Genetic Link Between Obesity and \textit{MMP14}-Dependent Adipogenic Collagen Turnover

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\textbf{OBJECTIVE—}In white adipose tissue, adipocytes and adipocyte precursor cells are ensheathed in a dense network of type I collagen fibrils. The fate of this pericellular collagenous web in diet-induced obesity, however, is unknown. This study seeks to identify the genetic underpinnings of proteolytic collagen turnover and their association with obesity progression in mice and humans.

\textbf{RESEARCH DESIGN AND METHODS—}The hydrolysis and degradation of type I collagen at early stages of high-fat diet feeding was assessed in wild-type or \textit{MMP14} (\textit{M}t\textit{t}1-\textit{MMP})-haploinsufficient mice using immunofluorescent staining and scanning electron microscopy. The impact of \textit{MMP14}-dependent collagenolysis on adipose tissue function was interrogated by transcriptome profiling with cDNA microarrays. Genetic associations between \textit{MMP14} gene common variants and obesity or diabetes traits were examined in a Japanese cohort (\textit{n} = 3,653).

\textbf{RESULTS—}In adult mice, type I collagen fibers were cleaved rapidly in situ during a high-fat diet challenge. By contrast, in \textit{MMP14} haploinsufficient mice, animals placed on a high-fat diet were unable to remodel fat pad collagen architecture and display blunted weight gain. Moreover, transcriptional programs linking type I collagen turnover with adipogenesis or lipogenesis were disrupted by the associated decrease in collagen turnover. Consistent with a key role played by \textit{MMP14} in regulating high-fat diet–induced metabolic programs, human \textit{MMP14} gene polymorphisms located in proximity to the enzyme’s catalytic domain were closely associated with human obesity and diabetes traits.

\textbf{CONCLUSIONS—}Together, these findings demonstrate that the \textit{MMP14} gene, encoding the dominant pericellular collagenase operative in vivo, directs obesogenic collagen turnover and is linked to human obesity traits. \textit{Diabetes} 59:2484–2494, 2010

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\textbf{RESEARCH DESIGN AND METHODS—}Mice, diets, and metabolic phenotyping. \textit{MMP14} \textsuperscript{-/-} mice were kindly provided by Dr. Motoharu Seiki (University of Tokyo) and maintained in C57BL/6 background (11). \textit{MMP14} haploinsufficient (\textit{MMP14} \textsuperscript{+/-}) and wild-type (\textit{MMP14} \textsuperscript{+/+}) male mice, aged 12 weeks, were used for all experiments. Each mouse was housed individually and placed on a high-fat (45% fat) or control (10% fat) diet (Research Diets) for the indicated periods of time. In selected studies, mice were individually housed in Oxymax/CLAMS metabolic chambers (Columbus Instrument) for metabolic studies. After 2 days’ acclimation, \textit{VCO}, food consumption, and x-y ambulation were measured consecutively for 4 days.
MMP-dependent collagenolysis in vivo. Adipose tissues were fixed with 4% formaldehyde and frozen sections prepared for analysis. MMP-dependent collagen degradation products were detected with C12-C antibody (IBEX) (12). Fibrillar collagens were stained with Sirius red and quantified with ImageJ (NIH) (7). Student’s t test (unpaired and two tailed) was used for statistical analysis.

Collagenolysis in vitro. Primary preadipocytes were isolated from the inguinal fat pads of 3- to 4-week-old male wild-type and haploinsufficient (MMP14+/−) mice (7). Type I collagen was extracted from rat tails and conjugated with Oregon Green 488 (Molecular Probe) (13). Preadipocytes were cultured atop fluorescently tagged type I collagen-polymers with or without an adipogenic milieu (0.2 μm/d insulin, 0.5 μm/d 3-isobutyl-1-methylxanthine, and 0.25 μmol/d dexamethasone) (7). Nuclei were stained with DAPI and zones of collagenolysis identified with a fluorescent microscope. Data were standardized according to the multiarray average expression measure (14). The paired t test was two tailed, and P < 0.01 was considered statistically significant. Repeated, minimum twofold differences were adopted as the threshold of differential expression. Gene ontology analysis was performed using GOSTats packages (BioConductor).

Scanning electron microscopy. Scanning electron microscopy was used to examine the architecture of fat pad–associated collagen. Inguinal fat pads were fixed with 2% glutaraldehyde/1.5% paraformaldehyde in 0.1 mol/l Na cacodylate buffer and postfixed in 1% osmium tetroxide. Samples were immersed in liquid N2 to fracture the inner mass of adipose tissues, and imaged with an ARAMIS 1910 field emission scanning electron microscope.

Human subjects. Japanese healthy individuals with no obvious medical conditions (n = 3,653) were recruited through random sampling (15). All subjects gave written informed consent prior to the study, and the study design (Millennium Genome Project) was approved by the institutional review board and the ethics committee of the National Cardiovascular Center, Osaka, Japan.

MMP14 gene SNP study. Genomic DNA samples were collected from peripheral leukocytes. MMP14 gene variations were detected by TaqMan PCR (ABI PRISM 7000HT) and verified in a subset of samples by direct sequencing (ABI PRISM 7900HT) and NGS analysis using an objective lens 40×/NA 0.65 (Olympus) at 25°C. GeneChip microarray analysis. Total RNA was isolated from adipose tissues with RNeasy (Qiagen). Gene expression data were obtained by hybridizing labeled cRNA to Affymetrix Mouse Genome 430 2.0 Array. For analysis, data were standardized using the robust multiarray average expression measure (14). The paired t test was two tailed, and P < 0.01 was considered statistically significant. Replicated, minimum twofold differences were adopted as the threshold of differential expression. Gene ontology analysis was performed using GOSTats packages (BioConductor).

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RESULTS

High-fat diet triggers acute collagenolytic activity in adipose tissue. To characterize the impact of nutritionally induced obesity on the remodeling of the extracellular matrix (ECM) in adipose tissues, 3-month-old C57BL/6 mice were placed on either a low-fat (control) or high-fat diet for 1 week. Subsequently, inguinal fat pads were isolated and type I collagen architecture was assessed by Sirius red staining. As expected, adipose tissues recovered from mice placed on a control diet displayed a web-like network of interlocking collagen fibrils (Fig. 1A). In marked contrast, a high-fat diet induced significant decreases in Sirius red staining consistent with an unexpectedly rapid activation of collagenolytic activity (Fig. 1A and B). Given the dominant role assigned to matrix metalloproteinases in type I collagen turnover in vivo (7,9), adipose tissues prepared from control or high-fat diet–challenged mice were probed for the generation of collagen cleavage products with a polyclonal antibody that recognizes type I collagen neoepitopes exposed following MMP-specific hydrolysis (12). While control fat pads contained only small amounts of immunoreactive material, collagen cleavage products increased more than threefold in the high-fat diet–challenged group (Fig. 1A and C). Consistent with these results, scanning electron microscopy confirmed that the high-fat diet challenge induces a marked loss in the adipocyte-associated meshwork of fibrillar collagen (Fig. 1A).

To assess the impact of high-fat diet–initiated ECM remodeling on the adipose tissue transcriptome, mRNA was isolated from inguinal fat pads of control or high-fat diet–challenged mice and analyzed with cDNA microarrays. Following 1 week’s feeding of a high-fat diet, a subset of 113 transcripts was upregulated by twofold or greater with 34 transcripts suppressed (Fig. 1D). Unique up- and downregulated genes in the inguinal fat pads of wild-type mice can be found in supplemental Table I (available in an online appendix [http://diabetes.diabetesjournals.org/cgi/content/full/db10-0073/DC1]). Gene ontology analysis of upregulated transcripts revealed the enrichment for biological processes related to collagen catabolism (P = 0.004), collagen fibril organization (P = 0.003), and integrin-mediated signaling (P = 0.002), a major transduction pathway for adipocyte-type I collagen interactions (19,20). Interestingly, gene programs consistent with acute changes in lipid biosynthesis (P = 0.001), steroid metabolism (P = 0.003), and biosynthesis (P = 0.003) were also upregulated in tandem with the recruitment of ECM-remodeling transcripts (Fig. 1E). Taken together, these data support a model wherein high-fat diet–induced changes in ECM remodeling are closely linked to the early transcriptional programs responsible for regulating lipid and cholesterol biosynthesis—a conclusion corroborated by recent studies linking a collagen subfamily member to the regulation of adipose tissue mass (21).

MMP14 mediates high-fat diet–induced collagenolytic activity in adipose tissues. While a number of secreted MMPs express type I collagenolytic activity (22), MMP-13, MMP-8, and MMP-2 expression were not altered following challenge with a high-fat diet. By contrast, expression levels of the membrane-anchored collagenase, MMP14, were increased twofold during the 1-week-long high-fat diet challenge as assessed by quantitative PCR (relative mRNA levels: high-fat diet 7.2 ± 0.95 vs. control 3.4 ± 0.10; n = 4). MMP14 is a membrane-anchored matrix metalloproteinase that has been identified as the dominant peri-cellular collagenase used by mesenchymal cells (7,13,23). As the collagenolytic activity of isolated mouse preadipocytes is enhanced in the presence of an adipogenic cocktail (7), the impact of MMP14 dosage on collagenolytic activity was first assessed in vitro. Under resting conditions, MMP14+/+/ or MMP14+/− preadipocytes cultured atop a three-dimensional bed of type I collagen fibrils degraded the underlying substrate comparably (Fig. 2A and B). When stimulated with an adipogenic mix, however, MMP14+/− cells displayed a two-fold increase in collagenolysis while MMP14−/− cells were unable to up-regulate their collagen degrading activity (Fig. 2A and B). Loss of adipogenic collagenolysis in the haploinsufficient state is consistent with a quantitative requirement for the full complement of MMP14 protein on the cell surface (24).

Given these in vitro findings, the role of MMP14 in regulating collagen turnover in adipose tissues in situ was assessed in haploinsufficient mice because MMP14-null mice fail to thrive from birth and exhibit a marked decrease in life span (9). MMP14 haploinsufficient mice were indistinguishable from wild-type littermates, and no significant differences in adipose tissue size or morphology could be detected between MMP14+/− and MMP14+/+ mice (supplemental Fig. 1). Furthermore, gene expression patterns of key adipogenic factors including peroxisome
proliferator–activated receptor γ, insulin receptor, Glut4, and lipoprotein lipase (25) were similar between the two groups (supplemental Fig. 1). When, however, \(MMP14^{+/−}\) mice were placed on a high-fat diet, only small decreases in fibrillar collagen content were detected relative to littermate controls (Fig. 2C and D). Furthermore, signifi-
FIG. 2. MMP14 gene dosage determines adipogenic collagenolysis in vitro and in vivo. A and B: Collagenolytic potential of MMP14+/+ vs. MMP14−/− preadipocytes. Preadipocytes isolated from the inguinal fat pads of wild-type (MMP14+/+) and haploinsufficient (MMP14−/−) mice were cultured atop a bed of fluorescent type I collagen (green), and subjacent degradation was monitored by the disappearance of fluorescent signal after a 3-day culture period in the absence or presence of an adipogenic cocktail. Representative zones of degradation are indicated by arrows. Nuclei stained with DAPI (blue). Bar = 100 μm. Collagenolytic activity was quantified by scanning the total area of degraded collagen. Cells were isolated from a cohort of three mice for each group (n = 3). *P < 0.05. C: MMP14 gene dosage modulates collagenolysis in vivo. High-fat diet–induced collagenolysis is almost completely blocked by MMP14 haploinsufficiency (fibrillar collagen detected with Sirius red staining and cleaved collagen by immunofluorescence) (red). Bar = 100 μm. Scanning electron microscopy revealed intact collagen fibers enwrapping adipocytes in MMP14+/+, but not MMP14−/−, inguinal fat pads. Bar = 100 μm. D: Cleaved collagen and fibrillar collagen contents in inguinal fat pads of MMP14+/+ and MMP14−/− mice following a 1-week control diet or high-fat diet challenge. Results are expressed as means ± 1 SD (n = 6). *P < 0.01. E: Following a 2-week high-fat diet, the inguinal and perigonadal fat pads were isolated from MMP14+/+ or MMP14−/− mice and tissue weights determined. Results are expressed as means ± 1 SD (n = 6). *P < 0.01. (A high-quality digital representation of this figure is available in the online issue.)
cant increases in collagen cleavage products following high-fat challenge were not observed in MMP14+/− mice, while the fibrillar collagen architecture (as determined by scanning electron microscopy) remained unchanged (Fig. 2C). In concert with the diminished ability of MMP14 haploinsufficient mice to remodel fat pad collagen architecture during high-fat feeding, the MMP14+/− mice were also unable to expand their adipose mass comparably with control (Fig. 2E). Whereas the weight of inguinal or perigonadal adipose tissue of MMP14+/− mice increased two- to threefold during the 1-week high-fat diet challenge, the haploinsufficient mice failed to mount a similar increase in adipose tissue mass (Fig. 2E).

**Dysregulated transcriptome coordination in MMP14 haploinsufficient mice.** To probe the functional impact of MMP14 in regulating the acute phase gene response to a high-fat diet, we examined the transcriptome profile of inguinal fat pads of MMP14 haploinsufficient mice placed on a high-fat diet for 1 week. In contrast with MMP14-sufficient mice, the haploinsufficient animals failed to induce the gene sets enriched for ECM remodeling or lipid biosynthesis (Fig. 3A and B). Further, as opposed to wild-type mice, 42 genes were uniquely upregulated and 91 genes downregulated in the heterozygote animals (Fig. 3A, supplemental Fig. 2, and supplemental Table 2). In these mice, the high-fat diet challenge led to a paradoxical deregulation in the gene expression profile of transcripts involved in a wide range of biological processes, including glycerol-3-phosphate metabolism, acetyl-CoA biosynthesis, and both humoral immune and acute phase responses (Fig. 3B). These results indicate that a reduction in MMP14 gene dosage leads to a disruption in the transcriptional link existing between ECM remodeling and lipid biosynthesis that coordinates the expansion of adipose tissue.

To next assess the long-term consequences of MMP14 haploinsufficiency on high-fat diet–induced obesity, wild-type and haploinsufficient mice were placed on a high-fat diet for 3–6 months. The average percentage of fat mass of MMP14 haploinsufficient and wild-type mice placed under a control diet was comparable (mean ± SD 5.29 ± 0.58 vs. 5.07 ± 0.78%; n = 6). On the high-fat diet, however, whereas wild-type mice displayed an approximate 20% and 60% weight gain after 3 and 6 months, respectively, MMP14+/− mice increased in total weight by less than one-half of that observed in the control (Fig. 3C). As expected, the dramatic changes in weight gain that occurred in the long-term, high-fat feeding of MMP14+/− mice was also reflected in the attenuated expansion of tissue mass in isolated inguinal and perigonadal fat pads (Fig. 3D). Changes in MMP14 expression did not, however, affect whole-body energy balance during high-fat feeding because the respiration rate, daily food intake, and locomotion of wild-type and heterozygous mice were not significantly different (Fig. 3F). These results stress the role of MMP14 as a proteolytic modifier that acts locally within adipose tissues without overtly affecting the hypothalamic regulation of metabolism.

**Human MMP14 SNPs associate with obesity and diabetes traits.** Obesity is driven by a complex process that is coordinated by a host of genetic, epigenetic, and environmental factors (26–28). To extend the findings of the genotype-phenotype link found in mice, the association of MMP14 SNP genotypes with human obesity and diabetes traits was examined. The human MMP14 gene is located at chromosome 14q11–12, comprising 10 exons and spanning a 10-kb region that contains 157 known SNPs (NCBI dbSNP). Using a preliminary group (n = 48) randomly sampled from a Japanese population (15), we assessed the minor allele frequencies of candidate MMP14 SNPs (Fig. 4A). Initially, 16 SNPs spanning human MMP14 gene (from rs17211964 at chr14:23,304,272 through rs2236307 at chr14:23,312,554) were screened to determine their pairwise linkage disequilibrium coefficients (supplemental Tables 1 and 2). Three SNPs located in exon 5 (rs2236302; allele 2, C > allele 1, G), intron 5 (rs2236304; allele 2, C > allele 1, G), and exon 6 (rs2236307; allele 2, T > allele 1, C) were chosen based on their frequency (>10%), proximity to the catalytic domain of MMP14, and pairwise linkage disequilibrium that allow diverse haplotypes in combination. The minor allele (allele 1) frequency for the three SNPs among the study population was 11.0, 44.8, or 38.5%, respectively (Fig. 4A).

Using a study population that included 3,653 individuals consisting of 1,708 men and 1,945 women, we assessed MMP14 haplotypes. The analysis of the pairwise linkage disequilibrium among the three SNPs suggested that they constitute a block of haplotypes (D’ > 0.977). However, the estimated square of the correlation coefficient (r2) among the studied SNPs were sufficiently low (Table 1) to allow for the assembly of at least four major haplotypes (Fig. 4B). The association of MMP14 haplotypes with obesity and diabetes traits, i.e., BMI, waist-to-hip ratio, body fat, A1C, fasting blood glucose and insulin levels, homeostasis model assessment (HOMA) of insulin resistance and β-cell function, was examined in the dominant or recessive genetic model with multiple logistic regression analysis (17,18,28). Among the major haplotypes of the MMP14 gene (212, 221, 122, and 222), the haplotype 122 (GCT) was found to positively associate with BMI (P = 0.0017) (Fig. 4C) and waist-to-hip ratio (P = 0.0079) (Table 2). Because of the dominant role played by rs2236302 in defining the link between obesity traits and MMP14 haplotypes, rs2236302 genotype was used to further delineate the link between MMP14 genotype and quantitative obesity traits. The distribution of C/C, C/G, and G/G genotypes were 0.79% (n = 2,908), 19.1% (n = 695), and 1.2% (n = 44) among the study population. Due to the low frequency of the G/G genotype, the quantitative association study was performed by comparing between homozygote C/C and heterozygote C/G genotype groups. The association with obesity traits was then examined in a genotype or dominant model with ANCOVA. In the multivariate analyses of the total population, BMI and waist-to-hip ratio were associated with age (P < 0.0001), sex (P < 0.0001), history of smoking (P = 0.0129), hypertension (P < 0.0001), diabetes (P = 0.0003), and hyperlipidemia (P < 0.0001). When analyzed with adjustment for these variables, a highly significant correlation was observed between rs2236302 genotype and obesity traits (Fig. 4D) (mean ± SEM BMI, C/C 22.74 ± 0.06 vs. C/G 23.16 ± 0.11 kg/m2; waist-to-hip ratio, C/C 0.901 ± 0.001 vs. C/G 0.906 ± 0.002). The increase of BMI caused by rs2236302 minor allele was 0.42 kg/m2 (Cohen d = 0.13).

Of note, the positive effect of rs2236302 genotype on BMI and waist-to-hip ratio was preferentially observed in women (Fig. 4D), suggesting the existence of sexual dimorphism in the link between MMP14 and obesity phenotype (women P = 0.0004, d = 0.199, vs. men P = 0.5419, d = 0.059). Finally, human MMP14 SNPs were found to be weakly associated with A1C in men (P =
0.0685) (Table 3), suggesting a paradoxical relationship of MMP14 SNPs with diabetes predisposition in males. Consistently, MMP14 haplotype (121) associated with increased fasting blood glucose ($P = 0.0069$) and HOMA of insulin resistance ($P = 0.0386$) (Table 3), supporting a potential link between MMP14 and diabetes in men.

**DISCUSSION**

In this study, we have demonstrated that a high-fat diet acutely initiates the MMP14-dependent degradation of the type I collagen network found in adipose tissues and induces a selective set of transcripts that link ECM-related remodeling to lipid/cholesterol biosynthesis. While one
allele of MMP14 is sufficient for postnatal adipose tissue development, our results highlight the quantitative requirement of this protease for diet-induced expansion of adipose tissues in vivo. MMP14 gene expression, however, is not confined to preadipocyte/adipocyte cell population but can be found in vascular endothelial cells, pericytes, or fibroblasts (13,29,30). Consequently, MMP14-dependent remodeling of fat pad architecture may well involve the cooperative interplay of multiple cell populations. Never-

![Table 1: Pairwise Linkage Disequilibrium](#)

**TABLE 1**

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<thead>
<tr>
<th>Pair of SNPs</th>
<th>D'</th>
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<tr>
<td>rs2236302 and rs2236304</td>
<td>0.977</td>
<td>0.107</td>
</tr>
<tr>
<td>rs2236302 and rs2236307</td>
<td>0.993</td>
<td>0.075</td>
</tr>
<tr>
<td>rs2236304 and rs2236307</td>
<td>0.995</td>
<td>0.580</td>
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D', linkage disequilibrium measure.
theless, the direct physical association of the collagenous web with preadipocytes and adipocytes, coupled with the deposition of collagen degradation products in the peri-adipocyte space, supports a dominant role for these cells in diet-induced collagen remodeling. Further, our in vitro results also support the importance of adipogenic regulation on preadipocyte-mediated collagenolysis. Of note, human mesenchymal stem cells have recently been shown to mobilize MMP14 for trafficking and differentiation in three-dimensional collagen environments (31). Given that adipocyte progenitors can reside in perivascular stromal tissues (32,33), it is intriguing to speculate that MMP14 may likewise support the migration and differentiation of adipocyte progenitors within adipose tissues.

In addition to the ability of MMP14 to remodel collagen in a diet-dependent manner, the enzyme’s role in regulating the high-fat diet–responsive early-onset transcriptome is notable. High-fat diet challenge rapidly—within a week—induces a selective set of genes enriched for ECM remodeling and lipid/cholesterol biosynthesis, including the previously described genes, MEST and Npr3 (34). By contrast, the enrichment of genes associated with ECM remodeling and lipid/cholesterol biosynthesis is ablated in MMP14 haploinsufficient mice, though induction of MEST and Npr3 gene expression remains intact (supplemental Tables 1 and 2). The precise molecular mechanism by which MMP14 gene selectively regulates the fat pad transcriptome is unknown; however, MMP14-dependent collagenolysis may regulate transcriptional programs by modulating cell shape and tension in collagen-rich microenvironments (7,20). Despite the marked changes in fat pad size and function observed in MMP14 haploinsufficient mice, V02 consumption, food intake, and physical activity in these mice appear to be comparable with controls. While white adipose tissues of high-fat diet–challenged MMP14 haploinsufficient mice are small in size, the efficiency with which they oxidize fat versus carbohydrate may have undergone adaptive alterations in the in vivo setting. Indeed, respiratory ratio (VCO2/V02), was relatively lower in MMP14 haploinsufficient mice, which is consistent with a preferred utilization of fat over carbohydrate. While severe lipodystrophy increases the risk of fatty liver (35), we were unable to detect increased triglyceride accumulation in the livers of MMP14 haploinsuffi-
MMP14 LINKS ECM REMODELING AND OBESITY

In parallel with our findings in a mouse model of diet-induced obesity, human MMP14 SNPs were found to be associated with quantitative traits of obesity and diabetes in a Japanese population. The link between MMP14 SNPs and obesity or diabetes traits found in this study may well be due to altered MMP14 gene expression, catalytic activity, exocytosis (36), or unknown effects on neighboring genes that are in linkage disequilibrium. Interestingly, however, a rare nonsynonymous polymorphism identified in exon 5 of MMP14 appears to alter collagenolytic activity and adipogenic potential in vitro (T.-H.C., unpublished observations), supporting its potential link with MMP14 catalytic activity. A sib-pair linkage analysis of black and Caucasian nuclear family volunteers (364 sib pairs) has pointed to chromosome 14q11, where MMP14 resides, as one of three candidate loci linked with BMI and fat mass (37). While genetic risks for obesity have recently been highlighted by the identification of FTO (38) and MC4R (39) gene variants by genome-wide association studies (GWAS), candidate-gene approaches are still needed to bridge the gaps that remain unfill ed by GWAS analysis alone (40–42). Our study of a Japanese cohort is of moderate size (n = 3,653) but demonstrates a significant increase of BMI with rs2236302 heterozygosity. The effect size of this risk allele is modest (∆BMI 0.42 kg/m²), which may not allow for its detection by GWAS. Though multiple genes affect obesity traits in mice without relevant findings in humans, MMP14 gene dose or polymorphism may define a genetic susceptibility for obesity traits that spans the gulf between mice and humans.

In humans, obesity and diabetes phenotypes of MMP14 gene variants display a sexual dimorphism. The stronger association of MMP14 genotype/haplotypes with obesity traits in women may relate their higher subcutaneous fat volume (43). Because subcutaneous fat depots contain higher concentrations of collagen fibers relative to other tissues, MMP14-dependent collagenolysis may contribute more to the size regulation of subcutaneous fat pads in women. Female mice in the C57BL/6 background, whether MMP14 wild-type or haploinsufficient, did not significantly change their fat mass in response to diet. However, unlike male mice, MMP14 haploinsufficient female mice displayed a ~30% reduction of fat mass even under the conditions of a control diet (mean ± SD 13.3 ± 1.9 vs. 9.5 ± 0.9% for MMP14+/− and MMP14−/− mice, respectively; n = 6). Of note, basal fat mass of female mice is more than two times higher than that of male mouse. As such, MMP14 might be fully engaged in maintaining adipose tissue mass in female mice—and perhaps in women as well. Under these conditions, MMP14 gene variants may be expected to demonstrate an increased linkage with obesity traits. Additionally, the association of rs2236302 with BMI found in women was reproduced in postmenopausal women (n = 1,503, P = 0.0008), suggesting that constitutional but not hormonal differences contribute to the sexual dimorphism. Conversely, in men, MMP14 gene variants are associated with diabetes but not obesity traits (Table 3). The genetic or epigenetic predisposition that determines obesity or diabetes phenotypes in men, therefore, may differentially interact with MMP14 gene variants.

Given the diverse biological functions of MMP family members interacting with an array of substrates (44), it is often difficult to pin down the causal relationship between a specific MMP and a selective substrate. For example, MMP3 gene expression and variations are associated with body fat in Pima Indian population (45). While MMP3 gene expression decreases in obesity, MMP3 has been shown to be necessary for adipocyte differentiation in a manner independent of ECM context (46). Indeed, MMP3 is not required for type I collagen hydrolysis (47), and the substrate targets for MMP3 that regulates adiposity are unknown. By contrast, MMP14-dependent regulation of adipocyte differentiation is restricted to collagenous microenvironments (7), suggesting that the MMP14−/−type I collagen axis is the dominant pathway operative in adipocytes in vitro as well as in mouse and human adipose tissues. However, additional interactions with other MMP14 substrates, or the involvement of additional MMP family members in this process, cannot be ruled out. Indeed, MMP13 and MMP2, whose latent forms can be activated by MMP14 (10,48), may play cooperative roles in regulating adipocyte function. Additional studies will be required to identify other pathogenic links that may exist between MMP family members and their respective substrates during obesity progression. Along these lines, the metabolic impact of undigested collagen in MMP14 wild-type and haploinsufficient male mice also bears consideration. For example, the transgenic expression of hypoxia-inducible factor-1α leads to increased fibrosis, inflammatory response, and insulin resistance (49). Hence, while targeting MMP14 in adipose tissue may prevent diet-induced fat expansion, the overall impact of reduced collagen remodeling on inflammation and metabolism remains to be determined. Nonetheless, our data lend further support to a model wherein MMP14 functions as a metabolic rheostat that controls the rate of collagen turnover in adipose tissues. Our in vitro and mouse studies, combined with SNP association analyses, point to a critical role for the MMP14−/−type I collagen axis in regulating adipose tissue mass in states of nutritional challenge.

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No potential conflicts of interest relevant to this article were reported.

T.-H.C. analyzed data and wrote the manuscript, M.I. analyzed data, H.M. analyzed data, I.Y. analyzed data, Y.M. contributed to data and discussion, T.O. contributed to data and discussion, K.S.-K. analyzed data, and S.J.W. analyzed data and wrote the manuscript.

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