2012

Matrix metalloproteinase-9 genetic variations affect MMP-9 levels in obese children

INTERNATIONAL JOURNAL OF OBESITY, LONDON, v. 36, n. 1, pp. 69-75, JAN, 2012
http://www.producao.usp.br/handle/BDPI/42622

International Journal of Obesity (2012) 36, 69–75

0307-0565/12
www.nature.com/ijo

PEDIATRIC ORIGINAL ARTICLE

Matrix metalloproteinase-9 genetic variations affect MMP-9 levels in obese children

VA Belo[1], DC Souza-Costa[2], MR Luizon[3], CMM Lanna[4], PC Carneiro[3], TC Izidoro-Toledo[3], KC Ferraz[1] and JE Tanus-Santos[3]

[1]*Department of Pharmacology, Faculty of Medical Sciences, State University of Campinas, Campinas, Brazil;* [2]*Department of Pharmacology, Federal University of Juiz de Fora, Juiz de Fora, Brazil;* [3]*Department of Pharmacology, Faculty of Medicine of Ribeirao Preto, University of Sao Paulo, Ribeirao Preto, Brazil and* [4]*Department of Physiology, Federal University of Juiz de Fora, Juiz de Fora, Brazil*

Objective: Matrix metalloproteinase-9 (MMP-9) is involved in the atherosclerotic process and functional polymorphisms in the MMP-9 gene affect MMP-9 expression/activity, and are associated with cardiovascular diseases. However, no study has tested the hypothesis that functional MMP-9 polymorphisms could affect MMP-9 levels in obese children. We investigated whether three MMP-9 gene polymorphisms (C-1562T (rs3918242), 90(CA)$_{(14-24)}$ (rs2234681) and Q279R (rs17576)), or haplotypes, affect MMP-9 levels in obese children.
Methods: We studied 175 healthy control children and 127 obese children. Plasma MMP-9, tissue inhibitor of MMPs (TIMP)-1 and adiponectin concentrations were measured using enzyme-linked immunosorbent assay.
Results: We found similar MMP-9 genotypes, allelic and haplotypes distributions in the two study groups ($P>0.05$). However, we found lower plasma MMP-9 concentrations in obese subjects carrying the CC or the QQ genotypes for the C-1562T and the Q279R polymorphisms, respectively, in obese children compared with children with the other genotypes, or with non-obese children with the same genotypes (all $P<0.05$). Moreover, we found lower MMP-9 levels and lower MMP-9/TIMP-1 ratios (which reflect net MMP-9 activity) in obese children carrying the H2 haplotype (which combines the C, H and Q alleles for the three polymorphisms, respectively) when compared with obese children carrying the other haplotypes, or with non-obese children carrying the same haplotype ($P<0.05$).
Conclusions: Our findings show that MMP-9 genotypes and haplotypes affect MMP-9 levels in obese children and adolescents, and suggest that genetic factors may modify relevant pathogenetic mechanisms involved in the development of cardiovascular complications associated with obesity in childhood.
International Journal of Obesity (2012) **36,** 69–75; doi:10.1038/ijo.2011.169; published online 16 August 2011

Keywords: matrix metalloproteinase-9; childhood obesity; polymorphisms; haplotype

Introduction

Obesity is reaching epidemic proportions worldwide, and its prevalence is increasing among children and adolescents, likely accelerating the development of cardiovascular diseases and promoting premature death in adults.[1] There is now evidence indicating that the extent of atherosclerotic lesions in children and young adults correlate positively with body mass index.[2] Therefore, the identification of key pathogenetic mechanisms and genetic markers underlying such mechanisms may help to prevent the cardiovascular complications associated with obesity.

Matrix metalloproteinase-9 (MMP-9) is an endopeptidase capable of degrading components of extracellular matrix and other substrates. Altered expression and activity of MMP-9 and its major endogenous inhibitor, the tissue inhibitor of MMPs (TIMP)-1, have been implicated in atherosclerotic vascular remodeling.[3] In fact, experimental and clinical studies have clearly shown that MMP-9 is highly expressed in the atherosclerotic plaques,[4,5] and elevated MMP-9 levels have been shown in patients with atherosclerotic diseases.[6,7] Importantly, functional genetic polymorphisms in the MMP-9 gene modify MMP-9 levels and prognosis of patients with cardiovascular diseases.[6,8,9]

Although MMP-9 is a major factor in cardiovascular diseases, there are few studies examining possible alterations

Correspondence: Professor JE Tanus-Santos, Department of Pharmacology, Faculty of Medicine of Ribeirao Preto, University of Sao Paulo, Avenue Bandeirantes, 3900, 14049-900 Ribeirao Preto, Sao Paulo, Brazil.
E-mail: tanus@fmrp.usp.br or tanussantos@yahoo.com
Received 28 February 2011; revised 1 June 2011; accepted 2 July 2011; published online 16 August 2011

in MMP-9 and TIMP-1 levels in obese children.[10–12] In addition, no previous study has tested the hypothesis that functional MMP-9 polymorphisms could affect MMP-9 levels in obese children and adolescents. In the present study, we aimed at investigating whether MMP-9 gene polymorphisms or haplotypes (combinations of genetic markers) affect MMP-9 levels in obese children and adolescents. We studied three functional MMP-9 polymorphisms that are known to modify MMP-9 expression or activity,[8,13,14] and have been associated with cardiovascular diseases:[6,8,9,15,16] the C-1562T (rs3918242) single nucleotide polymorphism and the microsatellite $(CA)_{14-24}$ at −90 position (rs2234681), both in the promoter region of the MMP-9 gene, and the Q279R single nucleotide polymorphism (rs17576) in the exon 6. In addition, we have also assessed plasma thiobarbituric acid-reactive species (TBARS) levels (an index of oxidative stress), the homeostatic model assessment insulin resistance (HOMA IR) index and adiponectin levels in the subjects included in the present study, because we hypothesized that MMP-9 polymorphisms would not significantly affect these relevant biomarkers, although we supposed that they would affect MMP-9 levels.

Methods

Subjects

This study was approved by the Institutional Review Board at the Federal University of Juiz de Fora, Juiz de Fora, Brazil. Parents and children were informed as to nature and purpose of the study. Parents gave their written consent and children gave their verbal consent.

Study populations consisted of 127 normotensive obese subjects (57 self reported as white and 70 self reported as black) recruited from the Endocrinology Ambulatory of the Adolescent and Child Institute at Juiz de Fora and from the Childhood Endocrinology Ambulatory of the IMEPEN Foundation at Juiz de Fora as outpatients. The control group consisted of 175 healthy children and adolescents (96 self reported as white and 79 self reported as black) recruited from the local community and unrelated to the obese patients.

All children underwent physical examination. Height was measured to the nearest 0.1 cm by using a wall-mounted stadiometer. Body weight was measured with a digital scale to the nearest 0.1 kg. Body mass index was calculated as the weight in kilograms divided by height in meters squared. Obesity was defined as body mass index greater than the 95th percentile, matched according to age and sex.[17] Systolic and diastolic blood pressures were measured at least three times after more than 15 min of rest, and the presence of hypertension was defined as systolic blood pressure and/or diastolic blood pressure exceeding the 95th percentile.[18] Children with hypertension were not included in this study, because hypertension may affect MMP concentrations.[19]

At the time of clinical attendance, venous blood samples were collected and genomic DNA was extracted from the cellular component of 1 ml of whole blood by a salting-out method and stored at −20 °C until analyzed.

Laboratory analyses

Glucose concentrations and lipid parameters (total cholesterol, triglycerides, high-density lipoprotein cholesterol) were determined in plasma and serum, respectively, with routine enzymatic methods using commercial kits (Labtest Diagnostic SA, Lagoa Santa, Brazil). Low-density lipoprotein concentration was calculated according to the Friedewald formula. TBARS levels were determined by a fluorimetric method as previously described.[20]

Enzyme immunoassays of MMP-9, TIMP-1, adiponectin and insulin

Venous blood samples were collected after overnight (8–12 h) fasting, immediately centrifuged at 2000 *g* for 10 min at room temperature, and plasma samples were stored at −70 °C until analyzed. MMP-9, TIMP-1 and adiponectin concentrations were measured in EDTA-plasma, using commercially available enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN, USA) according to manufacturer's instructions. Insulin concentrations were measured in EDTA-plasma using a kit (Genese Produtos Diagnosticos, Sao Paulo, Brazil). The estimate of insulin resistance obtained by HOMA IR was calculated as previously described.[21]

DNA isolation and genotype analyses

Genotypes for the (C-1562T) polymorphism of MMP-9 were determined by PCR amplification using the primers 5′-GCC TGG CAC ATA GTA GGC CC-3′ (sense) and 5′-CTT CCT AGC CAG CCG GCA TC-3′ (antisense), and PCR conditions as previously described.[22,23] The amplified products were digested with *Sph I* (New England Biolabs, Ipswich, MA, USA) overnight at 37 °C, producing fragments of 247 bp and 188 bp in the case of a polymorphic variant (allele T), or an undigested 435-bp fragment in the case of a wild-type allele (allele C). Fragments were separated by electrophoresis in 12% polyacrylamide gels and visualized by silver staining.

To determine the genotypes for the $-90(CA)_{14-24}$ polymorphism, a PCR was carried out using the primers: 5′-GAC TTG GCA GTG GAG ACT GCG GGC A-3′ (sense) and 5′-GAC CCC ACC CCT CCT TGA CAG GCA A-3′ (antisense). The PCR conditions were performed as previously described,[22,23] and the amplified products were separated in a 7% polyacrylamide-urea gel and visualized by silver staining. Differences in molecular weight (or number of bases), from 146 bp (CA 14 repeats) to 166 bp (CA 24 repeats), were determined by comparison with migration of a 10-bp DNA ladder (Invitrogen, Carlsbad, CA, USA) and with some samples from homozygotes that were sequenced. The alleles

for the microsatellite $-90(CA)_{14-24}$ polymorphism were classified as 'low' (L) count when the number of CA repeats was less than 21, and as 'high' (H) when the number of CA repeats was 21 or more.[22,23] Genotypes of Q279R (rs17576) were determined using TaqMan Allele Discrimination assay (Applied Biosystems, Foster City, CA, USA). The PCR conditions were performed according to the manufacturer's instructions.

Statistical analysis

The clinical and laboratorial characteristics of obese patients and controls were compared by unpaired *t*-test (normally distributed variables) or Mann–Whitney test (not normally distributed variables). The categorical variables were compared between groups by χ^2-tests. The distribution of genotypes for each polymorphism was assessed for deviation from the Hardy–Weinberg equilibrium, and differences in genotype frequency and in allele frequency between groups were assessed using χ^2-tests. A value of $P<0.05$ was considered statistically significant.

The Haplo.stats package (version 1.4.4; http://cran.r-project.org/web/packages/haplo.stats/index.html) was used to estimate the haplotype frequencies. The haplo.em function computes maximum likelihood estimates of haplotype probabilities using the progressive insertion algorithm. The haplo.score function was used to compute haplotype-specific score statistics to test for association.[24] Only the haplotypes with frequencies greater than 2% were taken into consideration for the haplotype-specific score analysis. The haplo.cc function was also used to calculate odds ratio and 95% confidence intervals for each haplotype. The haplotypes including genetic variants of three MMP-9 polymorphisms (C-1562T, $-90(CA)_{14-24}$ and Q279R) were: H1 (CLQ), H2 (CHQ), H3 (CHR), H4 (THR), H5 (TLR), H6 (CLR), H7 (THQ), and H8 (TLQ). Differences in haplotype frequency were further tested using a contingency table, and a value of $P<0.00625$ (0.05/number of haplotypes, 8; Bonferroni's correction) was considered significant to correct for the number of comparisons made.

To examine the effects of genotypes and haplotype on circulating levels of MMP-9, TIMP-1 and on MMP-9/TIMP-1 ratios, adiponectin, insulin and TBARs levels in the two study groups, we used the Kruskall–Wallis test followed by Dunn's multiple comparison test (not normally distributed variables) or analysis of variance, followed by Tukey's test (normally distributed variables). However, the H5, H6, H7 and H8 haplotypes were excluded from this analysis, because they were very rare.

Finally, to further examine the effects of MMP-9 haplotypes on MMP-9 levels, we have also performed an additional analysis. We compared MMP-9 haplotypes distributions in two groups of obese subjects: the lower quartile (LOWER-Q) group, which included subjects in the lower quartile of plasma MMP-9 distribution, and the upper quartile (UPPER-Q) group, which included subjects in the upper quartile of plasma MMP-9 distribution. We hypothesized that the haplotypes associated with lower MMP-9 levels in our initial analysis would be more commonly found in the LOWER-Q group than in the UPPER-Q group.

Results

The clinical and laboratorial characteristics of the study groups are presented in Table 1. Higher body mass index values were found in the obese group as compared with the control group ($26.1 \pm 4.3\,kg\,m^{-2}$ versus $18.3 \pm 2.9\,kg\,m^{-2}$; $P<0.05$). Obese subjects had higher arterial blood pressure, fasting insulin, HOMA IR index, TBARS, low-density lipoprotein cholesterol and triglycerides, and lower adiponectin and high-density lipoprotein cholesterol than control subjects (all $P<0.05$; Table 1).

We found no significant differences in MMP-9 concentrations and in MMP-9/TIMP-1 ratios when obese subjects were compared with controls ($P>0.05$; Figures 1a or c). However, obese subjects had lower plasma TIMP-1 concentrations than control subjects ($P<0.05$; Figure 1b).

Supplementary Table 1 shows MMP-9 genotypes and allele frequency distributions in the two study groups. The distribution of genotypes for each polymorphism studied here showed no deviation from Hardy–Weinberg equilibrium, except for the Q279R single nucleotide polymorphism in obese patients, a finding that may be expected in cases of case–control studies.[25] No deviation from Hardy–Weinberg equilibrium was observed when considering only black or only white subjects within each study group.

To examine possible effects of ethnicity on MMP-9 genotypes distribution, we carried out two different analyses. The first analysis included black and white children and adolescents, whereas the second analysis took into consideration

Table 1 Clinical characteristics of study participants

Variable	*Control*	*Obese*
N	175	127
Age (years)	11.9 ± 3.1	10.3 ± 2.7*
Ethnicity (% white)	56	48
BMI ($kg\,m^{-2}$)	18.3 ± 2.9	26.1 ± 4.3*
SBP (mm Hg)	105.6 ± 11.5	111.2 ± 10.0*
DBP (mm Hg)	65.6 ± 9.0	70.7 ± 8.8*
Fasting glucose ($mmol\,l^{-1}$)	4.6 ± 0.6	4.7 ± 0.5
Fasting insulin ($\mu U\,l^{-1}$)	9.3 ± 4.2	13.0 ± 6.7*
HOMA IR index	1.8 ± 0.9	2.8 ± 1.6*
Adiponectin ($ng\,ml^{-1}$)	$13\,730 \pm 578$	$11\,570 \pm 617$*
TBARS ($nmoL\,ml^{-1}$)	3.9 ± 2.1	5.0 ± 2.3*
Total cholesterol ($mg\,dl^{-1}$)	141.9 ± 42.6	147.3 ± 40.1
LDL cholesterol ($mg\,dl^{-1}$)	74.5 ± 23.1	89.0 ± 31.4*
HDL cholesterol ($mg\,dl^{-1}$)	43.6 ± 8.9	39.1 ± 10.2*
Triglycerides ($mg\,dl^{-1}$)	76.8 ± 28.7	88.2 ± 43.6*

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA IR, homeostasis model assessment insulin resistance; TBARS, thiobarbituric acid-reactive species; LDL, low-density lipoprotein; HDL, high-density lipoprotein. Values are mean ± s.d. *$P<0.05$ vs control.

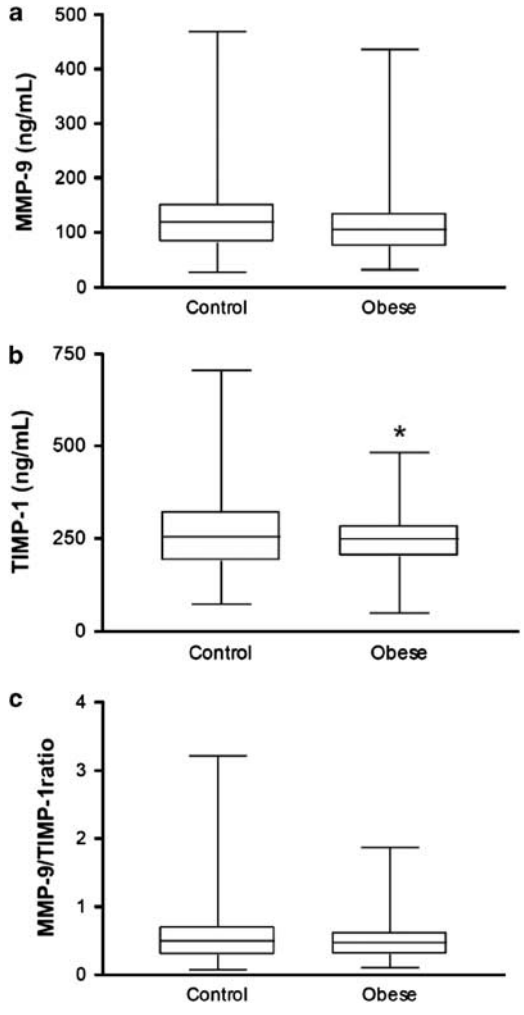

Figure 1 Plasma MMP-9 concentrations (**a**), TIMP-1 concentrations (**b**) and MMP-9/TIMP-1 ratios (**c**) in controls ($N=175$) and obese ($N=127$) children and adolescents. The box and whiskers plots show range and quartiles. The boxes extend from the 25th percentile to the 75th percentile, with a line at the median. The whiskers show the highest and the lowest values. *$P<0.05$ vs Control group.

only white children and adolescents, which corresponded to 48–56% of the subjects. Both analyses showed no significant differences in genotypes and alleles distributions for the three MMP-9 polymorphisms between the control group and obese group (all $P>0.05$; Supplementary Table 1), except for the Q279R polymorphism. The QR genotype was found with lower frequency in the obese group compared with controls ($P=0.038$; Supplementary Table 1), and this finding could be due to significant deviation from Hardy–Weinberg equilibrium observed in this group. Whereas some minor differences were detected, we found no statistically significant differences in haplotype frequency distributions when the two groups of subjects were compared ($P<0.05$; Supplementary Table 2).

Importantly, we examined the effects of MMP-9 genotypes on plasma MMP-9 concentrations. With respect to the C-1562T polymorphism, we found that obese patients with the CC genotype had lower MMP-9 levels than control subjects with the same genotype, and lower MMP-9 levels than obese patients with the CT or TT genotypes (Figure 2a; both $P<0.05$). With respect to the $-90(CA)_{14-24}$ polymorphism, we found that obese patients with the HL genotype had lower MMP-9 levels than control subjects with the same genotype (Figure 2b; $P<0.05$). Finally, with respect to the Q279R polymorphism, we found that obese patients with the QQ genotype had lower MMP-9 levels than control subjects with the same genotype, and lower MMP-9 levels than obese patients with the RR genotype (Figure 2c; both $P<0.05$).

In addition, we studied the effects of MMP-9 haplotypes on MMP-9 and TIMP-1 levels, and on MMP-9/TIMP-1 ratios. Although no significant differences were found in TIMP-1 levels when the different haplotype groups were compared (Figure 3b; $P>0.05$), we found lower MMP-9 levels and lower MMP-9/TIMP-1 ratios in obese subjects with the H2 haplotype compared with control subjects carrying the H2 haplotype, or with obese subjects with the H1, H3 or H4 haplotypes (Figures 3a and c; all $P<0.05$).

Further confirming the haplotype effects on MMP-9 levels, we found that the H2 haplotype was more commonly found in obese children and adolescents with lower MMP-9 levels than in obese subjects with higher MMP-9 levels (Table 2; $P=0.001$).

Finally, we found that MMP-9 genotypes for the three MMP-9 polymorphisms had no effects on TIMP-1, MMP-9/TIMP-1, adiponectin, insulin, HOMA IR index and TBARS levels (Supplementary Table 3; $P>0.05$). Moreover, MMP-9 haplotypes had no effects on adiponectin, insulin and TBARS levels, and on the HOMA IR index (Supplementary Table 3; $P>0.05$).

Discussion

The main novel finding reported here was that two functional MMP-9 genetic polymorphisms affect the circulating MMP-9 levels in obese children and adolescents, either when considered their single effects or when MMP-9 polymorphisms exert their combined effects within MMP-9 haplotypes. Particularly, lower MMP-9 levels were found in obese children with the CC genotype for C-1562T polymorphism, or with the QQ genotype for Q279R polymorphism, or in subjects carrying the H2 (C H Q) haplotype. Because MMP-9 levels are associated with cardiovascular diseases,[6,7] our findings suggest that obese children with these particular MMP-9 genotypes or haplotype may be at lower risk of developing cardiovascular diseases.

Although increased MMP-9 levels were shown in cardiovascular diseases,[4–7] we found similar MMP-9 concentrations and MMP-9/TIMP-1 in obese children compared with non-obese children. Although this finding confirms previous

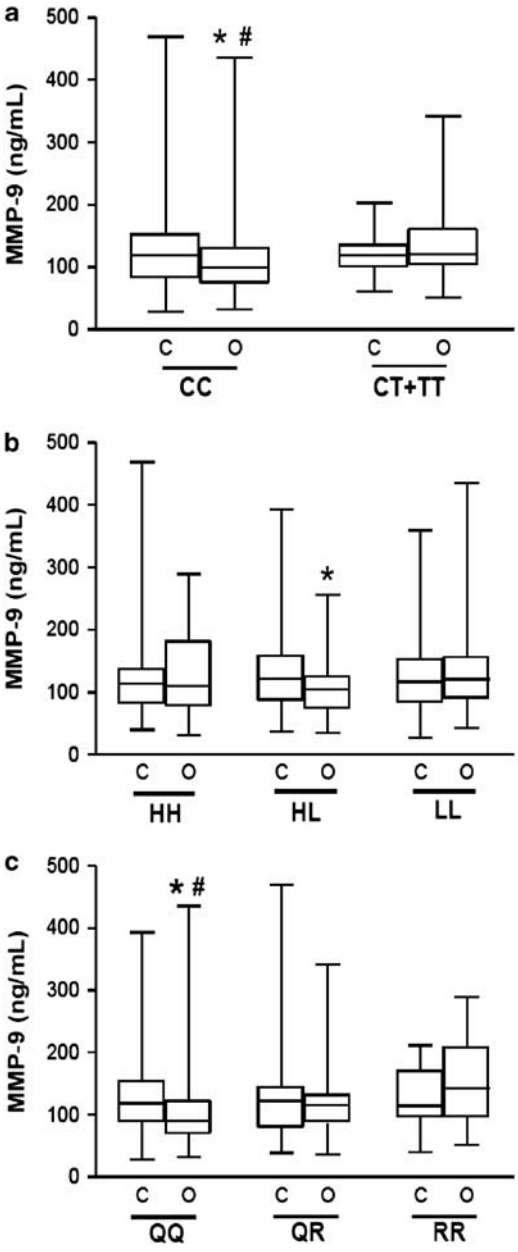

Figure 2 Plasma MMP-9 levels in the control group (C) and in obese children and adolescents (O) with different genotypes for MMP-9 polymorphisms. Subjects were grouped according to MMP-9 genotypes for the C-1562T (**a**), the $-90(CA)_{14-24}$ (**b**) and the Q279R (**c**) MMP-9 polymorphisms. The box and whiskers plots show range and quartiles. The boxes extend from the 25th percentile to the 75th percentile, with a line at the median. The whiskers show the highest and the lowest values. $^{*}P<0.05$ vs the respective control group. $^{\#}P<0.05$ vs obese patients with the CT + TT genotypes (**a**), or vs obese patients with the RR genotype (**c**).

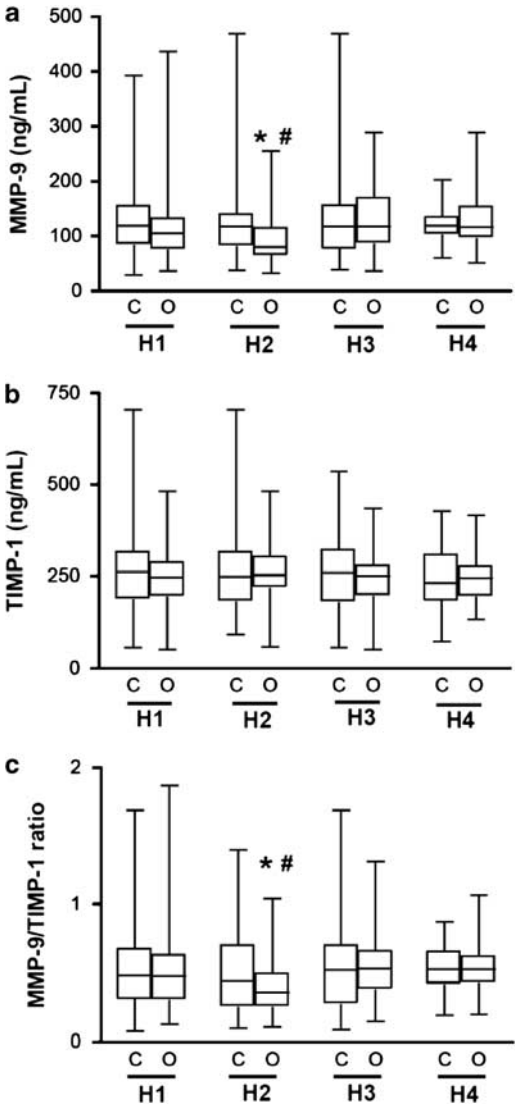

Figure 3 Plasma MMP-9 (**a**) and TIMP-1 (**b**) levels and MMP-9/TIMP-1 ratios (**c**) in the control group (C) and in obese children and adolescents (O) with different MMP-9 haplotypes. The box and whiskers plots show range and quartiles. The boxes extend from the 25th percentile to the 75th percentile, with a line at the median. The whiskers show the highest and the lowest values. $^{*}P<0.05$ vs the respective control group. $^{\#}P<0.05$ vs obese patients with the H1, H3 or H4 haplotypes.

results,[12] some authors found increased circulating MMP-9 and TIMP-1 levels in obese children with coexisting hypertension.[10,11] It is possible that hypertension may explain the discrepancies between studies, because hypertension may increase MMP-9 levels.[19] Interestingly, we found lower TIMP-1 concentrations and lower adiponectinemia in obese children compared with non-obese children, and these findings are consistent with the idea that adiponectin (an adipose-specific protein and anti-atherogenic factor) modulates TIMP-1 expression by macrophages.[26]

Although several polymorphisms have been detected in the MMP-9 gene, the C-1562T polymorphism increases MMP-9 expression as a result of loss of a nuclear repressor protein binding site when the T allele is present.[8] The lower MMP-9 levels that we found in obese subjects not carrying the T allele is supported by this previous finding. Conversely, the lack of significant effects of this polymorphism in

Table 2 MMP-9 haplotypes frequency distributions in the Lower-Q and in the Upper-Q groups of obese patients

Haplotype		*Hap-score*	P-*values*	*Frequency*	*Lower-Q*	*Upper-Q*	*OR (95% CI)*
H1	CLQ	−0.019	0.985	0.422	0.427	0.422	1.000 (Reference)
H2	CHQ	−3.443	0.001*	0.230	0.322	0.127	0.433 (0.211–0.891)*
H3	CHR	1.155	0.248	0.226	0.201	0.261	1.091 (0.539–2.206)
H4	THR	2.313	0.021	0.087	0.048	0.121	2.663 (0.899–7.886)
H5	TLR	NA	NA	—	NA	0	—
H6	CLR	1.682	0.093	0.021	0.000	0.037	—
H7	THQ	NA	NA	—	NA	0	—
H8	TLQ	NA	NA	—	NA	0	NA

Abbreviations: MMP-9, matrix metalloproteinase-9; Lower-Q, lower quartile; Upper-Q, upper quartile; CI, confidence intervals; OR, odds ratio; NA, not available. Global-stat = 20.178, df = 5. *$P = 0.0011573$. A value of $P < 0.00625$ (0.05/number of haplotypes) was considered significant, to correct for the number of comparisons made in haplotype analysis (Bonferroni's correction). The Lower-Q group included obese subjects in the lower quartile of plasma MMP-9 distribution, whereas the Upper-Q group included obese subjects in the upper quartile of plasma MMP-9 distribution.

non-obese subjects is in line with previous findings showing that this polymorphism does not affect MMP-9 levels in healthy subjects.[22,23] In addition, we found that absence of R allele for the Q279R polymorphism was associated with lower MMP-9 levels in obese children, and this finding agrees with previous results showing that this polymorphism affects MMP-9 activity.[14] Finally, we found no effects of the $(CA)_{14-24}$ polymorphism on MMP-9 levels, although there is evidence that this polymorphism affects MMP-9 expression.[13] Although it is clear that the adipose tissue is an active endocrine and paracrine organ that releases a large number of bioactive mediators that could modulate the expression of MMP-9 gene,[27] the present study has not been designed to define the mechanisms explaining the interactions of MMP-9 polymorphisms with childhood obesity.

The analysis of haplotypes, rather than single polymorphisms, may provide improved genetic information. Here, we show for the first time that MMP-9 haplotypes may modulate the circulating MMP-9 levels in obese children and adolescents. Particularly, obese children carrying the H2 (C H Q) haplotype had lower MMP-9 levels and lower MMP-9/TIMP-1 ratios (an index of net MMP-9 activity[28]) than those without these genetic markers. Further supporting this finding, the comparison of extreme quartiles of MMP-9 levels confirmed that the H2 haplotype is associated with lower circulating MMP-9 levels. Interestingly, these results are in line with a previous study showing that the H2 haplotype protects against the development of hypertensive left ventricle hypertrophy.[9] Together, these findings suggest that lower MMP-9 levels associated with the H2 haplotype could be associated with protective effects against cardiovascular diseases.

In the present study, we found no association between MMP-9 genotypes or haplotypes and childhood obesity, except for the QR genotype, which was apparently more common in non-obese than in obese subjects. Although this result is not statistically significant after correction for multiple comparisons, it could be explained by Hardy–Weinberg deviation for this polymorphism in obese subjects, and this finding may be expected in cases of case–control studies.[25] The essentially negative results with respect to the possible association between MMP-9 polymorphisms and obesity suggest that, although MMPs have an important role in the expansion of adipose tissue mass, with intense extracellular matrix remodeling in obesity,[29] genetic markers involving MMP-9 may not be very relevant to the development of childhood obesity.

We found that obese children and adolescents had higher TBARs levels (a marker of oxidative stress) when compared with non-obese controls. These results confirm that obesity is associated with increased oxidative stress and may have important implications. In fact, oxidative stress is a major MMPs activator[30] and therefore may potentiate MMPs activities in obese subjects. However, the lack of significant differences between genotype or haplotype groups, as reported here, suggest that MMP-9 polymorphisms do not interact significantly with oxidative stress to modulate MMP-9 levels in obese subjects.

Although there is evidence that plasma MMP-9 levels are clearly associated with prognosis in patients with cardiovascular diseases,[6] we are not sure about the origin of circulating MMP-9. Therefore, it is not clear at this time what does it mean to have variations in MMP-9 plasma levels. Although further studies linking MMP-9 levels with relevant clinical events are necessary, an important implication of the present findings is that obese children with the CC or with the QQ genotypes for the C-1562T and the Q279R polymorphisms, respectively, or those with the H2 haplotype, may be protected against the development of cardiovascular diseases associated with obesity. These findings may suggest that MMP-9 is a relevant pharmacological target in the prevention of complications associated with obesity. It remains to be proved whether MMPs inhibitors decrease the incidence of cardiovascular events in obese subjects without these genetic markers.

In conclusion, we found evidence indicating that MMP-9 genotypes and haplotypes affect MMP-9 levels in obese children. Our findings support the notion that genetic factors may modify relevant pathogenetic mechanisms involved in the development of cardiovascular complications associated with obesity in childhood. Further studies examining the effects of MMP-9 polymorphisms on the incidence of clinical events in obese children are warranted.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This study was supported by Fundaçao de Amparo a Pesquisa do Estado de Sao Paulo, Conselho Nacional de Desenvolvimento Cientifico e Tecnologico and IMEPEN Foundation at Juiz de Fora.

References

1 Adams KF, Schatzkin A, Harris TB, Kipnis V, Mouw T, Ballard-Barbash R *et al*. Overweight, obesity, and mortality in a large prospective cohort of persons 50 to 71 years old. *N Engl J Med* 2006; **355**: 763–778.
2 Berenson GS, Srinivasan SR, Bao W, Newman III WP, Tracy RE, Wattigney WA. Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. *N Engl J Med* 1998; **338**: 1650–1656.
3 Dollery CM, Libby P. Atherosclerosis and proteinase activation. *Cardiovasc Res* 2006; **69**: 625–635.
4 Galis ZS, Khatri JJ. Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. *Circ Res* 2002; **90**: 251–262.
5 Galis ZS, Sukhova GK, Lark MW, Libby P. Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. *J Clin Invest* 1994; **94**: 2493–2503.
6 Blankenberg S, Rupprecht HJ, Poirier O, Bickel C, Smieja M, Hafner G *et al*. Plasma concentrations and genetic variation of matrix metalloproteinase 9 and prognosis of patients with cardiovascular disease. *Circulation* 2003; **107**: 1579–1585.
7 Kai H, Ikeda H, Yasukawa H, Kai M, Seki Y, Kuwahara F *et al*. Peripheral blood levels of matrix metalloproteases-2 and -9 are elevated in patients with acute coronary syndromes. *J Am Coll Cardiol* 1998; **32**: 368–372.
8 Zhang B, Ye S, Herrmann SM, Eriksson P, de Maat M, Evans A *et al*. Functional polymorphism in the regulatory region of gelatinase B gene in relation to severity of coronary atherosclerosis. *Circulation* 1999; **99**: 1788–1794.
9 Lacchini R, Jacob-Ferreira AL, Luizon MR, Coeli FB, Izidoro-Toledo TC, Gasparini S *et al*. Matrix metalloproteinase 9 gene haplotypes affect left ventricular hypertrophy in hypertensive patients. *Clin Chim Acta* 2010; **411**: 1940–1944.
10 Glowinska-Olszewska B, Urban M. Elevated matrix metalloproteinase 9 and tissue inhibitor of metalloproteinase 1 in obese children and adolescents. *Metabolism* 2007; **56**: 799–805.
11 Glowinska-Olszewska B, Urban M, Florys B. Selected matrix metalloproteinases (MMP-2, MMP-9) in obese children and adolescents]. *Endokrynol Diabetol Chor Przemiany Materii Wieku Rozw* 2006; **12**: 179–183.
12 Belo VA, Souza-Costa DC, Lana CM, Caputo FL, Marcaccini AM, Gerlach RF *et al*. Assessment of matrix metalloproteinase (MMP)-2, MMP-8, MMP-9, and their inhibitors, the tissue inhibitors of metalloproteinase (TIMP)-1 and TIMP-2 in obese children and adolescents. *Clin Biochem* 2009; **42**: 984–990.
13 Shimajiri S, Arima N, Tanimoto A, Murata Y, Hamada T, Wang KY *et al*. Shortened microsatellite d(CA)21 sequence down-regulates promoter activity of matrix metalloproteinase 9 gene. *FEBS Lett* 1999; **455**: 70–74.
14 Allan JA, Docherty AJ, Barker PJ, Huskisson NS, Reynolds JJ, Murphy G. Binding of gelatinases A and B to type-I collagen and other matrix components. *Biochem J* 1995; **309**(Pt 1): 299–306.
15 Peters DG, Kassam A, St Jean PL, Yonas H, Ferrell RE. Functional polymorphism in the matrix metalloproteinase-9 promoter as a potential risk factor for intracranial aneurysm. *Stroke* 1999; **30**: 2612–2616.
16 Pollanen PJ, Karhunen PJ, Mikkelsson J, Laippala P, Perola M, Penttila A *et al*. Coronary artery complicated lesion area is related to functional polymorphism of matrix metalloproteinase 9 gene: an autopsy study. *Arterioscler Thromb Vasc Biol* 2001; **21**: 1446–1450.
17 Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, Flegal KM, Guo SS, Wei R *et al*. CDC growth charts: United States. *Adv Data* 2000; 1–27.
18 Update on the 1987 Task Force Report on High Blood Pressure in Children and Adolescents: a working group report from the National High Blood Pressure Education Program. National High Blood Pressure Education Program Working Group on Hypertension Control in Children and Adolescents. *Pediatrics* 1996; **98**: 649–658.
19 Martinez ML, Lopes LF, Coelho EB, Nobre F, Rocha JB, Gerlach RF *et al*. Lercanidipine reduces matrix metalloproteinase-9 activity in patients with hypertension. *J Cardiovasc Pharmacol* 2006; **47**: 117–122.
20 Cau SB, Dias-Junior CA, Montenegro MF, de Nucci G, Antunes E, Tanus-Santos JE. Dose-dependent beneficial hemodynamic effects of BAY 41-2272 in a canine model of acute pulmonary thromboembolism. *Eur J Pharmacol* 2008; **581**: 132–137.
21 Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; **28**: 412–419.
22 Demacq C, Vasconcellos VB, Marcaccini AM, Gerlach RF, Silva Jr WA, Tanus-Santos JE. Functional polymorphisms in the promoter of the matrix metalloproteinase-9 (MMP-9) gene are not linked with significant plasma MMP-9 variations in healthy subjects. *Clin Chem Lab Med* 2008; **46**: 57–63.
23 Demacq C, de Souza AP, Machado AA, Gerlach RF, Tanus-Santos JE. Genetic polymorphism of matrix metalloproteinase (MMP)-9 does not affect plasma MMP-9 activity in healthy subjects. *Clin Chim Acta* 2006; **365**: 183–187.
24 Schaid DJ, Rowland CM, Tines DE, Jacobson RM, Poland GA. Score tests for association between traits and haplotypes when linkage phase is ambiguous. *Am J Hum Genet* 2002; **70**: 425–434.
25 Wittke-Thompson JK, Pluzhnikov A, Cox NJ. Rational inferences about departures from Hardy-Weinberg equilibrium. *Am J Hum Genet* 2005; **76**: 967–986.
26 Kumada M, Kihara S, Ouchi N, Kobayashi H, Okamoto Y, Ohashi K *et al*. Adiponectin specifically increased tissue inhibitor of metalloproteinase-1 through interleukin-10 expression in human macrophages. *Circulation* 2004; **109**: 2046–2049.
27 Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with cardiovascular disease. *Nature* 2006; **444**: 875–880.
28 Demacq C, Vasconcellos VB, Marcaccini AM, Gerlach RF, Machado AA, Tanus-Santos JE. A genetic polymorphism of matrix metalloproteinase 9 (MMP-9) affects the changes in circulating MMP-9 levels induced by highly active antiretroviral therapy in HIV patients. *Pharmacogenomics J* 2009; **9**: 265–273.
29 Lijnen HR. Angiogenesis and obesity. *Cardiovasc Res* 2008; **78**: 286–293.
30 Castro MM, Rizzi E, Rodrigues GJ, Ceron CS, Bendhack LM, Gerlach RF *et al*. Antioxidant treatment reduces matrix metalloproteinase-2-induced vascular changes in renovascular hypertension. *Free Radic Biol Med* 2009; **46**: 1298–1307.

Supplementary Information accompanies the paper on International Journal of Obesity website (http://www.nature.com/ijo)