Frequency of Genetic Variants at the *MC1R* Locus in a Student Population

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Introduction

The Melanocortin-1 Receptor gene (*MC1R*) encodes a protein that is associated with pigmentation in vertebrate animals. An extraordinary number of variations in this gene have arisen over time due to the importance of pigmentation in camouflage, photosensitivity, vitamin D production, and other evolutionary factors. Variations in the *MC1R* gene sequence became important to survival as humans migrated out of Africa and into cooler climates with less sun exposure, where lighter skin pigmentation (and therefore more vitamin D production) was key to survival. These genetic variations (alleles) continue to exist in modern humans. Recent research into the *MC1R* gene variations shows that variants occur at different frequencies in different human populations. For example, a variant named "R151C" occurs in about 9% of humans of European descent but is not detected in humans of Asian descent.

For several years in the undergraduate Genetics course (BIOL 304L), students have collected and sequenced a portion of their *MC1R* gene to identify the presence or absence of variants. The results have previously been limited to the in-class analysis, and no data from the samples have been used in any type of publication or presentation prior to now. Here we analyze the de-identified aggregate data from eleven semesters (fall 2011 through spring 2016, representing roughly 500 individual sequences) to discern the frequency of variants in our data. Based on the reported demographics of the MSU student population, we anticipate our numbers will be comparable to those found in Europe based on the origins of the Appalachian settlers that comprise the ancestry of the majority of the students. We compare our findings to existing data from other populations.

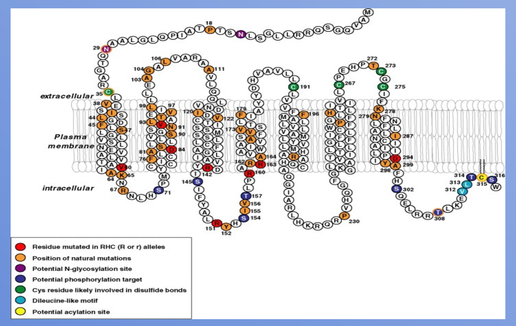

Figure 1. Illustration of the Melanocortin-1 Receptor amino acid primary structure, taken from García-Borrón *et al.* (2005).

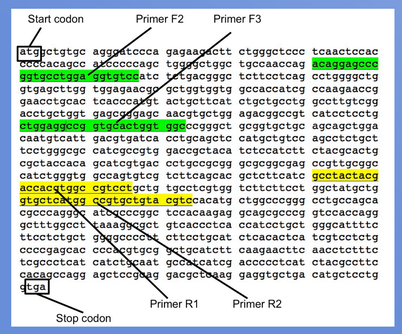

Figure 2. The human *MC1R* gene sequence showing the location of the PCR primers used for amplification.

Methods

The primary source of DNA for all samples came from buccal mouth swabs. Tissue samples from the swabs were boiled for 10 minutes in a solution of 10% Chelex, then centrifuged for 1 minute at 14,000 rpm. The supernatant was collected and used in the polymerase chain reaction (PCR) as the template DNA.

The PCR reaction consisted of the a total volume of 20 microliters (ml): 8 ml template DNA, 2 ml primers (forward and reverse), and 10 ml GoTaq mastermix. The PCR parameters were 30 cycles of the following phases: (1) 95^{O} C for 30 seconds; (2) 55^{O} C for 30 seconds; (3) 72^{O} C for 60 seconds. The PCR products were analyzed on 1.2% agarose gels and exhibited a band at length 324 basepairs.

We ligated PCR products to pGEM-T Easy plasmid vectors (Promega.com) and transformed plasmids into DH5-alpha *E. coli* cells. Cells were grown on tryptic soy agar plates containing ampicillin and X-gal; white colonies were selected and grown in tryptic soy broth. Plasmid DNA was subsequently purified from multiple bacterial clones using Qia-prep spin columns (Qiagen.com) and sequenced by ACTG, Inc. (ACTG inc.com).

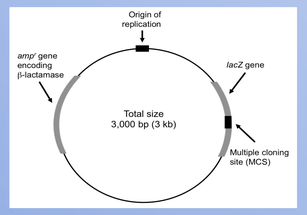

Figure 3. The pGEM-T Easy Plasmid vector used to subclone the *MC1R* amplicons.

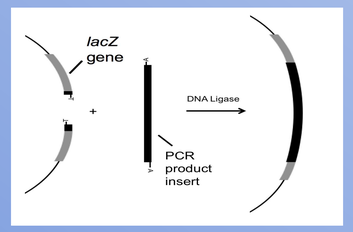

Figure 4. Insertion of the amplicon (PCR product) into the cloning site of pGEM-T Easy to facilitate Blue/White screening.

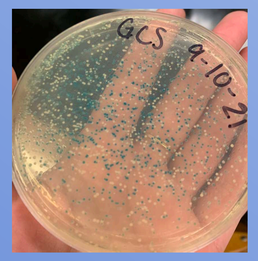

Figure 5. An example of DH5alpha *E. coli* cells showing blue and white clones.

Results

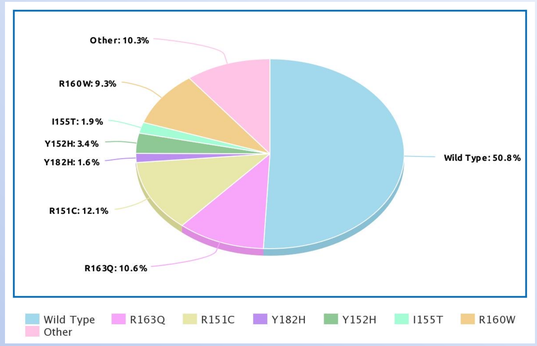

Figure 6. Allele frequencies for *MC1R* sequences from student class data. This data represents 321 distinct samples. Alleles categorized as “Other” occurred at a frequency of less than 1.5% (less than 5 occurrences in 321 samples).

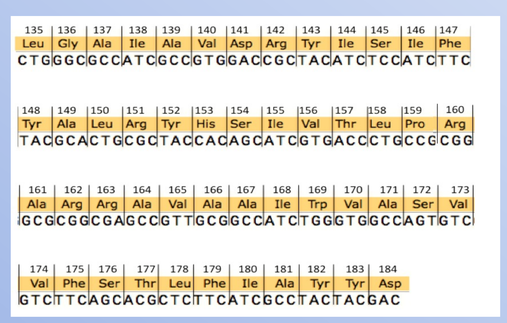

Figure 7. The region of the MC1R protein, from amino acids 135-184, that is being analyzed in this study.

Demographics of Morehead State University Student Population, as reported at www.datausa.io	
White	88.9%
African American	3.33%
2+ Races	2.62%
Hispanic or Latino	2.1%
Asian	0.715%
American Indian or Alaskan Native	0.124%
Native Hawaiian or Pacific Islander	0.0622%

Table 1. Demographics of Morehead State University Student Population.

Conclusions

A total of 33 distinct mutations were identified in the MSU student population. The alleles found in the MSU student population represent many of the same alleles seen in other studies, including three (R151C, R160W, R163Q) that represent the most common mutations found in the European population.

Allele Frequencies (%) of *MC1R* Mutations in Different Populations						
Mutation	Africa	Asia	India	Europe	U.S.	MSU Students
R151C	0	0	0	8.78	6.42	12.1
I155T	0	0	0	0.71	1.51	1.9
R160W	0	0	0	10.16	7.17	9.3
R163Q	0	75.51	4.72	6.23	9.15	10.6
Sample Size	117	343	53	1488	265	321

Table 2. Allele frequencies of *MC1R m*utations in different populations. Modified from Savage *et al.* (2008) with additional data from this study.

Appalachian ancestry consists largely of people who can trace their ancestry to Europe and specifically Scotland and Ireland, where there is a high frequency of *MC1R* mutations. We expected to see similar frequencies in the student population at MSU which is primarily derived from Appalachia. Three of the four most common mutant alleles in the MSU student population correspond to three of the most common mutations also seen in Europe.

The second most common mutant allele in the MSU student population was R163Q, at 10.6%. This allele is seen at high frequency in samples from Asia (Savage *et al.* 2008), and we hypothesize that its high frequency in an Appalachian population may represent introgression from Native Americans who can trace their ancestry to early human migrants that crossed into the Americas on the Beringian land bridge.

Bibliography

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