values); with that of older/unauthenticated batches which do not inhibit the enzyme. This comparison could quickly reveal the identity of the chemical marker(s) in the herb which are specifically associated with the ability to inhibit the enzyme in question.

(b) Study the chemoprofile of a closely related herb which does not inhibit the enzyme in question. In the case of Triphala, two of its components (*P. emblica* and *T. chebula*) inhibited hyaluronidase, whereas *T. bellerica* did not inhibit this enzyme[3]. Therefore, a close comparison of chemoprofiles of *T. bellerica* with that of *P. emblica* and *T. chebula* should permit identification of the chemical marker(s), of *P. emblica* and *T. chebula* fruit powders, which are uniquely associated with inhibition.

In principle, the experimental approaches described above, provide a workable protocol for the activity-based standardization of any herbal product which specifically inhibits the activity of a disease related enzyme. For example, activation of angiotensin converting enzyme (ACE)[10] is associated with certain types of cardiac disease. Thus, one could correlate a shift in IC50 values of inhibition of ACE by specific herbals; with specific changes in their chemoprofiles. These data can then be used for activity-based standardization of cardioprotective herbal drugs which inhibit ACE.

An additional advantage of this method is that it can be used for critical biochemical evaluation of the entire protocol used for processing the crude herb. For example, a big increase in IC50 value for hyaluronidase/collagenase inhibition occurring between two steps in the protocol would imply a significant loss of potency of the Triphala powder being tested. Therefore, one could modify the processing protocol to prevent this loss in potency of enzyme inhibition by Triphala.

So far, the discussion has been focused on herbals which inhibit the activity of a specific enzyme. However, there are herbal drugs which stimulate the activity of protective antioxidant enzymes (such as superoxide dismutase) or the cytoprotective enzyme, haeme-oxygenase. For natural products which specifically induce the activity of a protective enzyme, the EC50 value is defined as the effective concentration of the herbal drug at which the half maximal value of enzyme activity is observed. In principle, one could monitor changes in EC50 values for enzyme activation, and correlate it with the chemical profile of the herb in question. Such data may then be used in the activity-based standardization of the herbals which induce/stimulate the activity of protective enzymes.

There is an urgent need for robust but sensitive methods for activity-based standardization of herbal drugs. There is one report wherein *Piper longum* extracts were standardized based on the extract's ability to inhibit the activity of the alpha-glucosidase-I enzyme[11]. With the help of our published data on Triphala and its components, experiments using hyaluronidase and/or collagenase inhibition assays for the activity-based standardization of Triphala are explained. This approach can be extended to other herbal drugs which inhibit other disease-related enzymes or activate protective enzymes.

There are certain unique advantages in using enzyme assays for activity-based standardization of herbal drugs. The correlative data between IC50/EC50 values of enzyme inhibition/activation and a particular chemical marker provides a simple and definitive method for standardizing the potency of the herbal drug. This is because the enzyme chosen is strongly involved in the progression of the disease for which the herbal drug is prescribed. Also, the adaptability of enzyme assays to a high-throughput format makes this approach of activity-based standardization of herbal drugs scalable.

1. Goldman, P., *Ann. Intern. Med.*, 2001, **135**, 594–600.
2. Laakmann, G., Dienel, A. and Kieser, M., *Phytomedicine*, 1998, **5**, 435–442.
3. Sumantran, V. N. *et al.*, *J. Biosci.*, 2007, **32**, 755–761.
4. Smith, R. L., *Front. Biosci.*, 1999, **4**, 4–12.
5. Sawabe, Y. *et al.*, *Biochim. Biophys. Acta*, 1992, **1137**, 274–278.
6. Ende, C. and Gebhardt, R., *Planta Med.*, 2004, **70**, 1006–1010.
7. Demeule, M. *et al.*, *Biochim. Biophys. Acta*, 2000, **1478**, 51–60.
8. Kumar, M. S., Kirubanandan, S., Sripriya, R. and Sehgal, P. K., *J. Surg. Res.*, 2008, **144**, 94–101.
9. Sumitra, M. *et al.*, *Wound Repair Regen.*, 2009, **17**, 99–107.
10. Serra, C. P. *et al.*, *Phytomedicine*, 2005, **12**, 424–432.
11. Pullela, S. V. *et al.*, *J. Ethnopharmacol.*, 2006, **108**, 445–449.

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Explanation of genetic component of human height by recent genome-wide association studies

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Francis Galton's classical work[1] of finding a heritable component for human height has influenced various approaches towards determining the heritability of complex human traits and the contemporary genome-wide approach is one of them. Human height is polygenic, quantitative, one of the most accurately measured and highly heritable conventional variable of anthropometry. The recent success of genome-wide association studies (GWAS) in explaining a part of genetic spectrum of anthropometric traits especially human height in European populations could also be repeated in India with the help of physical anthropology literature pertaining to human growth and developmental research, thus facilitating the design and optimum utilization of the genome-wide approach.

Fisher's[2] viewpoint is that many variants of individually small effects explain the heritability of human height. However, the identification of robustly associated genetic variants that influence height in the general population through linkage and candidate gene association

studies has always remained a daunting task. The extreme changes in human height are known to be related to chromosomal aberrations and/or monogenic defects with recessive and dominant modes of inheritance. Fortunately, the heritable component of human height has been successfully explained with the help of recent GWAS by discovering mutations on genes related to previously unknown and often unanticipated biological pathways. Height is a common polygenic quantitative trait, reflecting the combined influence of multiple as-yet-undiscovered genetic factors. Its study is an ideal opportunity to dissect the architecture of a highly polygenic quantitative trait in humans because it is easily and accurately measured and relatively stable over a large part of the lifespan. Moreover, it is highly heritable as 90% of adult human height within a population is explained by genetic variation[3]. It is generally expected that loci associated with human height may be pleiotropic in their effects, and thus may also influence the risk or severity of other common disease expressions[3]. In the light of this fact, the overall goal of Genome-wide Investigation of ANthropometric Traits (GIANT)[4] consortium was initiated to combine data from large genetic studies to identify variants associated with anthropometric traits including height and obesity-related measures in order to unravel the role of these quantitative traits in the genetic dissection of common and complex diseases.

Weedon *et al.*[5] first identified common variants robustly associated with adult height variation in *HMGA2* gene (related to chromatin binding protein) and Sanna *et al.*[6] identified a single nucleotide polymorphism (SNP) at the GDF5-UQCC locus explaining overall variation of 0.3–0.7% of the total variance. Lettre *et al.*[3] in their efforts to increase the probability of detecting additional height loci performed a meta-analysis of dataset on more than 15,000 subjects scanned at genome-wide scale and validated 10 newly identified gene loci. The 12 most validated (p-value $< 5 \times 10^{-7}$) biological candidate gene regions for human height were ZBTB38, HMGA2, GPR126, HIST1H1D, GDF5, HHIP, TRP11-ATXN3, LIN288, DOT1L, SH3GL3-ADAMTSL3, CHCHD7-RDHE2 and CDK6, which explained around 2% of the population variation producing a difference in height of around 3.5 cm among individuals with ≤8 height-increasing alleles and ≥16 height-increasing alleles[3]. The SNPs detected in GWAS, when tested for additive model of inheritance were only able to explain a small fraction (0.1–0.8%) of the residual phenotypic variation in height[3]. Different biological processes involved in the human height development are mesoderm development, skeletal development, mitosis, nucleoside, nucleotide and nucleic acid metabolism, and intracellular signalling cascade[7], whereas Lettre *et al.*[3] identified gene sets on biological pathways including targets of the let-7 microRNA, chromatin remodelling proteins and hedgehog signalling as the important regulators of human stature. Thus, due to the advent of efficient GWAS, in addition to the replication of the two genes (*HMGA2*, *GDF5*), the positive signals for more than 20 loci have already been detected influencing adult human stature and provide new insights into human growth and developmental processes. The aforementioned investigators have also recommended testing of non-additive effects within and between loci and investigation of the role of these identified loci in individuals with non-European ancestry.

Physical anthropologists involved in research on human growth and development have shown the presence of remarkable differences in human height between different populations having defined cultural backgrounds in different eco-zones of India and this data could be utilized in designing research proposals for conducting efficient GWAS. Not only human height but various other pertinent morphological quantitative traits are available in the literature of anthropometry contributing to the field of biometrics and could be useful in dealing with genetic epidemiology of non-communicable common metabolic diseases like diabetes, cardio-vascular disorders, obesity, etc. Moreover, the longitudinal or cross-sectional studies of human height and related anthropometric traits on Indian population groups will prove to be a boon in designing contemporary GWAS. Weedon *et al.*[8] suggested that further studies are needed to investigate more thoroughly the presence of sex-specific effects in attaining final adult height because it is also significantly influenced by growth hormones and nutrition. In the Indian context, determining the small effect sizes that have characterized most of the variants recently identified for height may not pose a challenge to the study of polygenic diseases due to the presence of availability of endogamous population groups (defined by marriage between restricted clans). Very recently, Estrada *et al.*[9] anthropogenetically studied north-west Europeans who are the tallest among the world populations because body height in these people appears to have reached a plateau due to the ubiquitous presence of an optimal environment, thus genetic factors may have strong influence on human growth. They identified association of natriuretic peptide precursor type C (*NPPC*) gene with overgrowth and skeletal anomalies.

Identification of population informative markers in order to ascertain genetically homogenous populations, in terms of their common ancestors in the recent past, within an ethnic or racial group is the current theme in genome variation research. Classically, for population genetic research, evolutionarily neutral traits were considered as the standards. In light of the exponential developments in genomic research, like microarray technology for genotyping thousands of markers together, supported by science reference projects like International HapMap Project, SNPs responsible for human height and other anthropometric traits may act as golden standard in detecting population informative markers. This may act as a boon for large scale disease specific association studies by eliminating the limitations like population stratification, case-control matching, etc.

1. Francis Galton, *J. Anthropol. Inst.*, 1876, 174–180.
2. Fisher, R. A., *Philos. Trans. R. Soc. Edinburgh*, 1918, **52**, 399–433.
3. Lettre, G. *et al.*, *Nat. Genet.*, 2008, **40**, 584–591.
4. Weedon, M. N. and Frayling, T. M., *Trends Genet.*, 2008, **24**, 595–603.
5. Weedon, M. N. *et al.*, *Nat. Genet.*, 2007, **39**, 1245–1250.
6. Sanna, S. *et al.*, *Nat. Genet.*, 2008, **40**, 198–203.
7. Gudbjartsson, D. F. *et al.*, *Nat. Genet.*, 2008, **40**, 609–615.
8. Weedon, M. N. *et al.*, *Nat. Genet.*, 2008, **40**, 489–490.
9. Estrada *et al., Hum. Mol. Genet.*, 2009; doi:10.1093/hmg/ddp296 (advanced publication).

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