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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 East1. 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 individuals2. Bank et al. (1995)3 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 hydrolyze starch in the mouth.

AMY1 gene cluster variation

<table>
<thead>
<tr>
<th>AMY1 cluster repeats - AMY*H0 allele</th>
<th>2B</th>
<th>2A</th>
<th>1A</th>
<th>1B</th>
<th>P1</th>
<th>1C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 AMY1 cluster repeats - AMY*H1 allele</td>
<td>2B</td>
<td>2A</td>
<td>1A</td>
<td>1B</td>
<td>P1</td>
<td>1C</td>
</tr>
<tr>
<td>1 AMY1 cluster repeats - AMY*H2 allele</td>
<td>2B</td>
<td>2A</td>
<td>1A</td>
<td>1B</td>
<td>P1</td>
<td>1C</td>
</tr>
</tbody>
</table>

Hypothesis

- High AMY1 gene copy number became selectively advantageous with the adoption of high starch diets in human populations.
- Populations with a long tradition of high starch diets should have an increased frequency of high AMY1 repeat number alleles when compared to hunter gatherers or those populations which have adopted cereal agriculture more recently.
- High AMY1 repeat number alleles will have been lost to low frequency more quickly than expected for neutral alleles.

Methodology

- PCR based assays were designed:
  1. To amplify an area around deletions present in the AMY1 genes but not in AMY2
  2. To amplify an area around deletions present in the AMY2 genes but not in AMY1
- Each PCR reaction produces 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.
- The number of AMY1 genes can be estimated, as the AMY1/AMY2 product ratio indicates 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 is PCR'd twice and each PCR is run twice on a GeneScan gel.

AMY Microsatellite Protocols

- Multiplex PCR protocols were designed to amplify 6 microsatellite markers closely linked to the AMY1 gene cluster.
- ABI GeneScan technology was used to provide accurate sizing of the products.
- To determine the microsatellite repeat number alleles.

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*H0 allele. These results suggest that AMY1 gene copy number variation in humans arose after the split between humans and chimpanzees.

Summary

- The comparison of FST values among different populations 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. The chimpanzee data suggests that the most frequent allele in humans (AMY1*H1) may not be the ancestral allele in humans as was previously assumed.

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References