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Ayurgenomics: A New Way of Threading Molecular Variability for Stratified Medicine

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Modern humans evolved in Africa and spread across the world, successfully colonizing diverse geographical locations including extremes of latitudes, altitudes, arid and wet conditions as well as regions endemic for different pathogens. Besides geo-climatic conditions, human genomes have also been shaped by an aggregate of sociocultural factors such as admixture, mating patterns, food sources, and dietary habits. These footprints of human history in the form of genetic variations reside in our genomes, and many of them have become relatively fixed in the past millions of years in certain populations. Nearly 11 million single-nucleotide polymorphisms (SNPs) have now been catalogued in humans across diverse populations by the International HapMap Consortium. Coupled to this, the individual genome projects have also revealed a large fraction of variations that are specific to an individual. With this enormous amount of variability, it now seems that there are as many human genomes as there are humans. Though a majority of these variations might be neutral, a large number of these differences could contribute to adaptation, phenotypic variability, differences in disease susceptibility, and response to environment even within healthy individuals of a population. With global socioeconomic and cultural changes leading to altered lifestyles, diet patterns, and migration into non-native environments, the effects of advantageous variations could sometimes turn deleterious. A striking example of this is the increased prevalence of cardiovascular diseases in newer generations of African Americans compared to their African ancestors.[1] This further adds another level of complexity in interpreting the true effects of variations. It has now been proven both at the genetic and expression levels that most of the total variance is due to interindividual differences within populations.[2,3] Moreover, in any healthy population, we also observe a spectrum of physical and physiological phenotypes, as well as individuals who are differentially predisposed or protected from diseases. Thus, there is a need to mine and identify variations that are physiologically relevant and explain interindividual differences in phenotypes/disease susceptibility, prognosis, response to treatment and consequently study their effects in a context specific manner.

This forms the basic tenet for personalized medicine that aims to identify genetic variations that are linked to specific phenotypes and would be useful in

- prevention of disease and maintenance of health
- early detection, diagnosis, and screening in the prediseased state and customized screening based on personal risk
- development of a tailored treatment regime after the onset of disease to improve prognosis and quality of life

A key factor in determining an individual's susceptibility to disease as well as response to treatment should include the recognition of both the extrinsic (environmental) and the intrinsic (physiological and genomic) factors. There is a definite need and scope for evolving novel ways to stratify healthy individuals and develop a better understanding of normal phenotypic variation.[4]

Success in discovering markers for common diseases has been shadowed by the ever-increasing sample sizes required to yield statistical significance. This is, in part, due to the "small n large P" problem of biological studies, further inflated by technological advances in genotyping, resulting in massive parallelization of parameters (P) studied in a relatively small number of independent samples (n). In most of disease association studies, although the disease states are well-characterized, the control category is "controlled" only for sex, age, and ethnicity. The enormous heterogeneity in healthy state as described above may mask the true effects in disease associations. Also, the fact that human physiology is too complex to be pinned down to a few loci has been highlighted in the recent spate of Genome Wide Association Studies (GWAS).[5] Higher statistical power using smaller sample numbers in GWAS is expected to result from selecting more homogeneous controls that are not only stratified by population but also stratified into core groups on the basis of shared physiology. An integrative approach of stratifying and clustering physiological states on the basis of molecular functioning therefore seems pertinent. While this method of grouping is an interesting proposition, it is remarkably difficult to prove with the current tool kit available to modern medicine and biology. The multiscale nature of such an endeavor makes it nearly intractable to approaches usually undertaken.

We found this challenge very stimulating, and our understanding of population-wide variability across Indian populations (IGV Consortium)[6] provided a major thrust to further our quest for understanding the variability in healthy individuals. The head start came from the fact that there exists an exquisitely elaborate system of predictive and personalized medicine in India, i.e., Ayurveda, which has been practiced for over 3500 years. The system already has a built-in framework for stratifying healthy individuals who differ in susceptibility to disease and response to drug and environment. In contrast to the empirical approach of contemporary medicine, the Ayurveda therapeutic regimen is tailored to an individual's physiology. Though this system has fueled many drug discovery approaches and some attempts into its integration in pharmacogenetics have been made,[7,8] a systematic analysis of underlying principles has been lacking.

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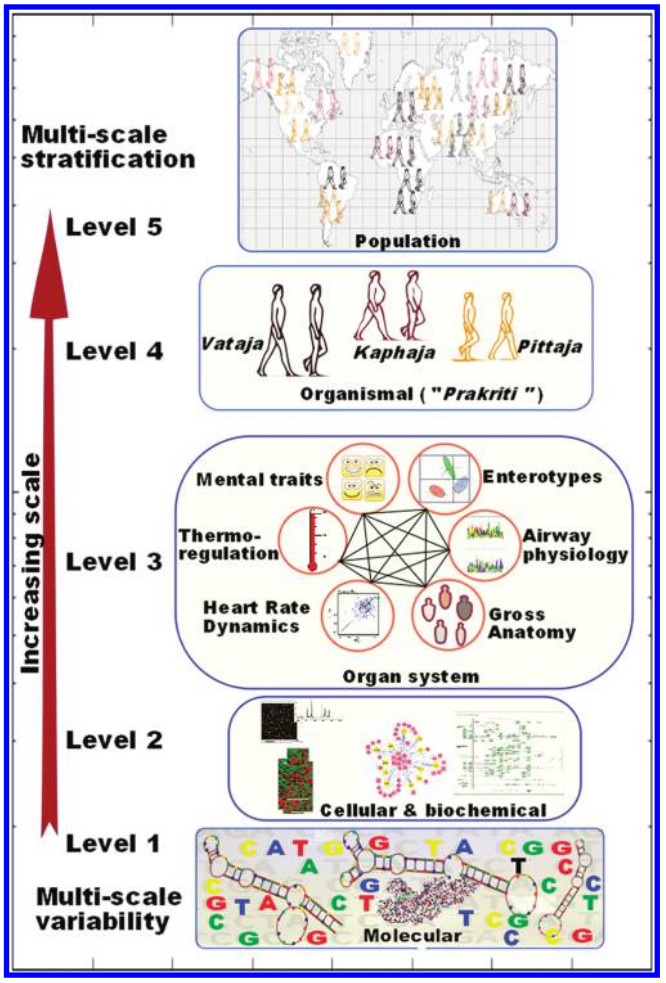

Figure 1. Weaving the threads of molecular variability through Ayurgenomics. Formation of distinct clusters are demonstrated here through the concept of *Tridoshas* (*Vata*, *Pitta*, and *Kapha*) manifesting in *Prakriti*, even in the presence of large interindividual variability present at the molecular, cellular, and organ physiology levels. Questionnaire assessment allowed us to select end-of-the-spectrum manifestations of *Tridoshas*, namely, *Vataja* (*V*), *Pittaja* (*P*), and *Kaphaja* (*K*) *Prakriti* types. *Prakriti* develops through the network of interactions within and between the organ systems as well as cellular processes and can be deconvoluted using observable and measurable phenotype-phenotype links. This could help stratify the "within-health variability" into core groups of individuals with shared physiological and cellular processes.

In order to undertake integration of this most ancient system of medicine, which is scripted in Sanskrit, with the language of modern genomics and medicine, we undertook this endeavor with a trans-disciplinary team of researchers. For the first time we could demonstrate molecular evidence for these concepts and build a framework for "Ayurgenomics", which can provide impetus to personalized medicine.[9,10] We provide a perspective of the concepts and also the further prospects in this field (Figure 1). The original Sanskrit verses with their meanings are available as supplementary online material in our prior publications.

AYURVEDA: OLD IS THE NEW "NEW"

Ayurveda describes the involvement of *Tridoshas*, which behave like latent variables (but not necessarily orthogonal) in the functioning of the organism. The three *Doshas*, *i.e.*, *Vata*, *Pitta*, and *Kapha*, work in harmony to create a state of good health in an individual while also regulating each other. An individual's basic constitution, *Prakriti*, is described to be a consequence of the relative proportion of *Vata*, *Pitta*, and *Kapha*. These proportions of *Tridoshas* are not only genetically determined (*Shukra Shonita*) but also influenced by the environment during development, especially maternal diet and lifestyle. *Prakriti* is fixed at the time of birth and remains invariant throughout the individual's lifespan. Ethnicity (*Jatiprasakta*), familial characteristics (*Kulanupatini*), and geo-climatic regions (*Deshanupatini*) are also implicated in influencing phenotypic variability through their effect on *Tridoshas* and *Prakriti*. Thus, most of the factors such as ethnicity, geography, and environment that contribute to interindividual variability at the genetic or epigenetic levels are embedded in Ayurveda's concept of *Prakriti*. In an individual, the *Tridoshas* work in conjunction and maintain homeostasis throughout the lifetime of the individual.

Distinct properties and functions have been ascribed to each *dosha*. For instance, *Vata* contributes to manifestation of shape, cell division, signaling, movement, cognition, *etc.*, and also regulates the activities of *Kapha* and *Pitta*. *Kapha* is responsible for growth and maintenance of structure, storage, and stability. *Pitta* is primarily responsible for metabolism, thermoregulation, energy homeostasis, pigmentation, vision, and host surveillance. Thus phenotypic diversity in a population, according to Ayurveda, is a consequence of a continuum of relative proportions of *Doshas*. Although no two individuals can be identical, ancient texts have categorized the baseline phenotypic variability into seven possible constitutional types, namely, *Vataja* (*V*), *Pittaja* (*P*), *Kaphaja* (*K*), *Vata-Pittaja* (*VP*), *Pitta-Kaphaja* (*PK*), *Vata-Kaphaja* (*VK*), and *Vata-Pitta-Kaphaja* (*VPK*). Among these, the first three, which have predominance of one of the three doshas, are considered as extremes, exhibiting readily recognizable phenotypes, and are more predisposed to specific diseases. The assessment of *Prakriti* is achieved through querying the different attributes of an individual such as physiology, anatomy, and mental aptitude. For instance, at the anatomical level, these constitutions differ with respect to body frame and build, skin, eye and hair color, texture and composition; at the physiological levels the differences are observed with respect to food habits and digestive capacity, tendency to gain weight, disease resistance and healing capacity, tolerance for specific weather, metabolism of toxic compounds, *etc.* Besides these, differences in taste preferences, memorization capabilities, response to stress as well as aptitude differences are also described. Ayurvedic practitioners deconvolute the "mixture impression" thus obtained to identify proportions of *Vata*, *Pitta*, and *Kapha* in an individual's *Prakriti*. This "subjective" assessment (which was objectivized to a scoring system through a questionnaire) considers different phenotypic attributes of an individual and links these multiple windows to create intraindividual phenotype-to-phenotype links. A disease according to Ayurveda is a perturbation of *Vata*, *Pitta*, and *Kapha* in an individual from his or her homeostatic state. Ayurvedic treatment aims to bring it back to its native state by appropriate dietary and therapeutic regime. Food or medicines including lifestyle factors have been described to enhance or reduce a particular *dosha*, and therefore an individual specific treatment is provided. Thus the beauty of Ayurveda lies in the fact that an individual, a disease condition, drug, diet as well as environment are described in terms of *doshic* components and appropriate customizations are provided to balance these states.

Ayurveda describes not only the functional attributes of *Vata*, *Pitta*, and *Kapha* but also their contribution on different scales in seven different constitutions. Therefore, Ayurveda already has a stratified approach as its basic tenet for personalizing therapy. Thus we felt that integration of this stratified approach could complement approaches to development of personalized medicine while gaining insights into systems biology. We realized that before making any attempt toward this endeavor we would have to address an ontological challenge in connecting together the literature from Ayurveda, modern medicine, and molecular biology. Broader yet clearer definitions were needed before any communication across them could be expected to be fruitful. We decided to work with normal states of health before progressing on to disease associations.

OLD AND NEW: DISCOVERY OF MOLECULAR CORRELATES OF *PRAKRITI*

We tried to explore the molecular basis of three most contrasting *Prakriti* types, predominantly *V*, *P*, and *K*. For this, we first designed a questionnaire that could capture the clinical features described in Ayurvedic literature for phenotyping of *Prakriti* in an objective manner. In order to minimize the effect of confounding factors on expression of *Prakriti*, we conducted our study on age- and sex-matched subjects from a genetically homogeneous Indo-European (IE) background. The homogeneity of the *V*, *P*, and *K* subjects and relatedness with the background population was confirmed through phylogenetic analysis using a set of unlinked markers studied in the Indian Genome Variation Consortium project.

We explored whether healthy individuals who have predominance of *V*, *P*, or *K* in their *Prakriti* exhibit differences in

- gross biochemical levels in peripheral blood
- expression at the genome-wide levels
- genetic level, *i.e.*, variations in DNA that are more stable than gene expression

We observed significant differences in biochemical profiles (otherwise within the normal laboratory range) between the *Prakriti* types that were further validated through bootstrap resampling. For instance, *P* males had higher values for most of hematological parameters such as hemoglobin, packed cell volume, and red blood cell count; *K* males had lower prothrombin time and HDL, higher levels of triglycerides, total cholesterol, VLDL, LDL, LDL/HDL ratio, and serum uric acid. Many of the parameters observed in *Kapha* are independent predictors of cardiovascular mortality and corroborate with disease descriptions associated with *Kapha*.

Transcriptional profiles of pooled RNA from *V*, *P*, and *K* revealed differences in core biological processes between these *Prakriti* groups. Some of these overlapped with the biochemical pathways mentioned previously, *e.g.*, hemostasis. This led us to hypothesize that there is indeed an underlying cellular system in each *Prakriti* type that can be assessed through the modern genomics approach. The Ayurvedic abstraction of *Kapha* as being the promoter of anabolic state overlapped with the overall upregulation of genes involved in cellular biosynthesis including ATP and cofactor biosynthesis and purine salvage pathway. Both male and female individuals of the *Vata* group showed enrichment of differentially expressed genes involved in cellular processes such as cell cycle, DNA repair, and recombination as well as transport functions. *Vata* governing manifestation of shape and cell division and transport has also been described in Ayurveda texts.

Interestingly, as might be expected from complex interplay in biology, correlation with other processes/pathways was not so straightforward. For instance, the expression of genes involved in olfactory transduction processes was observed to be significantly low in both male and female individuals of *Pitta Prakriti.* While striking differences with respect to the immune functions were observed, different facets of the immune function seemed to be differentially modulated in different *Prakriti* types. *P* had a higher expression of genes involved in innate immunity, whereas *K* had a higher expression of genes involved in adaptive immunity. Thus susceptibility to infections, atopy, and allergic reactions are likely to vary according to the constitution types. This is interesting as host adaptive and innate immune responses are being increasingly implicated in seemingly unrelated disorders such as cancer and obesity and also seem to shape the human systems biology through the diet-microbiota axis. Other differentially regulated pathways that are promising and could corroborate with canonical functions of *Prakriti* types include JAK-STAT signaling and cytokine−cytokine interaction that process information and regulation of actin cytoskeleton and MAPK signaling that regulates growth.

Also noteworthy among the differentially expressed genes was a significant over-representation of hub and housekeeping genes. Typically, hub genes are defined to have 10 or more interacting partners. Differential expression of hub genes could therefore modulate a series of networks and pathways and could have system-wide effects. *P* had an overexpression of hub genes involved in pro-apoptotic functions and positive regulation of innate immune response. Apoptosis is finely tuned by the body to maintain health and has wide-ranging implications in organogenesis, immune tolerance, tissue remodelling, *etc.* Thus inherent variability in this process could confer interindividual differences in diseases; for instance, more apoptosis could enhance aging and neurodegeneration, and too little could promote cancer.

Gene expression is a dynamic process and reflects the ongoing execution of cellular functions. Variation in DNA might represent more stable changes in the genome and hence form a canvas over which more dynamic patterns develop in an individual's lifetime. Therefore, we carried out genetic analysis on a subset of differentially expressed genes to test whether common variations in these genes exhibit differences between the constitution types. After performing corrections for false discovery rates, we observed 14 SNPs from 5 genes to differ significantly between the *Prakriti* types. The genes that distinguish these groups are involved in development (*FAS*, *AKT3*, *FBN2*), multiple signaling pathways (*FAS*, *AKT3*), interact with multiple proteins (*RAD51*, *FAS*, *INSR*), or are prominent drug targets (*EGLN1*, *FAS*, *AKT3*). This makes these genes ideal candidates for understanding systems biology. The observed genotypic differences also corroborated with expression differences between the same *Prakriti* groups. Most importantly, once the genotypes of all the constitution types were pooled, the combined allele frequency was similar to the Indo-European background population that they were derived from. This indicates that inherent genetic differences within a heterogeneous mix of healthy individuals get masked in the absence of a method that allows us to stratify them. Thus, we decided to prove the hypothesis that mixing endophenotypes of health might mask the true effect of variations in association studies. Each of these genes is well studied and interesting to pursue. We selected *EGLN1,* a gene that was relatively less studied at the genetic level.

EGLN1, popularly referred to as *PHD2*, is a key oxygen sensor gene, and we hypothesized that variations in this gene could have meaningful physiological effects. Oxygen sensing and appropriate induction of the hypoxia response are very important in people living at high altitudes. We observed significant differences in frequency of *EGLN1* variations that distinguished *P* from *K* across a representative set of 24 Indian populations residing at different altitudes. Surprisingly, the *P* group of normal individuals who were otherwise "lowlanders" had a significant over-representation of the alleles that were more frequent in high-altitude population subgroup. It was clear from our analysis that *EGLN1* was able to partition even closely related populations based on altitude. To explore whether this gene could be a common thread uniting the physiology across all high-altitude populations of the world, we analyzed *EGLN1* variations in the *HGDP-CEPH* Human Genome Diversity Panel, which has sampled populations from various geographical locations all around the world. Interestingly, there was significant correlation between altitude and the same allele as well as three other *SNP*s spanning the same region, irrespective of genetic relatedness between the populations. Recently, *EGLN1*, has been linked to high-altitude adaptation in different regions of China and the Andes, by other groups.[11−14] This further highlights the merit of our approach.

We hypothesized that if the variations in *EGLN1* linked to the *P* group are over-represented in natives of high altitude, then the other allele that is linked to the *K* type might be involved in maladaptation to similar altitudes. Analysis of genotype frequencies of *EGLN1* in sojourners from Indo-European background who developed high-altitude pulmonary edema (HAPE) revealed a significant over-representation of the genotype linked to *K*. This genotype was also associated with higher expression of *EGLN1*, which could thus be correlated to lower expression of hypoxia-responsive genes. Thus we speculate that individuals of *K* constitution are likely to mal-adapt to high-altitude conditions. Interestingly, Ayurveda assigns *Prakriti* also to environment, *P* constitution being more protected at high altitudes is consistent with the Ayurvedic school of thought that considers mountains mainly as *Kapha-Vata* dominant regions and having more prevalence of *Kapha-Vata* diseases.

Enthused by results of *EGLN1* in HAPE, we explored whether variability in EGLN1 levels could also be consequential in diseases that show hypoxic response such as in asthma.[15] Ongoing studies in our institute utilizing siRNA and other small molecule PHD inhibitors in an *Ova*-challenged mouse model of asthma are yielding interesting insights into the mechanisms of *EGLN1* in asthma (unpublished observations). These studies exemplify the use of "informed hypothesis" to functionally validate the role of putative genes through chemical biology approaches.

AYURGENOMICS: THE WAY FORWARD

It also seems from our observations that analyzing extreme constitution types that have phenotype-phenotype linkages within them might allow us to identify important axes such as hypoxia, apoptosis, inflammation, *etc.* that could contribute to system-wide changes. For instance, differences in hypoxia-inducible factors (HIF) through expression differences in *EGLN1* could not only contribute to differential prognosis in various diseases such as cancer, asthma, chronic obstructive pulmonary disease, ischemia, stroke, *etc.* where hypoxia is implicated but could also lead to variability in processes such as inflammation, metabolism, erythrocytosis, oxidative stress, and other downstream targets of HIF.[16−18] The outcome of these differences could be assessed through physiological and biochemical measurements. Some of these parameters could connect to features that are described for *Prakriti* assessment and thereby help objectivize them for global applicability. Enrichment for genes belonging to the key cellular pathways in a single data set strengthened our belief that categorizing into Ayurvedic phenotypes captures the differential regulation of these processes and hence must be testable at the level of multiorgan physiology. Therefore the next challenge is in threading the intraindividual physiological and molecular attributes through *Prakriti* phenotypes.

This approach fits well with "systems theory" which implies that the "whole is greater than the sum of its parts". We propose that identification of functional axes in healthy individuals would be a key to uncovering intraindividual cryptic phenotype-phenotype links. For our purpose we define some salient features of such axes. These axes could be

- highly connected to many organs; just as in gene networks, hubs in physiological functioning would be key in organizing the system
- easily quantifiable in a relatively noninvasive manner
- well-defined and characterized in the modern system of medicine and physiology
- known to have diverse disease associations

We propose to measure more than one axis in the same individual, each one measuring a slightly different yet physiologically connected function. These measures could then be collapsed into latent variables by using dimensionality reduction techniques that could be overlaid with *Prakriti* information for supervised classification tools to develop objective classifiers of *V*, *P*, and *K*. A blind approach such as hierarchical clustering can also be applied to assess the clusters formed on the basis of modern anatomical-physiological measurements and the concordance of these with *Prakriti* driven clusters formed through a supervised machine learning approach. On the basis of our present understanding of *Prakriti*, we propose that all or some of the following axes could be explored:-

Cardiovascular function and its regulation. Assessment of the cardiovascular axis is a logical extension of biochemical findings as well as expression and genetic differences. For example, autonomic regulation of heart rate and endothelial function might help us elucidate the correlates of variability in oxygen-sensing mechanisms. Effects on heart rate variability have been reported in otherwise pathological states such as chronic intermittent hypoxia in obstructive sleep apnea.[19] Sympathetic activation or disturbed sympathovagal balance, which results from chronic stress conditions,[20] might be captured in the one of the *Prakriti* groups. For more detailed mechanistic understanding of sympathetic stress, additional measurements of related systems such as the renal-angiotensin-aldosterone (RAA) system are expected to support our working hypothesis of "measurable" stratification.

Systemic inflammation. It is known that low grade systemic inflammation is a feature of many lifestyle diseases such as Metabolic Syndrome.[21] Measurement of cytokines such as IL-6, fibrinogen and C-reactive protein could be done and used for both supervised and unsupervised classification of individuals. Recent studies linking these markers with heart rate variability[22] and hypoxia encourages us to attempt identification of common threads running beneath multiorgan physiology.

Apoptosis Axis. A delicate balance between pro- and anti-apoptotic mechanisms drives the healthy functioning of tissues.

Since we observed a large number of differentially expressed hub genes to be over-represented in apoptosis, tissue-specific assays that measure this balance might yield differential regulation in supervised and unsupervised clusters formed in the study population.

Microbiota Axis. There is increasing evidence that dietary factors interact with the host gut microflora to modulate gut motility, nutrient absorption, and immune system.[23] Differences in digestive capacity, immune differences, and dietary habits among *Prakriti* types has opened up interesting possibilities to characterize *Prakriti*-specific microbiomes through metagenomic analysis. This is especially relevant in the light of a recent report of existence of three major enterotypes of human gut microbiome across global populations.[24] This could be a major step toward therapeutic regimens tailored according to an individual's microbiota.

Anthropometry. Second-digit to fourth-digit ratio in an individual has been linked to physiological and behavioral manifestations.[25] Since Ayurveda takes into account many anthropometric features in phenotypic assessment, we anticipate measures like these to be informative in objectivising *Prakriti*.

Given the diversity of the human genome, the most practical approach in development of personalized medicine would be to identify individuals on the basis of shared physiology and obtain stratified groups that would respond more or less similarly to external environment including diet and drugs. Thus, this integrative approach might allow us to identify groups of individuals that share core physiological variations, a requirement for development of stratified medicine. It might be worthwhile to mention that in Ayurveda, selection of a suitable drug and dietary regime is made on the basis of clinical assessment of the individual with respect to disease endophenotype, the basic constitution as well as the status of health at the time of their administration. For this purpose it describes its subject matter into three major heads termed as "TRISUTRA", *viz.*, causes (*hetu*), features (*linga*), and therapeutics (*aushadha*) both for healthy and diseased. We have in this commentary touched just the "tip of the iceberg".

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REFERENCES

(1) Moalem, S., and Prince, J. (2007) The cholesterol also rises. In *Survival of the Sickest: A Medical Maverick Discovers Why We Need Disease*, 1st ed., pp 49−70, HarperCollins Publishers, India.

(2) Witherspoon, D. J., Wooding, S., Rogers, A. R., Marchani, E. E., Watkins, W. S., Batzer, M. A., and Jorde, L. B. (2007) Genetic similarities within and between human populations. *Genetics 176* (1), 351–9.

(3) Storey, J. D., Madeoy, J., Strout, J. L., Wurfel, M., Ronald, J., and Akey, J. M. (2007) Gene-expression variation within and among human populations. *Am. J. Hum. Genet. 80* (3), 502–9.

(4) Olson, M. V. (2011) Genome-sequencing anniversary. What does a "normal" human genome look like? *Science 331* (6019), 872.

(5) Frazer, K. A., Murray, S. S., Schork, N. J., and Topol, E. J. (2009) Human genetic variation and its contribution to complex traits. *Nat. Rev. Genet. 10* (4), 241–51.

(6) Indian Genome Variation Consortium (2008) Genetic landscape of the people of India: a canvas for disease gene exploration. *J. Genet. 87* (1), 3−20.

(7) Bhushan, P., Kalpana, J., and Arvind, C. (2005) Classification of human population based on HLA gene polymorphism and the concept of Prakriti in Ayurveda. *J. Altern. Complement. Med. 11* (2), 349–53.

(8) Patwardhan, B., and Mashelkar, R. A. (2009) Traditional medicine-inspired approaches to drug discovery: can Ayurveda show the way forward? *Drug Discovery Today 14* (15−16), 804–11.

(9) Prasher, B., Negi, S., Aggarwal, S., Mandal, A. K., Sethi, T. P., Deshmukh, S. R., Purohit, S. G., Sengupta, S., Khanna, S., Mohammad, F., Garg, G., Brahmachari, S. K., Indian Genome Variation Consortium, Mukerji, M. (2008) Whole genome expression and biochemical correlates of extreme constitutional types defined in Ayurveda. *J. Transl. Med. 6*, 48.

(10) Aggarwal, S., Negi, S., Jha, P., Singh, P. K., Stobdan, T., Pasha, M. A., Ghosh, S., Agrawal, A., Prasher, B., and Mukerji, M. (2010) EGLN1 involvement in high-altitude adaptation revealed through genetic analysis of extreme constitution types defined in Ayurveda. *Proc. Natl. Acad. Sci. U.S.A. 107* (44), 18961–6.

(11) Peng, Y., Yang, Z., Zhang, H., Cui, C., Qi, X., Luo, X., Tao, X., Wu, T., Ouzhuluobu, B., Ciwangsangbu, D., Chen, H., Shi, H., and Su, B. (2011) Genetic variations in Tibetan populations and high-altitude adaptation at the Himalayas. *Mol. Biol. Evol. 28* (2), 1075–81.

(12) Xu, S., Li, S., Yang, Y., Tan, J., Lou, H., Jin, W., Yang, L., Pan, X., Wang, J., Shen, Y., Wu, B., Wang, H., and Jin, L. (2011) A genome-wide search for signals of high-altitude adaptation in Tibetans. *Mol. Biol. Evol. 28* (2), 1003–11.

(13) Bigham, A., Bauchet, M., Pinto, D., Mao, X., Akey, J. M., Mei, R., Scherer, S. W., Julian, C. G., Wilson, M. J., López Herráez, D., Brutsaert, T., Parra, E. J., Moore, L. G., and Shriver, M. D. (2010) Identifying signatures of natural selection in Tibetan and Andean populations using dense genome scan data. *PLoS Genet. 6* (9), e1001116.

(14) Storz, J. F. (2010) Evolution. Genes for high altitudes. *Science 329* (5987), 40–1.

(15) Huerta-Yepez, S, Baay-Guzman, G. J., Bebenek, I. G., Hernandez-Pando, R, Vega, M. I., Chi, L, Riedl, M, Diaz-Sanchez, D, Kleerup, E, Tashkin, D. P., Gonzalez, F. J., Bonavida, B, Zeidler, M, and Hankinson, O. (2011) Hypoxia Inducible Factor promotes murine allergic airway inflammation and is increased in asthma and rhinitis. *Allergy66* (7), 909–18.

(16) Semenza, G. L. (2000) HIF-1 and human disease: one highly involved factor. *Genes Dev. 14* (16), 1983–91.

(17) Smith, T. G., Robbins, P. A., and Ratcliffe, P. J. (2008) The human side of hypoxia-inducible factor. *Br. J. Haematol. 141* (3), 325–34.

(18) Eltzschig, H. K., and Carmeliet, P. (2011) Hypoxia and inflammation. *N. Engl. J. Med. 364* (7), 656–65.

(19) Shiomi, T., Guilleminault, C., Sasanabe, R., Hirota, I., Maekawa, M., and Kobayashi, T. (1996) Augmented very low frequency component of heart rate variability during obstructive sleep apnea. *Sleep 19*, 370–377.

(20) Schubert, C., Lambertz, M., Nelesen, R. A., Bardwell, W., Choi, J. B., and Dimsdale, J. E. (2009) Effects of stress on heart rate complexity—a comparison between short-term and chronic stress. *Biol. Psychol. 80* (3), 325–32.

(21) Das, U. N. (2004) Metabolic syndrome X: an inflammatory condition? *Curr. Hypertens. Rep. 6*, 66–73.

(22) Haensel, A., Mills, P. J., Nelesen, R. A., Ziegler, M. G., and Dimsdale, J. E. (2008) The relationship between heart rate variability and inflammatory markers in cardiovascular diseases. *Psychoneuroendocrinology 33*, 1305–1312.

(23) Kau, A. L., Ahern, P. P., Griffin, N. W., Goodman, A. L., and Gordon, J. I. (2011) Human nutrition, the gut microbiome and the immune system. *Nature 474* (7351), 327–36.

(24) Arumugam, M., Raes, J., Pelletier, E., Le Paslier, D., Yamada, T., Mende, D. R., Fernandes, G. R., Tap, J., Bruls, T., Batto, J. M., and

Bertalan, M. (2011) Enterotypes of the human gut microbiome. *Nature* 473 (7346), 174–80.

(25) Manning, J. T., and Bundred, P. E. (2001) The ratio of second to fourth digit length and age at first myocardial infarction in men: a link with testosterone? *Br. J. Cardiol.* 8 (12), 720–3.