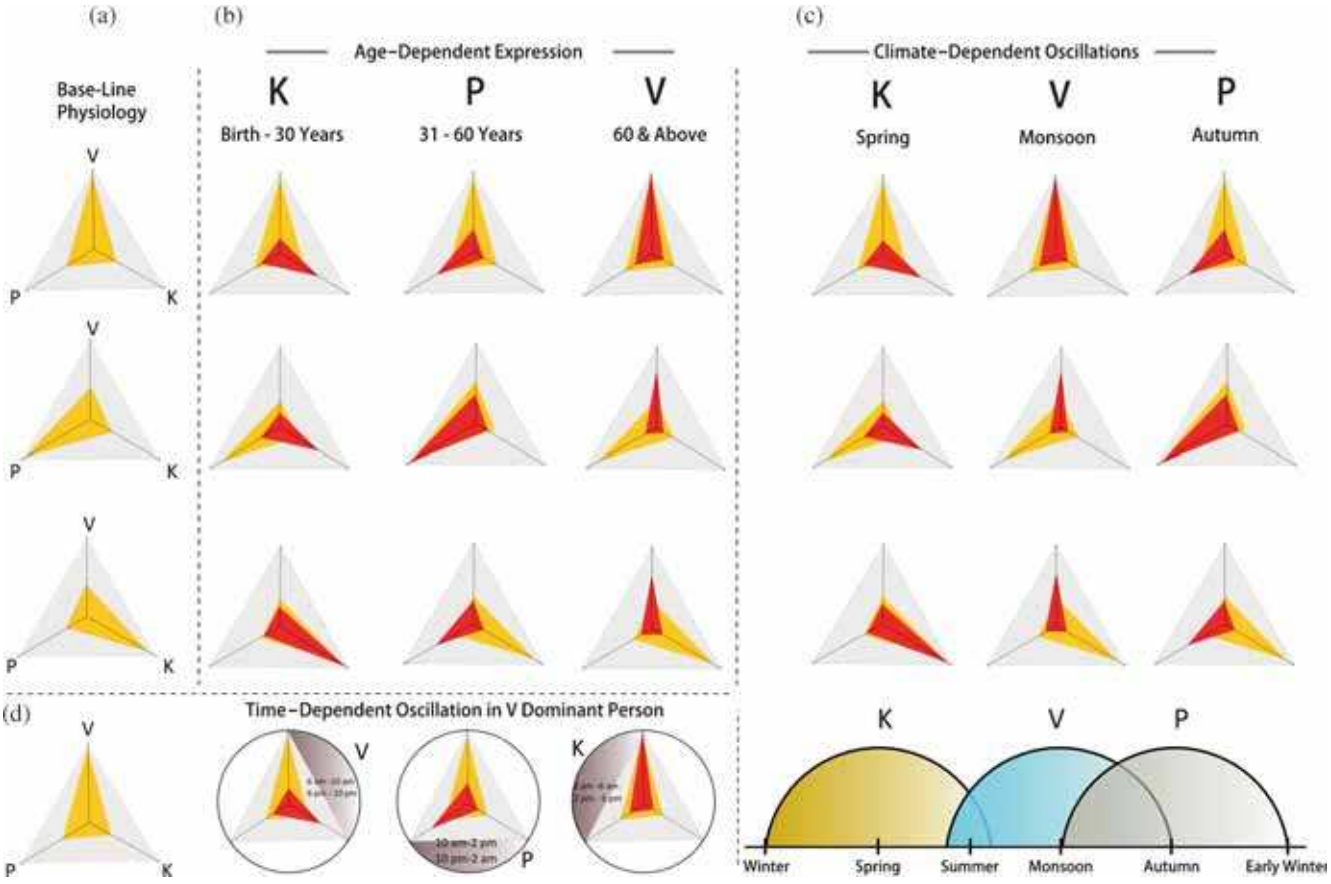

Figure 3. Spatio-temporal dynamics of *tridosha*. (a) Three baseline patterns of *tridosha* in *vata*, *pitta* and *kapha* dominant *prakriti* types. (b) Age-dependent expression of *kapha*, *pitta* and *vata* determined by baseline *prakriti doshas*. This is not equal for all *prakriti* as depicted by the size of red triangle. (c) Temporal responsiveness of *tridosha* to different seasons in a rhythmic manner. The gradual rise, peak and fall of VPK in different seasons is also depicted (lower panel). (d) Time-dependent oscillation of VPK during 24-h day night cycle in a *vata* dominant individual. The shaded area denotes the peak time of *dosha*. The static state (*praakrit*) and the dynamic (*vaikarik*) are represented by yellow and red triangles, respectively.

(*Samprapti - Vyadhi janaka dosha vyapar vishesha*) of *Doshas* and their site of interaction and manifestation (*dushya* and *sthana*) (1: *C.Ni.1*). The process of disease development takes six major steps (*shat kriyakaala)* and exhibits some preclinical features that provide opportunities for intervention at every step. The steps include initiation of *dosha* accumulation (*sanchaya*), *dosha* aggravation at a site (*prakopa*), spread of *dosha* into other tissues/systems (*prasara*), their interaction and effect on local target systems (*sthana samshraya*), leading to disease manifestation (*vyakti*), and further differentiation (*bheda*) (2: *S.Su.21*).

Initiation of the disease process and its development into specific subtypes depend upon the nature and severity of external etiological factors and their interaction with VPK. This leads to perturbation of specific aspects of *dosha* or their combinations. The origin and mechanism of perturbation of *doshas*, strength and kinetics of their interaction with body tissues/organs and systems are the major determining factors for clinical manifestation of disease, its severity and progression. This again depends on baseline robustness or vulnerability of the target systems as well as specific affinity of etiological factors to target them. Other influencing factors include the time and place during which the *dosha* involved might assume strength as a natural consequence of its rhythmicity (1: *C.Su.28* & *C.Ni.4*).

In the absence of intervention at any of these steps, perturbation continues to progress until the disease has manifested, or until advanced complications (*upadrava*)/comorbidities (*vyadhi sankara*) appear, through emerging entropy of *tridosha* and involvement of other systems in the process (1: *C.Ni.8*). Interestingly, comorbidities have also been described as an outcome of faulty management of disease without proper consideration of personalized aspects of clinical medicine (1: *C.Ni.8*). This is in contrast to the common belief that traditional medicines can all be administered 'off the shelf' without any concern for their side-effects.

While this conceptualization forms the common underlying principle of pathogenesis, each disease has been described to have a specific set of etiological factors, *dosha* subtypes, with characteristic involvement of particular organ systems leading to presentation of defined clinical phenotypes and advanced complications. For example, *prameha*, a broad group of disorders that include diabetes, can be caused by *kapha* with 10 subtypes, *pitta* with six subtypes, and *vata* with four subtypes, giving different clinical presentations in colour and odour of the urine (1:*C.Ni.4* & *C.Ci.6*). Also, in

Table 3. Details of attributes included in the questionnaire for assessment of different phenotypic aspects of *prakriti* (1: *C.Vi.8*; 2: *S.Sa.4*; 3: *A.S.Sa.8*).

A. Clinical assessment parameters for physical body parts		
Shape, symmetry, size, height/ length/breadth	Overall body frame, as well as individual body parts	Head, forehead, face, eyebrows, eyes, lips, jaws, teeth, hands, palms, legs, soles, joints, shoulder, chest, nails, etc.
Appearance	Skin	Lustrous, wrinkled, moles, marks, pimples, freckles, cracks, etc.
	Eye	Shiny/dim/dry or dull Sclera with milky white, reddish tinge or muddy appearance
Colour	Teeth	Milky white/yellowish/dull or blackish
	Eye	Light brown/dark brown/black/grey/green/ blue
	Palate, palms and soles	Dark/reddish/pale yellow/pink
	Skin	Fair/dark/reddish/pale yellow/pink/wheatish/golden/fresh colour/dusky
	Body hair	Black/dark brown/light brown/dusky/copper/ blonde
	Scalp hair	Black/dark brown/light brown/dusky/copper/blonde
Nature	Skin	Dry/oily/normal/seasonal or variable Type: thick/thin
	Lips, palm and sole	Smooth/soft/rough; firm; cracked; wrinkled
	Nails	Smooth/soft/rough; firm; cracked (brittle); flat/convex
	Scalp hair	Dry/oily/normal/seasonal or variable
Texture	Skin	Smooth/rough/coarse Firm/loose soft/hard
	Scalp hair	Type: thick/thin; straight/wavy/fizzy or curly Feel: coarse/smooth; soft/hard
Response	Scalp hair	Graying/falling/breaking
Growth/ bulk		Dense/moderate/scanty; baldness (yes/no)
B. Physiological parameters: body metabolism and excretion-related clinical parameters		
Metabolism	Appetite, thirst and digestive power	Frequency, regularity, amount, variability in response to diet
	Bowel and bladder habits	Frequency, regularity, amount, variability in response to diet
	Body temperature, prespiration and odour	Low, medium, high, variable
Sleep	Quantity	Low, medium, high, variable
	Quality	Shallow, deep, sound
Body weight changes		Frequent fluctuations, difficulty in gaining, difficulty in loosing
C. Response to diet and environment		
Food	Like/do not like Suit/do not suit	Sweet/sour/salty/bitter/pungent/astringent; cold/warm; dry/oily
Weather	Preference Health problem	Temperature/humidity
Season	Preference Health problem	Summer/early winter/late winter/autumn/ spring/rainy/none/season transition
D. Physical activities and movements		
Walking and working	Speed, style/accuracy, amount and quality Eyes, eyebrows, jaw, lips, tongue, head, shoulder, hands, legs	
Overall strength assessment	Physical, mental, resistance power, healing power	
E. Voice, speech and communication		
Voice	Quality	Low/feeble/weak/broken/rough/deep/good tone/sharp/clear/high pitched/loud/soft, pleasing
	Amount	Less/moderate/excessive
Speech	Speed	Quick or fast or brisk/medium/slow/variable
	Consistency	Consistent/inconsistent/moderate
Content of speech	Thoughtfulness	Well guarded well thought of/ wavering easily deviated/ sharp accurate spontaneous

Table 3 (*contd*)

	Style	Convincing/argumentative/sweet & pleasing to ears/avoid confrontations/deviated from main topic/irrelevant in between
F. Higher functions of brain and psycho-social behaviour		
Speed	Memorizing, forgetfulness, recalling, initiation, making new friends, anger, irritability Retaining, planning, execution, achieving ends, retaining friends, anger, forgiveness, generosity, faith and beliefs	
Memory type	Olfactory, auditory, tactile, gustatory, visual	

Details of attributes included in the questionnaire for assessment of different phenotypic aspects of *prakriti* (1: *C.Vi.8*; 2: *S.Sa.4*; 3: *A.S.Sa.8*). The numbers 1,2 and 3 refer to the English translations of Sharma 1981, Sharma 1999 and Srikanthamurthy 1992 respectively.

spite of the existence of 20 different varieties of *prameha*, this group of disorders analogous to metabolic syndrome has the initial involvement of *dosha* (*kapha*) and premature fat/adipose tissue (*bahu abaddha meda*) as the seat of pathogenesis, which can then take alternate trajectories to different subtypes. Ayurveda examines the variability in disease not only in terms of stage and severity, but also differences in triggers causing onset of pathogenesis that could result in specific subtypes of the disease (1: *C.Su.19*, C.*Ni.1* & *C.Vi.8*). This is in contrast to the practice of modern medicine where the clinical symptoms are analysed in great detail, but baseline variability of the individual as well as the initial triggers of pathogenesis leading to differential course of disease are not well understood.

Tridosha and prakriti

Personalized management of health: Ayurveda advocates special types of therapy for enhancement of regenerative potential (*rasayana*) and reproductive health (*vajikarana*) of an individual (1: *C.Ci.1*). These therapies are administered only after cleansing the body of toxins, and accumulated *tridoshas*. Dynamic states of health are maintained through personalized recommendations of diet, exercise, rest, sleep and other lifestyle practices with respect to time and amount based on an individual's *prakriti*, also considering his or her age, place, season etc. (1:*C.Sa.6* & *C.Vi.1*). This includes periodic cleansing of the body during rhythmic peaks of VPK (*kapha* during spring; *pitta* during autumn and *vata* during monsoon season, figure 3) to prevent accumulation of excess *tridoshas* and other excretory metabolites (*malas*) (1: *C.Su.6* & *C.Su.7*; 2: *S.Su.6*). This is carried out following specific protocols which include a preparatory phase for the system to expel the toxins, followed by a postprocedural care that ensures the proper restoration and rejuvenation (1: *C.Su.15* & *C.Si.1*). This preemptive approach of Ayurveda towards maintenance of health is aimed at preventing the manifestation of diseases to which an individual is predisposed (*ajaatanam vikaranam anutpatti*) (1: *C.Su.7*).

Rasayana therapy by definition is meant to enhance the strength and robustness of the systems by augmenting their cellular functions (1: *C.Ci.1*; 2: *S.Ci.27*). It improves the higher functions of brain and mental faculties like cognition, memory, speech, intelligence, etc. It thus acts as a preventive therapy for ageing and age-related disorders, increasing the longevity of an individual. This at times is also administered in the advanced stages of diseases where the recovery from them is expected to come through tapping the regenerative potential of the system rather than through corrective mechanisms of drugs (Sharma 1981; Srikanthamurthy 1992; Sharma 1999).

Personalized management of diseases: The goal of medical treatment is the alleviation of disorder in a manner that does not provoke pathogenesis in others or disturb healthy tissues. For this purpose, Ayurveda describes clinical examination points pertaining to disease and diseased individual, by a physician, to analyse not only the nature and strength of disease as well as that of the individual to select the appropriate line of treatment and usage of drug (1: *C.Vi.1* & *C.Vi.8*; 2: *S.Su.35*). Thus a triad of disease, diseased and drug, (*roga, rogi, aushadha*) as described below is analysed for delivery of personalized medicine:

- Examination of variables related to disease (*vikriti parikSha*) which include presentation of clinical subtypes, severity and stage (*vyadhi avastha*), strength and multiplicity of triggers, etiological factors both extrinsic (*hetu*) and intrinsic (*dosha-dushya*).
- Diseased individual-related baseline *prakriti*, suitability towards therapy and drugs (*satmya, agni, koshtha*), age of the individual.
- Present status of health-physical (*deha bala-sara, vyayama shakti* etc.), psychological (*chetas bala*) and individual's present status of metabolism and waste clearance organs (*agni bala* and *koshtha*), including external environmental factors like geoclimatic (*desha)* and time (*kaala)*.

Ayurveda has also laid down guidelines for integration of a personalized approach even in the process of development of drugs (1: *C.Vi.8*). This is done through basic understanding of nature and activities of drugs, employment of pharmaceutical processes for development of specific dosage form keeping in view of the desired biological activity, with methods for their storage and preservation (1:*C.Su.4*, *C.Su.26*, *C.Ka.1-12*). The details of specification with which a pharmacological action of the drug is to be understood is as follows: 'such a drug formulation when administered in a given dose, for specific type of disease, in a particular individual, will bring the perturbed *dosha*

back to homeostasis either through excretion or titration' (1: *C.Vi.8.87*). Concurrent advancements in the area of pharmaceutical sciences and pharmacology enabled the physicians to make personalized variations also in the delivery of therapeutics with respect to routes, time and modes of administration.

Trisutra thus is an operational framework of network medicine both in the context of healthy and diseased. It integrates the understanding of networks in physiology in the context of spatio-temporal dynamics and translates them for the development of predictive as well as personalized preventive and therapeutic medicine.

Integration of *Trisutra* framework to systems biology and network medicine

Understanding human individuality: threading dimensions of variability

The unanticipated extent of human genome variation as catalogued in numerous genomic databases has nearly ruled out the possibility of defining or reconstructing a reference healthy human from mere reading of the genomic sequences (The 1000 Genomes Project Consortium 2012; Olson 2011, 2012). There are now nearly 38 million SNPs, 1.4 million indels, 14 k deletions and 20 k structural variations represented in the variation databases (The 1000 Genomes Project Consortium 2012). The majority of the common variations are shared across world populations (The 1000 Genomes Project Consortium 2010, 2012). However, the frequencies of these variations differ between populations and among individuals as a consequence of migration, admixture, natural selection, pathogen load or cultural practices (Tishkoff and Verrelli 2003; Tishkoff *et al*. 2007; Coop *et al*. 2009; The 1000 Genomes Project Consortium 2010; Jablonski and Chaplin 2010; Fumagalli *et al*. 2011; Hancock *et al*. 2011; Patterson *et al*. 2012; Fu and Akey 2013). Genetic variability gives rise to enormous combinatorial possibilities whose effects ramify through the genetic, transcriptional, biochemical, proteomic, metabolic and higher order physiological network levels, impacting the entire health of an individual (Jeong *et al.* 2000; Rual *et al.* 2005; Stelzl *et al.* 2005; Pan *et al.* 2008; Park *et al.* 2009; Hawkins *et al.* 2010; Lusis and Weiss 2010; Vidal *et al.* 2011; Baryshnikova *et al.* 2013). The networks are also responsive to external and internal cues (Hancock *et al*. 2008, 2011; Coop *et al*. 2009; Jackson and Bartek 2009). Further dimensions to this variability is added by epigenetic changes and the enormous human microbial diversity (Dolinoy and Jirtle 2008; Human Microbiome Jumpstart Reference Strains Consortium 2010; Human Microbiome Project Consortium 2012; Cho and Blaser 2012; Pflughoeft and Versalovic 2012). In addition to heritable variation, it is now acknowledged that an individual's genome is also patterned by a large number of prezygotic *de novo* mutations, whose incidence is influenced by the paternal age effect (PAE), as well as by transgenerational epigenetic inheritance (Goriely and Wilkie 2012; Kong *et al.* 2012; Sharma 2013). Each individual is thus an ecosystem harbouring a unique subset of variations and the phenotype of an individual is the net outcome of the ecosystem. A major challenge of systems biology is to differentiate meaningful and functional variations from the neutral ones, comprehend their cumulative effects at the systemwide level, thereby linking them to phenotypes (Olson 2011, 2012; Gibson and Visscher 2013; Visscher and Gibson 2013).

Major efforts have been made to link variation information to many quantitative phenotypic traits like skin pigmentation, anthropomorphic features, physiological and metabolic attributes and human adaptations (Katzmarzyk and Leonard 1998; Hancock *et al.* 2008; Jablonski and Chaplin 2010). All such studies have provided a catalogue of genes and linked biological processes as well as pathways, many of which are assumed to shape human phenotypic attributes (Blake *et al.* 2009; Robinson and Mundlos 2010; Suhre *et al.* 2011). However, how they shape or integrate systems is still not well understood.

Another aspect gaining importance is differences in the association of biological variability with temporal cues such as circadian, seasonal and other biological rhythms in an individual (Roenneberg *et al.* 2007; Okamoto-Mizuno and Mizuno 2012). It is now well established that there are different biological clocks which are primarily set through the suprachiasmatic nucleus (SCN) and mediated by per-arnt-sim (PAS) domain containing proteins, while distinct ontological processes have been observed to be enriched at different times of the day (Merrow *et al.* 2005). Circadian rhythms are intricately linked to metabolic homeostasis which in turn is important for maintenance of cellular rhythmicity. Studies have revealed that there are inter-individual differences in rhythmicity of expression of genes during different times of the day, season as well as with age (Touitou *et al*. 1986; Okamoto-Mizuno and Mizuno 2012; Chua *et al*. 2013). For instance, variations in clock genes that associate differently with sleep duration have been associated with different human chronotypes who have eveningness or morningness tendencies (Katzenberg *et al*. 1998; Roenneberg *et al*. 2003; Wittmann *et al*. 2006). A recent RNA sequencing study carried out on individuals throughout the year have identified genes that exhibit different patterns of expression during different seasons (Dopico *et al.* 2015).

Inter-individual differences in diurnal and metabolic rhythms leading to different metabotypes have also been reported (Morgan *et al.* 1998; Chua *et al.* 2013). It has also been shown that intake of a high-fat diet not only disrupts the normal circadian cycle but also causes a large scale genesis of *de novo* oscillating transcripts, resulting in reorganization of the coordinated oscillations between coherent transcripts and metabolites (Hatori *et al*. 2012; Eckel-Mahan *et al*. 2013). Besides nutrition, irregularities in sleep, temperature and so forth, lead to disruptions in these rhythms and have been associated with susceptibility to diabetes, obesity and cardiac disorders (Morgan *et al.* 1998; Wittmann *et al.* 2006; Okamoto-Mizuno and Mizuno 2012). Genetic variants are also linked to differences in the interaction between genes, environment and nutrition (Lampe *et al.* 2013; Sales *et al.* 2014). Many genes that are responsive to temporal as well as

environmental and nutrition factors also overlap with those that shape human phenotypes and physiology. Despite this huge body of information on genes, variation and phenotypes, the connectivity from genetic determinants to phenotypic attributes of a system, its robustness and responsiveness to perturbations in an individualized perspective is still lacking. Understanding human individuality would be a key to defining baseline health states that is a prelude to preclinical and diseased states. This would enable development of individualized tailored therapies that could help manage health and disease.

East meets West: why prakriti should have genomic correlates?

There is much resistance and skepticism concerning the idea that concepts of individuality as well as the basis for personalized medicine in Ayurveda can be explained by contemporary views of gene function and notions of causality in disease. We see no fundamental contradiction between the traditional medical practices outlined here, and genomic medicine, and in fact consider it natural to expect that there will be molecular genetics reason for the existence of *prakriti*. One objective of personalized medicine is to classify individuals with respect to their risk of disease, which is exactly the basis of ayurvedic practice. It may be argued that Western medicine assumes that clinical traits are somewhat independent and continuous such that there is no broad categorization of risk across multiple domains of health. However, we increasingly recognize that some people are at high risk for metabolic disease or immune disease or mental health problems. These categorizations are not necessarily equivalent to those based on the *dosha*, but they point to an increasing recognition that it may be possible to identify subclasses of healthy people with subclinical tendencies.

From the reverse perspective, if we accept that in Ayurveda, which has been practiced in India for over 5000 years, people adopt different diet/lifestyle practices and even places and season most suitable to their *prakriti* to remain healthy, then it is beholden upon us to look for the underlying biochemical and physiological basis of this individuality.

Strong candidates could be for instance, differences in metabolic activity particularly relating to the flux of gluconeogenesis, oxidative phosphorylation, lipogenesis and protein biosynthesis; differences in the counts and state of activity of diverse blood, immune and neuronal cell subtypes; and variation in endocrine, cytokine and other systemic signalling systems. Variability in these core pathways contribute to physiological differences between individuals and connect to all major diseases. It is anticipated that these could also differ between *prakriti* as they resonate with the physiological attributes of *vata*, *pitta* and *kapha*. All of these can be studied with high-throughput techniques such as transcriptomics, metabolomics, lipidomics and flow cytometry. Indeed, it is becoming apparent that there is very strong structure to gene expression profiles such that suites of hundreds or even thousands of genes are coordinately regulated, and relatively stable over time, unless perturbed under conditions of disease (Chaussabel *et al.* 2010; Chen *et al.* 2012; Preininger *et al.* 2013).

Finally, as outlined above, the *prakriti* are strongly determined at birth as a result of mixing of parental contributions, which we can now interpret as genotypes. It seems to us perfectly reasonable to suppose that given a large enough sample size, likely in the hundreds of thousands given the scope of contemporary meta-analyses that genomewide association studies (GWAS) would start to identify genetic variants that associate with *prakriti*. In the following, we review the one which is already discovered variant in EGLN1 that was identified through a candidate gene approach to fit this expectation. Although it is clear how genotypes can regulate individual endophenotypes, it is not so conceptually straight forward to appreciate how genotype effects combine together to yield a small number of somewhat distinct physiological states. This is where network biology needs to be integrated with classical statistical genetics, which largely focusses on the additive effects of individual genotypes on individual traits.

Human phenotype phasing: need for reference scaffold

To comprehensively define a healthy individual in a population, approaches are needed that can help apportion an individual's phenotypic variability into phenotypic phases that could be (i) explained by ancestry, heritability, geographical and climatic adaptations, (ii) due to *de novo* events, or (iii) shaped by diet, environment and life style factors. Phenotypic phasing on these lines would reduce the dimensionality of human variation to fewer axes of expression linked to regulatory networks and aspects of human physiome.

It is evident that although the reductionist approach of understanding the consequence of a genetic variant in a model system often provides valuable mechanistic insights, it is not contextual and is usually unable to provide explanation for the whole integrated system (Noble 2002, 2008; 2011; Auffray *et al.* 2009; Loscalzo and Barabasi 2011). However, it is also true that with much complexity and connectivity in readouts, identifying the parts that comprise the whole is even a bigger challenge of integrating information of different dimensions and scales across various cellular hierarchies (Loscalzo and Barabási 2011; Olson 2012). This problem of system biology is not trivial. Assembling a system from its parts to reconstruct human phenotypes is analogous to, if not more challenging than, sequencing and *de novo* assembly of a genome. The task of this assembly becomes relatively easier in the presence of a reference scaffold (Olson 2012). However, at present there are no phenotypic scaffolds available and threading the networks from the genome to a phenome is mostly a heuristic exercise.

Prakriti scaffolds for defining human individuality

Ayurveda phenotyping provides comprehensive analysis and classification of individuals into seven broad *prakriti* groups. These phenotype scaffolds could allow identification and assembly of physiologically connected variations that can

anchor networks from different scales and explain human individuality. As described above, the diverse aspects that have been dealt and observed independently to affect human phenotypes and its responses are all considered cumulatively and threaded together in defining an individual's *prakriti*.

The second major aspect of this approach as described in the earlier sections is the inherent weight given to background factors that are considered during *prakriti* assessment. For instance, assessment of height, skin pigmentation and other traits are considered in the context of the baseline of the population from a particular ethnic background, ecocline and from a particular age group. Thus, a person who would be qualified as tall or short, dark or fair, is considered in the context of the population background and not just as an objective measure. Appreciation for evolving relative measures of baseline values is gaining importance post GWAS (Yang *et al.* 2010; Olson 2012; Turchin *et al.* 2012). The most striking example is of height. There is nearly 80% heritability in height. GWAS on height in a quarter million individuals have identified over 400 loci that explains just 20% variance in height (Yang *et al*. 2010; Olson 2012; Turchin *et al*. 2012; Wood *et al*. 2014). Similar-sized studies of BMI and waist-to-hip ratio have uncovered even less of the genetic, ∼100 and 50 variants each respectively, explaining less than 3% of the phenotypic variance. While much has been written about the missing heritability problem, now it appears that one of the major problems is simply lack of statistical power to observe very small effect sizes.

It can be argued that one reason for the small effect sizes is environmental (or epigenetic) heterogeneity. We recently showed by simulation that if it is assumed that people live in two different environments (for example, some adopt a more healthy low calorie high activity lifestyle, others adopt the typical modern high calorie sedentary one), then genotype–environment interactions might readily be expected at the level of genetic risk scores (that is, the cumulative effect of alleles) even when it is not detectable for individual genotypes, which is overwhelmingly the case (Marigorta and Gibson 2014). Importantly, it also appears that under these mixed environment conditions, the power to detect genetic associations is decreased substantially because of the inflation of phenotypic variance in the mixed population. The point is that if in mixed environments, GWAS underperforms, the ability to stratify individuals into subpopulations that are more homogeneous should greatly increase the ability to discover variants, some of which may be specific to subpopulations. It should be apparent that *prakriti* can be considered as alternate environments within which genotypes affect disease risk. Consequently, we propose that phenotypic stratification based on principles of Ayurveda could feasibly increase the resolution of GWAS under some circumstances (Juyal *et al.* 2012). A second aspect for these low risk scores could be due to the difficulty in dissecting QTLs or more important response QTLs in cross-sectional studies. Since, *prakriti* of an individual also predicts the trajectory of responsiveness to environmental cues, stratifying individuals through *prakriti* could help resolve QTLs from a heterogeneous background. This can be tested through metaanalysis of large expression datasets using *prakriti* specific signatures.

Additionally, in meta-analysis of GWAS, it has been observed that power of association studies increases in magnitude when the results are weighed on the basis of selection signatures in associated variants (Kumar *et al.* 2011; Dudley *et al.* 2012; Karlsson *et al.* 2014). Thus, it is possible that many signals are already present in the data but the contextuality of these variations needs to be uncovered (Olson 2012). Many of these common variations have been linked to human phenotypes and diseases that are also considered during *prakriti* assessment. Characterization of different population cohorts by *prakriti* methods could facilitate improved identification of genomic polymorphisms linked to independent phenotypic attributes, and their assembly into phenotype scaffolds.

A third aspect that Ayurveda considers in assessing the *prakriti* of an individual is adaptability to environment, lifestyle, diet and drugs. Any deviation from an individual's *prakriti* level is the perturbed state for that person and the knowledge of the same is used for the treatment of the individual. Since specific treatment regimes are considered to balance VPK as described earlier, therapeutic interventions also need to be personalized. Thus, an individual serves as a control for himself, and deviation from his or her threshold provides an actionable point for each individual.

Various methods for phenotypic classification such as somatotypes, chronotypes, metabotypes and personality types are available (Sheldon *et al.* 1940; Myers and McCaulley 1988; Roenneberg *et al.* 2003; Chua *et al.* 2013). Axes of human gene expression variation on the basis of variability in peripheral blood RNA expression have also been reported recently (Preininger *et al.* 2013). In healthy adults, seven common axes of variation are consistently observed, and capture over half of the total variance in peripheral blood gene expression. Each axis involves the coordinated regulation of hundreds to thousands of genes that have functions enriched for subtypes of immune function, from T-cell or B-cell signalling to innate immunity and interferon response. Each of us have measurable average level of expression of genes in each axis, and these measures are relatively stable over time. In pilot experiments, we have observed some correspondence between these measures and *prakriti*, but more work is needed to establish the robustness of the correlations. It is also important to note that coordinated regulation of gene expression along the axes is observed in other tissues such as adipocytes and fibroblasts (Preininger *et al.* 2013), but the scores are only mildly correlated across individuals. This suggests that comprehensive matching of gene expression to *prakriti* would require profiling of multiple tissue types, which may not be practical. Certainly, a comprehensive assessment of individuality as described in Ayurveda that encompasses different systems and connects it explicitly to outcome in health and disease, not to mention their relation with personalized therapeutics, is not currently available (Prasher *et al.* 2008).

Linking human individuality to the natural evolution of disease states

There have been a number of surprises in the course of identification of genes involved in monogenic and complex disease even where heritability of phenotypes and disease is clearly observed (Loscalzo *et al.* 2007). For instance, the concept of a single gene explaining a substantial fraction of phenotypes in monogenic disorders has been challenged (Kato *et al.* 2007, 2009). This is exemplified by the study of variation in the β-globin gene implicated in sickle-cell anaemia. Though this is the single largest implicated locus in every sickle-cell patient, not all individuals carrying this mutation exhibit the same clinical phenotypes. Thus there is incomplete penetrance and variable expressivity (Raj *et al.* 2010). Roles for modifier genes interacting with the primary mutation, leading to intermediate pathophenotypes in different environment of hypoxia, infection and dehydration have been implicated in this phenomenon (Kato *et al.* 2007, 2009). Depending on the intermediate pathophenotypes there is a difference in the course of the disease in an individual leading to variability in symptoms like painful crisis, anaemia, stroke chest pain, infarction etc. With the 1000 genomes sequencing project it has become even more evident that predicting the phenotypic outcome of a pathogenic mutation is going to be a difficult exercise as a healthy individual on an average harbours nearly 100–200 predicted pathogenic variations (O'Roak *et al.* 2011). Some, perhaps the majority of these variants are false positive predictions but a large number of them certainly have variable penetrance consistent with extensive studies in model organisms (Polaczyk *et al.* 1998; Barkoulas *et al.* 2013; Chari and Dworkin 2013). All these factors reduce the predictive value of a primary mutation in the absence of knowledge of modifiers, whose effects will often be as complex as the genetics of traits such as height.

For complex diseases, a spate of GWAS has been successful in explaining only a minor fraction of burden in diseases. Notably, by definition, the explanation for risk to diseases by the associated variants is for population rather than the individual, i.e. it explains how many of the variation among individuals can be attributed to the genetic polymorphism, but does not say how many of the phenotype observed in any given person is due to their genotypes. GWAS have not only revealed that widely different pathways are linked to the same disease but also different diseases to the same genes with extensive cross talk between seemingly nonoverlapping pathways. These observations could be best explained by the existence of broad endo-phenotypes (corresponding genotypes) that are differently predisposed to more than one disease and evolve into more discrete disease states through a systemic response to stochastic and environmental cues. PheWAS studies and big data mining from EMR records are providing corroborating observations (Shah *et al.* 2009; Baryshnikova *et al.* 2013; Marx 2013). However, there is as yet no working road map to address health to disease transitions and to predict which state of health could take which course of disease. It is plausible that the thresholds for risk conferred by variations are individual specific (Gibson 2012). The average effects observed in GWAS do not necessarily predict effect sizes at the $N = 1$ level, as the same variant cannot effect in some people and a large effect in others, averaging to a small effect.

Since diseased individuals can be stratified on the basis of *prakriti*, the integration of *prakriti* could afford a type of phenotypic stratification that might facilitate identification of core components of variation that could determine the differential involvement of intermediate pathophenotypes and thereby the course of disease (Sethi *et al.* 2011). For instance, if reexamined from the perspective of Ayurveda, the pathophenotypes described for sickle-cell anaemia, such as painful crisis, thrombosis and haemolytic crisis, are also described as predisposed phenotypes of aggravated *vata*, *kapha* and *pitta* types respectively (1: *C.Su.20*; 2: *S.Su.21*). It is therefore possible that inherent susceptibilities might determine different phenotypic manifestation of the diseases and therefore the integration of *prakriti* concepts into preclinical condition might have a higher predictive value. It would be worthwhile to reiterate that *prakriti doshas* can confer differential robustness/susceptibility to the tissue or organ systems physiology. Therefore, the outcome of the samedisease in different *prakriti* can have different manifestations.

Ayurgenomics approach for integration of human individuality with P4 medicine

To integrate the *trisutra* concept of Ayurveda with genomic methods, the first step would be to use a shared vocabulary to denote the properties and interrelationships of these concepts (shared ontological descriptions) in the language of systems biology or modern network medicine. Therefore, we hypothesize that the *tridosha* assessed using *prakriti* methods of phenotypic characterization of healthy individuals can be associated with molecular and genomic correlates. As a first step in testing this idea, we have studied genetic, gene expression and biochemical profiles from peripheral blood amongst *prakriti* types, to analyse and probe the ontological links between *doshas* and molecular signatures (Prasher *et al.* 2008). Similarly, others have observed differences in immune cell type abundance associated with the *prakriti* (Rotti *et al.* 2014) and a recent study also reports epigenetic differences between *prakriti* types.

The study which was the first of its kind was carried out in unrelated healthy individuals of predominant *prakriti* belonging to an Indo-European genetic background from north India in an age group of 19–40 years (Prasher *et al.* 2008). Genetic homogeneity in terms of the ethnic background of these individuals was established by a set of genomewide neutral markers. Following this, genomewide expression profiling was carried out on these individuals and both the genders were analysed separately. At the biochemical level, there were significant differences in lipid profiles, liver functions, haematocrit, and blood clotting between the constitution types, albeit within normal range. This highlights that the normal range of biochemical parameter for different constitution types may be different, so also their

subclinical ranges. Significant differences with respect to genomewide expression were also observed between contrasting *prakriti* types. The genes that varied between the groups mapped to core biological processes such as the cell cycle, immune response, apoptosis and regulation of physiological processes, metabolism and haemostasis. Many of the differences resonated with the descriptions of Ayurveda (1: *C.Su.12*, *C.Su.18*). It has been observed that although interaction between genetic and environmental factors can lead to multiple diseases and diversity of clinical phenotypes, this may only happen through a finite number of intermediate pathophenotypes. Interestingly, all the processes that we observed to differ between *prakriti* types overlap with the intermediate pathophenotypes. The intermediate pathophenotypes have been implicated in determining the progression and subphenotypes in different diseases. This core result has been observed in a replication cohort from a different ethnic background (unpublished results).

It is thus possible that the baseline differences in healthy individuals which are captured through *prakriti* phenotyping could help us to classify individuals already at the preclinical stage before they take a specific course through intermediate pathophenotypes. Since *prakriti* concepts are linked to health management, knowledge of an individual harbouring a primary mutation with a given *prakriti* could also be useful in preventive aspects at the preclinical level. This assumes more importance in scenarios where there is lack of availability of appropriate drugs and the quality of life is severely compromised with the progression of disease. Early identification of perturbation could thus lead to preventive management during preclinical stages, for instance in the context of metabolic diseases such as atherosclerosis and type 2 diabetes or late onset neurodegenerative disorders.

We observed significant overrepresentation of hubs and housekeeping genes in the differentially expressed gene sets in the *prakriti* groups. Hub genes are central to gene networks and variability in them could impact a large number of functions and the genes linked in a network are good candidates to act as genetic modifiers (Barabási *et al.* 2011). Since the method of *prakriti* phenotyping captures multiple seemingly unconnected systems, genetic variation underlying *prakriti* could enable identification of hub genes that would have systemwide effects. Analysis of genetic variations in a subset of differentially expressed genes revealed a significant set that differed between the *prakriti* types. Incidentally, some of the genes that remained significant even after FDR correction were core regulatory genes (*FAS*, *AKT3*, *EGLN1*, *RAD51*, *FBN2*), variations in which are likely to impact multiple phenotypes and functions (Aggarwal *et al.* 2010). It is now thought that primary disease mutations are often members of peripheral nodes of disease gene networks that interact with highly connected hubs, as a consequence of which they have the capacity to contribute to diverse disease processes. Since mutations in hubs are often lethal, probing the involvement of hubs becomes a challenge. It is being realized that methods needed to identify individuals at the preclinical stage who are likely to progress differently to the intermediate pathophenotypes are not available (Goh *et al.* 2007; Vidal *et al.* 2011). We propose that integration of *prakriti* methods can help identify core and hub genes that connect to multiple system phenotypes and are also responsive to extrinsic and intrinsic cues. The readouts would be the intermediate pathophenotypes but at the preclinical stage that would decide the susceptibility and progression to diseases depending on the expression of the peripheral genes. Management of the disease would be more tractable if such interconnectivities are identified.

We followed up *EGLN1* (PHD2), a key oxygen sensor which differed between *pitta* and *kapha* both at the expression and genetic level and was associated with high altitude adaptation (97). *EGLN1* expression was lower in *pitta* compared to *kapha* and *vata*. The genotype linked to higher expression in *kapha* was overrepresented in individuals who suffered from high altitude pulmonary edema. The *pitta*-linked genotype which correlates with higher basal levels of HIF1a, was nearly fixed in natives of high altitude. The observation of this gene being linked to high altitude adaptation has since been replicated in many global populations (Pagani *et al.* 2012; Simonson *et al.* 2012; Bigham *et al.* 2013).

Maintenance of oxygen homeostasis is intricately linked to development, growth and survival of an organism (Semenza 2011). The majority of the cellular responses in hypoxia are mediated by a group of transcription factors called the hypoxia inducible factors (HIFs). EGLN1 belongs to the prolyl hydroxylase (PHD) group of proteins that under well-oxygenated conditions become active and hydroxylate HIF1a, which is then targeted for degradation by the von Hippel-Lindau (VHL) proteasomal machinery (Ivan *et al.* 2001; Greer *et al.* 2012). In hypoxic conditions, EGLN1 is inactive which in turn leads to stabilization of HIF1a. Activation of HIF1a leads to switching on of nearly 100–200 genes whose functions are required to cope with low oxygen conditions (Kaelin and Ratcliffe 2008) (figure 4).

From our observation of differences in *EGLN1* between *pitta* and *kapha prakriti*, as well as from the growing literature, we propose a conceptual similarity of *EGLN1* with the organizing principle of *tridosha*. Table 4 and figure 4 highlight the multiple dimensions and scales at which EGLN1 functions and lead us to argue that it satisfies the criteria (as described in section 'Ayurveda: translational medicine with systems approach') for it to be called a molecular equivalent of *tridosha*. Briefly, EGLN1 has a role right from foetal development through homeostasis in health and disease (Kaelin and Ratcliffe 2008; Cheng *et al.* 2014; Duan *et al.* 2014). Its rhythmic nature and responsiveness to intrinsic and extrinsic cues and autofeedback loop ensures its expression and activity in physiological limits set by its genetic make-up (Kaelin and Ratcliffe 2008; Hatori *et al.* 2012; Matsuura *et al.* 2013; Nguyen *et al.* 2013). Dysregulation of this gene has been linked to diseases of multiple systems (Ladroue *et al.* 2008; Sen Banerjee *et al.* 2012; Franke

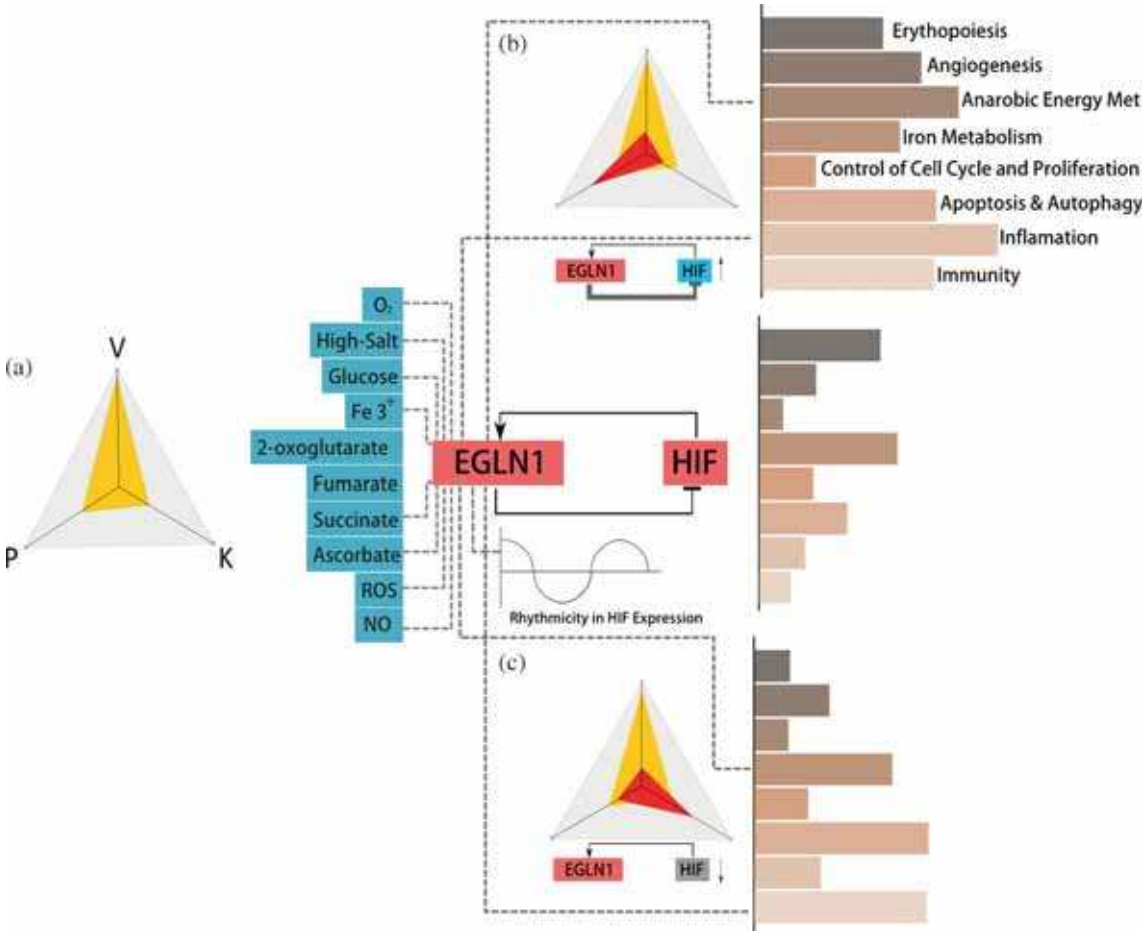

Figure 4. Determinants and dynamics of EGLN1-HIF axis. (a) Triangle represents the baseline conditions in which EGLN1-HIF axis normally operates with an auto-regulatory feedback mechanism in a temporal rhythmic manner. Ambient oxygen and cellular cues impact the EGLN1-HIF axis which in turn modulates a large number of pathways. (b) In low oxygen condition, where EGLN1 is inactive, HIF is stabilized leading to upregulation of pathways depicted. These resonate with the *pitta* functions. (c) In normoxic condition, EGLN1 is active which leads to degradation of HIF resulting in down-regulation of HIF targeted pathways. The illustration depicts the various aspects of EGLN1 given in table 4.

et al. 2013; Fujita *et al.* 2014). Model system studies indicate that the biphasic nature of the PHD-HIF could modulate the outcome of many diseases (Natarajan *et al.* 2006; Lee *et al.* 2008; Ahmad *et al.* 2012; Kiss *et al.* 2012; Sen Banerjee *et al.* 2012). The extent of modulation of these processes depends on the tissue, its oxygenation status, presence of cofactors, levels of PHD2 and HIF, and other tissue-specific transcriptional factors that work in conjunction with HIF. It has also been shown to be a therapeutic target for several diseases (Ziello *et al.* 2007; Haase 2010; Harten *et al.* 2010; Nagel *et al.* 2010; Ong and Hausenloy 2012; Kalucka *et al.* 2013; Selvaraju *et al.* 2013; Zhao and Wu 2013; Soni 2014). Lower expression and subsequent stabilization of HIF produces *pitta*-like molecular phenotypes whereas higher expression might regulate *kapha* attributes (Aggarwal *et al.* 2010; Simonson *et al.* 2012).

Differential responsiveness to drugs is not only due to differences in drug metabolizing enzymes or transporters (pharmacokinetics), but also attributed to genetic differences in the target proteins (pharmacodynamics) and more importantly due to differential involvement of those targets/pathways in pathogenesis of the disease occurring in an individual. This is exemplified by the *EGLN1* study, where different disease conditions modulation of its expression can either favour recovery like in ischaemia or aggravate the disease as in cancer. Genetic differences leading to inherent differences in expression of this gene could not only modulate the disease but could also be relevant in deciding the optimum dosage for management of the disease. This is substantiated by our recent observation wherein we have demonstrated a genetic link between *EGLN1* and *VWF* variations which can modulate the thrombotic outcome in response to hypoxic condition (Aggarwal *et al.* 2015). This functional link assumes importance both in high altitude adaptation as well as conditions of cellular hypoxia. Thus, integration of our understanding of the principles of Ayurveda in drug discovery development

Table 4. *EGLN1* as a molecular correlate of *tridosha*. The table depicts the various characteristics of *tridoshas* which are fulfilled by the functions of *EGLN1* at the molecular level (Ivan *et al.* 2001; Natarajan *et al.* 2006; Ziello *et al.* 2007; Ladroue *et al.* 2008; Lee *et al.* 2008; Aggarwal 2010; Harten *et al.* 2010; Semenza 2011; Ahmad *et al.* 2012; Kiss *et al.* 2012; Sen Banerjee *et al.* 2012, Franke *et al.* 2013; Kalucka *et al.* 2013; Matsuura *et al.* 2013; Selvaraju *et al.* 2013. Duan *et al.* 2014; Fujita *et al.* 2014; Soni 2014).

Tridosha concordance	Functional attribute	Physiological activity	Outcome
As an organizing principle	Pervasive across systems	Maintenance of oxygen homeostasis	Growth, development and survival of organism
Active from initiation	Development	Role in development from embryonic stages	Involved in development of multiple systems like heart, vasculature, hepatic, retinal and skeletal system
Rhythmicity	Pulsatile expression of HIF1a modulated by PHD2	Controls temporal dynamics of HIF	Maintenance of dynamics of protein levels, its quantity and/or localization through PHD-HIF axis
Involved in maintenance of homeostasis throughout lifetime	• steady state sensing of extrinsic environment and intrinsic factors	Response to • Low ambient oxygen • Cellular hypoxia, low-ferrous iron, TCA cycle intermediates - fumarate, succinate and 2 oxoglutarate, NO, ROS, hormones • Response to ischemia	• Affects global transcription, translation, stress response and signalling through PHD2-HIF axis • Modulation of core physiological process regulated by HIF1a ➢ Oxygen homeostasis in adult vascular system ➢ Erythropoiesis ➢ Angiogenesis, ➢ Anaerobic energy metabolism, ➢ Iron metabolism ➢ Control of cell cycle and proliferation ➢ Apoptosis and autophagy ➢ Redox homeostasis, ➢ Inflammation ➢ Immunity ➢ Hematopoietic stem cell in steady state and stress
	Auto-feedback	Regulation of PHD2 expression by HIF or activity by cellular metabolites	Reversal to homeostasis
	Responsiveness to diet and life style	Inhibition of PHD2 • By high salt intake • Fat and glucose	• Induction of antihypertensive mechanism via HIF1 activation • Decreased glucose level and reduced lipid accumulation
Genetic variability	Adaptation and adaptability	Baseline variability in *EGLN1* gene confers differences in PHD2 expression	Adaptation to high altitude is mediated through ancestral allele linked to low expression of PHD2
Individuality	Interindividual variability within the population	Genotypes linked to expression differs between *prakriti*	Might explain several linked phenotypic attributes of *prakriti*
Underlying cause of health and disease	Disease susceptibility progression, prognosis, responsiveness to therapy	Baseline variability, in response to extrinsic and intrinsic stimuli and their tissue-specific effects	Protective where hypoxia is favoured and deleterious where its responses favour pathogenesis
Role in intervention	PHD-HIF axis as a therapeutic target	Relevant in diseases where hypoxia is a cause or is consequential in pathogenesis	1. Cartilage repair 2. Wound healing improvement 3. Arteriogenic phenotype 4. Brain tumour 5. Renal anaemia 6. Cardiovascular disease 7. Therapeutic revascularization after visceral surgery 8. Myocardial ischemia 9. Recovery from stroke 10. Treatment of inflammatory diseases

holds enormous potential, not just for predictive health but also for personalized therapeutics (Patwardhan and Mashelkar 2009; Dwivedi *et al.* 2012).

Ayurgenomics: an operational framework for integrating the *trisutra* concepts with modern medicine

1. Basic tenets of Ayurveda are highlighted, showing how the personalized, predictive, preventive and participatory aspects parallel P4 medicine
2. Network medicine with a systems approach includes discussion of how:
   a. interlinks between cause (*hetu*), feature (*linga*) and therapeutics (*aushadha*) in *trisutra* form the basis for translational medicine
   b. a common organizing principle, *tridosha,* connects the three axes of *trisutra*
   c. expression of *tridoshas* is evident at different levels of organization
   d. variability in *tridosha* during development governs inter-individual differences leading to seven broad *prakriti* types in a population
   e. dynamic components of *tridosha* link health and disease in response to external and internal environmental agents
   f. understanding of human individuality forms the core for personalized management of health and disease
3. *Prakriti* provide phenotype scaffolds through P-P links for
   a. understanding human individuality
   b. stratification of individuals irrespective of population labels
4. *Prakriti* are likely to have genomic correlates e.g immune, metabolic and neurophysiological gene activity
5. Genetic and genomic analyses show how EGLN1 could be an example of a molecular contributor to *tridosha*

Concluding remarks

The identification of genes and pathways involved in development and manifestation of variable states of health, disease and responsiveness to drugs within and across populations will be crucial to integration of personalized approaches in drug discovery and development. This would also simultaneously facilitate development of biomarker-based drug delivery in a personalized manner. *Trisutra*, thus is an operational framework for translational aspects of network medicine with systems understanding. It can also provide a theoretical framework for integrating basic understanding at the systems level with outcomes in health and disease and development of personalized prevention and therapeutics.

Acknowledgements

We would like to acknowledge CSIR for funding the work (MLP901). We also would like to thank Rohit Vashisht for help with illustrations and Dr Rajesh S. Gokhale, Dr Ram Niwas Prasher, Dr Bharat Khuntia, Dr Arvind Kumar and Dr Mahua Maulik for their valuable and critical comments.

References

Aggarwal S., Negi S., Jha P., Singh P. K., Stobdan T., Pasha M. A. *et al.* 2010 EGLN1 involvement in high-altitude adaptation revealed through genetic analysis of extreme constitution types defined in Ayurveda. *Proc. Natl. Acad. Sci. USA* **107**, 18961–18966.

Aggarwal S., Gheware A., Agrawal A., Ghosh S., Prasher B., Mukerji M. *et al.* 2015 Combined genetic effects of EGLN1 and VWF modulate thrombotic outcome in hypoxia revealed by Ayurgenomics approach. *J. Transl. Med.* **13**, 1–11.

Agustí A. 2013 Phenotypes and disease characterization in chronic obstructive pulmonary disease: toward the extinction of phenotypes? *Ann. Am. Thorac. Soc.* **10** suppl., S125–S130.

Agustí A., Sobradillo P. and Celli B. 2011 Addressing the complexity of chronic obstructive pulmonary disease: from phenotypes and biomarkers to scale-free networks, systems biology, and P4 medicine. *Am. J. Respir. Crit. Care Med.* **183**, 1129–1137.

Ahmad T., Kumar M., Mabalirajan U., Pattnaik B., Aggarwal S., Singh R. *et al.* 2012 Hypoxia response in asthma: differential modulation on inflammation and epithelial injury. *Am. J. Respir. Cell Mol. Biol.* **47**, 1–10.

Auffray C., Chen Z. and Hood L. 2009 Systems medicine: the future of medical genomics and healthcare. *Genome Med.* **1**, 2.

Baffy G. and Loscalzo J. 2014 Complexity and network dynamics in physiological adaptation: an integrated view. *Physiol. Behav.* **131**, 49–56.

Barabási A.-L. 2007 Network medicine–from obesity to the "diseasome". *N. Engl. J. Med.* **357**, 404–407.

Barabási A.-L., Gulbahce N. and Loscalzo J. 2011 Network medicine: a network-based approach to human disease. *Nat. Rev. Genet.* **12**, 56–68.

Barkoulas M., van Zon J. S., Milloz J., van Oudenaarden A. and Felix M. A. 2013 Robustness and epistasis in the *C. elegans* vulval signaling network revealed by pathway dosage modulation. *Dev. Cell* **24**, 64–75.

Baryshnikova A., Costanzo M., Myers C. L., Andrews B. and Boone C. 2013 Genetic interaction networks: toward an understanding of heritability. *Annu. Rev. Genomics Hum. Genet.* **14**, 111–133.

Bigham A. W., Wilson M. J., Julian C. G., Kiyamu M., Vargas E., Leon-Velarde F. *et al.* 2013 Andean and Tibetan patterns of adaptation to high altitude. *Am. J. Hum. Biol.* **25**, 190–197.

Blake J. A., Bult C. J., Eppig J. T., Kadin J. A. and Richardson J. E. 2009 The mouse genome database genotypes:phenotypes. *Nucleic Acids Res.* **37(suppl 1)**, D712–D719.

Chari S. and Dworkin I. 2013 The conditional nature of genetic interactions: the consequences of wild-type backgrounds on mutational interactions in a genome-wide modifier screen. *PLoS Genet.* **9**, e1003661.

Chaussabel D., Pascual V. and Banchereau J. 2010 Assessing the human immune system through blood transcriptomics. *BMC Biol.* **8**, 84.

Chen R., Mias G. I., Li-Pook-Than J., Jiang L., Lam H. Y., Chen R. *et al.* 2012 Personal omics profiling reveals dynamic molecular and medical phenotypes. *Cell* **148**, 1293–1307.

Cheng S., Xing W., Pourteymoor S. and Mohan S. 2014 Conditional disruption of the prolyl hydroxylase domain-containing protein 2

(Phd2) gene defines its key role in skeletal development. *J. Bone Miner. Res.* **29**, 2276–2286.

Cho I. and Blaser M. J. 2012 The human microbiome: at the interface of health and disease. *Nat. Rev. Genet.* **13**, 260–270.

Chua E. C.-P., Shui G., Lee I. T.-G., Lau P., Tan L.-C., Yeo S.-C. *et al.* 2013 Extensive diversity in circadian regulation of plasma lipids and evidence for different circadian metabolic phenotypes in humans. *Proc. Natl. Acad. Sci. USA* **110**, 14468–14473.

Coop G., Pickrell J. K., Novembre J., Kudaravalli S., Li J., Absher D. *et al.* 2009 The role of geography in human adaptation. *PLoS Genet.* **5**, e1000500.

Dolinoy D. C. and Jirtle R. L. 2008 Environmental epigenomics in human health and disease. *Environ. Mol. Mutagen.* **49**, 4–8.

Dopico X. C., Evangelou M., Ferreira R. C., Guo H., Pekalski M. L., Smyth D. J. *et al.* 2015 Widespread seasonal gene expression reveals annual differences in human immunity and physiology. *Nat. Commun.* **6**, 7000.

Duan L.-J., Takeda K. and Fong G.-H. 2014 Hematological, hepatic, and retinal phenotypes in mice deficient for prolyl hydroxylase domain proteins in the liver. *Am. J. Pathol.* **184**, 1240–1250.

Dudley J. T., Chen R., Sanderford M., Butte A. J. and Kumar S. 2012 Evolutionary meta-analysis of association studies reveals ancient constraints affecting disease marker discovery. *Mol. Biol. Evol.* mss079.

Dwivedi V., Anandan E., Mony R. S., Muraleedharan T., Valiathan M., Mutsuddi M. *et al.* 2012 *In vivo* effects of traditional ayurvedic formulations in *Drosophila melanogaster* model relate with therapeutic applications. *PLoS One* **7**, e37113.

Eckel-Mahan K. L., Patel V. R., de Mateo S., Orozco-Solis R., Ceglia N. J., Sahar S. *et al.* 2013 Reprogramming of the circadian clock by nutritional challenge. *Cell* **155**, 1464–1478.

Fabbri L. M., Beghé B. and Agustí A. 2012 COPD and the solar system: introducing the chronic obstructive pulmonary disease comorbidome. *Am. J. Respir. Crit. Care Med.* **186**, 117–119.

Franke K., Gassmann M. and Wielockx B. 2013 Erythrocytosis: the HIF pathway in control. *Blood* **122**, 1122–1128.

Fu W. and Akey J. M. 2013 Selection and adaptation in the human genome. *Ann. Rev. Genomics Hum. Genet.* **14**, 467–489.

Fujita N., Hirose Y., Tran C. M., Chiba K., Miyamoto T., Toyama Y. *et al.* 2014 HIF-1-PHD2 axis controls expression of syndecan 4 in nucleus pulposus cells. *FASEB J.* **28**, 2455–2465.

Fumagalli M., Sironi M., Pozzoli U., Ferrer-Admettla A., Pattini L. and Nielsen R. 2011 Signatures of environmental genetic adaptation pinpoint pathogens as the main selective pressure through human evolution. *PLoS Genet.* **7**, e1002355.

Gibson G. 2012 Rare and common variants: twenty arguments. *Nat. Rev. Genet.* **13**, 135–145.

Gibson G. and Visscher P. 2013 From personalized to public health genomics. *Genome Med.* **5**, 1–2.

Goh K.-I., Cusick M. E., Valle D., Childs B., Vidal M. and Barabási A.-L. 2007 The human disease network. *Proc. Natl. Acad. Sci. USA* **104**, 8685–8690.

Goriely A. and Wilkie A. O. 2012 Paternal age effect mutations and selfish spermatogonial selection: causes and consequences for human disease. *Am. J. Hum. Genet.* **90**, 175–200.

Greer S. N., Metcalf J. L., Wang Y. and Ohh M. 2012 The updated biology of hypoxia-inducible factor. *EMBO J.* **31**, 2448–2460.

Haase V. H. 2010 Hypoxic regulation of erythropoiesis and iron metabolism. *Am. J. Physiol. Renal Physiol.* **299**, 1–13.

Hancock A. M., Witonsky D. B., Gordon A. S., Eshel G., Pritchard J. K., Coop G. *et al.* 2008 Adaptations to climate in candidate genes for common metabolic disorders. *PLoS Genet.* **4**, e32.

Hancock A. M., Witonsky D. B., Alkorta-Aranburu G., Beall C. M., Gebremedhin A., Sukernik R. *et al.* 2011 Adaptations to climate-mediated selective pressures in humans. *PLoS Genet.* **7**, e1001375.

Harten S. K., Ashcroft M. and Maxwell P. H. 2010 Prolyl hydroxylase domain inhibitors: a route to HIF activation and neuroprotection. *Antioxid. Redox Signal.* **12**, 459–480.

Hatori M., Vollmers C., Zarrinpar A., DiTacchio L., Bushong E. A., Gill S. *et al.* 2012 Time-restricted feeding without reducing caloric intake prevents metabolic diseases in mice fed a high-fat diet. *Cell Metabol.* **15**, 848–860.

Hawkins R. D., Hon G. C. and Ren B. 2010 Next-generation genomics: an integrative approach. *Nat. Rev. Genet.* **11**, 476–486.

Hood L. and Friend S. H. 2011 Predictive, personalized, preventive, participatory (P4) cancer medicine. *Nat. Rev. Clini. Oncol.* **8**, 184–187.

Human Microbiome Jumpstart Reference Strains Consortium 2010 A catalog of reference genomes from the human microbiome. *Science* **328**, 994–999.

Human Microbiome Project Consortium 2012 Structure, function and diversity of the healthy human microbiome. *Nature* **486**, 207–214.

Ivan M., Kondo K., Yang H., Kim W., Valiando J., Ohh M. *et al.* 2001 HIFα targeted for VHL-mediated destruction by proline hydroxylation: implications for O2 sensing. *Science* **292**, 464–468.

Jablonski N. G. and Chaplin G. 2010 Human skin pigmentation as an adaptation to UV radiation. *Proc. Natil. Acad. Sci.* **107** suppl. 2, 8962–8968.

Jackson S. P. and Bartek J. 2009 The DNA-damage response in human biology and disease. *Nature* **461**, 1071–1078.

Jeong H., Tombor B., Albert R., Oltvai Z. N. and Barabási A.-L. 2000 The large-scale organization of metabolic networks. *Nature* **407**, 651–654.

Juyal R. C., Negi S., Wakhode P., Bhat S., Bhat B. and Thelma B. 2012 Potential of ayurgenomics approach in complex trait research: leads from a pilot study on rheumatoid arthritis. *PLoS One* **7**, e45752.

Kaelin Jr W. G. and Ratcliffe P. J. 2008 Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. *Mol. Cell* **30**, 393–402.

Kalucka J., Ettinger A., Franke K., Mamlouk S., Singh R. P., Farhat K. *et al.* 2013 Loss of epithelial hypoxia-inducible factor prolyl hydroxylase 2 accelerates skin wound healing in mice. *Mol. Cell Biol.* **33**, 3426–3438.

Karlsson E. K., Kwiatkowski D. P. and Sabeti P. C. 2014 Natural selection and infectious disease in human populations. *Nat. Rev. Genet.* **15**, 379–393.

Kato G. J., Gladwin M. T. and Steinberg M. H. 2007 Deconstructing sickle cell disease: reappraisal of the role of hemolysis in the development of clinical subphenotypes. *Blood Rev.* **21**, 37–47.

Kato G. J., Hebbel R. P., Steinberg M. H. and Gladwin M. T. 2009 Vasculopathy in sickle cell disease: biology, pathophysiology, genetics, translational medicine, and new research directions. *Am. J. Hematol.* **84**, 618–625.

Katzenberg D., Young T., Finn L., Lin L., King D. P., Takahashi J. S. *et al.* 1998 A CLOCK polymorphism associated with human diurnal preference. *Sleep* **21**, 569–576.

Katzmarzyk P. T. and Leonard W. R. 1998 Climatic influences on human body size and proportions: ecological adaptations and secular trends. *Am. J. Phys. Anthropol.* **106**, 483–503.

Kiss J., Kirchberg J. and Schneider M. 2012 Molecular oxygen sensing: implications for visceral surgery. *Langenbeck's Arch. Surg.* **397**, 603–610.

Kong A., Frigge M. L., Masson G., Besenbacher S., Sulem P., Magnusson G. *et al.* 2012 Rate of *de novo* mutations and the importance of father's age to disease risk. *Nature* **488**, 471–475.

Kumar S., Dudley J. T., Filipski A. and Liu L. 2011 Phylomedicine: an evolutionary telescope to explore and diagnose the universe of disease mutations. *Trends Genet.* **27**, 377–386.

Ladroue C., Carcenac R., Leporrier M., Gad S., Le Hello C., Galateau-Salle F. *et al.* 2008 PHD2 mutation and congenital erythrocytosis with paraganglioma. *N. Engl. J. Med.* **359**, 2685–92.

Lampe J. W., Navarro S. L., Hullar M. A. and Shojaie A. 2013 Inter-individual differences in response to dietary intervention: integrating omics platforms towards personalised dietary recommendations. *Proc. Nutr. Soc.* **72**, 207–208.

Lee K., Lynd J. D., O'Reilly S., Kiupel M., McCormick J. J. and LaPres J. J. 2008 The biphasic role of the hypoxia-inducible factor prolyl-4-hydroxylase, PHD2, in modulating tumor-forming potential. *Mol. Cancer Res.* **6**, 829–842.

Li L., Ruau D. J., Patel C. J., Weber S. C., Chen R., Tatonetti N. P. *et al.* 2014 Disease risk factors identified through shared genetic architecture and electronic medical records. *Sci. Transl. Med.* **6**, 234–257.

Loscalzo J. and Barabási A. L. 2011 Systems biology and the future of medicine. *Wiley Interdiscip. Rev. Syst. Biol. Med.* **3**, 619–27.

Loscalzo J., Kohane I. and Barabasi A. L. 2007 Human disease classification in the postgenomic era: a complex systems approach to human pathobiology. *Mol. Syst. Biol.* **3**.

Lusis A. J. and Weiss J. N. 2010 Cardiovascular networks systems-based approaches to cardiovascular disease. *Circulation* **121**, 157–170.

Marigorta U. M. and Gibson G. 2014 A simulation study of gene-by-environment interactions in GWAS implies ample hidden effects. *Front. Genet.* 5.

Marx V. 2013 Biology: the big challenges of big data. *Nature* **498**, 255–260.

Matsuura H., Ichiki T., Inoue E., Nomura M., Miyazaki R., Hashimoto T. *et al.* 2013 Prolyl hydroxylase domain protein 2 plays a critical role in diet-induced obesity and glucose intolerance. *Circulation* **127**, 2078–2087.

Merrow M., Spoelstra K. and Roenneberg T. 2005 The circadian cycle: daily rhythms from behaviour to genes. *EMBO Report* **6**, 930–935.

Morgan L., Arendt J., Owens D., Folkard S., Hampton S., Deacon S. *et al.* 1998 Effects of the endogenous clock and sleep time on melatonin, insulin, glucose and lipid metabolism. *J. Endocrinol.* **157**, 443–457.

Myers I. B. and McCaulley M. H. 1988 *Myers-Briggs type indicator*. MBTI: Consulting Psychologists Press, Palo Alto, California, USA.

Nagel S., Talbot N. P., Mecinović J., Smith T. G., Buchan A. M. and Schofield C. J. 2010 Therapeutic manipulation of the HIF hydroxylases. *Antioxid. Redox Signal.* **12**, 481–501.

Natarajan R., Salloum F. N., Fisher B. J. and Kukreja R. C. 2006 Hypoxia inducible factor-1 activation by prolyl 4-hydroxylase-2 gene silencing attenuates myocardial ischemia reperfusion injury. *Circ. Res.* **98**, 133–140.

Nguyen L. K., Cavadas M. A., Scholz C. C., Fitzpatrick S. F., Bruning U., Cummins E. P. *et al.* 2013 A dynamic model of the hypoxia-inducible factor 1α (HIF-1α) network. *J. Cell Sci.* **126**, 1454–1463.

Noble D. 2002 Modeling the heart—from genes to cells to the whole organ. *Science* **295**, 1678–1682.

Noble D. 2008 *The music of life: biology beyond genes*. Oxford University Press, New York, United States.

Noble D. 2011 Neo-Darwinism, the modern synthesis and selfish genes: are they of use in physiology? *J. Physiol.* **589**, 1007–1015.

Okamoto-Mizuno K. and Mizuno K. 2012 Effects of thermal environment on sleep and circadian rhythm. *J. Physiol. Anthropol.* **31**, 14.

Olson M. V. 2011 What does a "normal" human genome look like? *Science* **331**, 872.

Olson M. V. 2012 Human genetic individuality. *Annu. Rev. Genomics Hum. Genet.* **13**, 1–27.

Oltvai Z. N. and Barabási A.-L. 2002 Life's complexity pyramid. *Science* **298**, 763–764.

Ong S.-G. and Hausenloy D. J. 2012 Hypoxia-inducible factor as a therapeutic target for cardioprotection. *Pharmacol. Ther.* **136**, 69–81.

O'Roak B. J., Deriziotis P., Lee C., Vives L., Schwartz J. J., Girirajan S. *et al.* 2011 Exome sequencing in sporadic autism spectrum disorders identifies severe *de novo* mutations. *Nat. Genet.* **43**, 585–589.

Pagani L., Ayub Q., MacArthur D. G., Xue Y., Baillie J. K., Chen Y. *et al.* 2012 High altitude adaptation in Daghestani populations from the Caucasus. *Hum. Genet.* **131**, 423–433.

Pan Q., Shai O., Lee L. J., Frey B. J. and Blencowe B. J. 2008 Deep surveying of alternative splicing complexity in the human transcriptome by high-throughput sequencing. *Nat. Genet.* **40**, 1413–1415.

Park J., Lee D. S., Christakis N. A. and Barabási A. L. 2009 The impact of cellular networks on disease comorbidity. *Mol. Syst. Biol.* 5.

Patterson N., Moorjani P., Luo Y., Mallick S., Rohland N., Zhan Y. *et al.* 2012 Ancient admixture in human history. *Genetics* **192**, 1065–1093.

Patwardhan B. and Mashelkar R. A. 2009 Traditional medicine-inspired approaches to drug discovery: can Ayurveda show the way forward? *Drug Discov. Today* **14**, 804–811.

Pflughoeft K. J. and Versalovic J. 2012 Human microbiome in health and disease. *Annu. Rev. Pathol.* **7**, 99–122.

Polaczyk P. J., Gasperini R. and Gibson G. 1998 Naturally occurring genetic variation affects *Drosophila* photoreceptor determination. *Dev. Genes Evol.* **207**, 462–70.

Prasher B., Negi S., Aggarwal S., Mandal A. K., Sethi T. P., Deshmukh S. R. *et al.* 2008 Whole genome expression and biochemical correlates of extreme constitutional types defined in Ayurveda. *J. Transl. Med.* **6**, 48.

Preininger M., Arafat D., Kim J., Nath A. P., Idaghdour Y., Brigham K. L. *et al.* 2013 Blood-informative transcripts define nine common axes of peripheral blood gene expression. *PLoS Genet.* **9**, e1003362.

Raj A., Rifkin S. A., Andersen E. and van Oudenaarden A. 2010 Variability in gene expression underlies incomplete penetrance. *Nature* **463**, 913–918.

Robinson P. N. and Mundlos S. 2010 The human phenotype ontology. *Clin. Genet.* **77**, 525–534.

Roenneberg T., Wirz-Justice A. and Merrow M. 2003 Life between clocks: daily temporal patterns of human chronotypes. *J. Biol. Rhythms* **18**, 80–90.

Roenneberg T., Kumar C. J. and Merrow M. 2007 The human circadian clock entrains to sun time. *Curr. Biol.* **17**, R44–R45.

Rotti H., Guruprasad K. P., Nayak J., Kabekkodu S. P., Kukreja H., Mallya S. *et al.* 2014 Immunophenotyping of normal individuals classified on the basis of human dosha *prakriti*. *J. Ayurveda Integr. Med.* **5**, 43–9.

Rual J.-F., Venkatesan K., Hao T., Hirozane-Kishikawa T., Dricot A., Li N. *et al.* 2005 Towards a proteome-scale map of the human protein–protein interaction network. *Nature* **437**, 1173–1178.

Sales N., Pelegrini P. and Goersch M. 2014 Nutrigenomics: definitions and advances of this new science. *J. Nutr. Metabol.*, Article ID 202759.

Selvaraju V., Parinandi N. L., Adluri R. S., Goldman J. W., Hussain N., Sanchez J. A. *et al.* 2013 Molecular mechanisms of action and therapeutic uses of pharmacological inhibitors of HIF-prolyl 4-hydroxylases for treatment of ischemic diseases. *Antioxid. Redox Signal.* **20**, 2631–2665.

Semenza G.L. 2011 Oxygen sensing, homeostasis, and disease. *N. Engl. J. Med.* **365**, 537–547.

Sen Banerjee S., Thirunavukkarasu M., Rishi M. T., Sanchez J. A., Maulik N. and Maulik G. 2012 HIF-prolyl hydroxylases and cardiovascular diseases. *Toxicol. Mech. Methods* **22**, 347–358.

Sethi T. P., Prasher B. and Mukerji M. 2011 Ayurgenomics: a new way of threading molecular variability for stratified medicine. *ACS Chem. Biol.* **6**, 875–880.

Shah N. H., Jonquet C., Chiang A. P., Butte A. J., Chen R. and Musen M. A. 2009 Ontology-driven indexing of public datasets for translational bioinformatics. *BMC Bioinformatics* **10** suppl 2, S1.

Sharma P. 1981 *Charaka Samhita: text with english translation,* pp. 240. Chaukambha Orientalia Publisher, Varanasi, India.

Sharma P. 1999 *Susruta-Samhita: with english translation of text and Dalhana's commentary along with critical notes*. Chaukamba Vishwa Bharati, Varanasi, India.

Sharma A. 2013 Transgenerational epigenetic inheritance: focus on soma to germline information transfer. *Prog. Biophys. Mol. Biol.* **113**, 439–446.

Sheldon W. H., Stevens S. S. and Tucker W. B. 1940 *The varieties of human physique*, pp. 347. Harper & Brothers, New York, USA.

Simonson T. S., McClain D. A., Jorde L. B. and Prchal J. T. 2012 Genetic determinants of Tibetan high-altitude adaptation. *Hum. Genet.* **131**, 527–533.

Soni H. 2014 Prolyl hydroxylase domain-2 (PHD2) inhibition may be a better therapeutic strategy in renal anemia. *Med. Hypotheses* **82**, 547–550.

Srikanthamurthy K. R. 1992 *Astanga Samgraha* of Vagbhata: text with English translation. Chaukhambha Orientalia, Varanasi, India.

Stelzl U., Worm U., Lalowski M., Haenig C., Brembeck F. H., Goehler H. *et al.* 2005 A human protein-protein interaction network: a resource for annotating the proteome. *Cell* **122**, 957–968.

Suhre K., Shin S.-Y., Petersen A.-K., Mohney R. P., Meredith D., Wägele B. *et al.* 2011 Human metabolic individuality in biomedical and pharmaceutical research. *Nature* **477**, 54–60.

Suthram S., Dudley J. T., Chiang A. P., Chen R., Hastie T. J. and Butte A. J. 2010 Network-based elucidation of human disease similarities reveals common functional modules enriched for pluripotent drug targets. *PLoS Comput. Biol.* **6**, e1000662.

The 1000 Genomes Project Consortium 2010 A map of human genome variation from population-scale sequencing. *Nature* **467**, 1061–1073.

The 1000 Genomes Project Consortium 2012 An integrated map of genetic variation from 1,092 human genomes. *Nature* **491**, 56–65.

Tian Q., Price N. and Hood L. 2012 Systems cancer medicine: towards realization of predictive, preventive, personalized and participatory (P4) medicine. *J. Intern. Med.* **271**, 111–121.

Tishkoff S. A. and Verrelli B. C. 2003 Patterns of human genetic diversity: implications for human evolutionary history and disease. *Annu. Rev. Genomics Hum. Genet.* **4**, 293–340.

Tishkoff S. A., Reed F. A., Ranciaro A., Voight B. F., Babbitt C. C., Silverman J. S. *et al.* 2007 Convergent adaptation of human lactase persistence in Africa and Europe. *Nat. Genet.* **39**, 31–40.

Touitou Y., Touitou C., Bogdan A., Reinberg A., Auzeby A., Beck H. *et al.* 1986 Differences between young and elderly subjects in seasonal and circadian variations of total plasma proteins and blood volume as reflected by hemoglobin, hematocrit, and erythrocyte counts. *Clin. Chem.* **32**, 804.

Turchin M. C., Chiang C. W., Palmer C. D., Sankararaman S., Reich D., Hirschhorn J. N. *et al.* 2012 Evidence of widespread selection on standing variation in Europe at height-associated SNPs. *Nat. Genet.* **44**, 1015–1019.

Vidal M., Cusick M. E. and Barabasi A.-L. 2011 Interactome networks and human disease. *Cell* **144**, 986–998.

Visscher P. M. and Gibson G. 2013 What if we had whole-genome sequence data for millions of individuals? *Genome Med.* **5**, 80.

Wittmann M., Dinich J. and Roenneberg T. 2006 Social jet-lag: sleep, well-being and stimulus consumption of different chronotypes. *Chronobiol. Int.* **23**, 497–509.

Wood A. R., Esko T., Yang J., Vedantam S., Pers T. H., Gustafsson S. *et al.* 2014 Defining the role of common variation in the genomic and biological architecture of adult human height. *Nat. Genet.* **46**, 1173–1186.

Yang J., Benyamin B., McEvoy B. P., Gordon S., Henders A. K., Nyholt D. R. *et al.* 2010 Common SNPs explain a large proportion of the heritability for human height. *Nat. Genet.* **42**, 565–569.

Zhao S. and Wu J. 2013 Hypoxia inducible factor stabilization as a novel strategy to treat anemia. *Curr. Med. Chem.* **20**, 2697–2711.

Ziello J. E., Jovin I. S. and Huang Y. 2007 Hypoxia-inducible factor (HIF)-1 regulatory pathway and its potential for therapeutic intervention in malignancy and ischemia. *Yale J. Biol. Med.* **80**, 51.

Received 6 April 2015, in final revised form 16 June 2015; accepted 26 June 2015
Unedited version published online: 6 July 2015
Final version published online: 4 March 2016