



Determining potential causal mechanisms by genetic fine mapping, genomic annotation and functional characterization at obesity and fat distribution loci

Supervisors: [Associate Prof Cecilia Lindgren](#)^{1,2}
[Prof Roger Cox](#)^{1,3}

Departments: ¹ [Wellcome Trust Centre for Human Genetics \(WTCHG\)](#),
[Nuffield Department of Medicine](#)
² [Big Data Institute](#)
³ [MRC Harwell](#)

Background

Obesity is an urgent global health challenge with no imminent preventive solutions. Genetic factors influencing obesity and related traits can be used to better understand disease aetiology and mechanisms, leading to improvements in prevention and treatment. Recent studies by our group and collaborators have shown that fat distribution (as measured by waist-to-hip ratio, adjusted for BMI (WHR)) has a distinct underlying genetic underpinning from that of overall obesity. Our recent studies identified 49 loci associated with fat distribution. While we have seen very little evidence of sexual dimorphism in overall obesity, almost half of the 49 fat distribution loci show significant sexual dimorphism, almost all of which display a stronger effect in women. The identified loci were largely located in non-coding regions, enriched for genes expressed in adipose and for putative regulatory elements.

We hypothesise that fat distribution determining genes and variants contribute to insulin resistance as well as type 2 diabetes risk and fundamentally affect the biology of adipose tissue development and response to excess energy intake, in a sex- and depot-dependent manner.

The broad aims of the work that is to be performed by the research fellow are to:

1. Use “big data” including GWAS for fat distribution (WHR, ectopic fat *etc.*) and rich functional annotation data from adipose and mesenchymal lineage-relevant tissues (i.e. chromatin marks, conformation capture, accessible chromatin, DNA methylation, transcription factor (TF) binding sites and transcript expression data) to identify obesity signals and resolve these into likely causative variants, their mechanistic underpinning and their target genes and transcripts;
2. To map the transcriptional drivers of sex-specific and depot-specific differences;
3. To functionally validate causative SNPs, regulatory elements, TF binding sites and effector genes/transcripts *in vitro* (human and mouse) and *in vivo* using mouse models.

Description of the work to be undertaken

Big data: We will start with public and proprietary GWAS data to identify the 'credible' sets of variants that are likely to drive each association signal through in-silico fine mapping of the significantly associated fat distribution loci. We will integrate these summary statistics with publicly available genome annotation data, such as RNA sequencing data from GTEx, TF binding ChIP-seq, ATAC-seq data, open chromatin and chromatin state data from ENCODE, NIH Roadmap and other public repositories. We will test for statistical enrichment of these annotations among variants influencing fat distribution traits at established loci as well as genome-wide using both published and proprietary methods. These analyses will be performed across all tissues as well as within specific cell-types. We will also perform these analyses in combined, female-only and male-only datasets separately, from which we will determine whether the observed sexual dimorphism can be explained by enrichment of specific annotations. Similarly we will generate data to study depot specific effects. Finally, we will identify genes mapping to loci with enriched annotations and determine whether these genes are involved in specific pathways or biological systems.

Adipose data sets: There is currently a lack of genomic data sets in adipose tissue that could be used to study depot- and sex-specific effects of WHR-associated variants. We are producing data sets in mouse primary pre-adipocytes during differentiation from visceral and subcutaneous adipose depots (including ATACseq and ChIPseq). We will generate similar parallel data sets for human cells to supplement the limited human adipose data available.

Functional validation *in vitro*: Three approaches will be used:

- (i) Predicted functional SNPs will be tested for the effect of alternate sequences on TF binding using electrophoretic mobility shift assays (EMSA).
- (ii) Potential regulatory elements defined in the analysis and marked by alternate SNPs/sequence haplotypes associated with WHR will also be tested in enhancer or promoter assays as appropriate.
- (iii) Potential elements will be tested *in vitro* using lentiviral delivery of CRISPR/Cas constructs to edit or delete variants/regulatory elements with the aim to phenotypically characterize the resulting consequences in gene expression and differentiation.

Functional validation *in vivo*: Comprehensive comparative analysis of human and mouse genomic and epigenomic data will allow the identification of conserved regulatory sequences for functional assessment using genome editing (CRISPR/Cas9) in mice.

Taken together, the research we set out in this application, seeks to advance understanding of the mechanisms involved in the regulation of differential central/visceral fat accumulation. This knowledge should support translational advances by providing biological pathways and effector genes that might serve as targets for drug intervention.

Supervisor's recent relevant publications (* and ‡ indicates authors contributed equally)

1. Shungin D*, Winkler TW*, Croteau-Chonka DC*, Ferreira T*, Locke AE*, Mägi R*, [470 authors], Heid IM‡, Loos RJ‡, Cupples LA‡, Morris AP‡, **Lindgren CM‡**, Mohlke KL‡ (2015). [New genetic loci link adipose and insulin biology to body fat distribution](#). *Nature* **518**: 187-96. PMID: 25673412.
2. Locke AE*, Kahali B*, Berndt SI*, Justice AE*, Pers TH*, [483 authors], **Lindgren CM**, Beckmann JS, Barroso I, North KE, Ingelsson E, Hirschhorn JN, Loos RJ, Speliotes EK (2015). [Genetic studies of body mass index yield new insights for obesity biology](#). *Nature* **518**: 197-206. PMID: 25673413.
3. Heid IM*, Jackson AU*, Randall JC*, Winkler TW*, Qi L*, Steinthorsdottir V*, Thorleifsson G* [291 authors], Fox CS‡, Mohlke KL‡, **Lindgren CM‡** (2010). [Meta-analysis identifies 13 new loci associated with waist-hip ratio and reveals sexual dimorphism in the genetic basis of fat distribution](#). *Nat Genet* **42**: 949-960. PMID: 3000924.
4. Goldsworthy M, Bai Y, Li C, Ge H, Lamas E, Hilton H, Esapa CT, Baker D, Baron W, Juan T, Véniant MM, Lloyd DJ and **Cox RD** (2016). [Haplo-Insufficiency of the Insulin Receptor in the presence of a splice-site mutation in Ppp2r2a results in a novel di-genic mouse model of type 2 diabetes](#). *Diabetes* **65**: 1434-46. PMID:26868295.
5. Merkestein M*, Laber M*, McMurray F, Andrew D, Sachse G, Sanderson J, Li M, Usher S, Sellayah D‡, Ashcroft FM‡, **Cox RD‡** (2015). [FTO Influences Adipogenesis by Regulating Mitotic Clonal Expansion](#). *Nat Commun* **6**: 6792. PMID:25881961