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Expression of *FBN1* during adipogenesis: Relevance to the lipodystrophy phenotype in Marfan syndrome and related conditions

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ABSTRACT

Fibrillin-1 is a large glycoprotein encoded by the *FBN1* gene in humans. It provides strength and elasticity to connective tissues and is involved in regulating the bioavailability of the growth factor TGFβ. Mutations in *FBN1* may be associated with depleted or abnormal adipose tissue, seen in some patients with Marfan syndrome and lipodystrophies. As this lack of adipose tissue does not result in high morbidity or mortality, it is generally under-appreciated, but is a cause of psychosocial problems particularly to young patients. We examined the role of fibrillin-1 in adipogenesis. In inbred mouse strains we found significant variation in the level of expression in the *Fbn1* gene that correlated with variation in several measures of body fat, suggesting that mouse fibrillin-1 is associated with the level of fat tissue. Furthermore, we found that *FBN1* mRNA was up-regulated in the adipose tissue of obese women compared to non-obese, and associated with an increase in adipocyte size. We used human mesenchymal stem cells differentiated in culture to adipocytes to show that fibrillin-1 declines after the initiation of differentiation. Gene expression results from a similar experiment (available through the FANTOM5 project) revealed that the decline in fibrillin-1 protein was paralleled by a decline in *FBN1* mRNA. Examination of the *FBN1* gene showed that the region commonly affected in *FBN1*-associated lipodystrophy is highly conserved both across the three human fibrillin genes and across genes encoding fibrillin-1 in vertebrates. These results suggest that fibrillin-1 is involved as the undifferentiated mesenchymal stem cells transition to adipogenesis but then declines as the developing adipocytes take on their final phenotype. Since the C-terminal peptide of fibrillin-1 is a glucogenic hormone, individuals with low fibrillin-1 (for example with *FBN1* mutations associated with lipodystrophy) may fail to differentiate adipocytes and/or to accumulate adipocyte lipids, although this still needs to be shown experimentally.

1. Introduction

Fibrillin-1 is a large glycoprotein encoded in humans by the *FBN1* gene (MIM 134797). It is strongly expressed in tissues of mesenchymal origin and localises to the extracellular matrix (ECM) [1,2] where it contributes to strength and elasticity of tissues [3] and regulates the bioavailability of transforming growth factor beta (TGF-β) [4–7]. Mutations in *FBN1* result in multisystem abnormalities of connective tissues, most frequently manifesting as Marfan syndrome (MFS) in humans (MIM 154700). MFS affects the skeletal, ocular and cardiovascular systems, with major morbidity and mortality arising from dilatation and dissection of the ascending aorta. In some individuals with *FBN1* mutations, there is also a marked lack of subcutaneous adipose tissue, resulting in an abnormally thin phenotype (for example, see [8]).

Abbreviations: ADSMC, adipose derived mesenchymal stem cells; BMI, body mass index; CAGE, cap analysis of gene expression; ECM, extracellular matrix; GO, gene ontology; MFS, Marfan syndrome.

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Extreme cases of lipodystrophy with or without Marfanoid features have been associated with mutations at the 3′ end of the *FBN1* gene [9–14]. Although this phenotype is not specifically associated with mortality in MFS patients, it causes considerable psychosocial stress, particularly to vulnerable youths who are already struggling to adjust to the diagnosis of a life-threatening condition and have significant body image issues [15,16]. This body morphology impacts on self-image and on the way patients interact with their peers. At the other end of the scale, obesity affects nearly 10% of the world's population [17] and is a major financial and psychological burden to high income countries. Understanding the normal role of fibrillin-1 in generating adipose tissue could lead to therapies to ameliorate problems relating to both overweight and underweight.

The function of fibrillin-1 in determining the level of adipose tissue has not been rigorously addressed, although a recent paper describes a glucogenic hormone produced from the 170 C-terminal amino acids [13]. The *Fbn1* gene is strongly expressed by mouse cells of adipocyte lineage [1]. Fibrillin-1 is secreted by rat adipocytes [18] and, as mentioned above, the phenotype associated with human *FBN1* mutations frequently (but not always) involves depletion of subcutaneous adipose tissue. A genotype-phenotype correlation exists between the lipodystrophy phenotype and frameshift mutations at the 3′ end of the *FBN1* gene, in the second last exon, coding Exon 64 (exon numbering from http://www.umd.be/FBN1/; shown as Exon 65 in the Ensembl Genome Browser) [9–14]. Reduced subcutaneous tissue and abnormal adipocytes can also be associated with mutations in central exons, for example in our patient with a mutation in exon 25 [8], clearly indicating that fibrillin-1 is involved in determining the formation and maturation of adipocytes.

Adipose tissue develops through a cascade of events leading to conversion of mesenchymal stem cells to preadipocytes which undergo terminal differentiation into adipocytes. This process is regulated by key transcription factors CEBP (CCAAT enhancer binding protein) and PPARG (peroxisome proliferator activated receptor gamma) [19,20]. Once proliferation of adipocyte precursors has ceased, lipid-filled storage vacuoles form in the intracellular space [21]. Adipocytes are constantly replenished in adult tissues (approximately 10% per annum [22]) and response to alterations in nutritional status can involve changes in both cell size and number (for example, [23–25]). Understanding the factors regulating adipocyte differentiation offers the potential of treatments for obesity (adipose excess) as well as lipodystrophy (adipose deficiency).

Adipogenesis can be seen as having two phases (reviewed extensively by [26]). In the early phase cells become committed to differentiation and in the later phase cells expand to accommodate the requirements of lipid storage. The ECM is extensively reorganised during this process, with down-regulation of most secreted proteins and up-regulation of basement membrane and basal lamina. Over 65 proteins make up the ECM of adipose tissue [26] including fibrillin-1, fibronectin, a range of collagen subunits, osteonectin and latent transforming growth factor binding protein 1 (LTBP1), a member of the fibrillin gene superfamily. During preadipocyte formation from mesenchymal stem cells collagen type VI increases in amount and provides a scaffold for a lipid monolayer. The extracellular matrix of the preadipocytes then undergoes gradual up-regulation of collagen type IV [27], which interacts with collagen type VI, and collagen type V. Fibrillar collagens (type I and type III), fibronectin and other ECM components may peak early in differentiation before being down-regulated. As differentiation progresses, the ECM is reorganised to provide storage space for lipid vacuoles. The ECM in mature adipose tissue is under constant turnover to ensure that adequate lipid storage space is available [26,28].

The process of adipogenesis can be recreated *in vitro* using primary mesenchymal stem cells, treated with growth factors to promote differentiation along the adipose lineage. In this paper we describe investigations of the role of fibrillin-1 in adipogenesis *in vitro* and adipose expansion *in vivo*, based on independent experiments using either microarray or promoter expression analysis derived from the FANTOM5 project [29,30].

2. Materials and methods

2.1. Analysis of mouse gene expression across strains

Mouse gene expression data were downloaded from BioGPS [31]. Gene expression in mouse epididymal adipose tissue was derived from data presented in [32], based on a customised microarray platform GNF1M. There was one probeset for *Fbn1* (gnf1m00711_a_at) and one for *Fbn2* (gnf1m02242_a_at). Available results were from pooled RNA obtained from mice at 25 weeks of age, of the following mouse strains: C57BL/6J ($n = 3$), C3H/HeJ ($n = 4$), CBA/J ($n = 2$), DBA/2J ($n = 3$). To find genes with similar expression patterns to *Fbn1*, the Correlation function in BioGPS was used, with minimum correlation coefficient set at 0.75. Mouse phenotype data were obtained from the Mouse Phenome Database (MPD; [33]) which provides results for mice scanned immediately post-mortem using mouse densitometer dual energy X-ray absorptiometry (PIXImus mouse densitometer (LUNAR, Madison, WI). Details of the mouse husbandry are available at http://phenome.jax.org/db/q?rtn=projects/docstatic&doc=Jaxpheno1/Jaxpheno1_Animal. Results for MPD data set Jaxpheno1 were downloaded for body weight, body fat tissue weight and body fat percentage for males and females aged 8 and 16 weeks. Results for single nucleotide polymorphisms for C57Bl/6J, C3H/HeJ, CBA/J and DBA/2J strains were retrieved from MPD and the Mouse Genome Informatics database (MGI; [34]). The mouse *Fbn1* transcription start site region was identified using data from the FANTOM3 [35] and FANTOM5 projects and the coordinates on the current build of the mouse genome (GRC m38.p4) determined by a BLASTN search in Ensembl [36].

2.2. Analysis of human adipose tissue gene expression data

Gene expression data from the adipose tissue of a previously analysed cohort of 30 obese (BMI > 30 kg/m^2) and 26 non-obese (BMI < 30 kg/m^2) women [37] was subjected to Significance Analysis of Microarrays [38]. Affymetrix microarray data from 114 adult Swedish women without diabetes [39] was examined for correlations between markers of adiposity and *FBN1* expression with the statistical package Statview (SAS Institute Inc., NC, USA). The human *FBN1* transcription start site was identified using data from the FANTOM3 and FANTOM5 projects [2,29,35].

2.3. Detection of fibrillin-1 protein during adipogenesis

To assess further the impact of adipogenesis on fibrillin1, cryopreserved adipose derived mesenchymal stem cells (ADSMC) from a single female donor who had undergone abdominal liposuction were obtained from the Edinburgh Adipose Tissue Bank, University of Edinburgh, UK [40]. The research was approved by the South East Scotland Research Ethics Committee 03 (Reference 1-0/S1103/45). The cells were cultured initially in DMEM (Gibco, Life Technologies Lit, Paisley UK) with 10% heat inactivated foetal bovine serum (GE Healthcare, Little Chalfont, UK), 1 X GlutaMAX (Gibco), 1 μg/μL bFGF (PeproTech, Rocky Hills, NJ, USA), at 37 °C and 5% CO_2. Culture vessels were coated with 0.1% gelatin. Seeding density was 20,000 cells per well when using 8-well chamber slides (NUNC) and 50,000 cells per well when using 6 well plates. StemPro® Adipogenesis Differentiation Medium (Fisher Scientific, Loughborough UK) was prepared according to manufacturer's instructions and filtered through a 500 mL 75 mm 0.45 μm filter unit (Thermo Scientific, Leicestershire, UK). Cells were transferred to this medium after 24 h when 75–80% confluency had been achieved. 100 μL (chamber slides) or 1 mL (6 well plates) of StemPro® Adipogenesis Differentiation Medium was then added to the cells while DMEM with foetal bovine serum, GlutaMAX and bFGF as above was added to the control