The role of ACVR1C signalling in human fat distribution

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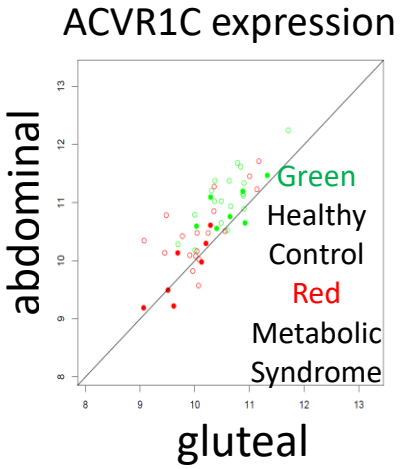
Background

Human fat distribution is a strong and independent determinant of metabolic disease risk such as Type 2 Diabetes (T2D), cardiovascular and fatty liver disease, but the molecular determinants regulating site-specific fat storage are still poorly understood. TGFβ signalling plays an important role in adipose tissue (AT) development and function. ACVR1C is a transmembrane receptor for TGFβ family ligands. Animal studies have shown that ACVR1C functions as a negative regulator of lipolysis (PMID:25161195, PMID: 22933117). In humans the expression of *ACVR1C* is highest in AT of all 44 tissues examined in GTEX. Furthermore, GWAS have shown that low-frequency (N150H) and rare (I195T) coding variants in ACVR1C predicted to lead to loss-of-function are associated with increased lower body fat accumulation and protection from T2D (bioRxiv 352674, bioRxiv 372128, PMID: 30389748).

Pilot data

Using global transcriptomic (Affymetrix) data from the Oxford Biobank (OBB) (subset n=72) we observe that *ACVR1C* is differentially expressed between abdominal and gluteal AT. There are n=165 heterozygous carriers of the ACVR1C$_{N150H}$ variant in the OBB which demonstrates feasibility for recall studies of carriers (typical recall numbers are n=10-15). Detailed characterisation of human fat distribution (DXA scan) in the OBB (n=4,600) shows a sex-specific strong effect (~10% gain) on leg fat content in male carriers of the ACVR1C$_{N150H}$.



Hypothesis

ACVR1C exerts fat depot specific effects on mature adipocyte and/or adipose progenitor function to regulate human fat distribution.

Aims

1. Characterise ACVR1C gene and protein expression in adipose tissues and cells.
2. Perform *in vitro* functional studies in depot-specific human adipocyte progenitor (AP) cells and *in vitro* differentiated adipocytes with modified ACVR1C function.
3. Whole body human metabolic phenotyping of carriers of the ACVR1C$_{N150H}$ variant.

Description of the work to be undertaken

1. Existing abdominal and gluteal samples from a human whole AT bank (100 males and 100 females) will be used to quantify gene and protein expression of ACVR1C. Cellular distribution

will be ascertained in fractionated samples by Real-Time PCR and immunohistochemistry (samples in existence). Follow *ACVR1C* expression during abdominal and gluteal adipocyte differentiation.

2. Examine the effects of stable knock-down and over-expression of ACVR1C on proliferation, differentiation, and post-receptor signalling (SMADs) in abdominal and gluteal APs. Using existing dox-inducible vectors we will also determine the effects of acute (48 hrs) knockdown of ACVR1C in differentiated adipocytes on basal and stimulated lipolysis and glucose uptake (established techniques). Finally, we will obtain adipose biopsies from ACVR1C$_{N150H}$ variant carriers (recall-by-genotype from the OBB), immortalise APs using the HPV/hTERT system, and thereafter using CRISPR we will edit the ACVR1C$_{N150H}$ variant to wild-type. The generated cells will undergo detailed phenotypic characterisation as above. Reciprocal experiments will also be conducted by editing wild-type ACVR1C to ACVR1C$_{N150H}$ in existing immortalised abdominal and gluteal APs.
3. Male and female carriers (and age- and BMI-matched non-carriers) of ACVR1C$_{N150H}$ will be recruited from the OBB to conduct whole body phenotypic characterisation. This will include oral glucose tolerance tests with measurement of glucose, insulin and NEFA to ascertain glucose tolerance, insulin sensitivity and systemic lipolytic regulation. Standardised abdominal and gluteal AT samples will also be taken using gun biopsies for analysis of adipocyte size and *ex vivo* tissue quantification of lipolysis. If lipolysis experiments are positive it will also be possible to conduct in vivo AT adrenergic lipolytic regulation studies using specific adipose arterio-venous sampling as previously demonstrated from our group.

Future

Conceptually, targeting pathways that can alter regional fat storage efficiency is likely to impact on multiple chronic metabolic diseases. With ACVR1C there are opportunities for drug target screens to identity ACVR1C inhibitors since ACVR1C is a cell surface receptor with some known ligands.

Supervisors' recent relevant publications:

1. Loh NY, Neville MJ, Marinou K, Hardcastle SA, Fielding BA, Duncan EL, McCarthy MI, Tobias JH, Gregson CL, **Karpe F**, **Christodoulides C**. LRP5 regulates human body fat distribution by modulating adipose progenitor biology in a dose- and depot-specific fashion. *Cell Metabolism.* 2015; 21:262-72.
2. Small KS, Todorčević M, Civelek M, El-Sayed Moustafa JS, Wang X, Simon MM, Fernandez-Tajes J, Mahajan A, Horikoshi M, Hugill A, Glastonbury CA, Quaye L, Neville MJ, Sethi S, Yon M, Pan C, Che N, Viñuela A, Tsai PC, Nag A, Buil A, Thorleifsson G, Raghavan A, Ding Q, Morris AP, Bell JT, Thorsteinsdottir U, Stefansson K, Laakso M, Dahlman I, Arner P, Gloyn AL, Musunuru K, Lusis AJ, Cox RD, **Karpe F**, McCarthy MI. Regulatory variants at KLF14 influence type 2 diabetes risk via a female-specific effect on adipocyte size and body composition. *Nature Genetics.* 2018; 50:572-580.
3. Denton N, Pinnick KE, **Karpe F**. Cartilage oligomeric matrix protein is differentially expressed in human subcutaneous adipose tissue and regulates adipogenesis. *Mol Metab*. 2018; 16:172-179.
4. **Karpe F**, Vasan SK, Humphreys SM, Miller J, Cheeseman J, Dennis AL, Neville MJ. Cohort Profile: The Oxford Biobank. *Int J Epidemiol*. 2018; 47:21-21.
5. **Karpe F**, Pinnick KE. Biology of upper-body and lower-body adipose tissue-link to whole-body phenotypes. *Nature Reviews Endocrinology.* 2015;11:90-100.