Genetic insights into the role of brown adipose tissue in human metabolic disease

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Background

Obesity is associated with the development of type 2 diabetes and cardiovascular disease. The rising prevalence of obesity has focused attention on the discovery of safe and effective drugs promoting weight-loss and lowering glucose and lipid levels. A promising approach is to stimulate energy expenditure through thermogenesis by increasing the activity of brown adipocytes. The presence of brown adipose tissue (BAT) in adult humans is now established. BAT activity correlated inversely with visceral adiposity and fasting plasma glucose. Chronic cold exposure also led to increased BAT activity, concomitant with a reduction in body fat mass and enhancement in insulin sensitivity. These findings suggest that pharmacologic inducers of adipocyte browning may ameliorate obesity and related metabolic diseases. Supporting this premise, the *FTO* locus, the strongest genetic signal for BMI, was shown to be mechanistically linked to obesity via impaired adipocyte thermogenesis (Claussnitzer et al, 2016).

Using RNA seq and unbiased genome-wide expression analyses Kajimura's lab identified a 'core' set of genes whose expression is highly specific for human (and mouse) brown adipocytes (Shinoda et al, 2015). Notably, variants within/near a number of these (e.g. *KCNK3*, *KDM4C*, *EYA2, RARB*) have been associated with BMI, serum lipids and/or waist-hip ratio in GWAS. Spiegelman's group further showed that KCNK3 functions to dampen adrenergic signalling, thus attenuating thermogenesis in murine brown adipocytes. Accordingly, adipose-specific *Kcnk3* knockout mice had increased energy expenditure and were resistant to obesity, glucose intolerance and hepatosteatosis (Chen et al, 2017). *Kdm4c* knockout mice are similarly lean with reduced cholesterol levels (mousephenotype.org).

Aim

Investigate the role of human BAT in susceptibility to obesity and related metabolic diseases.

Experimental strategy

1. *Ascertain the biological relevance of human BAT in metabolic disease pathogenesis.* To interrogate the genetic contribution of BAT in the development of obesity and related diseases we will generate RNA seq and ATAC seq data from human brown preadipocytes, *in vitro* differentiated brown adipocytes and BAT nuclei which have been derived from surgical neck biopsies. No chromatin state map data from these cells or tissue exist. Thereafter we will examine for enrichment of obesity, glycaemic and lipid-

associated GWAS variants in open chromatin regions in these cells and tissue. This is a means of defining cell and tissue-specific causal involvement in a disease/trait (Scott et al, 2017; Farh et al, 2015).

2. *Determine the genetic contribution of brown adipocyte enriched genes in human obesity and related diseases.* We will focus on a GWAS signal within/near a 'core' brown adipocyte gene (e.g. *KCNK3*, *KDM4C*) overlapping open chromatin in brown adipocytes/BAT as identified in aim 1. Utilising fine mapping data from GIANT we will localise the GWAS-associated causal variant(s) at this locus. These studies will be complemented with promoter-reporter and chromatin conformational capture (3C) experiments in brown adipocytes. We will additionally utilise CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa) to determine the effects of the causal variant(s) on the core brown adipocyte gene expression. Finally, we will employ lentiviral vector systems to inducibly knock-down and over-express the BAT gene of interest in immortalised human brown adipocytes available in our lab. The effects of these genetic manipulations on basal and cAMP-stimulated adipocyte O2 consumption (respirometry) and lipolysis and mitochondrial content will subsequently be determined. There is also scope to recruit-by-genotype from the Oxford Biobank for assessment of BAT function using infrared thermography.

Summary

Collectively, the studies outlined will establish the biological importance of human BAT in susceptibility to human obesity and related diseases. This work will also determine whether manipulation of BAT activity to stimulate energy expenditure through thermogenesis constitutes a promising avenue for the treatment of these disorders. Finally, by exploring the phenome-wide association study (pheWAS) characteristics of any genetic variant(s) found to alter brown adipocyte function, this study will also generate predictions about any wider benefits and/or potential side effects of therapeutically targeting BAT genes of interest.

Supervisor's recent relevant publications

1. Carrat GR, Hu M, Nguyen-Tu MS, et al (2017) Decreased STARD10 Expression Is Associated with Defective Insulin Secretion in Humans and Mice. *Am J Hum Genet* **100**: 238-256.
2. Todorčević M, Hilton C, McNeil C, et al (2017). A cellular model for the investigation of depot specific human adipocyte biology. *Adipocyte* **6**: 40-55.
3. Scott RA, Scott LJ, Mägi R, et al (2017) An Expanded Genome-Wide Association Study of Type 2 Diabetes in Europeans. *Diabetes* **66**: 2888-2902.
4. Loh NY, Neville MJ, Marinou K, et al (2015) LRP5 regulates human body fat distribution by modulating adipose progenitor biology in a dose- and depot-specific fashion. *Cell Metab* **21**: 262-72.
5. Fuchsberger C, Flannick J, Teslovich TM, et al (2016). The genetic architecture of type 2 diabetes. *Nature* **536**: 41-47.

References

1. Claussnitzer M, Hui CC, Kellis M (2016) FTO Obesity Variant and Adipocyte Browning in Humans. *The New England Journal of Medicine* **374**:192-3.
2. Shinoda K, Luijten IH, Hasegawa Y, et al (2015) Genetic and functional characterization of clonally derived adult human brown adipocytes. *Nature Medicine* **21**: 389-94.

3. Chen Y, Zeng X, Huang X, et al (2017) Crosstalk between KCNK3-Mediated Ion Current and Adrenergic Signaling Regulates Adipose Thermogenesis and Obesity. *Cell* **171**: 836-848.
4. Scott RA, Scott LJ, Mägi R, et al (2017) An Expanded Genome-Wide Association Study of Type 2 Diabetes in Europeans. *Diabetes* **66**: 2888-2902.
5. Farh KK, Marson A, Zhu J, et al (2015) Genetic and epigenetic fine mapping of causal autoimmune disease variants. *Nature* **518**: 337-43.