AMY1 Gene Copy Number, Metabolic Syndrome Parameters and Ethnicity - a Pilot Study.

Authors: Dr Ferguson WGL¹, Dr (PhD) Beaver PW²,³, Professor Schofield G⁴, O'Dwyer T¹, Dr Joseph N¹, Zinn C⁴ & Ass. Professor Chan C-K⁵

Author affiliations: Kumeu Medical Centre¹, Auckland, New Zealand, Latrobe University School of Allied Health, Dietetics and Nutrition,² Melbourne, Australia, Fitgenes Pty Ltd³, Melbourne, Australia, Human Potential Centre, Auckland University of Technology, Auckland, New Zealand⁴ and Nazarbayev University, Dept. of Biomedical Sciences, School of Medicine, Astana, Kazakhstan⁵.

Introduction:
The copy number of AMY1, the gene that codes for salivary amylase, varies substantially between individuals and population groups¹. Research shows that individuals with a low copy number of AMY1 may have a decreased capacity for aspects of carbohydrate metabolism and be predisposed to being overweight / obesity.²,³ It is likely that these individuals may be at increased risk of metabolic syndrome and Type 2 Diabetes if chronically exposed to high carbohydrate loads.⁴,⁵ This relationship may prove important for understanding how dietary advice can be optimised for individuals, but little is known about the association between parameters of the metabolic syndrome and AMY1 gene copy number, especially in New Zealand ethnic groups, such as Maori and Pacific people. We conducted a cross sectional pilot study to explore the relationship between AMY1 copy number, HbA1c, BMI and ethnicity.

Method:
A sample of 49 patients were enrolled in the study, all aged 35-55 stratified by HbA1c either 40 or less (27) or greater than 46 (22), 26 of whom were European and 23 were of Maori or Pacific ethnicity. Type 1 Diabetes and other endocrine disorders were excluded. Basic metabolic and anthropomorphic parameters were recorded and the AMY1 copy number analysed. Dietary interventions with respect to carbohydrate load were then tailored to patients’ individual copy numbers.

Discussion:
We found the expected association between HbA1c and increased BMI. When low copy numbers (4 or less) were compared with high copy numbers (6 or more) the patients with high copy numbers had an average HbA1c of 34.4 and an average BMI of 37.0. The patients with a low copy number had a higher HbA1c of 47.3 and yet their BMI was considerably less averaging 31.9. That is a low AMY1 copy number appears to show a greater degree of difficulty with glycaemic control, that relates directly to the starch component of the diet and furthermore this is of considerably more significance than BMI in predicting a higher HbA1c. These results were independent of ethnicity. Preliminary results for the dietary interventions with respect to carbohydrate load based on the patients’ individual copy number resulted in significant reductions in metabolic parameters, such as Glucose levels and HbA1c.

Conclusions:
1. AMY1 gene Copy number may be more significant than BMI in predicting HBA1c and hence risk of Type 2 Diabetes.
2. Dietary interventions based on the patient’s AMY1 copy number can have a significant effect on metabolic parameters, and hence risk of Type 2 Diabetes.

References:
AMY1 Gene Copy Number, Metabolic Syndrome Parameters and Ethnicity - a Pilot Study.

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Presenter: Dr Paul Beaver (PhD)

Author affiliations: Kumeu Medical Centre\textsuperscript{1}, Auckland, New Zealand, Latrobe University School of Allied Health, Dietetics and Nutrition,\textsuperscript{2} Melbourne, Australia, Fitgenes Pty Ltd\textsuperscript{3}, Melbourne, Australia, Human Potential Centre, Auckland University of Technology, Auckland, New Zealand\textsuperscript{4} and Nazarbayev University, Dept. of Biomedical Sciences, School of Medicine, Astana, Kazakhstan\textsuperscript{5}.

Conflict of Interest: Dr Paul Beaver is the co-founder, Chief Scientific Officer and a shareholder in Fitgenes Australia Pty Ltd, as well as an Honorary, Senior Research Fellow, School of Allied Health, Dietetics and Nutrition, La Trobe University, Melbourne, Australia.
Introduction

- Currently a global obesity & Type 2 Diabetes (T2D) epidemic.
- The 'one size fits all' approach is not working so now looking to the human genome for answers.
- Meta-analyses have identified certain gene variations (SNPs*) associated with T2D, such as for FTO and MC4R genes.
- FTO SNP is the strongest and most replicated association with BMI (OR =1.67)\(^1\)

*Single Nucleotide Polymorphisms


Structural Variations in Genomic DNA

- Different types of genetic structural variations.
- SNPs are the most abundant, the most researched and have long been associated with phenotype variations.
- Recently, the importance of copy-number variations (CNVs) has been realised.
- Recent research shows strong associations between CNVs and disease states, and hence our health.\(^1\)

AMY1 COPY NUMBER and OBESITY

- The AMY1 gene, which codes for salivary amylase, has CNVs.
- CV values vary substantially between individuals and population groups (CNV from 1 – 20).
- The AMY1 CN is the genome’s largest influence on obesity.
- Independently verified to have the greatest correlation to a physiological parameter.
- The AMY1 CN is linearly related to amount of salivary amylase.
  - CN = 2 - salivary amylase 4 IU/L
  - CN = 9 - salivary amylase 28 IU/L


AMY1 CN - testing

- Genotyping
  - SNPs - mature and relatively straight forward
  - CNV - newer, more complex, and requires more precision and sensitivity to discriminate between the CN values

- Is there any other way to quantify the effect of AMY1 CNs?

- Can we use AMY1 SNPs as surrogates for AMY1 CNs even if structurally they are different?
AMY1 CNs compared with SNPs

- Phenotypically CNs have a much greater effect than SNPs
- Any AMY1 SNP only explains a very small fraction of the variation in the AMY1 CN,\(^1,2\) i.e. only on average 1 CN per minor allele \(^1\).
- AMY1 CN range is from 2 to 20+
- In the GIANT Meta-analysis, none of the 17 AMY1 SNPs studied even reached nominal significance with regard to BMI\(^1\)


### AMY1 Copy Number - overview

<table>
<thead>
<tr>
<th>LOW (1-4)</th>
<th>INTERMEDIATE (5-8)</th>
<th>HIGH (9+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Hunter - gatherers</td>
<td>• Moderate salivary amylase production</td>
<td>• Agricultural societies</td>
</tr>
<tr>
<td>• Low salivary amylase production</td>
<td>• Moderate starch sensitivity</td>
<td>• High salivary amylase production</td>
</tr>
<tr>
<td>• High starch sensitivity</td>
<td>• Moderate risk of obesity and T2D</td>
<td>• Low starch sensitivity</td>
</tr>
<tr>
<td>• Increased risk of insulin resistance</td>
<td>• Up to 800%(^1) increased risk of obesity &amp; T2D</td>
<td>• Decreased insulin resistance</td>
</tr>
<tr>
<td>• Up to 800%(^1) increased risk of obesity &amp; T2D</td>
<td>• Increased gluten sensitivity</td>
<td>• Lower risk of obesity and T2D</td>
</tr>
<tr>
<td>• Increased gluten sensitivity</td>
<td>• Lower perception of oral starch and satiety.</td>
<td>• Lower gluten sensitivity</td>
</tr>
<tr>
<td>• Higher risk of infection</td>
<td>• Higher risk of infection</td>
<td>• Foods taste sweeter and richer</td>
</tr>
<tr>
<td>• More sensitive to current western diet.</td>
<td>• More sensitive to current western diet.</td>
<td>• Lower risk of infection</td>
</tr>
</tbody>
</table>

## Results

<table>
<thead>
<tr>
<th>CN</th>
<th>LOW CN=2,3 or 4</th>
<th>INTERMEDIATE CN = 6,7, 8 or 9</th>
<th>HIGH CN = 10+</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of patients (N)</td>
<td>18 (62%)</td>
<td>10 (34%)</td>
<td>1* (4%)</td>
</tr>
<tr>
<td>BMI (average)</td>
<td>31.9</td>
<td>37.0</td>
<td>34.0</td>
</tr>
<tr>
<td>HbA1c (average) (mmol/m)</td>
<td>47.3</td>
<td>34.4</td>
<td>61</td>
</tr>
</tbody>
</table>

*Patient had been suffering from severe depression for 20+ years.
In humans salivary amylase activity (s-AA) is regarded as a surrogate stress marker as amylase responds faster to acute psychological stress than cortisol.

**CONCLUSION**

- The T2D Patients with the lower copy numbers were on average much smaller patients with much higher HbA1c levels than the patients with the higher copy numbers, who on average were bigger patients but had significantly lower HbA1c levels.

- This suggests that the patients with lower AMY1 copy numbers could not tolerate starch as well as those with the higher copy numbers, and were at a higher risk of becoming T2D, based on their HbA1c values, irrespective of their ethnicity.

## Case Study #1

- 49-year-old woman, BMI 36, Metabolic Syndrome, T2D, & on Insulin.
- Struggled with her weight despite totally committed to diet and exercise, optimal medication and careful blood sugar monitoring.
- On a low carbohydrate/Paleo diet her sugars swung quite wildly between 7 and 29, with very few within the normal range.
- In early June 2016 received results of her AMY1 Copy Number = 2.
- Modified the quantity and type of starch in her diet.
- Glucose results suddenly much more tightly controlled, with about half the results in the normal range.
- Also feeling much better in herself.
- Without this vital piece of her genomic puzzle the patient’s doctor would have not normally considered such severe carbohydrate (starch) restriction,
- Would have assumed other metabolic reasons for her difficult insulin resistance and would have not persevered in trying to solve the problem with further dietary modification.
Case Study #1

Modified diet based on AMY1 Copy Number CN = 2

Case Study #2

- 53 year old man - Non Insulin Dependent Diabetes Mellitus (NIDDM) in 2000 and Coronary Artery Disease in 2006
- Despite reasonably rigorous dietary measures and increasing medication his glycaemic control deteriorated.
- Diabetic medication - slow release Insulin, Glicazide and Metformin.
- His diet at this time was a prudent low carb/low fat.
- In May 2016 he had a HbA1c reading of 71 mmol/mol.
- Despite being totally compliant was not making progress.
- On 23rd May 2016 found out that he had a low AMY1 copy number (CN=2) and modified diet accordingly.
Case Study #2

After 1 week his doctor stopped his insulin and halved his T2D medication. After week 2 the patient stopped remaining medication. He is no longer on any medication.

Case Study #2

<table>
<thead>
<tr>
<th>MACRONUTRIENTS</th>
<th>BEFORE</th>
<th>AFTER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>56.6%</td>
<td>19.90%</td>
</tr>
<tr>
<td>Protein</td>
<td>22.4%</td>
<td>42.15%</td>
</tr>
<tr>
<td>Fats</td>
<td>21.0%</td>
<td>37.95%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Results 23rd May 2016 (prior to receiving AMY1 results)</th>
<th>Results 29th August 2016 (After changing diet based on his AMY1 result of CN = 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (Kg)</td>
<td>127</td>
<td>115</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>140</td>
<td>124</td>
</tr>
<tr>
<td>BMI</td>
<td>39.2</td>
<td>35.1</td>
</tr>
<tr>
<td>HbA1c(mmol/mol)</td>
<td>71</td>
<td>41</td>
</tr>
<tr>
<td>Blood Pressure</td>
<td>144/91</td>
<td>120/70</td>
</tr>
</tbody>
</table>
Conclusions

1. The AMY1 copy number (CN) test is a powerful clinical tool for use as a biomarker.
2. AMY1 CN values have a very strong association with metabolic parameters and the risk of T2D.
3. AMY1 gene CN was more significant than BMI in predicting HbA1c and hence risk of Type 2 Diabetes.
4. Dietary interventions based on the patient’s AMY1 CN can have a very significant effect on metabolic parameters, and hence risk of Type 2 Diabetes.
5. The effects of the AMY1 CN were independent of ethnicity, whether Caucasian, Maori or Pacific people.
6. The AMY1 CN can assist in stratifying overweight and / or insulin resistant patients who are more likely to respond to a starch modified diet.