Quantitative Genetic Analysis of Morphometric Data: Challenges and Considerations

Richard J. Sherwood[1,2], Kieran McNulty[3], Dana L. Duren[1]

[1] Division of Morphological Sciences and Biostatistics,
Boonshoft School of Medicine, Wright State University, Dayton, OH 45420
[2] Dept. of Orthodontics, Case Western Reserve University, Cleveland, OH 44106
[3] Evolutionary Anthropology Lab, University of Minnesota, Minneapolis, MN 55455

Introduction

The characterization and quantification of craniofacial form has long been a primary interest of comparative anatomists. While modern morphometric techniques are proving very successful at describing craniofacial shape, the dissection of the genetic determinants of that shape is more elusive. The current research seeks to elucidate the genetic underpinnings of variation in the complex craniofacial and dentognathic regions.

When approaching the genetic analysis of a complex shape, consideration must be given to the mode of acquisition of phenotypic characters. While simple methodologies may provide only a cursory description of shape compared to more sophisticated geometric techniques, the former may outperform the latter in elucidating the genetic underpinnings of trait variation. Comparisons of differing morphometric techniques, and their relative power in quantitative genetic analyses, illustrate the challenges encountered when trying to maximize the strength of both the morphologic descriptors and the genetic signal.

To explore the efficacy of different modes of phenotypic description we present, 1) a genetic analysis of 10 craniofacial traits described as simple linear and angular metrics; 2) genetic analysis of latent variables, derived via principal components analysis, encompassing larger regions of craniofacial anatomy; 3) comparison with previous results identifying latent variables derived via geometric morphometric analysis.

Primary Hypotheses

Hypothesis 1: Methodological approaches providing a comprehensive morphologic descriptor of a discrete anatomical region (e.g., an analysis of mandibular form) are associated with a stronger genetic signal than methods providing less comprehensive descriptors (e.g., a single measure of mandibular height).

Hypothesis 2: As morphologic descriptors incorporate larger anatomical regions, (e.g., mandible and maxilla) the effect of individual genes will be sufficiently diluted that statistical genetic techniques are less successful at localizing chromosomal regions harboring genes influencing variation.

Study Sample

The Fels Longitudinal Study began in 1929 in Yellow Springs, Ohio (Roche 1992). Data for the current analysis were obtained from lateral cephalographs of 1381 Fels participants (656 male, 725 female) ranging in age from 8.0 to 91.9 years (mean = 26.1 years) at the time of examination.

The 1381 individuals with cephalometric data come from 159 nuclear and extended families. In addition to parent-offspring and full sib-pairs, the larger pedigrees contain 17 other relative pair classes: e.g., avuncular (n pairs = 993), first cousins (n=760), second cousins (n=338), and so on. In all, there are 6,035 relative pairings represented among the 1381 participants.

Phenotyping

Radiographs were scanned using an Epson Expression 10000XL, and assessed for overall quality (positioning, exposure, artifacts, etc.) and only those deemed acceptable by the assessor were included.

Nemoceph (CDIimaging) was used for rapid and accurate collection of cephalometric data. Markers are placed on predefined cephalometric points, and a rough outline of the external and internal aspects of the skull, central incisors, and first molars is provided by the program. These outlines are fit to the cranial contours and teeth to provide an exact tracing of the craniofacial features. Measurements are made based on the craniometric points identified. For this presentation we have chosen 10 facial metrics.

Genetic Analysis

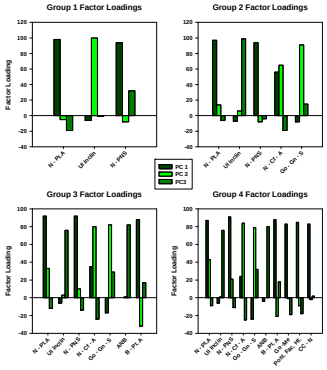
A variance components-based linkage analysis method (SOLAR; Almasy and Blangero, 1998) was used to obtain heritabilities and univariate genome-wide multipoint LOD scores for craniofacial measures.

Results

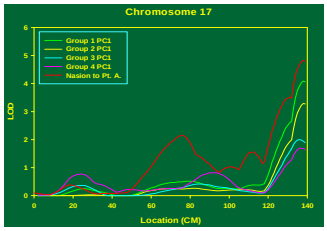
Nasion to Pt. A	h^2=0.49 (0.05)	53% VAR* P=1.26E-26
	LOD=4.83	Chromosome 17 @ 138 cM
Upper Incisor Inclination	h^2=0.39 (0.06)	2.2% VAR P=1.27E-14
	LOD=3.61	Chromosome 9 @ 65 cM
Nasion to Pns Maxillary Depth	h^2=0.49 (0.05)	50% VAR P=5.10E-28
	LOD=3.04	Chromosome 1 @ 117 cM
Maxillary Height	h^2=0.53 (0.05)	15% VAR P=2.16E-30
	LOD=2.88	Chromosome 2 @ 21 cM
Gonion-Gnathion-Skull	h^2=0.54 (0.05)	3.3% VAR P=1.78E-32
	LOD=2.69	Chromosome 8 @ 57 cM
Point A to Nasion to Pterygoid	h^2=0.35 (0.05)	2.8% VAR P=1.32E-15
	LOD=2.38	Chromosome 13 @ 57 cM
Ramus to Point A	h^2=0.48 (0.05)	33% VAR P=1.52E-26
	LOD=2.12	Chromosome 2 @ 179 cM
Gonion to Menton	h^2=0.41 (0.05)	39% VAR P=2.67E-18
	LOD=1.95	Chromosome 1 @ 221 cM
Posterior Facial Height	h^2=0.45 (0.05)	50% VAR P=2.30E-27
	LOD=1.12	Chromosome 18 @ 59 cM
Cranial Length	h^2=0.42 (0.05)	28% VAR P=1.30E-23
	LOD=1.97	Chromosome 1 @ 168 cM

*% variance explained by covariates. Covariates in the analysis were sex, age, sex x age, age^2, sex x age^2

Result 1: Traits analyzed are shown along with heritability estimates (h^2), standard error of those estimates, and the percent of variance explained by covariates (sex, age, sex x age, age^2, sex x age^2). Results of genome-wide linkage are also shown with maximum Log-Odds score (LOD) and chromosomal location of maximum LOD. In this instance, LOD > 2.87 is considered evidence for genome-wide significance, while a LOD >1.67 is considered suggestive evidence. The first four traits show strong evidence for significant linkage.



Result 2: To evaluate the effects of increasing the complexity of morphologic descriptors on genetic analyses, principal component (PC) analyses were run on four groups of craniofacial traits containing 3, 5, 7, and all 10 traits respectively. In all cases, three PCs were extracted and the above figure shows the factor loading scores for the four groups. The factor loadings were then used to calculate PC scores for individuals for the first three PCs. Those scores were then subjected to quantitative genetic analyses.

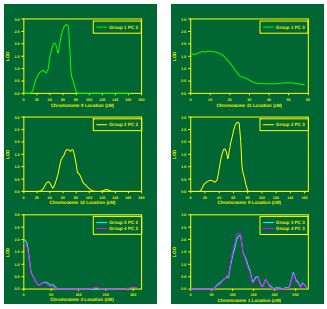


Result 3: The figure above shows the genome-wide linkage results for the trait "Nasion to Pt. A" and for PC1 for each of the four trait groups.

Genome-wide linkage scans identified a significant linkage (LOD=4.83) for Na – Pt. A on chromosome 17 at 138 cM. A secondary peak also occurs at approximately 75 cM. Genome-wide linkage scans of PC1 scores for the four groups of traits all showed maximal linkage at the same location on chromosome 17. LOD scores for PC1 scores decreased as the number of variables included in the analysis increased. The secondary peak identified at 75 cM for the individual trait is not evident with any of the PC1 scores although a slight peak around 22 cM becomes more defined as the number of traits increase in the PC analysis.

Inferences: The factor loading scores for each PC group identify the trait Na – Pt. A as a significant influence on PC1 scores. It may not be surprising that the strong linkage signal identified for that trait is retained in the PC1 scores. The reduction of that signal as more traits are added to the PC analysis supports the contention that additional morphologic descriptors may adversely effect quantitative genetic analyses by effectively diluting the available genetic signal.

The secondary linkage peak associated with the addition of more traits to PCA may indicate a novel genetic signal.



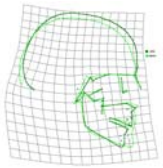
Result 4: The figures above show the genome-wide linkage results for the second and third principal component for each of the four groups. Again, in some cases, results may be driven by a single trait. QTL on Chromosome 9 match the location for the QTL associated with "Upper incisor inclination," while those on Chromosome 1 match the location for "Nasion to PNS". In both cases the magnitude of the LOD score is reduced relative to that of the individual trait. The QTL identified on Chromosomes 4, 10, and 21 may identify novel linkage regions not clear from individual traits.

Inferences: As previously noted, results of linkage analysis on PC-derived traits are similar to the individual traits loading heavily on the components. These analyses identify the potential for this approach to identify, or at least emphasize, chromosomal regions not noted in analysis of individual traits.

Future Explorations

We have previously explored the utility of a geometric morphometric approach in characterization of craniofacial phenotypes for subsequent quantitative genetic analyses (McNulty et al., 2009; Sherwood and McNulty, 2011). Genome-wide linkage analyses identified several suggestive linkages associated with the derived phenotypes. We are in the process of planning additional, large scale investigations into the utility of geometric methodologies for quantitative genetic analyses.

Points used in our geometric morphometric analysis of human cranial shape are standard anatomical landmarks with the exception of the series of points on the superior aspect of the brain case. These semi-landmarks were allowed to slide during Procrustes superimposition, and thereafter were considered geometrically homologous points.



Cranial shape differences described by PC1 from geometric morphometric analysis of human cranial shape. To visualize shape change on PC1, the mean landmark configuration was calculated and then warped according to the first eigenvector by adding the vector to or subtracting it from the mean coordinates. The grid represents the thin-plate spline interpolation of the displacement vectors from the "minus" configuration to the "plus" configuration (the shape change associated with the positive end of PC1).

Discussion

This work originated after a series of discussions with colleagues regarding the utility of a geometric morphometric approach in quantitative genetic analyses. The current presentation is intended to merely provide an example using a set of craniofacial traits we had available. This approach has limitations and future work will explore additional methods of phenotypic refinement including partial least squares analysis. The results of this particular example are as follows:

Hypothesis 1: Hypothesis 1 is rejected. Specifically, less comprehensive descriptors, e.g., a single cephalometric trait, provides a stronger genetic signal than more comprehensive descriptors (i.e., PC scores). This may result from the coincident relationship that the trait loading most heavily on PC1 (Na - Pt. A) was also the single trait with the largest single LOD score in linkage scans. It has been shown that bivariate linkage scans may result in amplification of the linkage signal above the individual traits (e.g., Duren et al., 2008; Havill et al., 2003).

Hypothesis 2: Hypothesis 2 is accepted. As additional traits were incorporated into the PCA the strength of the linkage signal was reduced relative to that of single traits or fewer traits. This result may not be altogether surprising given that QTL identified for individual traits were spread across the genome with little overlap between traits. If we had initially chosen traits all with demonstrated linkage to a single chromosomal region, these results may have been very different.

Advances in genetics, and characterization of anatomical form, have accrued at a tremendous rate providing powerful tools for anatomists interested in the determinants of anatomical form. If the primary goal of statistical genetic analyses is to identify the genes influencing variation, the likelihood of achieving that goal is related to the number, and effect size, of the genes involved. It is typically more difficult to localize and identify the genetic influences of polygenic traits because each gene, by itself, may exert a relatively small influence. With proper consideration, the goals of both the maximal description of biological shape, and the dissection of the genetic architecture influencing that shape, can be accomplished.

Acknowledgements and References

The Authors would like to thank the organizers of this symposium for the invitation to participate. It is an honor to help celebrate the work of Robert Sokal. R.J. Sherwood would like to thank Eric Delson for providing the initial suggestion regarding phenotypic refinement for genetic analysis of the craniofacial complex.

This work was supported by NIH grants DE018497, DE016692, and HD012252.

Literature Cited

1. Almasy, L., and J. Blangero, 1998 Multipoint quantitative-trait linkage analysis in general pedigrees. *American Journal of Human Genetics* **62**: 1198-1211.
2. Duren, D. L., J. Blangero, R. J. Sherwood, J. E. Curran, T. Dyer *et al.* 2008 Childhood cortical bone and skeletal age show bivariate genetic linkage to chromosome 2p. *BMRI* **23**: 550.
3. Havill, L. M., M. C. Mahaney, and J. Rogers, 2003 A QTL with pleiotropic effects on serum levels of osteocalcin and bone-specific alkaline phosphates in baboons maps to a region corresponding to human chromosome 6p21.1-21.3. *J Bone Miner Res.* **18**: F122.
4. McNulty, K. P., D. L. Duren, J. Blangero, T. Dyer, S. A. Cole *et al.* 2009 The geometry and architecture of craniofacial inheritance. *American Journal of Physical Anthropology* **138**: 289.
5. Sherwood, R. J., and K. P. McNulty, 2011 Dissecting the genetic architecture of craniofacial shape, pp. 145-171 in *Biological Shape Analysis: Proceedings of the 1st International Symposium*, edited by P. E. Lestrel. World Scientific, Singapore.