Establishing the Causal Relationionship between Obesity, Insulin Resistance and Autophagy

Introduction & Significance

Type 2 diabetes is a metabolic disorder that hinders the body's ability to process insulin and disrupts metabolic homeostasis. It **affected over 382 million people in 2013** (1), and has no known cure. **Obesity**, a cause of Type 2 diabetes, leads to metabolic stress that activates autophagy (5). **Autophagy**, a catabolic process that regulates energy homeostasis, **correlates with fluctuations in both insulin resistance and obesity** (2), but the causal relationship between this process and metabolic disorders is not clear. Using genetic mapping, we have scrutinized the **relationships between autophagy related (Atg) genes and the phenotypes exhibited in Type 2 diabetes**, and found that **Atg5, Atg7, and Atg10 expression in the islet tissue and Insulin levels all are linked to chromosome 2**. Using conditional scans and mediation analysis, we discovered that **Ell3 is a likely mediator of Atg5 and Atg10** expression in the islet tissue **and insulin levels**, and, when downregulated, **causes an increase in both autophagy and insulin levels.**

Dataset

The data used for this research came from mice from a **BTBR x B6 F2 cross**. Alan D. Attie's Lab at the University of Wisconsin-Madison recorded this data set. The BTBR set results from a C57BL/6 and BTBR T+ tf strains of mice with Lepob

Specifications

> 516 mice
> 144 quantitative phenotypes
> 16,677 genes
> 2057 genomic markers

Methods

During this research, our team utilized **QTL analysis**. A QTL is a quantitative trait locus, which is a location on a chromosome that may be linked to a specific phenotype. QTL analysis is used to identify QTLs for phenotypes of interest.

Bayesian Information Criterion is a statistical method for model selection and the lowest BIC score is the preferred model. In our analysis, the models were:

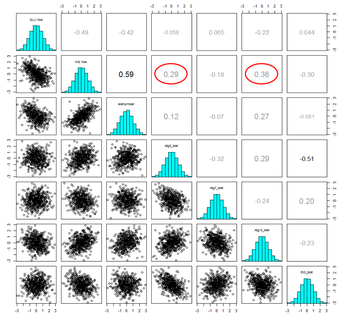
Independant
Expression of Gene of Interest
↕
Genotype
↕
Clinical Phenotype

Reactive
Genotype
↓
Clinical Phenotype
↓
Expression of Gene of Interest

Causal
Genotype
↓
Expression of Gene of Interest
↓
Clinical Phenotype

Complex
??

Pairwise Scans 1



A pairwise scan plots each element of a cross object's correlation with every other element.

In this example, the **correlation value of 0.29 between Atg5 and insulin levels** suggests there is a relation between these two traits.

Other correlation values of note include a **0.36 correlation between Atg10 and insulin levels.**

Mediation Analysis 2

The **anova** function allows one to determine causality to some extent.

```
> anova(lm(Atg5_islet ~ Sex + Q2, data = f2g$pheno))
Analysis of Variance Table

Response: Atg5_islet
           Df Sum Sq Mean Sq F value   Pr(>F)
Sex         1   1.03  1.0313  1.1258   0.2892
Q2          2  33.21 16.6057 18.1273 2.15e-08 ***
Residuals 487 446.12  0.9161
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> anova(lm(Ell3_islet ~ Sex + Q2, data = f2g$pheno))
Analysis of Variance Table

Response: Ell3_islet
           Df Sum Sq Mean Sq F value Pr(>F)
Sex         1   0.06   0.062  0.0737 0.7861
Q2          2  70.98  35.490 42.2251 <2e-16 ***
Residuals 487 409.32   0.840
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> anova(lm(Atg5_islet ~ Sex + Ell3_islet + Q2, data = f2g$pheno))
Analysis of Variance Table

Response: Atg5_islet
           Df Sum Sq Mean Sq  F value Pr(>F)
Sex         1   1.03   1.031   1.4307 0.2322
Ell3_islet  1 126.55 126.549 175.5664 <2e-16 ***
Q2          2   2.47   1.234   1.7119 0.1816
Residuals 486 350.31   0.721
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> anova(lm(Ell3_islet ~ Sex + Atg5_islet + Q2, data = f2g$pheno))
Analysis of Variance Table

Response: Ell3_islet
           Df Sum Sq Mean Sq  F value    Pr(>F)
Sex         1   0.00   0.062   0.0937    0.7596
Atg5_islet  1 126.80 126.805 191.7372 < 2.2e-16 ***
Q2          2  32.08  16.039  24.2513 9.136e-11 ***
Residuals 486 321.41   0.661
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

The above anova function output shows that **Atg5 and Ell3 influence each other**, and that **Ell3 influences Atg5**, not the other way around.

Baysian Informaiton Criterion

The lowest BIC score is the likely model. If it does not differ by at least 5 from every other score, the test is inconclusive.

```
> with(f2g$pheno,
+      triple.fit(Ell3_islet, INS.10wk, Q2))
independent    reactive      causal     complex
   2721.423    2747.453    2685.302    2695.148
> with(f2g$pheno,
+      triple.fit(Ell3_islet, Atg5_islet, Q2))
independent    reactive      causal     complex
   2701.133    2623.136     580.950    2589.633
```

Ell3 is casual for both Atg5 and insulin, suggesting that it is a strong mediator of the two traits.

```
> with(f2g$pheno,
+      triple.fit(Ell3_islet, Atg10_islet, Q2))
independent    reactive      causal     complex
   2725.791    2769.810    2702.968    2713.164
```

Ell3 is also causal for Atg10.

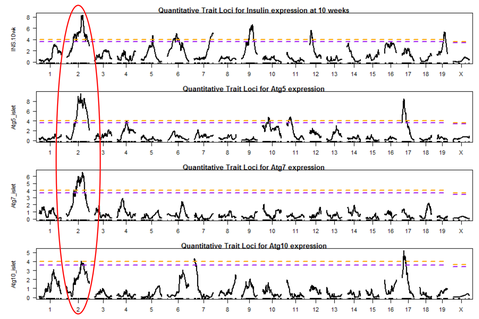
```
> with(f2g$pheno,
+      triple.fit(Ell3_islet, Atg7_islet, Q2))
independent    reactive      causal     complex
   2717.094    2763.245    2717.764    2715.314
> with(f2g$pheno,
+      triple.fit(Atg5_islet, INS.10wk, Q2))
independent    reactive      causal     complex
   2753.522    2750.169    2722.139    2728.715
```

It's relationship with Atg7 is inconclusive.

Genome Scans 3

A **LOD (logarithm of odds) Score** is an estimate of the linkage between two genes. For a complex process like genetic trait analysis, 4 is an acceptable threshold.

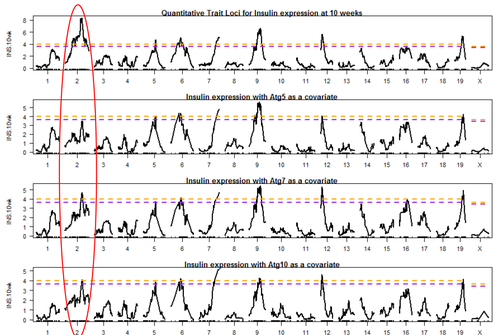
Genome scans like the one below display the LOD Scores for a trait at each point along the genome.



We wanted to discover which Atg proteins shared peaks with either insulin, glucose, or islet turnover, all of which are phenotypes associated with type 2 diabetes. Three different Atg proteins share a peak with insulin on chromosome 2 (seen above), which suggests that there are gene(s) on that chromosome that mediate both autophagy and insulin levels.

Conditional Scans 4

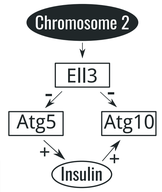
A **conditional scan** is a genome scan where a certain trait or gene is run as a covariate. This results in a LOD peak drop in magnitude proportional to the covariate's influence on the gene's expression.

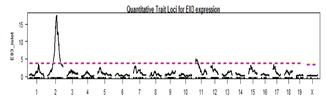


In this example, Insulin's peak on chromosome 2 is dropped below the significance threshold when run with covariates Atg5, Atg7, or Atg10, meaning that **there is a gene on chromosome 2 that links Atg5, Atg7, Atg10 and insulin levels.**

Conclusion

After using mediation analysis to determine which genes are causal, it is likely that Ell3 is a strong mediator for Atg5, Atg10, and insulin in islet tissue.



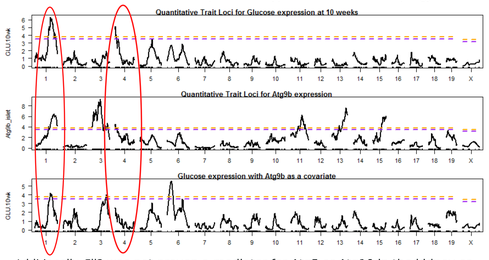


This is the likely QTL pathway, based on our project's results.

Ell3 is a protein coding gene that is thought to bind enhancers in stem cells, but also behaves like Ell (eleven-nineteen lysine-rich leukemia gene), which acts as a negative regulator of p53 and regulates cell proliferation and survival (3). Ell3's relation to autophagy also explains the result of a certain study, which showed that autophagy increases cell life and combats cancer formation (4). The latter provides evidence for the underlying idea behind our original hypothesis; that autophagy could help to maintain islet function in the face of Type 2 diabetes by increasing islet cell function and lifespan.

Future Research

Most research surrounding type 2 diabetes focuses on insulin levels and islet turnover, but glucose is another phenotype that is associated with diabetes. Shown by this genome scan, Atg9b causes glucoses' LOD peaks on chromosomes 1 and 4 to drop, implying a correlation between Atg9b and glucose. Finding a mediator for both of these would further reveal the relatiomship between type 2 diabetes and autophagy.



Additionally, Ell3 was not proven a mediator for Atg5 or Atg10 in the kidney or the liver. This is unsurprising based on the research that backed our original hypothesis. However, it is definitely worth examining activity in other tissues.

Literature Cited

1. "Type 2 Diabetes Statistics and Facts." Healthline. N.p., n.d. Web. 16 Nov. 2015.
2. Kosvan, Jerry. "Altered Autophagy in Human Adipose Tissues in Obesity."National Center for Biotechnology Information. U.S. National Library of Medicine, 3 Nov. 2010. Web. 16 Nov. 2015.
3. HJ, Ann, Kim G & Park KS. "Ell3 stimulates proliferation, drug resistance, and cancer stem cell properties of breast cancer cells via a MEK/ERK-dependent signaling pathway (Abstract)." Biochemical and Biophysical Research Communications (2013). 557-564. Web. 14 March 2016.
4. Tavassoly, Iman. "Dynamics of Cell Fate Decision Mediated by the Interplay of Autophagy." Springer. Springer International Publishing, 2015. Web. 16 Nov. 2015.
5. Codogno, Patrice and Alfred Meijer. "Autophagy. A Potential Link between Obesity and Insulin Resistance." Cell Metabolism 1.11 (2010): 449-451. Web. 16 Nov. 2015.

Additional Research

Patel, Avignat, et al. "Autophagy in Idiopathic Pulmonary Fibrosis." PLOS ONE. Plos One, 18 July 2012. Web. 16 Nov. 2015.

Levine, Beth, Noboru Mizushima, and Herbert W. Virgin. "Autophagy in Immunity and Inflammation." Nature. U.S. National Library of Medicine, n.d. Web. 16 Nov. 2015.

Deretic, Vojo, et al. "Autophagy in Immunity Against Mycobacterium Tuberculosis: A Model System to Dissect Immunological Roles of Autophagy." Current Topics in Microbiology and Immunology. U.S. National Library of Medicine, n.d. Web. 16 Nov. 2015.

He, Congcong, et al. "Exercise-induced BCL2-regulated Autophagy Is Required for Muscle Glucose Homeostasis." Nature. U.S. National Library of Medicine, 18 Jan. 2012. Web. 16 Nov. 2015.

Kosvan, Jerry. "Altered Autophagy in Human Adipose Tissues in Obesity."National Center for Biotechnology Information. U.S. National Library of Medicine, 3 Nov. 2010. Web. 16 Nov. 2015.

Mandal, Dr. Ananya. What is autophagy? News Medical. Web. 1.Dec.2015Falconer, Douglas S., MacKay, Trudy F.C. Introduction to Quantitative Genetics. Benjamin Cummins, 1996.Haim, et al. "Elevated autophagy gene expression in adipose tissue of obese humans: A potential non-cell-cycle-dependent function of E2F1." Autophagy. 11.11 (2015): 2074-2088. Web. 16 Nov. 2015.Images:Jax.org. The Jackson Laboratory, 2016. Web. 13 March 2016.