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Elizabeth Phillips

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# Copy Number Variation in Salivary Amylase: A Participant-Based Study on Genetic Variation

Elizabeth Phillips

Amylase (AMY1) is an enzyme found in the mouth that is used to help digest carbohydrates. It has been found that the copy number of AMY1 has been positively associated with protein levels within an individual and also that individual's population. This information can correspond to the positive ancestral linkage of high starch consumption within agricultural and hunter-gatherer societies. A high starch consumption means that the AMY1 enzyme will be more prevalent within their bodies, and the presence of AMY1 could both help bodies process starches better and prevent future conditions or intestinal diseases. The amylase gene is conclusively connected to the AMY1 copy number production. I hypothesized that individuals within a population will have a similar copy number of the AMY1 gene to each other. Twenty-five high school students located in Norman, Oklahoma were asked to retrieve buccal swabs from the inside of their cheek. DNA then was abstracted from these samples, and a quantitative polymerase chain reaction (qPCR), a machine used to detect the amount of genetic material found in the DNA, was completed in order to determine the copy number within each salivary sample. The qPCR was completed two different times in order to ensure correct results when the data was presented. Results indicated that the copy number within the population were similar to each other, and ranged from 1-12. This means that individuals located in this population have a lower production of amylase, and this provides indication that they are more likely to become obese than in previous research papers located in Arizona. Research shows that a smaller production of AMY1 may contribute to the chances of obesity in the future.

*Keywords:* AMY1, obesity, copy number variation

## Introduction

Salivary a-amylase is an enzyme found in the mouth that processes and breaks down carbohydrates. A-amylase is coded and produced by the AMY1 gene presented on the 1p21 chromosome in a DNA sequence.[1] The AMY1 gene has one of the highest copy number variations in a human DNA sequence, ranging from 1 to 18 diploids.[2] Copy number is defined as the number of times the section of a gene is repeated in a DNA sequence. Studies have shown a positive correlation between copy number of the AMY1 gene and the production of salivary a-amylase.[3] As copy number of the AMY1 gene increases, this means that more salivary a-amylase is produced. The same thing occurs to low copy number variation in retrospect to amylase. Understanding how amylase is produced and found in society could help the human population by having a positive comprehensive effect in which it changes the body and its functions. It is important to understand this because low copy number has been associated with a predisposition to obesity.[4] Identifying key aspects in copy number variation within salivary amylase such as evolution, geographical location,

1. Yang, Ze-Min, Jing Lin, Long-Hui Chen, Min Zhang, Wei-Wen Chen, and Xiao-Rong Yang. 2015. "The roles of AMY1 copies and protein expression in human salivary α-amylase activity." *Physiology & Behavior* 138, 173-178. *Academic Search Elite*, EBSCO*host* (accessed January 9, 2017).

2. Ibid. 1-2.

3. Perry, George H., Nathaniel J. Dominy, Katrina G. Claw, Arthur S. Lee, Heike Fiegler, Richard Redon, and Anne C. Stone, et al. 2007. "Diet and the evolution of human amylase gene copy number variation." *Nature Genetics* 39, no. 10: 1256-1260. *Academic Search Elite*, EBSCO*host* (accessed January 9, 2017).

as well as exercise could help provide reasoning as to what plays an important role in its production.

### Amylase Copy Number V. AMY1 Gene

Copy number variation has been directly linked to the production of salivary amylase, as well as the AMY1 gene. By showing the breakdown of starch viscosity and the amount of AMY1 present in an individual, it is shown that there is a positive correlation between the amount of salivary a-amylase produced in comparison to the AMY1 gene.[5] In order to find copy number of the AMY1 gene, a quantitative polymerase chain reaction (qPCR) is performed. A qPCR is used to amplify and detect or quantify a targeted DNA molecule, which occurs through DNA replication by a series of temperature changes around 25-35 times. Mentioned previously, if there is a lower copy number of the AMY1 gene, then this means that there will most likely be a lower production of salivary a-amylase in general. This correlation would apply to the AMY1 gene with a higher production as well, meaning a higher output of salivary a-amylase.[6]

### Evolution

During the evolutionary process, how genes become different, copy number can be influenced by "selective pressures".[7] Selective pressure includes purifying selection, gene duplication, and positive selection. Purifying selection, or negative selection, is the removal of alleles from a section of DNA that could be potentially harmful. Gene duplication is when a specific section of DNA is replicated. Positive selection is when new beneficial genetics arise in order to benefit a population as a whole.[8] These selective pressures provide fundamental information in order to better understand evolution in either populations or individuals.

Copy number variation can occur for multiple reasons; however, one of the more prevalent reasons is evolution. One idea is that cultural evolution is one of the more rapidly moving genetic changes. This has been happening, recently due to new innovations and cultural changes and this makes human genes adapt to their environment.[9] In the past three decades, there has been an increase in starch production due to a greater consumption of foods such as beer, fruit juices, corn syrup, and manufactured food. The increase in starch consumption has made a-amylase enzymes overcompensate for the change in dietary needs.[10] The production of amylase is not just because of the circumstances that are placed in the environment, stress levels, and circadian rhythm. Amylase has evolved independently over the years, and these populations are found from all over the world.[11]

There is a considerable amount of starch consumption variation among different human populations. George Perry took a sample size of 50 European-Americans and compared their copy number to high starch consumption populations: the Japanese and the Hadza hunter-gatherers. The higher starch popu-

4. Falchi, Mario, Julia Sarah El-Sayed Moustafa, Petros Takousis, Francesco Pesce, Amélie Bonnefond, Johanna C Andersson-Assarsson, and Jane Skinner, et al. 2014. "Low copy number of the salivary amylase gene predisposes to obesity." Nature Genetics 46, no. 5: 492-497. Academic Search Elite, EBSCOhost (accessed January 9, 2017).

5. Mandel, Abigail L., Catherine Peyrot des Gachons, Kimberly L. Plank, Suzanne Alarcon, and Paul A. S. Breslin. 2010. "Individual Differences in AMY1 Gene Copy Number, Salivary α-Amylase Levels, and the Perception of Oral Starch." Plos ONE 5, no. 10: 1-9. Academic Search Elite, EBSCOhost (accessed January 9, 2017).

6. Ibid. 1256.

7. Feng, Zhang, Gu Wenli, Matthew E. Hurles, and James R. Lupski. 2009. "Copy Number Variation in Human Health, Disease, and Evolution." Annual Review Of Genomics & Human Genetics 10, no. 1: 451-481. Academic Search Elite, EBSCOhost (accessed January 9, 2017).

8. Ibid. 1-2.

9. Richerson, Peter J., Robert Boyd, and Joseph Henrich. 2010. "Gene-culture coevolution in the age of genomics." Proceedings Of The National Academy Of Sciences Of The United States Of America107, no. S2: 8985-8992. Academic Search Elite, EBSCOhost (accessed January 9, 2017).

10. Van der Maarel, Marc J.E.C., Bart van der Veen, Joost C.M. Uitdehaag, Hans Leemhuis, and L. Dijkhuizen. 2002. "Properties and applications of starch-converting enzymes of the α-amylase family." Journal Of Biotechnology 94, no. 2: 137. Academic Search Elite, EBSCOhost (accessed January 9, 2017).

lations were compared to the low starch consumers including Biaka and Mbuti who are rainforest hunter-gatherers. Perry's findings suggest that copy number of individuals are more likely comparable in dietary habits than in geographical proximity.[12] Perry suggested that "natural selection may have influenced AMY1 copy number in certain human populations."[13] This observation could suggest a change in the AMY1 gene between populations, suggesting the gene is either becoming more predominant or becoming eclipsed.

Evolutionary changes have affected the a-amylase production among dogs as well. Taking samples from multiple breeds of domesticated dogs and comparing the results to wolves, there was a positive correlation between an increase in amylase production. Domesticated dogs seemed to have an increase in starch-rich diets, and over time the copy number variation changed to compensate for the change in diet.[14] Reiter's findings suggest that this type of dietary intake affects humans as well, not just dogs. Changes in their copy number becoming a positive correlation with starch intake after domestication mean that humans are the reason that amylase production has changed.

Multiple perspectives show how amylase copy number can change because of genetic variation, evolution, and geographical location. Other factors play a key role in the significance of amylase copy number, as well as salivary a-amylase production.

## Obesity

There appears to be a link between obesity and salivary amylase levels. It has been found that the AMY1 gene is one of the largest genetic influences on obesity as a whole.[15] Recent research has found that each copy of the AMY1 gene reduces the risk of obesity by 1.2 fold, which appears to contribute a genetic risk of 11% to the development of obesity.[16] This information suggests that the higher one's amylase production, the less predisposed an individual is to become obese. On the other hand, the lower the amount of amylase a person produces, the higher their predisposition is to becoming obese. Understanding how amylase plays an important role in obesity can help to prohibit the overall effect, making the human population understand why and how it works.

A different study looked at the AMY1 gene and how it corresponded to individuals who were in the normal weight range for their age, and those who were considered obese. By this comparison, it was found that individuals who had a lower copy number were significantly more likely to be obese.[17] This study showed that the difference in the AMY1 gene becomes about an eightfold difference in risk compared to individuals with the most copy number, and those with the least.[18] Amount of AMY1 gene is an important factor in human health, especially when considering the ties that it has to obesity. However, recent studies have suggested that there could be a possible change in the production of salivary a-amylase, combating the risk of obesity.

Amylase is influenced by other factors than the AMY1 gene, "Physical exercise is a strong activator of the sympathetic nervous system".[19] The amount of saliva produced in an individual is obtained through the sympathetic nervous system. Studies have predicted that exercise may be a factor that could help stimulate the production of salivary a-amylase. One study

11. Ibid. 2.

12. Ibid. 1258.

13. Ibid. 1258.

14. Reiter, Taylor, Evelyn Jagoda, and Terence D. Capellini. 2016. "Dietary Variation and Evolution of Gene Copy Number among Dog Breeds." PloS ONE 11, no. 2: 1-19. Academic Search Elite, EBSCOhost(accessed January 9, 2017).

15. Usher, Christina L, Carlos N Pato, Michele T Pato, Mark I McCarthy, David M Altshuler, Robert E Handsaker, and Andres Metspalu, et al. 2015. "Structural forms of the human amylase locus and their relationships to SNPs, haplotypes and obesity." Nature Genetics 47, no. 8: 921-925. Academic Search Elite, EBSCOhost (accessed January 9, 2017).

16. Ibid. 921.

17. Ibid. 492-493.

18. Ibid. 492.

19. Ligtenberg, Antoon J.M., Henk S. Brand, Petra A.M. van den Keijbus, and Enno C.I. Veerman. 2015. "The effect of physical exercise on salivary secretion of MUC5B, amylase and lysozyme." *Archives Of Oral Biology* 60, no. 11: 1639-1644. *Academic Search Elite, EB*SCO-

investigated this hypothesis: eight well-trained males were asked to perform a 60-minute cycle exercise. Afterward, saliva was retrieved from each participant in order to test for amylase. Findings showed that exercise did not change the saliva concentration but did increase the salivary amylase production by five-fold.[20] This finding is supported by previous work.[21]

## Previous Research

Previous research has been conducted on adults in order to determine the amount of salivary a-amylase produced. However, a more diverse genetic testing could assist researchers in understanding the gene that is present with amylase. All of the previously stated research has been adults, both male and female, from a variety of races. Animal testing also has proved to be beneficial in understanding the interaction of the AMY1 gene with amylase. Studies done on bonobos, also known as apes, has shown that sex identification plays a role in the production of salivary amylase.[22] While this study does not specify how this could relate to human interaction, like the previously mentioned studies done on humans, it can provide more in-depth information on how salivary amylase performs.

When discussing genetic research findings, genotypic differences among races is often a confounding issue. While this could be beneficial, it is also important to take into consideration the background information of each individual. Researchers have looked at salivary samples from individuals with a similar starch consumption, and have found that this is more reliable than race.[23] It is important to analyze more than ethnicity when considering copy number variation, due to outside factors that could counteract an accurate representation of the population as a whole. With this being said, the defined European-American samples found in Perry's paper were only located in Arizona and were most likely only adults. There is a lack of diversity within the sample size when considering the youth, and also different European- Americans found in different parts of the country. For this research purpose, high school students, specifically located in Norman, Oklahoma will be included as participants. Looking at this information could provide more in-depth analysis of starch consumption in different geographical locations and how that affects the production of salivary a-amylase.

Identifying age and comparing geographical location in comparison to George Perry's paper could help to further understand the presence of the AMY1 gene. Interpreting data collection in the production of amylase of high school students in comparison to adults in Arizona could either support or disprove Perry's hypothesis, that dietary needs play a more important role than geographical proximity. Further understanding of the AMY1 gene could help researchers develop more perspectives towards the genetic predisposition of obesity.

## Methods

An ex post facto research design on genetic variation within salivary amylase was approved by the Internal Review Board (IRB) at Norman Public Schools (NPS). The current ex post facto study was conducted between February 2017 to March 2017.

### Collection Protocol

To begin collection, safety measures were taken in order to ensure samples were taken as per the protocol and patient health identification was protected. Gloves were worn for the duration of the collection process to ensure no cross-contamination occurred, as well as keep the researcher safe. Materials included

*host* (accessed January 9, 2017).

20. Walsh, N.P., A.K. Blannin, A.M. Clark, L. Cook, P.J. Robson, and M. Gleeson. 1999. "The effects of high-intensity intermittent exercise on saliva IgA, total protein and alpha-amylase." *Journal Of Sports Sciences* 17, no. 2: 129-134. *Academic Search Elite, EB*SCO*host* (accessed January 9, 2017).

21. Ibid. 1639-1640.

22. Behringer, Verena, Claudia Borchers, Tobias Deschner, Erich Möstl, Dieter Selzer, and Gottfried Hohmann. "Measurements of Salivary Alpha Amylase and Salivary Cortisol in Hominoid Primates Reveal Within-Species Consistency and Between-Species Differences." *PLoS ONE* 8, no. 4 (2013). doi:10.1371/journal.pone.0060773.

a buccal swab, 2mL containers to put the buccal swabs in, and both demographic questionnaire and anonymous consent form.

Students were asked to review the full disclosure letter addressed to them, and they were asked to sign an informed consent form if they were willing to consent to participate. To see disclosure, please refer to Appendix A. Then, if they chose to participate in the study, they were asked to fill out demographic questions. Within the demographic questionnaire, there will be a random number written on it corresponding to their sample number in order to ensure anonymity as well as the correct demographic information. To see demographic questions, please refer to Appendix A as well. An announcement was made to students in a class about the study, and if they wished to participate they came into another room and give us their samples. Students approached me one at a time in order to minimize exposure to other students and to keep track of the samples. They were then asked to swab their cheek for 30 seconds with a sterile Catch-All Sample Collection Swab without it touching their teeth. Samples were then put in a container and a number was written on the swab to correspond with the demographic questions. The privacy of the students were not exposed throughout this process, which was reassured in the consent form. The swabs were then placed in a 2 mL tube, frozen, and transported to a lab located at the University of Oklahoma under the direction of Cecil Lewis. Here, the samples were be kept in a fridge to maintain appropriate conditions.

### DNA Extraction

In order to minimize risk to the researcher as well as the DNA, an overhead LED light was used, preventing DNA from being exposed to the elements. Different pipette covers were used for each sample to guarantee that there was no DNA corruption. One blank sample was used as an outlier to make sure that there was no DNA corruption throughout this process.

DNA was extracted using the QIAamp DNA mini extraction kit. This kit allows for DNA extraction by using heat lysis as well as chemical lysis. A lysis is a method used to break open a cell membrane and extract DNA. With this, the steps include adding the different chemicals to the DNA and incubating the sample at 56 degrees Celsius. Multiple chemicals were added, vortexed, and centrifuged (in order to push the solid unwanted material to the bottom and the DNA to the top). This continued four more times, as well as including filtration steps, this allowed proper separation of the DNA from unwanted cellular material. Final DNA extracted samples were placed in a 2mL sample collection container with the identification number written on top. After this, they were placed in a freezer so that way they are kept safe from any unwanted harm.

### Quantitative Polymerase Chain Reaction (qPCR)

The DNA was thawed out for approximately 10-15 minutes. Next, a probe kit was utilized to assure that the salivary samples would amplify in the qPCR machine. Samples were then put in a labeled container that would fit into the qPCR machine. One sample was left blank as a control, as well as one sample having a known copy number (E. coli). Amplification occurred through multiple heating and cooling processes (about 25-35 times) within a qPCR machine, until the exact concentration of the AMY1 gene was detected. The amplification process within a qPCR lasted roughly 1 to 2 hours. Data was then collected on a computer to show/display the amplification process and the copy number presented within each sample. Information found was then interpreted and put in a spreadsheet and various graphs. At the end of the data collection, samples were destroyed through proper disposal into a biohazard waste container.

## Results

Numerical information is presented below for the copy number of the AMY1 genes. This information was gathered and put together within an Excel spreadsheet. The graphs will be shown through the format of copy number being presented on the x-axis, and proportion of individuals with that copy number being shown on the y-axis. Each graph will be divided up in relation to race, however this data could also show repetition of people who are more than one race. Graphs will then be compared to those found in the supplement of George Perry's paper regarding a similar topic.

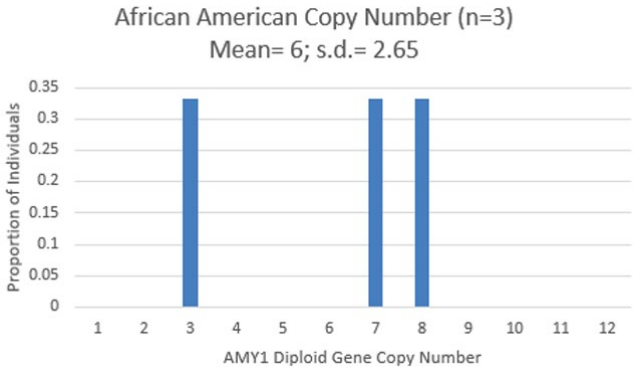


*Figure 1*
*This graph shows the copy number present with high school students who consider themselves African American. It is shown that there was a limited sample in this test group, however the mean copy number is close to that presented in George Perry's paper.*

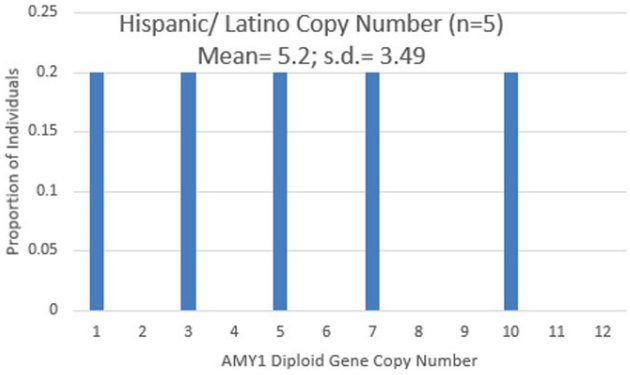


*Figure 2*
*This graph shows the copy number presented with high school students who consider themselves Hispanic or Latino. The sample size while still on the small size is at five, and shows a great variety between the copy number presented within the group as a whole.*

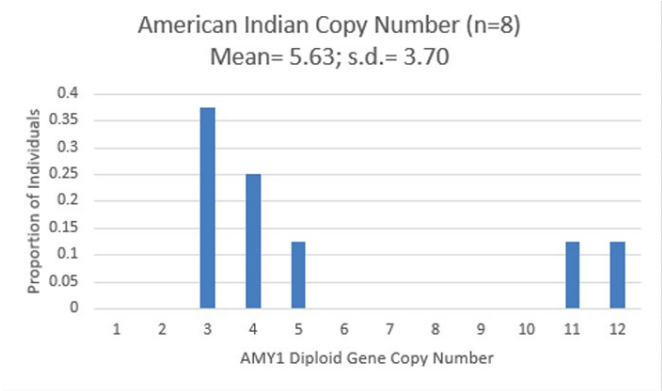


*Figure 3*
*This graph shows the copy number presented with high school students who consider themselves American Indian. This group of data has copy numbers which are closer together and more correlated. The two most common copy numbers were three and four, showing the similarity between copy number within this group.*

# Discussion

## Race Relation

When considering the data collection there was not a lot of variation within race. Caucasian being the highest group of samples is simple in comparison to George Perry's paper. The mean copy number found within high school Caucasians (European-Americans) was 5.33 (Figure 4), while the copy number found in Perry's paper was 6.80 (Figure 6).

This shows that high school European-Americans located in Oklahoma tend to have a lower copy number than adults in Arizona. While there are limitations considering the smaller sample size, it is still valid to look at the correlation between races as well as the copy number as a whole. With this being said there was a computed p-value of 2.26*10^(-5). This shows that since the p-value is smaller than a reasonable cut off, it is concluded that they are different i.e. sufficient evidence against the claim that the Arizona and Oklahoma means are equal. The p-value was found using the separate (unpooled) variances for inferences in two-population means. Even with a small sample size through the p-value it is shown that there is a difference between copy numbers in Arizona, and Oklahoma. Looking at Perry's values shows that the closest mean copy number within race is the African American subgroup. The copy number for this group was 6, which means it is the closest to Perry's being 6.80. It would be expected that Caucasians would have the closest copy number to the European-American group found in Perry's paper; however, this was not the case. While the African American group had a very small sample size it still shows an accurate representation when comparing race in genetic studies

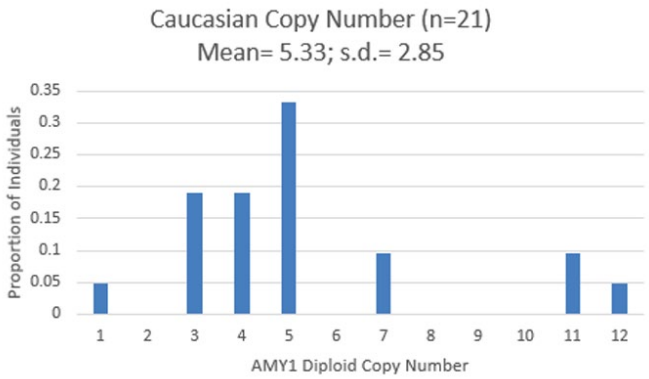


*Figure 4*
*This graph shows the copy number presented with high school students who consider themselves Caucasian. The data group presented shows shows a wide variety of individuals tested with 21 test subjects. Overall there is a widespread of copy numbers, however there seems to be a closely correlated amount between three and five.*

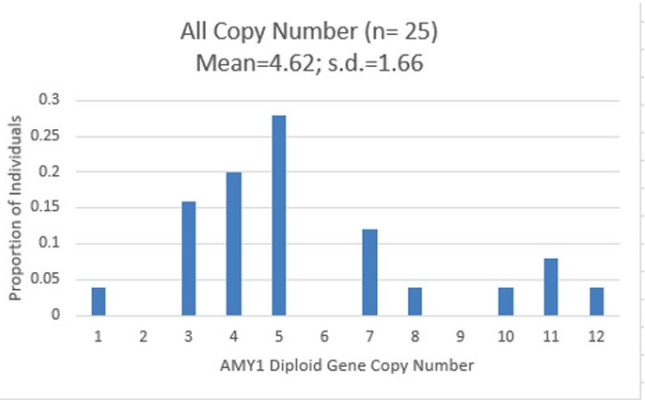


*Figure 5*
*This graph represents all of the high school test subjects who were tested. Overall it is the best representation of data considering that it has the highest amount of individuals, as well as having the most accurate amount of correlation for each section of individuals. In comparison it shows the most accurate representation for copy number presented in high school students.*

*Overall the sample size was cut in half compared to the paper done by George Perry, especially when looking at race individually. Looking at the copy number of high school students as a whole shows a better representation of the copy number variation presented in Norman.*

like this one. There is no correlation between race and copy number considering that Caucasian would have the closest copy numbers in relation to Perry's European-Americans, this was however not true. Considering this it would be most beneficial to look at high school students as a whole in comparison to the copy number found in Perry's paper. Given the numerical values it is realistic to believe that copy number is found in relation to geographic location, specifically when considering dietary intake of individuals.

## Copy Number Variation

Relating the information gathered from the experiment to previous studies is important when considering the future implications of these findings. When looking at Figure 5 and Figure 6 together it is shown how copy number variations range from either 1-12, or 1-15. Considering this information and the graphic information when looking at the proportion of individuals, it is shown that copy number variation has a wide range, and is very widespread within the samples provided. While the mean in Figure 5 is 4.62 copies, and the one in Figure 6 is 6.80 copies, there is much diver-

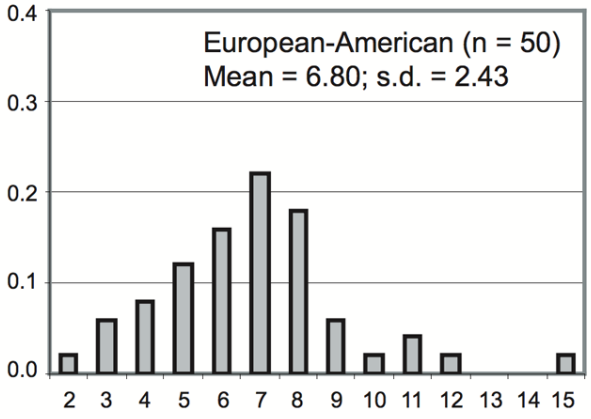


*Figure 6*
*This graph represents the copy number variation found in European Americans done by George Perry. Individuals in this paper ranged from age, and were located in Arizona. The mean copy number appears to be higher than the copy number found in Norman, Oklahoma.* [24]

23. Ibid. 1256-1257.

24. Perry, George. "Supplementary information for: GH Perry, NJ Dominy, KG Claw, AS Lee, H Fiegler, R Redon, J Werner, FA Villanea, JL Mountain, R Misra, NP Carter, C Lee, and AC Stone Diet and the evolution of human amylase gene copy number variation." *Nature*

sity in the population who maintain the copy number variation itself. A random sample of the copy numbers within Perry's paper is presented within Table 1 and shows how there is a variation within copy number, however, the majority of copy numbers are between 6-8. This means that there is no definite copy number that will fit as a generic number for the population that it is in. Considering this information the results found from the high school students in Oklahoma are accurate, despite their wide range in variation.

Looking at each figure individually, it is shown that race is not a significant variable, and all Norman High students in Oklahoma seem to have a lower copy number. Perry's adult samples from Arizona tend to have a higher copy number of about two each time. This information could provide a possible correlation between starch consumption being a factor in the lower copy number being presented in Oklahoma. Oklahoma could tend to consume less starch in the past than in Arizona, with this genetically speaking their copy number would not have had a chance to become higher.

## Obesity Risk

Oklahoma as a whole tends to have a lower copy number than Arizona. As this is shown there has been a positive correlation between low copy number and

**Supplementary Table 1. qPCR, protein quantification, and aCGH data for high- and low-starch population samples.**

| Population | Sample | Starch level (high/low) | Diploid *AMY1* copies (qPCR) | Standard Deviaion | Diploid *AMY1* copies (integer) | AMY1 protein mg/mL | Chr1tp-6D2 log2 ratio | Chr1tp-30C7 log2 ratio |
|---|---|---|---|---|---|---|---|---|
| European-American | EUR001 | High | 7.30 | 0.50 | 7 | 2.83 | | |
| European-American | EUR002 | High | 4.26 | 0.49 | 4 | 1.65 | | |
| European-American | EUR003 | High | 7.10 | 0.93 | 7 | 3.85 | | |
| European-American | EUR004 | High | 4.91 | 0.21 | 5 | 1.09 | | |
| European-American | EUR005 | High | 8.15 | 0.78 | 8 | 1.63 | | |
| European-American | EUR006 | High | 11.73 | 0.55 | 12 | 5.17 | | |
| European-American | EUR007 | High | 6.50 | 0.38 | 6 | 3.24 | | |
| European-American | EUR008 | High | 8.44 | 0.70 | 8 | 2.80 | | |
| European-American | EUR009 | High | 5.73 | 0.39 | 6 | 3.30 | | |
| European-American | EUR010 | High | 8.36 | 0.93 | 8 | 4.28 | | |
| European-American | EUR011 | High | 7.63 | 0.45 | 8 | 2.91 | | |
| European-American | EUR012 | High | 6.89 | 0.51 | 7 | 2.89 | | |
| European-American | EUR013 | High | 11.20 | 0.80 | 11 | 3.76 | | |
| European-American | EUR014 | High | 6.18 | 0.31 | 6 | 2.65 | | |
| European-American | EUR015 | High | 7.94 | 1.12 | 8 | 1.70 | | |
| European-American | EUR016 | High | 5.56 | 0.76 | 6 | 3.20 | | |
| European-American | EUR017 | High | 8.53 | 0.75 | 9 | 2.96 | | |
| European-American | EUR018 | High | 9.67 | 0.56 | 10 | 4.87 | | |
| European-American | EUR019 | High | 7.46 | 1.00 | 7 | 4.00 | | |
| European-American | EUR020 | High | 3.41 | 0.56 | 3 | 0.93 | | |
| European-American | EUR021 | High | 2.21 | 0.50 | 2 | 0.22 | | |
| European-American | EUR022 | High | 5.27 | 0.54 | 5 | 1.65 | | |
| European-American | EUR023 | High | 9.14 | 0.64 | 9 | 2.72 | | |
| European-American | EUR024 | High | 7.64 | 0.22 | 8 | 2.46 | | |
| European-American | EUR025 | High | 5.87 | 0.36 | 6 | 1.35 | | |

Table 11

This table shows random samples taken from Perry's paper, which show a closer look at the genetic variation presented within each individual. The table allows for an expanded look into each person's individual copy number. Overall the sample size was cut in half compared to the paper done by George Perry, especially when looking at race individually. Looking at the copy number of high school students as a whole shows a better representation of the copy number variation presented in Norman.

*Genetics*. September 9, 2007. Accessed September 18, 2016. http://www.nature.com/ng/journal/v39/n10/extref/ng2123-S1.pdf.

25. Ibid. 7.

26. "Oklahoma." Oklahoma State Obesity Data, Rates and Trends: The State of Obesity. Accessed March 11, 2017. http://stateofobesity.org/states/ok/.

27. "Arizona." Arizona State Obesity Data, Rates and Trends: The State of Obesity. Accessed March 11, 2017. http://stateofobesity.org/states/az/.

a higher chance at obesity. Individuals with a lower copy number will not be able to process starches as well as those with a higher copy number, this is why they have a higher chance at obesity. Through this information it is accurate to assess that high school students in Oklahoma, as most likely the population as whole, will tend to have a higher chance of obesity. This assertion is back up by the statistics showing that Oklahoma has an obesity rate of 33.9% among adults.[24] In comparison the obesity rate of adults in Arizona is 28.4%.[25] Considering that Arizona has a higher copy number it is reasonable to see the correlation between Oklahoma having a higher chance of obesity than in Arizona. However, this information does not mean that people will necessarily become obese in Oklahoma, it means that they have a higher risk than people located in Arizona.

## Limitations and Future Research

Limitations within this study mainly included sample size. The sample size taken was of 25 high school students in Oklahoma. With this it was not possible to have more than 25 samples given the amount of time available. However, there was not a wide range of diversity within different races, while this was not a big factor in the research proposal, it would have been beneficial to have a bigger group of different races, as well as a wider age group in order to have a more concrete view of Oklahomans as a whole. In comparison to Perry's sample size of 50, the sample size of 25 within this research presented a good beginning representation within Oklahoma.

Future research within this subject could be presented within multiple options. Asking demographic questions such as weight and height and doing a Body Mass Index (BMI), would help to provide a more concrete insight on the findings that this study showed. Looking at Oklahoma as a whole instead of taking a subsection of it in Oklahoma would be interesting. This would provide a wider scope to see if widespread copy number variation was different in the state of Oklahoma as a whole. As this is considered looking at adults in Oklahoma not only high school students could help to provide a deeper understanding of copy number. Any of these possible scenarios could provide a deeper insight on copy number as a whole.

## Conclusion

In conclusion, it is shown that the AMY1 copy number is in correlation between the production of salivary amylase. This is shown through multiple studies done by other researchers as well as within this study. Looking at previous studies done by George Perry, and taking similar samples within subgroups, I was able to compare the copy number of individuals. There seemed to be no correlation between race related groups, possibly because of the small sample size, but rather that each copy number appeared to be closely associated with starch ingestion within past generations. Through this information it was shown that high school students in Norman, Oklahoma tend to have a lower mean copy number than adults in Arizona. This is shown in correlation of the mean value, as well as the p-value being 2.26*10^(-5), showing that Oklahoma and Arizona have a difference in copy number. With this, Oklahomans may have a greater chance at becoming obese than individuals in Arizona. Information like this can help to combat obesity by providing the public with a deeper insight on the need for them to protect their bodies with healthy food and exercise. As shown earlier in this paper exercise increases the production of salivary amylase. Understanding bodily functions like this could provide researchers with a deeper insight on the way in which bodies work, and the best way to protect them.

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# Appendix A

The informed consent as shown to participants is shown below as well as the demographic questions:

ANONYMOUS/CONFIDENTIAL RESEARCH

## Dear Student:

I am a senior here at Norman High. I am conducting this research for my AP Capstone class, under the advisement of Sarah DeWitt. I am conducting a research study entitled Amylase Copy Number. The purpose of this study is look at amylase copy number of high school students in Norman, Oklahoma. Amylase is a salivary enzyme found in the mouth that processes and breaks down carbohydrates. Copy number is defined as the number of times the section of a gene is repeated in a DNA sequence. This corresponds with the amount of salivary amylase produced. However it would be beneficial to see the amount of amylase produced, given that small amounts have corresponded to obesity in previous studies. The data that I will be retrieving from this study will be compared to that in a previous study done by George Perry. I will compare the copy number found in high school students in Oklahoma to that in Perry's paper, which he looked at in a different part of the United States. This comparison will give me initial data in order to compare my research and data to. Perry's null hypothesis was that individuals of the same race will have similar copy numbers, however it was found that this was not the case. This will help to prove my hypothesis with copy number variation being present because of diet and evolution.

Your participation will involve filling out a paper with your demographic information, as well as providing 2 mL of saliva and should only take about 10 minutes of your time. Your involvement in the study is voluntary, and you may choose not to participate or to stop at any time. The results of the research study may be published, but your name will not be used. Your identity will not be associated with your responses in any published format.

The findings from this project will provide information on amylase copy number, and the presence within different individuals, with no cost to you other than the time it takes for the survey.

If you have any questions about this research project, please feel free to call me, Elizabeth Phillips, at [redacted] or [redacted]. Questions about your rights as a research participant or concerns about the project should be directed to the Institutional Review Board at Norman Public Schools at [redacted].

Thanks for your consideration!

Sincerely,
Elizabeth Phillips

# Demographic Questions

The information used in this questionnaire will remain anonymous in association with your saliva. Please answer the following questions to your best abilities. You may leave a question blank if you do not feel comfortable answering it.

1. Subject Number :_____
2. Age:_____
3. Gender (Circle): Male Female
4. Ethnicity (Circle): African American Hispanic/ Latino Asian Caucasian American Indian/ Alaskan Native Other:_____
5. How many hours a week do you exercise? (Circle): 0 Hours 1-3 Hours 4-6 Hours More Than 6 Hours
6. How long has your family lived in Oklahoma (Circle): 1st Generation 2nd Generation 3rd Generation 4th Generation 5th Generation or more