Genetic Link Between Obesity and *MMP14*-Dependent Adipogenic Collagen Turnover

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OBJECTIVE—In white adipose tissue, adipocytes and adipocyte precursor cells are enmeshed 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.

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 *MMP14 (MT1-MMP)*-haploinsufficient mice using immunofluorescent staining and scanning electron microscopy. The impact of *MMP14*-dependent collagenolysis on adipose tissue function was interrogated by transcriptome profiling with cDNA microarrays. Genetic associations between *MMP14* gene common variants and obesity or diabetes traits were examined in a Japanese cohort ($n = 3,653$).

RESULTS—In adult mice, type I collagen fibers were cleaved rapidly in situ during a high-fat diet challenge. By contrast, in *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 *MMP14* in regulating high-fat diet–induced metabolic programs, human *MMP14* gene polymorphisms located in proximity to the enzyme's catalytic domain were closely associated with human obesity and diabetes traits.

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

Obesity is closely linked to the development of diabetes and negatively affects life expectancy (1–3). At early stages of obesity, gene programs are activated within fat depots that support tissue expansion, which arises as a combined result of the hypertrophic growth of adipocytes and the recruitment of adipocyte precursors to the adipogenic process (4,5). Within adipose tissues, both adipocytes and adipocyte precursors are enveloped by a dense network of type I collagen (6,7). This collagenous web is presumed to provide adipose tissues with structural integrity and elasticity necessary to maintain optimal form and function (8). Matrix metalloproteinase (MMP)14 (MT1-MMP) is a pericellular type I collagenase that is critical for the postnatal development of the mesenchyme, including adipose tissues (7). Whether the developmental role played by *MMP14* during adipogenesis is relevant to the pathogenesis of adult obesity remains unclear. The premature morbidity and mortality observed in *MMP14* knockout mice (7,9) preclude attempts to assess the impact of nutritional interventions on adult obesity in these animals. Moreover, it has not been determined whether the diet-induced expansion of fat mass is functionally linked to the remodeling of the type I collagen network.

Here, we demonstrate that the type I collagen architecture of adipose tissues undergoes rapid degradative remodeling in response to a high-fat diet challenge in vivo via a proteolytic process mediated by MMP14 (10). A reduction in *MMP14* dosage not only prevents the diet-induced cleavage of the type I collagen network in vivo but also disrupts the regulation of the transcriptional programs linked to adipogenesis and lipogenesis. Consistent with insights obtained from analyses of genetically modified mice, human *MMP14* single nucleotide polymorphisms (SNPs) are identified that positively associate with human obesity and diabetes traits. Taken together, these data support a new model wherein *MMP14* not only participates in obesity pathogenesis but also serves as a potential genetic modifier of obesity and diabetes predisposition in humans.

RESEARCH DESIGN AND METHODS

Mice, diets, and metabolic phenotyping. $MMP14^{+/-}$ mice were kindly provided by Dr. Motoharu Seiki (University of Tokyo) and maintained in C57BL/6 background (11). *MMP14* haploinsufficient ($MMP14^{+/-}$) and wild-type ($MMP14^{+/+}$) 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, Vo_2, 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 C1-2C 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 fluoresceinated type I collagen polymers with or without an adipogenic mix (0.2 μmol/l insulin, 0.5 mmol/l 3-isobutyl-1-methylxanthine, and 0.25 μmol/l dexamethsone) (7). Nuclei were stained with DAPI and zones of collagenolysis identified with a fluorescent microscope (model no. IX71; Olympus) 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).

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 N_2, fractured to expose the inner mass of adipose tissues, and imaged with an AMRAY 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 7900HT) and verified in a subset of samples by direct sequencing (ABI 3700). Haplotype distribution was estimated with an EM algorithm (16). The association of $MMP14$ gene haplotypes with age- and sex-adjusted quantitative traits was tested with the QTLHAPLO program (17) using logistic regression analysis (18). The genotype-phenotype association with rs2236302 was performed with one-way ANCOVA adjusted for age, history of smoking, drinking, diabetes, hypertension, and hyperlipidemia.

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). Uniquely up- and downregulated genes in the inguinal fat pads of wild-type mice can be found in supplemental Table 1 (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 biologic 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 collagenolysis 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-tethered matrix metalloproteinase that has been identified as the dominant pericellular 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 upregulate 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
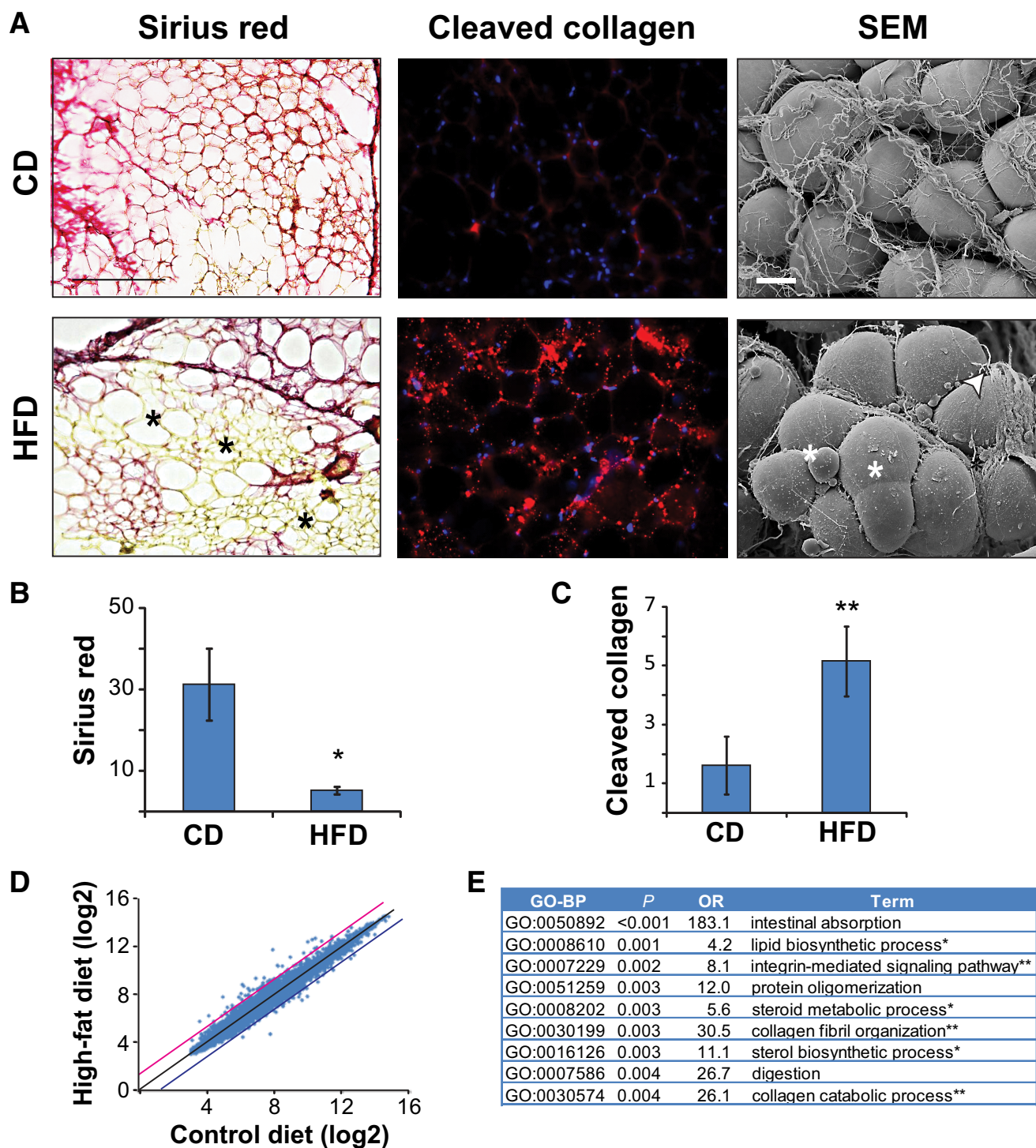
FIG. 1. Type I collagen degradation induced by high-fat feeding. *A*: Type I collagen cleavage induced by a 1-week regimen of high-fat diet feeding. Fibrillar collagen is detected with Sirius red staining in inguinal fat pads of 3-month-old C57BL/6 mice under a control diet (CD) (*top row*) or high-fat diet (HFD) (*bottom row*) feeding. Type I collagen cleavage products are detected with C1, 2C antibody (cleaved collagen, red). Nuclei are stained with Hoechst dye (blue). The fibrillar status of type I collagen fibrils encircling adipocyte clusters is disrupted when mice are placed on an HFD (scanning electron microscopy [SEM]). Disrupted collagen fibers (*arrowhead*) are found in association with variably sized adipocytes (*asterisks*). Bar = 10 μm. *B* and *C*: Quantification of Sirius red–positive staining and cleaved collagen in the inguinal fat pads of mice after 1 week of control diet or high-fat feedings. For each group, images were collected from more than six randomly selected fields. *$P < 0.01$; **$P < 0.01$. *D*: Genome-wide transcriptome change induced by a 1-week high-fat diet challenge. While more than 99% of transcripts did not display significant changes (*x*-axis, control diet; *y*-axis, high-fat diet), a specific subset of genes display a significant increase in expression. Replicated dataset from two independent experiments. Slopes with *y*-intercepts 0, 1, and −1 (log 2 scale) are shown. *E*: Overrepresented gene ontology (GO) pathways. High-fat diet–dependent transcriptome changes after a 1-week high-fat diet challenge are categorized following validation in two or more independent experiments. Bar = 100 μm. *Lipid/cholesterol biosynthesis pathways; **ECM remodeling pathways. (A high-quality digital representation of this figure is available in the online issue.)

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. 2*C* and *D*). Furthermore, signifi-
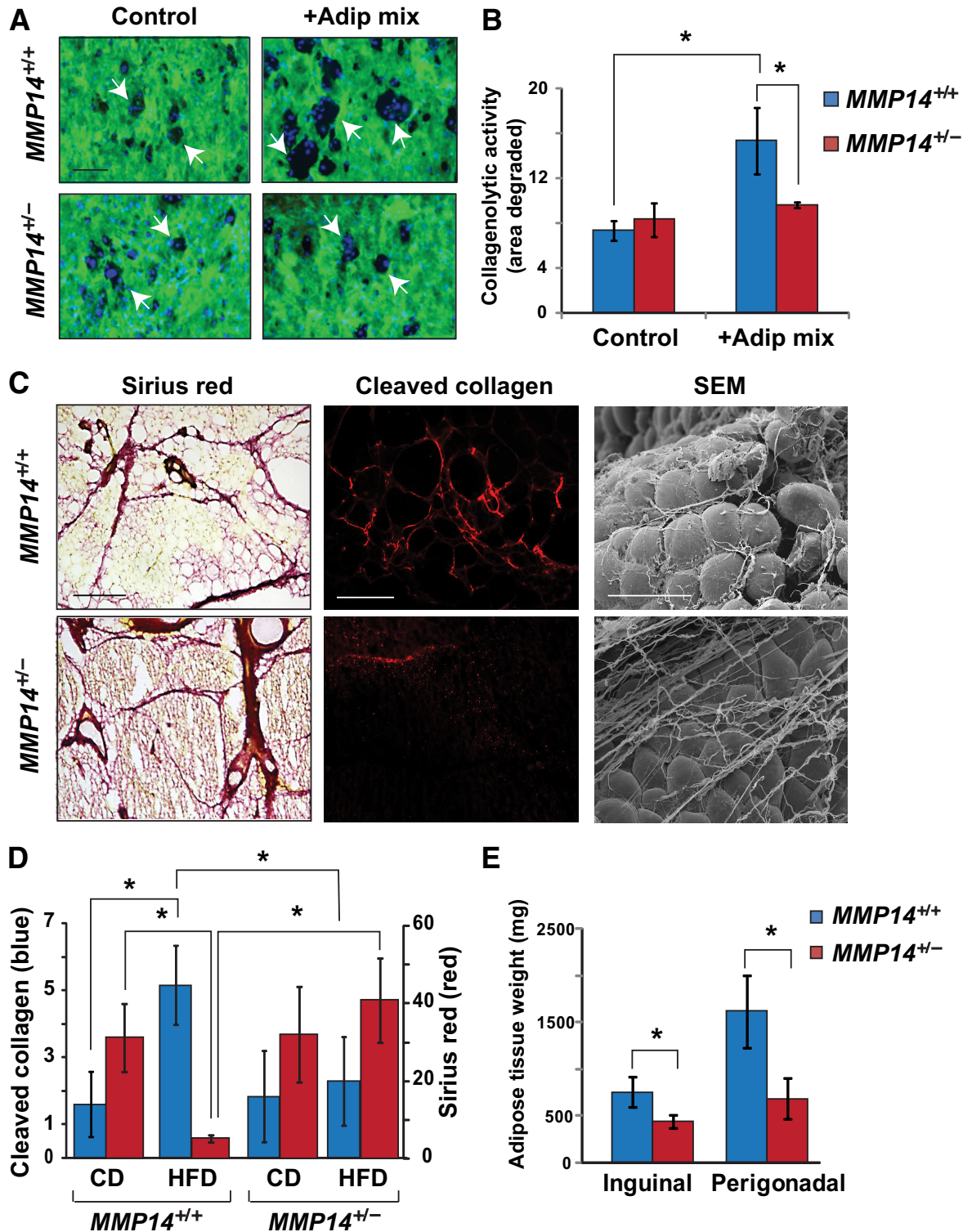
FIG. 2. *MMP14* gene dosage determines adipogenic collagenolysis in vitro and in vivo. *A* and *B*: Collagenolytic potential of $MMP14^{+/+}$ vs. $MMP14^{+/-}$ preadipocytes. Preadipocytes isolated from the inguinal fat pads of wild-type ($MMP14^{+/+}$) and haploinsufficient ($MMP14^{+/-}$) mice were 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. 2*C*). 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. 2*E*). 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. 2*E*).

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. 3*A* and *B*). Further, as opposed to wild-type mice, 42 genes were uniquely upregulated and 91 genes downregulated in the heterozygote animals (Fig. 3*A*, 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. 3*B*). 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 $\pm$ SD 5.29 $\pm$ 0.58 vs. 5.07 $\pm$ 0.7%; $n = 6$). 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. 3*C*). As expected, the dramatic changes in weight gain that occured 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. 3*D*). 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. 3*F*). 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. 4*A*). 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. 4*A*).

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 (r^2) among the studied SNPs were sufficiently low (Table 1) to allow for the assembly of at least four major haplotypes (Fig. 4*B*). 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 four 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. 4*C*) 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 79.7% ($n = 2,908$), 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. 4*D*) (mean $\pm$ SEM BMI, C/C 22.74 $\pm$ 0.06 vs. C/G 23.16 $\pm$ 0.11 kg/m^2; waist-to-hip ratio, C/C 0.901 $\pm$ 0.001 vs. C/G 0.906 $\pm$ 0.002). The increase of BMI caused by rs2236302 minor allele was 0.42 kg/m^2 (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. 4*D*), 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 = $

A

B

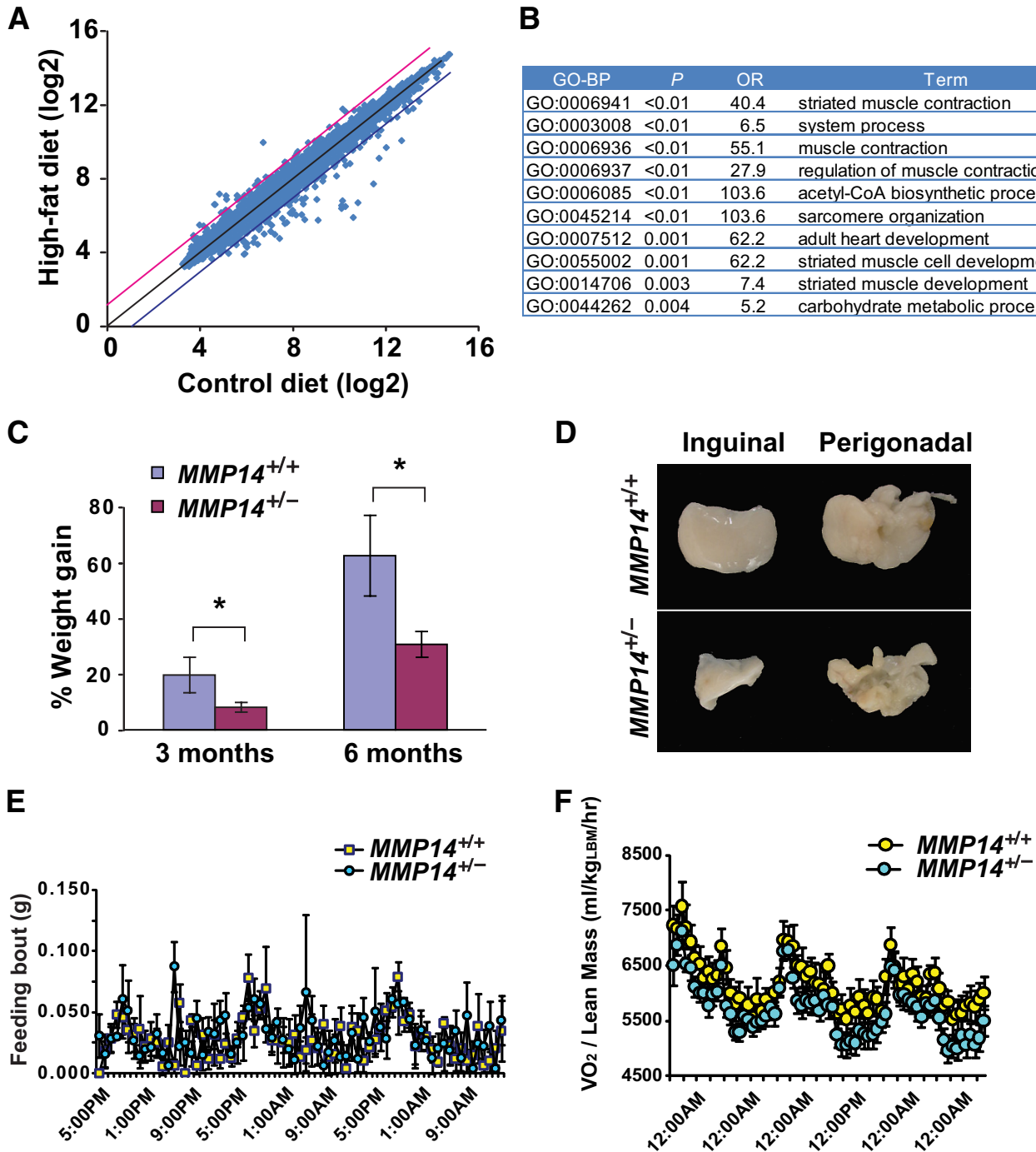| GO-BP	P	OR	Term
GO:0006941	<0.01	40.4	striated muscle contraction
GO:0003008	<0.01	6.5	system process
GO:0006936	<0.01	55.1	muscle contraction
GO:0006937	<0.01	27.9	regulation of muscle contraction
GO:0006085	<0.01	103.6	acetyl-CoA biosynthetic process*
GO:0045214	<0.01	103.6	sarcomere organization
GO:0007512	0.001	62.2	adult heart development
GO:0055002	0.001	62.2	striated muscle cell development
GO:0014706	0.003	7.4	striated muscle development
GO:0044262	0.004	5.2	carbohydrate metabolic process*

C

D

E

F

FIG. 3. *MMP14* gene dosage controls nutritional transcriptome response and obesity development. *A* and *B*: Disrupted high-fat diet–induced transcriptome changes in *MMP14* haploinsufficient mice. A scatter plot is shown of mRNA expression values (log2 scale) in inguinal fat pads isolated from $MMP14^{+/-}$ mice fed a control diet (*x*-axis) vs. high-fat diet (*y*-axis). In comparison with gene expression profiles obtained in wild-type mice (Fig. 1*B*), genes involved in a diverse set of metabolic pathways critical to adipose tissue function (*asterisks*) are misregulated in $MMP14^{+/-}$ mice. GO, gene ontology. *C* and *D*: *MMP14* haploinsufficiency protects mice from diet-induced increase of fat mass after 3 or 6 months of high-fat diet feeding (means ± SD; *n* = 8). Representative images of inguinal and perigonadal fat pads are shown following isolation from $MMP14^{+/+}$ and $MMP14^{+/-}$ mice that had been placed on high-fat feeding for 3 months. *E* and *F*: Feeding bouts (g/day) and metabolic rates adjusted for lean mass (Vo_2/kg lean mass) were determined in metabolic cages during high-fat diet feeding. (A high-quality digital representation of this figure is available in the online issue.)

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
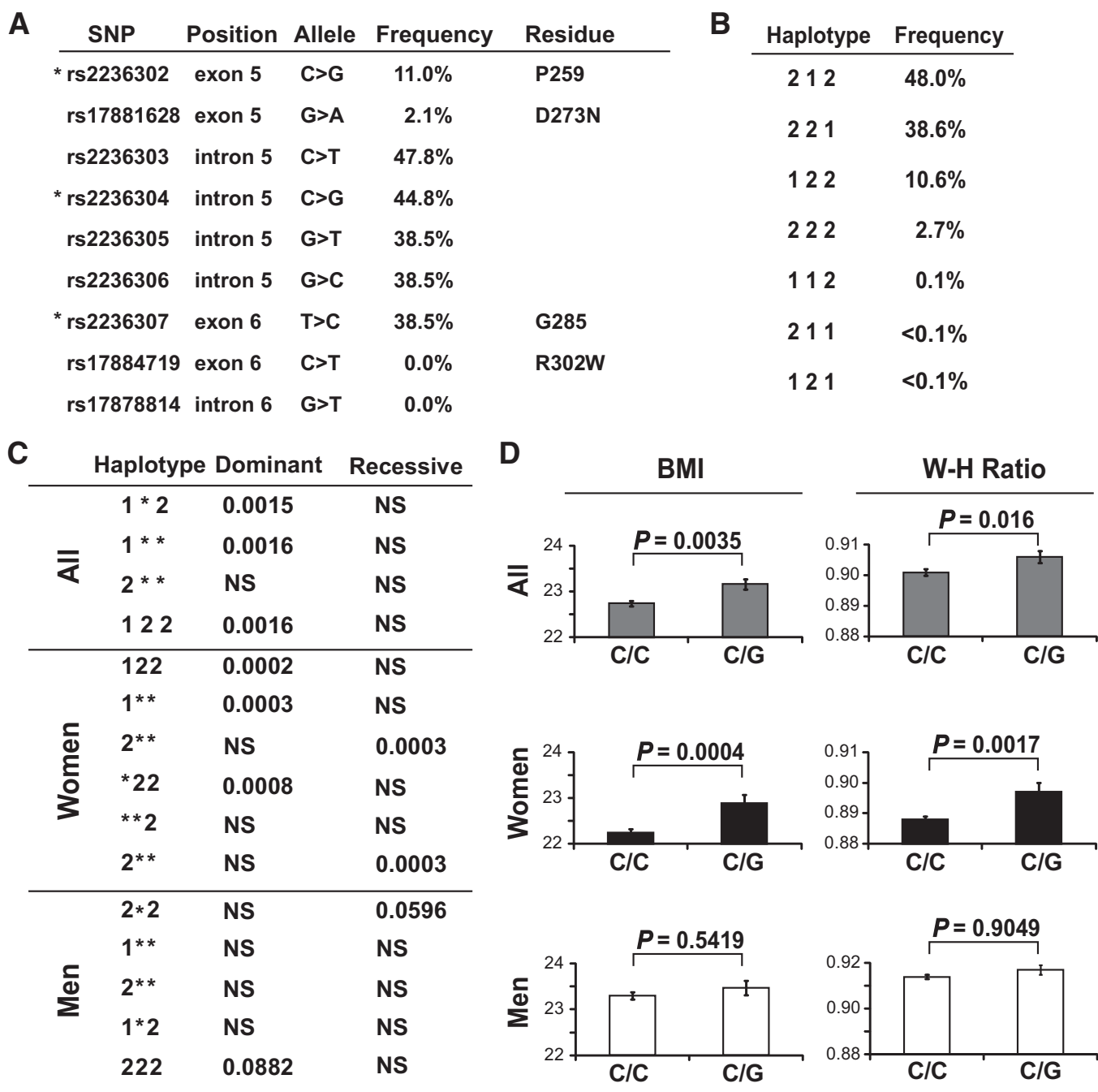
A

SNP	Position	Allele	Frequency	Residue
* rs2236302	exon 5	C>G	11.0%	P259
rs17881628	exon 5	G>A	2.1%	D273N
rs2236303	intron 5	C>T	47.8%	
* rs2236304	intron 5	C>G	44.8%	
rs2236305	intron 5	G>T	38.5%	
rs2236306	intron 5	G>C	38.5%	
* rs2236307	exon 6	T>C	38.5%	G285
rs17884719	exon 6	C>T	0.0%	R302W
rs17878814	intron 6	G>T	0.0%	

B

Haplotype	Frequency
2 1 2	48.0%
2 2 1	38.6%
1 2 2	10.6%
2 2 2	2.7%
1 1 2	0.1%
2 1 1	<0.1%
1 2 1	<0.1%

C

	Haplotype	Dominant	Recessive
All	1 * 2	0.0015	NS
	1 * *	0.0016	NS
	2 * *	NS	NS
	1 2 2	0.0016	NS
Women	122	0.0002	NS
	1**	0.0003	NS
	2**	NS	0.0003
	*22	0.0008	NS
	**2	NS	NS
	2**	NS	0.0003
Men	2*2	NS	0.0596
	1**	NS	NS
	2**	NS	NS
	1*2	NS	NS
	222	0.0882	NS

D

FIG. 4. Human *MMP14* SNP associations with obesity and diabetes traits in a Japanese cohort. *A*: Human *MMP14* SNPs located in the proximity of the region encoding MMP14 catalytic domain. Minor allele frequency for each SNP was determined. *SNPs used for haplotype analyses. *B*: *MMP14* haplotype determinations were based on the combination of rs2236302, rs2236304, and rs2236307. *C*: Association of human *MMP14* haplotypes with BMI. Haplotype associations with BMI of the total population ($n = 3,531$), women ($n = 1,944$), and men ($n = 1,587$) were assessed in both dominant and recessive inheritance models. The results of ANCOVA analyses are shown with *P* values. *Any genotype. NS, *P* value > 0.1. *D*: Association of rs2236302 genotype with BMI and waist-to-hip (W-H) ratio. Means ± SEM of BMI (*left panel*) and waist-to-hip ratio (*right panel*) are shown for the total population ($n = 3,647$), women ($n = 1,944$), and men ($n = 1,703$), respectively.

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

Pair of SNPs	D′	r^2
rs2236302 and rs2236304	0.977	0.107
rs2236302 and rs2236307	0.993	0.075
rs2236304 and rs2236307	0.995	0.580

D′, linkage disequilibrium measure.

TABLE 2
Association of *MMP14* haplotype with obesity/diabetes traits

	Dominant		Recessive	
	Haplotype	*P*	Haplotype	*P*
BMI	1*2	0.0016	2**	0.0016
	1**	0.0016		
	122	0.0017		
	12*	0.0017		
	*22	0.0211		
Waist-to-hip ratio	122	0.0079	2**	0.0086
	12*	0.0080		
	1*2	0.0084		
	1**	0.0086		
Leptin	NS		222	0.0394
A1C	NS		NS	
Fasting blood glucose	121	0.0113	NS	
	222	0.0460		
Fasting insulin	*2*	0.0158	*12	0.0125
	12*	0.0335	*1*	0.0158
	122	0.0404	212	0.0160
	*22	0.0419	21*	0.0200
	1**	0.0483	2**	0.0483
			2*2	0.0494
HOMA-IR	12*	0.0259	*12	0.0272
	122	0.0327	212	0.0322
	2	0.0343	*1*	0.0343
	1**	0.0358	2**	0.0358
	1*2	0.0445	2*	0.0404
	121	0.0496		
HOMA-β	*2*	0.0029	*12	0.0026
	*22	0.0604	*1*	0.0029
	22*	0.0725	212	0.0041
			21*	0.0045
			2*2	0.0236

NS, $P > 0.1$. *Any genotype. HOMA-β, HOMA of β-cell function; HOMA-IR, HOMA of insulin resistance.

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 micro-environments (7,20). Despite the marked changes in fat pad size and function observed in *MMP14* haploinsufficient mice, V_{O_2} 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 (V_{CO_2}/V_{O_2}), 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-

TABLE 3
Sexual dimorphism in the link between *MMP14* and obesity/diabetes traits

	Men				Women			
	Dominant		Recessive		Dominant		Recessive	
	Haplotype	*P*	Haplotype	*P*	Haplotype	*P*	Haplotype	*P*
BMI	NS		NS		122	0.0002	2**	0.0004
					1**	0.0004		
					*22	0.0008		
Waist-to-hip ratio	NS		2*2	0.0417	122	0.0009	2**	0.0015
					1**	0.0015		
					*22	0.0037		
Leptin	222	0.0369	222	0.0157	*22	0.0630	NS	
			2*2	0.0365	112	0.0944		
A1C	22*	0.0685	NS		NS		NS	
Fasting blood glucose	121	0.0069	NS		NS		NS	
	222	0.094						
Fasting insulin	NS		NS		*22	0.0361	NS	
HOMA-IR	121	0.0386	NS		*22	0.0379	NS	
HOMA-β	*2*	0.0075	*12	0.0065	222	0.0253	NS	
			1	0.0075				
			212	0.0090				

*Any genotype. HOMA-β, HOMA of β-cell function; HOMA-IR, HOMA of insulin resistance; NS, $P > 0.1$.

cient mice (total glycerol 14.4 $\pm$ 2.7 vs. 11.8 $\pm$ 1.7% in wild-type and $MMP14^{+/-}$ mice, respectively; $n = 6$).

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 unfilled 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 (ΔBMI 0.42 kg/m^2), 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 $\pm$ SD 13.3 $\pm$ 1.9 vs. 9.5 $\pm$ 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 mice. 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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