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Salivary amylase gene copy number: Have humans adapted to high starch diets?

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Background
Cereal crop domestication began approximately 10,000 years ago in the Middle East. Wild varieties were already being used for food from 16,000 years ago. Rice was domesticated in China and Maize was domesticated in South America, both around 7,000 years ago. These global changes in subsistence led to an overall reduction in protein and fat intake and an increase in carbohydrate intake, especially starch. Salivary Amylase is an enzyme found in saliva which catalyses the initial stage of starch digestion in the mouth. Humans show variation in salivary amylase (AMY1) gene copy number between 2 and 18 gene copies in different individuals. Bank et al. (1992) demonstrated that variation in AMY1 gene copy number results in differing levels of salivary amylase enzyme expression. High levels of AMY1 enzyme expression confers an increased ability to hydrolyse starch in the mouth.

AMY1 gene cluster variation

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<tr>
<th>AMY1 cluster repeats</th>
<th>AMY*H0 allele</th>
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<tr>
<td>2B 2A 1C</td>
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0 AMY1 cluster repeats - AMY*H1 allele

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<th>AMY1 cluster repeats</th>
<th>AMY*H1 allele</th>
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<td>2B 2A 1A 1B P1</td>
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1 AMY1 cluster repeats - AMY*H2 allele

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<tr>
<th>AMY1 cluster repeats</th>
<th>AMY*H2 allele</th>
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<td>2B 2A 1A 1B P1 1C</td>
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Hypothesis

High AMY1 gene copy number became selectively advantageous with the adoption of high starch diets in human populations.

Methodology

Two PCR based assays were designed:

1) To amplify an area around a deletion present in the AMY1 genes but not in AMY2.
2) To amplify an area around a deletion present in the AMY2 genes but not in AMY1.

Each PCR reaction produced 2 fragments of different size, one originating from AMY1 and the other from AMY2.

ABI GeneScan technology was used to quantify the ratio of AMY1 products to AMY2.

As the number of AMY1 genes are always constant, the AMY1:AMY2 product ratio indicated the number of AMY1 genes present.

Experiments were carried out to assess the accuracy of the quantification method and to determine how many times the assay needed to be repeated to obtain reliable results. Each sample was PCRd twice and each PCR was run twice on a GeneScan gel.

AMY Microsatellites Protocol

A multiple PCR protocol was designed to amplify 6 microsatellites markers closely linked to the AMY1 gene cluster.

ABI GeneScan technology was used to determine the microsatellite repeat number alleles.

Determining Phase

In addition to using families to establish AMY1 repeat number alleles, software was developed for estimating haplotypes, as well as for the accurate prediction of AMY1 allele frequencies.

Microsatellite haplotypes were established by typing family samples.

Identifying Selection (1)

Differences in allele frequencies among populations

The proportion of allele frequency variation due to differences between populations was estimated using FST. Directional selection on certain alleles in some, but not in other populations, will lead to an increase in FST for that locus, when compared to values for neutral loci (a null-distribution). Balancing selection leads to a decrease in FST when compared with neutrally evolving loci. When compared against a published null-distribution of FST between European and East Asian populations for assumed neutral markers, the highest pairwise FST obtained for the AMY1 data (Mongolia vs Saamis=0.056) was not an outlier on the distribution (See Fig 3).

Identifying Selection (2)

Intra-allelic variability

Intra-allelic variability is the joint distribution of the frequency of an allele and the extent of variability at closely linked loci. Under the neutral model, the frequency of an allele should be related to its age, because it takes a long time for rare (including new) alleles to drift to high frequencies in populations. Therefore, if a young allele is at high frequency in a population then this suggests that selection has been operating. The relative ages of different alleles can be estimated by looking at the variation in closely linked markers (e.g. microsatellites). Preliminary analysis of the AMY1 repeat alleles and variance at six closely linked microsatellites indicates that there has been some selection in the AMY1*H1 allele compared to the AMY1*H0 allele in European populations.

Chimpanzee AMY1 alleles?

The AMY1 quantification protocol was redesigned to quantify chimpanzee AMY1 variation. The 5 chimp tested were found to be homozygous for the AMY1*H10 allele. These results suggest that AMY1 gene copy number variation in humans arose after the split between humans and chimpanzees.

Summary

Based on the comparison of FST values among different populations it was not possible to identify different selection forces acting on AMY1 alleles in different populations. This could be because selective pressure has not been acting on the AMY1 alleles. Alternatively, a lack of power in the method and the data may also explain our observations. The FST method is known to be relatively insensitive. Using data from six closely linked human microsatellites in an analysis of intra-allelic variability, some evidence was found for positive selection acting on the AMY1*H1 allele in Europeans.

Further work is in progress to estimate a date for the emergence of the various AMY1 repeat number alleles.

Acknowledgements

Many thanks to Dr. M. Stumpf for the development of MstMulti (interallelic variability analysis software).

References


Fig 2: Geographical frequency distribution of mean number of AMY1 repeats per individual.