Alu Dimorphism at the PV92 Locus of Chromosome 16 is in Equilibrium for University Student Population

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Alu is a retrotransposable element, which refers to its ability to be copied and move from one region of DNA to another DNA region. At the PV92 locus of chromosome 16, Alu is a 300 bp dimorphic insert that can either be present or absent. It does not encode a protein product and has lost the ability to transpose. It is specific to primates and the PV92 locus of chromosome 16 to humans. Alu is a member of the family of short, interspersed elements (SINEs). It is approximately 300 nucleotides in length, but with an estimated one million copies on a person’s chromosomes, it makes up about 11% of the human genome. At the PV92 locus of chromosome 16, Alu can be either present or absent, and has lost the ability to transpose. An individual may test homozygous present having the Alu on two chromosomes, heterozygous having the Alu on one chromosome, or homozygous absent lacking PV92 Alu on both chromosomes. Alu is believed to have inserted into the human genome during the last million years during dispersion of modern humans. Therefore, differences in genotype and allele frequencies between human populations are an important tool in understanding human evolution.

Alu insertion polymorphisms are excellent markers for studying the genetic structure and relationships among human populations. Several reviews focusing on Alu elements have been published. Although most Alu inserts do not cause disease, some deleterious Alu activity is associated with a number of disorders in humans. The Alu insert at the PV92 locus of chromosome 16 along with other Alu genetic marker locations are widely used to estimate genetic diversity of human populations. In addition to other geographic locations, researchers have examined PV92 polymorphisms in populations from Africa, Russia, the Caucasus, England, Spain, Eurasian Regions, South Morocco, Argentina, Malaysia, Uruguay, Ivory Coast, Siberia, and Nigeria. One preliminary study examined the PV92 locus of 60 university students from African-American and Japanese populations as well as a control. Nonetheless, to the best of our knowledge no large-scale study has been published on the PV92 locus of chromosome 16 for a university student population or focused on Hardy-Weinberg equilibrium.

Hardy-Weinberg equilibrium is a state where allele and genotype frequencies in a population remain constant from generation to generation. In Hardy-Weinberg equilibrium populations are not evolving in terms of a specific gene. There are certain factors that can affect equilibrium including mutations, natural selection, nonrandom mating, genetic drift, and gene flow. Small populations are more susceptible to changes and are typically not in equilibrium. Large populations are more likely to be in equilibrium, as the effects of mutations, mating, and genetic drift are negligible. Nevertheless, disequilibrium is still frequent in large populations because natural selection and gene flow are common.

This research was performed at Charleston Southern University (CSU), which is a liberal arts four-year private university in North Charleston, South Carolina. According to the school’s website, as of 2021, there were approximately 3,350 students enrolled. Roughly 15% of the undergraduate students are age 25 and older. The international student population makes up about 2% of the student body and comes from 41 different countries. The full-time CSU undergraduate population is made up of 64% females and 36% males. CSU has a range of ethnicities represented on campus, which according to Data USA include White (59.6%), Black (19.8%), Hispanic/Latino (4.1%), Asian (1.6%), and other or unknown (14.9%). The purpose of this research is to find the PV92 Alu genotypes of a racially diverse university student population and determine if it is in equilibrium. We obtained and analyzed data from 269 students at CSU belonging to four different races; Asian, Black, Hispanic/Latino, and White.

Methods

Sample collection

All samples came from undergraduate students enrolled in an introductory genetics lab course at CSU. DNA was collected at random from volunteers. Samples were not linked to specific individuals, but were grouped by gender and race, which was determined by phenotypic appearance. This research was approved by the Institutional Review Board at CSU.

DNA isolation

DNA was isolated following the methods established by Dolan with a few modifications. Cells were collected by vigorously rinsing cheek pockets for 30 seconds with 10 mL of 0.9% saline solution. A 1.5 mL aliquot was then centrifuged at 5,000 rpm for 90 seconds to pellet the cells. The supernatant was removed, and the pellet was suspended in 30 µL of 0.9% saline solution. The sample was then placed into a 1.5 mL tube along with 100 µL of 10% Chelex resin (Carolina Biological Supply Company) and heated for 10 minutes at 95 °C in a heat block. Heat lysed the cells, and the Chelex resin removed metal contaminating ions. After heating, the sample was vigorously shaken for 5 seconds and centrifuged at 5,000 rpm for 90 seconds. DNA was collected by taking 30 µL of the clear supernatant and stored at -20 °C until used in the next step.
Polymerase chain reaction (PCR)

PCR was performed using PuReTaq Ready-To-Go™ PCR Beads with a primer loading dye mix from Carolina Biological Supply. Primer sequences were 16S-F: 5’- GGATCTCAGGTTGGTAGCAGTCG-3’ and 16S-R: 5’-GAAAGGCAAGCTACGAGAGCCTCC-3’. A 2.5 µL aliquot of isolated DNA was mixed with 22.5 µL primer loading dye mix and one Ready-To-Go™ Bead per sample. The thermocycler was programmed for an initial denaturation of 2 minutes and 94 °C, followed by 35 cycles of denaturation for 1 minute at 94 °C, hybridization for 1 minute at 68 °C, and extension for 2 minutes at 72 °C, with a final extension of 5 minutes at 72 °C. PCR products were stored at -20 °C until used in the next step.

Gel electrophoresis

PCR products were separated on a 1.5% agarose gel by electrophoresis along with a pBR322/BsrNI DNA ladder (Carolina Biological Supply Company) and stained with SYBR™ Safe DNA gel stain (Invitrogen™) using previously established protocols. Gels were visualized and photographed using a Gel Doc™ XR system (Bio-Rad Laboratories). A 4 µL aliquot of a positive control was added to the far left lane and a 4 µL aliquot of negative control to the next lane of each gel. In separate wells 10 µL of PCR product were added per sample, as described by Dolan.

Results

In this research, 269 individuals were tested for their PV92 Alu genotypes. Table 1 shows the data organized by race, gender, and genotype. It reveals the majority of students are White, there are more females than males tested, and homozygous absent is the most common genotype. Figure 1 is an example of a standard gel, illustrating each of the genotypes and controls. The Alu insert can be present (+) or absent (-) on each person’s two chromosomes. If absent, a DNA band of about 380 bp is seen. If present, a larger DNA band around 680 bp is seen because the Alu insert is approximately 300 bp in length. Figure 2 shows the percent of individuals with each of the three genotypes to compare the numbers within and between races and genders. Comparing the genotypes in terms of percent allows for an easier evaluation of trends since each category has a different number of participants. Asian students have the highest percent of homozygous present, Hispanic/Latino students have the highest percent of heterozygous, and White students have the highest percent of homozygous absent genotypes. Other apparent trends are the similarity in the distribution of genotypes between male and female White students and between Black and White female students. Goodness of fit between genders and races was determined using chi square tests. Analyses indicated no significant differences between males and females or between Black and White students, but there was a significant difference between other races (Table 2). Hardy-Weinberg calculations (Table 3) suggested the student population is close to equilibrium, and chi square analysis showed no significant difference between the CSU student population tested and equilibrium (Table 2).

Discussion and Conclusions

Figure 1 shows a standard gel, demonstrating each of the genotypes and controls. The SYBR™ Safe DNA gel stain used is more sensitive than traditional stains such as ethidium bromide or CarolinaBLU™. Therefore, faint bands of the incorrect size caused by nonspecific amplification were observed and ignored when interpreting the gels. It was also common to see insoluble material remaining in the wells and diffuse smaller sized bands towards the bottom of the gel caused by primer dimmers. However, only prominent bands of the correct sizes were used to determine PV92 Alu genotypes.

The data is difficult to compare in terms of numbers, as the majority of participants in the research were White females (Table 1). Nevertheless, the data collected is representative of the student population at CSU. After comparing the genotypes in terms of percent (Figure 2), chi square calculations were used to see if any relationships were a good fit between the races or genders. When comparing males...
In conclusion, this is likely the first large-scale study that has been published on the PV92 locus of chromosome 16 for a university student population. Among the 269 students tested at CSU, homozygous absent is the most common genotype. Asian students have the highest percent of homozygous present, Hispanic/Latino students the highest percent of heterozygous, and White students the highest percent of homozygous absent genotypes. Consistent with other studies, the highest PV92 allele frequencies are in Asian and Hispanic/Latino populations. No significant genotype differences were found between male and female or between Black and White students. Nevertheless, there are significant differences between all other race combinations. Hardy-Weinberg calculations indicate that the overall student population at CSU is in equilibrium.

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Notes and References

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Table 2. Goodness of fit for gender and race data.

<table>
<thead>
<tr>
<th>Genotype Comparisons</th>
<th>Ratio</th>
<th>Chi Square</th>
<th>Significant Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male vs Female</td>
<td>0.358</td>
<td>0.0817</td>
<td>No</td>
</tr>
<tr>
<td>Asian vs Black</td>
<td>0.256</td>
<td>20.88</td>
<td>Yes</td>
</tr>
<tr>
<td>Asian vs Hispanic or Latino</td>
<td>1.429</td>
<td>23.571</td>
<td>Yes</td>
</tr>
<tr>
<td>Asian vs White</td>
<td>0.047</td>
<td>61.917</td>
<td>Yes</td>
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<tr>
<td>Black vs Hispanic or Latino</td>
<td>5.571</td>
<td>34.41</td>
<td>Yes</td>
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<tr>
<td>Black vs White</td>
<td>0.183</td>
<td>1.88</td>
<td>No</td>
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<tr>
<td>Hispanic or Latino vs White</td>
<td>0.033</td>
<td>313.26</td>
<td>Yes</td>
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<tr>
<td>Observed vs Hardy-Weinberg</td>
<td>1.000</td>
<td>0.26</td>
<td>No</td>
</tr>
</tbody>
</table>

Table 3. Calculations for frequencies and equilibrium.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Allele Frequency</th>
<th>Hardy-Weinberg Equilibrium</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-,-)</td>
<td>54.60%</td>
<td>N.A.</td>
</tr>
<tr>
<td>(+,-)</td>
<td>37.20%</td>
<td>N.A.</td>
</tr>
<tr>
<td>(+,+)-</td>
<td>8.20%</td>
<td>N.A.</td>
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<tr>
<td>-</td>
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<td>73.20%</td>
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<tr>
<td>+</td>
<td>N.A.</td>
<td>26.80%</td>
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