AIMA Conference – Auckland, NZ, 6th & 7th April, 2019

Importance of AMY1 Copy Number in Influencing Metabolic Health and Hence Risk of Obesity and Type-2 Diabetes

Dr Paul Beaver (PhD)

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Introduction

- Global healthcare crisis due to an epidemic in chronic diseases, such as obesity, Type 2 Diabetes (T2D) and Cardiovascular Diseases (CVDs).
- The 'one size fits all' approach is not working.
- Now looking to the human genome for answers.
- Meta-analyses certain gene variations associated with T2D i.e. FTO & MC4R genes.
- Top 97 risk SNPs (from GWS studies) only explain about 2.7% of the 40–70% BMI hereditability[1].
- Need to look at gene–gene, gene–environment interactions & other structural variations.

REF 1: Locke AE, Kahali B, Berndt SI, Justice AE, Pers TH, Day FR, Powell C, Vedantam S, Buchkovich ML, Yang J, Croteau-Chonka DC. Genetic studies of body mass index yield new insights for obesity biology. Nature. **2015** Feb;518(7538):197.

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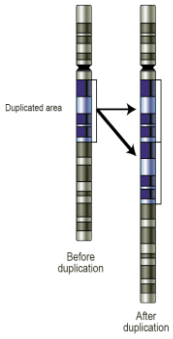
Structural Variations in Genomic DNA

Different types of genetic structural variations.

- SNPs* - are a gene variation that consists of alteration in a single genetic "letter", or base.
- Copy-number variations (CNVs) - the whole gene is repeated
- Recent research strong associations between CNVs, disease states, and our health.[1]



*Single Nucleotide Polymorphisms

REF 1: **Zarrei, M.,** MacDonald, J.R., Merico, D. and Scherer, S.W.,.A copy number variation map of the human genome. *Nature Reviews Genetics*, **2015**, *16*(3), pp.172-183.

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AMY1 Copy Numbers and Salivary Amylase

- Salivary α-amylase (sAA), encoded by the AMY1 gene, is produced by the salivary glands under the control of the autonomic nervous system.
- It is responsible for initiating starch digestion in the mouth producing a mixture of maltose, isomaltose and glucose.
- sAA levels are directly related to serum Amylase levels.
- Low serum Amylase levels have been associated with metabolic abnormalities ,such as obesity, diabetes non-alcoholic fatty liver disease .

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AMY1 Copy Number and Obesity

- The AMY1 gene, which codes for salivary amylase, has CNVs.
- CV values vary substantially between individuals and population groups[1] (1 – 20+ CNVs).
- The AMY1 CN is the genome's largest influence on obesity[2].
- Independently verified to have the greatest correlation to a physiological parameter.[3]
- 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

REF 1: **Novembre, J.,** Pritchard, J. K., & Coop, G.. Adaptive drool in the gene pool. Nat Genet, **2007,** 39(10), 1188-1190. doi:10.1038/ng1007-1188.

REF 2: Usher, C.L., Handsaker, R.E., Esko, T., Tuke, M.A., Weedon, M.N., Hastie, A.R., Cao, H., Moon, J.E., Kashin, S., Fuchsberger, C. and Metspalu, A. Structural forms of the human amylase locus and their relationships to SNPs, haplotypes and obesity. Nature genetics, **2015**, 47(8), pp.921-925

REF 3: Falchi M, Moustafa JS, Takousis P, Pesce F, Bonnefond A, Andersson-Assarsson JC, Sudmant PH, Dorajoo R, Al-Shafai MN, Bottolo L, Ozdemir E. Low copy number of the salivary amylase gene predisposes to obesity. Nature genetics. **2014** May;46(5):492..

AMY1 Copy Numbers compared with SNPs

- Structurally AMY1 copy numbers(CNs)and AMY1 SNPs are different.
- Phenotypically CNs have a much greater effect cf SNPs.
- Any AMY1 SNPs only explain a small fraction of the variation in the AMY1 CN variation.[1]
- Example, AMY1 SNP (rs4244372)
  - Change in CN per minor allele is -1.24
- However AMY1 CNs range is from **2 to 20+**
- In the GIANT Meta-analysis, **none** of the 17 AMY1 SNPs even reached nominal significance with regard to BMI[1]

REF 1: Usher, C.L., Handsaker, R.E., Esko, T., Tuke, M.A., Weedon, M.N., Hastie, A.R., Cao, H., Moon, J.E., Kashin, S., Fuchsberger, C. and Metspalu, A., . Structural forms of the human amylase locus and their relationships to SNPs, haplotypes and obesity. Nature genetics**, 2015,** 47(8), pp.921-925.

AMY1 Copy Number - overview

LOW (1-4)	INTERMEDIATE (5-8)	HIGH (9+)
• Hunter – gatherers • Low salivary amylase production • High starch sensitivity • Increased risk of insulin resistance • Up to 800%[1] increased risk of obesity & T2D • Increased gluten sensitivity • Lower perception of oral starch and satiety. • Higher risk of infection • More sensitive to current western diet.	• Moderate salivary amylase production • Moderate starch sensitivity • Moderate risk of obesity and T2D	• Agricultural societies • High salivary amylase production • Low starch sensitivity • Decreased insulin resistance • Lower risk of obesity and T2D • Lower gluten sensitivity • Foods taste sweeter and richer • Lower risk of infection • More adaptable to current western diet.

REF 1: Falchi M, Moustafa JS, Takousis P, Pesce F, Bonnefond A, Andersson-Assarsson JC, Sudmant PH, Dorajoo R, Al-Shafai MN, Bottolo L, Ozdemir E. Low copy number of the salivary amylase gene predisposes to obesity. Nature genetics. **2014** May;46(5):492..

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Case Study #1- patient of Dr William Ferguson

- 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.

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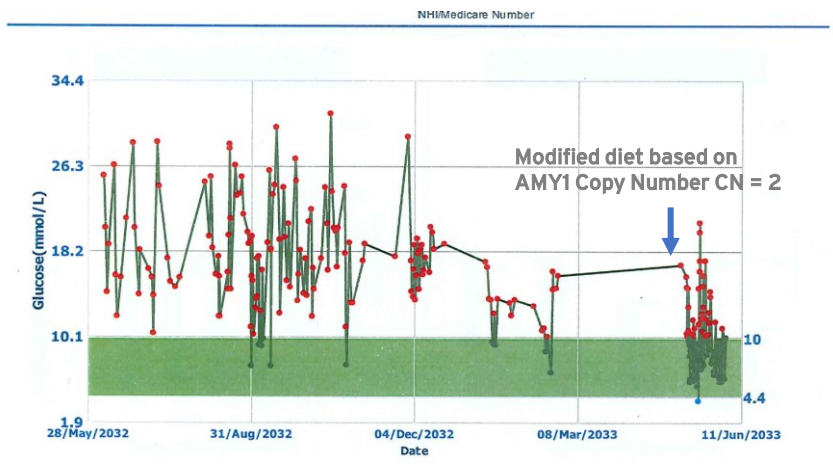
Case Study #1 (cont.)



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Case Study #2 - patient of Dr William Ferguson

- 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 23rdMay 2016 found out that he had a low AMY1 copy number (CN=2) and modified diet accordingly.

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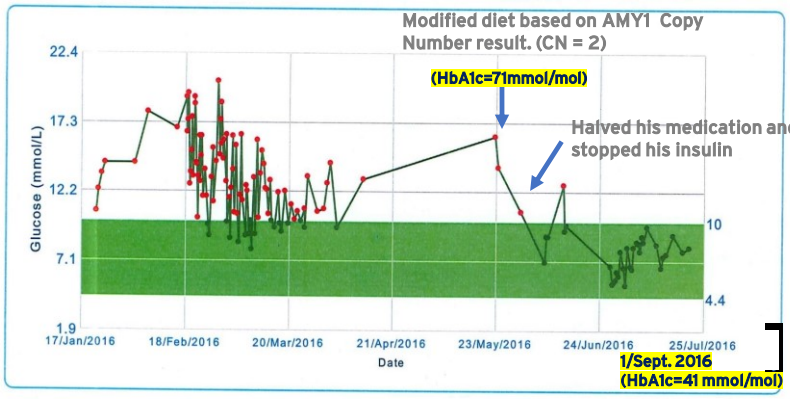
Case Study #2 (cont.)



- After being on 3 types of T2D medication for over 16 years the patient was off all medication within 2 weeks of making the appropriate dietary changes. In addition, he able to stop taking his heart medication within 10 weeks after being on this for 10 years.
- Over 2 two years later he is still not on medication and the savings from not having to purchase medications is estimated to be approximately $8,628.00 (NZ) per year.

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Case Study #2 (cont.)

MACRONUTRIENTS	BEFORE	AFTER
Carbohydrates	56.6%	19.90%
Protein	22.4%	42.15%
Fats	21.0%	37.95%

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

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Interventions

- Not as simple as just cutting out /severely reducing starch – people still need some carbohydrates to function i.e. athletes.

- Timing of starch intake should be when amylase production is at highest:
  - At lowest 40 minutes after waking
  - Peaking around 5pm.

- You can increase your amylase production through interventions which can allow for carbohydrate intake.

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Increasing Amylase

Foods containing citric acid have been shown to increase the production of amylase.

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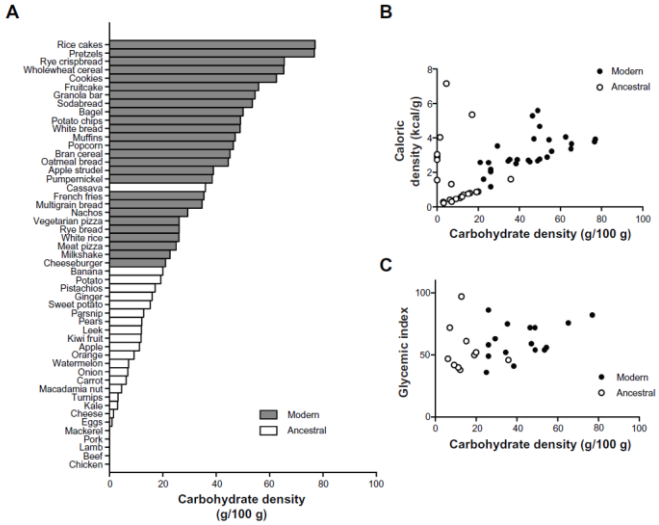
Carbohydrate densities of ancestral foods c.f. Westernised Diets



REF; Spreadbury I. Comparison with ancestral diets suggests dense acellular carbohydrates promote an inflammatory microbiota, and may be the primary dietary cause of leptin resistance and obesity. Diabetes, metabolic syndrome and obesity: targets and therapy. **2012**;5:175-189.

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High Starch Foods

Grains and Cereals

CORN
Including corn starches, popcorn, corn cakes, corn chips, and maize flours used to make corn bread, tortillas and tacos

PASTA
Almost all pasta is made from durum wheat, which is rich in carbohydrates, including starch

TAPIOCA
Tapioca is a starch extracted from cassava root, a tuber native to South America

BREAKFAST CEREALS
Cereals typically contain starches in the form of rice, grains, dried fruit and sugar and other sweeteners.

RICE
Particularly Jasmine rice which has a higher glycaemic response than Basmati rice, rice crackers, rice cakes, rice flour.

WHEAT
Wheat is the major component of flour, bread, biscuits, and cakes.

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Low Starch Foods

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Amylase Inhibitors and ATIs

- Certain foods contain both amylase and trypsin (an enzyme that digests protein) inhibitors and are referred to as ATI's (amylase trypsin inhibitors).
- ATIs naturally occur in plants.
- The mechanism plants use to protect themselves from being eaten /over eaten by pests.
- Genetic modification of grains for pest resistance has led to a deliberate increase of ATIs in our grains.
- The new varieties of wheat that have been bred for pest resistance, have increased levels of ATIs.
- Other Amylase Inhibitors include the following;

Beverages	Other	Supplements	Herbs
Black tea	Ginger	Grape Seed Extract	Baical skullcap
Green tea		Green Tea	Bilberry
Red Wine		Quercetin	Dandelion
			Ginkgo biloba
			Lemon Balm
			Rhodiola
			Rosemary

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Dietary Interventions

- Starches - type i.e. resistant (Amylose) rather than Amylopectin starches.
  - i.e. for CNVs 1-4, decrease to 20 - 25% under normal conditions.
- Don't eat starch carbs by themselves.
- Eat protein and non starch vegetables before any starch foods.
- Before starting a meal drink either lemon juice, lime juice or cider vinegar in water.
- Follow traditional ways of eating i.e. Mediterranean, Japanese etc
- Chew food slowly.

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Lifestyle Interventions

Stop smoking – 1 cigarette reduces salivary amylase by about 44%.

Avoid / severely limit alcohol before eating a meal.

Significantly higher salivary amylase levels have been observed for individuals reporting a positive mood and calmness.

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Exercise Interventions

Exercise has consistently been shown to significantly increase mean salivary α-amylase activity i.e.

- bicycle ergometer - 50 – 70% VO_2max for 30 -90 minutes, or to exhaustion
- Treadmill running - 50 – 70% VO_2max for 30 minutes

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Impact of Medication on Salivary Amylase Levels

- The most common cause of salivary gland hypofunction is prescription medication.
- Xerostomia (dry mouth) - associated with 80% of the most commonly prescribed medications.
- Salivary flow rates decrease as the number of these prescription medications increase, along with duration of use.
- Medications can result in salivary gland hypofunction, such as:
  - Antihistamines
  - Sedatives
  - Anxiolytics Morphine-based analgesics
  - Tricyclic Antidepressants
  - SSRI Antidepressants
  - Neuroleptics
  - Alpha one receptor blocking antihypertensives
  - Beta blockers (decrease protein secretion).[1]

- It may be important to support the levels and activity of amylase, for patients on these medications

REF 1; Hofer E, Jensen SB, Pedersen AM, Bardow A, Nauntofte B. Oral microflora in patients with salivary gland hypofunction. Oral Biosci Med. 2004;1(2):93-108.

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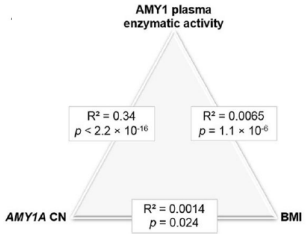
Relation Between AMY1 CN, AMY1 activity and BMI

A longitudinal study with N=5,000 and a 9 year follow-up.

- Strong associations between AMY1 or AMY2 activity and lower BMI.
- Significant contribution of AMY1A copy number to lower BMI and obesity risk.
- Causal 'negative' effect of BMI on AMY1 and AMY2 activities.
- Significant negative contribution of AMY1 activity at baseline to the change in BMI during the 9-year follow-up, and
- A significant contribution of AMY1A copy number to lower obesity risk in children, suggesting a 'bidirectional' relationship between AMY1 activity and adiposity.



REF: Bonnefond A, Yengo L, Dechaume A, Canouil M, Castelain M, Roger E, Allegaert F, Caiazzo R, Raverdy V, Pigeyre M, Arredouani A. Relationship between salivary/pancreatic amylase and body mass index: a systems biology approach. BMC medicine. **2017** Dec;15(1):37.

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Waist-to-height ratio is a better screening tool than BMI for cardiometabolic risk factors (CMFRs).

- Body mass index (BMI) - proxy for obesity for many years.
- Waist-to height ratio (WHtR) is receiving much attention.
- Why? - high metabolic and inflammatory activity associated with the visceral fat deposits within the abdominal cavity and the effect on CMFRs.
- Extensive research - the best global clinical screening tool for CMFRs is now WHtR
- Should be less than 0.5 to stay healthy.

REF: Ashwell M., Ashwell M, Gunn P, Gibson S. Waist-to-height ratio is a better screening tool than waist circumference and BMI for adult cardiometabolic risk factors: systematic review and meta-analysis. Obesity reviews. **2012** Mar 1;13(3):275-86.

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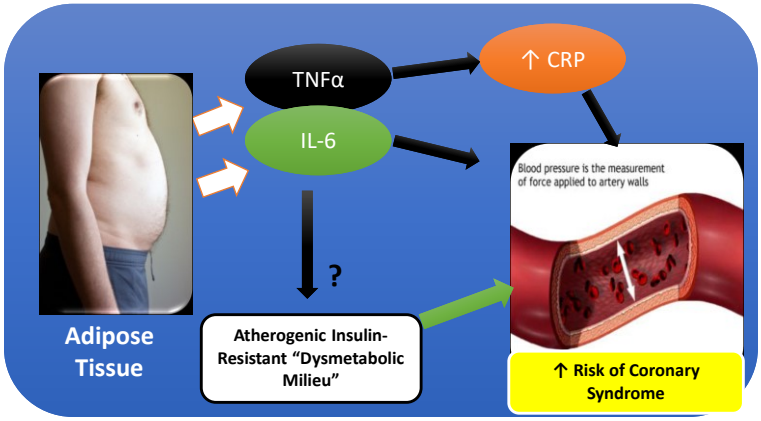
Inflammation, Obesity and Cardiovascular Disease: Is Abdominal Obesity the Missing Link?



CRP = C-reactive protein; IL-6 = interleukin 6; TNFα = tumor necrosis factor alpha
Adapted from Després JP. *Int J Obes Relat Metab Disord.2003;27:S22-S24,*

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Inflammation causes Abnormal Lipids

- AMY1 activity is associated with higher HDL cholesterol in health individuals.[1]
- During chronic inflammatory diseases, inflammation and infections can also induce a variety of alterations in lipid metabolism, including decreases in serum HDL cholesterol, and increases in triglycerides, lipoprotein(a), and LDL levels. [2]
- Systemic concentrations of pro-inflammatory mediators are higher in obese (BMI> 30) than in normal weight individuals.[3]
- The concentrations of most of the mediators, such as C-reactive protein are at least 10-fold higher in obese compared with normal weight individuals. [3]
- Positive relationship between BMI, waist circumference and serum CRP levels as well as other pro-inflammatory markers. [3]

REF 1: Bonnefond A, Yengo L, Dechaume A, Canouil M, Castelain M, Roger E, Allegaert F, Caiazzo R, Raverdy V, Pigeyre M, Arredouani A. Relationship between salivary/pancreatic amylase and body mass index: a systems biology approach. BMC medicine. **2017** Dec;15(1):37.

REF 2: Tsoupras A, Lordan R, Zabetakis I. Inflammation, not cholesterol, is a cause of chronic disease. Nutrients. **2018** May;10(5):604-642.

REF 3: Calder PC, Ahluwalia N, Brouns F, Buetler T, Clement K, Cunningham K, Esposito K, Jönsson LS, Kolb H, Lansink M, Marcos A. Dietary factors and low-grade inflammation in relation to overweight and obesity. British Journal of Nutrition. **2011** Dec;106(S3):S1-78.

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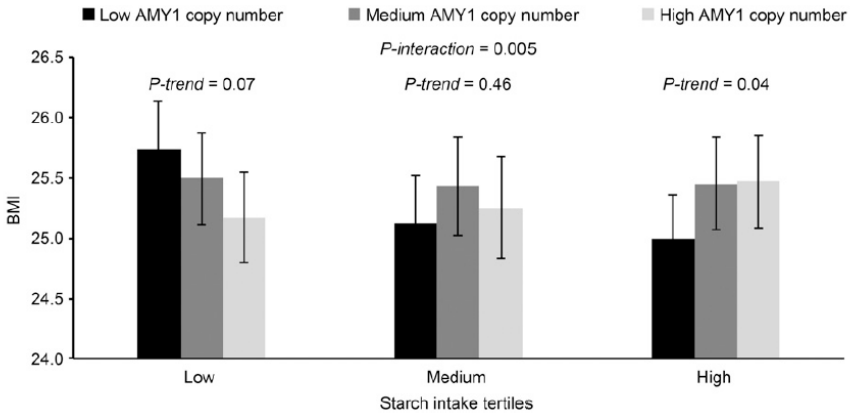
Dietary Starch, AMY1 Copy Number and BMI



REF; Rukh G, Ericson U, Andersson-Assarsson J, Orho-Melander M, Sonestedt E. Dietary starch intake modifies the relation between copy number variation in the salivary amylase gene and BMI. The American journal of clinical nutrition. 2017 May 24;106(1):256-62.

Influence of AMY1 CN on Gut Health

- Cohort of relatively lean, healthy and young individuals (N=201).
- No association between AMY1 CN, weight status, glucose tolerance, HOMA-IR or glucose tolerance.
- Individuals with higher AMY1 CNs had higher postprandial glycemia (+15-40%) after consuming starchy food but not sugary foods c.f. those with low CNs.
- In comparison, individuals with low AMY1 CNs had up to 600% increased breath methane production c.f. those with high CNs, suggesting differences in large bowel microbial metabolism.

REF: **Atkinson FS**, Hancock D, Petocz P, Brand-Miller JC. The physiologic and phenotypic significance of variation in human amylase gene copy number. The American journal of clinical nutrition. **2018** Sep 18;108(4):737-48.

Impact of AMY1 CN and Gut Microbiota

- A gut microbiota analysis of Mexican children and adults showed a positive correlation between AMY1 Copy Number and the Prevotella genus
- Prevotella is one of the most abundant enterotypes in the intestinal microbiome and has enzymes essential for fermentation and utilisation of complex polysaccharides.
- Prevotella is metabolically favourable for weight loss in response to certain dietary interventions,
- Prevotella abundance was not significantly associated with nutritional status or with total dietary carbohydrates (including fibre and starch).

- **REF: León-Mimila** P, Villamil-Ramírez H, López-Contreras B, Morán-Ramos S, Macias-Kauffer L, Acuña-Alonzo V, del Río-Navarro B, Salmerón J, Velazquez-Cruz R, Villarreal-Molina T, Aguilar-Salinas C. Low Salivary Amylase Gene (AMY1) Copy Number Is Associated with Obesity and Gut Prevotella Abundance in Mexican Children and Adults. Nutrients. **2018** Nov 1;10(11):1607.

AMY1 CNV and Insulin Response

- AMY1 CNV impacts **pre-absorptive insulin response** [1].
- Amylase begins the process of starch digestion in the mouth.
- **50%** of bread starch digestion occurs in the mouth.
- This breakdown of starch in the mouth can be viewed almost as an early warning signal to the body, to prepare itself for carbohydrates and the subsequent rise in blood glucose levels.
- The body responds by releasing a small amount of insulin, this is known as the '**pre-absorptive' or 'cephalic' insulin release (CPIR)[1].**
- The little bit of insulin released early means that the amount of insulin needed later is greatly reduced.

REF 1: Mandel AL, Breslin PA. High endogenous salivary amylase activity is associated with improved glycemic homeostasis following starch ingestion in adults. The Journal of nutrition. **2012** Apr 4;142(5):853-8.

Gluten 'sensitivity'

Starch ?

- Globally many people are embracing a gluten - free diet to try and improve their health and well-being.
- Individuals with low AMY1 CNs (2-4) are less able to metabolise and tolerate gluten (Lenander-Lumikari 2000)(Barera 2001) (Dempsey, 2012)(Junker, 2012)
- Possibly some of the intolerances that people are experiencing are due to starch sensitivity and therefore gluten free products (rice, corn) may not be helping them.

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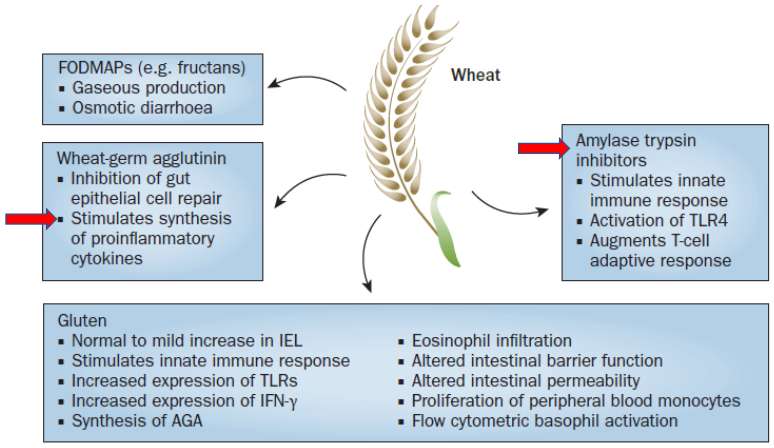
Effects of wheat based constituents that trigger clinical symptoms of non-coeliac gluten sensitivity



REF: Aziz I, Hadjivassiliou M, Sanders DS. The spectrum of noncoeliac gluten sensitivity. Nature Reviews Gastroenterology & Hepatology. **2015** Sep;12(9):516.

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Effect of AMY1 CN on Lipid Metabolism

- The was a difference in lipid metabolism between the healthy normal-weight women with AMY1 CNs 4 or less (LAs) compared with those with CNs 8 or more (HAs).
- Women with AMY1 CNs 4 or less showed lower levels of long- and medium-chain fatty acids, and higher levels of dicarboxylic fatty acids and 2-hydroxbutrate (a known marker of glucose malabsorption).
- Molecular and cellular functions significantly influenced by the AMY1 CN included carbohydrate metabolism, energy production and lipid metabolism.
- This research suggests that the women with the LAs had an increased reliance on fatty acids through ß- and ω- oxidation, and reduced cellular uptake of glucose, with consequent diversion of acetyl-CoA into ketogenesis

REF: Arredouani A, Stocchero M, Culeddu N, Moustafa JE, Tichet J, Balkau B, Brousseau T, Manca M, Falchi M, DESIR Study Group. Metabolomic profile of low–copy number carriers at the salivary α-amylase gene suggests a metabolic shift toward lipid-based energy production. Diabetes. **2016** Nov 1;65(11):3362-8.

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AMY1 CN Distribution in Modern Human Populations

Population	AMY1 CNV	
	Mean	Range
European	6.6	1 - 17
Japanese	7.8	2 - 18
Chinese	8.1	2 - 20
Malay	8.0	4 - 24
Korea	6.6	2 - 19
European - USA	6.8	2 - 15
Mexican	6.5	2 - 16

REF: Fernández CI, Wiley AS. Rethinking the starch digestion hypothesis for AMY1 copy number variation in humans. American journal of physical anthropology. **2017** Aug;163(4):645-57.

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Clinical Relevance - Copy numbers (2 – 4)

- Low production of amylase
- low ability to digest carbohydrates (Choi 2015)(Jimenez 2014)(Perry 2007)
- Less able to metabolise and tolerate gluten (Lenander-Lumikari 2000)(Barera 2001) (Dempsey, 2012)(Junker, 2012)
- 800% increased risk of obesity (Mejia-Benitez 2015)(Falchi 2014) (Perry, 2007)
- Increased risk of insulin resistance (Bencharit 2013)
- Increased risk of diabetes (Bencharit 2013)(Mandel, 2012)
- Increased risk of gut dysbiosis (Aoki, 2010)(Barret, 2010)(Kau, 2011)(Foxx-Orenstein, 2012)
- Increased risk of infection (Scannapeico, 1989)(Spear, 2014)(Reid, 2001)
- High starch food may taste less sweet (Engelen, 2007)(Santos, 2012)

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Clinical Relevance - Copy numbers (9+)

- High production of amylase
- Greater ability to digest carbohydrates (Choi 2015)(Jimenez 2014)(Perry 2007)
- Able to tolerate and metabolise gluten better (Lenander-Lumikari 2000)(Barera 2001) (Dempsey, 2012)(Junker, 2012)
- 800% decreased risk of obesity (Mejia-Benitez 2015)(Falchi 2014)
- Decreased diabetes risk (Bencharit 2013) (Mandel, 2012)
- Decreased insulin resistance risk (Bencharit 2013)
- Lower risk of gut dysbiosis (Aoki, 2010)(Barret, 2010)(Kau, 2011)(Foxx-Orenstein, 2012)
- Lower risk of infection (Scannapeico, 1989)(Spear, 2014)(Reid, 2001)
- High starch foods may taste sweeter (Engelen, 2007)(Santos, 2012)
- Higher chance of failure when attempting to quit smoking (Duskova, 2010)

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In Conclusion

The AMY1 Copy Number (CN) is;

- The genomes largest influence on obesity.
- Associated with many metabolic abnormalities.
- Influences lipid metabolism as well as carbohydrate and energy metabolism.
- Influences gut health.
- Targeted nutrition, exercise and lifestyle interventions can be used to significantly influence the impact of the AMY1 CN on our metabolic health.
- α-amylase, encoded by the AMY1 CN is a very important clinical bio-marker.

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Acknowledgements

Contribution to funding of the in-clinic trial in Auckland

- Professor Grant Schofield
- Fitgenes Australia Ltd

Doctors and Practitioners involved in the in-clinic trial

- Dr William Ferguson
- Tamara O'Dwyer (Nutritionist)
- Dr Nua Tupai

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Gusi Peace Prize International Awards -2018

Dr Michael Nobel and Ambassador Gusi presenting the Gusi Peace Prize International award to Dr Paul Beaver.

This award is also a recognition of the my wife Leigh, Professor Chee Kai Chan, and the tireless work by 100's of committed and dedicated integrative medical doctors, specialists and allied healthcare practitioners I have had the privilege of working with over the past 12+ years.

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Thank you.

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