Genetic Determinants of Orofacial Shape and Relationship to Cleft Lip/Palate

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Specific Aims

1. Determination of heritable midfacial morphometric variation among 8 mouse strains of the mouse Collaborative Cross

2. Fine-map major quantitative trait loci (QTLs) responsible for those major heritable midfacial morphometric phenotypes using the mouse Collaborative Cross > Instant mouse models

3. Carry out GWAS of four homologous human midfacial morphometric phenotypes in Tanzanian children (n=3700). Carry out replication study in Tanzanian children (n=2600). [Test association of confirmed loci in USA EUR, USA Hispanic/Latino, (Asian) populations]
The Collaborative Cross

Analysis of the Parental and F1 Collaborative Cross Mice
Variation tends to be highly structured

F1 crosses deviate non-randomly from the mid-parental values

Total Dominance Variance (magnified 7X)
Project Collection Sites

Calgary (Hallgrimsson)

San Francisco (Klein)

Denver (Spritz)

Mwanza, Tanzania (Manyama)
Tanzania 3D Photo / Data / Saliva Collection
# Summary of Enrollment

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1. 3701 Tanzanian Bantu children selected for genotyping (including duplicates) at CIDR using Illumina HumanOmni25Exome-8v1_A array (2,567,845 variants); 3671 passed CIDR QC
2. 3557 unique Tanzanian children genotyped, data sent to CBS at Univ Washington (Cathy Laurie)
3. Median call rate 99.9%; error rate ~1.4E-05 (duplicates)
4. Two XXY individuals identified and filtered
5. Final subjects 2199 unrelated individuals (KC<0.98; = half-sibs) + 563 families (424 w/2, 97 w/3, 42 w/4 relatives) discovered by analyses of relatedness
6. Minimal other population structure, few outliers, inbreeding coefficient very low
7. No batch effects or other significant artifacts; 99.8% of SNPs pass QC filters
8. 28% SNPs MAF <2% for autosomes, 0.5% for X-chr
9. 17.9% of SNPs filtered (mostly as monomorphic)
10. Genomewide imputation (Impute2) pending
Principal Components Analysis of Population

The graph shows the distribution of principal components analysis results for different populations. The density plot on the top left indicates the distribution of values along the EV1 axis. The scatter plot in the middle displays the relationship between EV1 and EV2, with different colors representing different populations. The right side of the graph shows a density plot for EV1.
Analysis of Relatedness in Population

IBD Estimates

Red line corresponds to $\hat{\Pr}(\text{IBD}=0) + \hat{\Pr}(\text{IBD}=1) = 1$
Head Circumference Data

Head Circumference for Tanzanian males Age 6-17

Head Circumference Tanzania Females Age 6-17
1. Two XXY individuals excluded
2. Four outliers excluded (probable measurement errors)

Top hits:
- kgp6832430 chr10; P=4.46E-07
- 3 SNPs chr 4; P=5.03E-07
- kgp6930620 chr6; P=7.72E-07
- kgp6397840 chr5; P=1.05E-06
- 2 SNPs chr 10; P=1.92E-06
Phenotyping

A 3D camera captures six digitized facial scans of each individual
Phenotyping

- Scans are compiled into a single mesh object for landmarking
- Landmarks are placed for 29 common morphometric facial points
Principal Components Analysis

- X, Y, Z variable coordinates for each landmark
- Calculate the covariance between each set of variables
- The top principal components (PC) represent the axes of variation with the largest magnitudes
Preliminary Analysis

PCs 1-4; PC1 accounts for 15% of total variance in shape.
Multivariate Regression

Quantification of shape variation associated with age

Human Facial Ontogenetic Allometry

Regression Score 2

Self Reported Age
Tanzanian Growth Charts: Height-for-age

Females

Males
Tanzanian vs. WHO 2007 Growth Charts: Height-for-age

Females

Males

Height (cm)

WHO 2007
Tanzania

Age
Tanzanian Growth Charts: BMI-for-age

Females

Males
Tanzanian vs. WHO 2007 Growth Charts:
BMI-for-age

**Females**

**Males**
Current status: 2791 images reprocessed
Automated Landmarking
Washington Mio; Florida State University

Meanshape (n=30)  Meanshape landmarked
Automated Landmarking
Manual and Automated Landmarking on Same Individual
Error Analysis of Automated vs. Manual Landmarking

![Graph showing principal component analysis for automated and manual landmarking. The graph plots principal component 1 against principal component 2, with different markers and colors representing different categories such as Automated, FB2570_Bad, FB2570_Good, and Manual.]
Analytic Plans

1. Complete image processing GWAS
2. Manually review all images
3. Landmark
4. Derive principal components
5. Genetic analysis using unrelated individuals
6. Genetic analyses using all (including related) subjects
7. Complete DNA QC for replication study
8. Genotyping for replication study at CIDR
9. Do image analysis for replication study
10. Landmark for replication study
11. Derive PCs for replication study
12. Genetic analysis replication study