RESEARCH NOTE

TIMP2 gene polymorphisms are associated with hypertension in patients with myocardial infarction

JOSÉ MANUEL RODRÍGUEZ-PÉREZ[1], NANCY MARTÍNEZ-RODRÍGUEZ[1], GILBERTO VARGAS-ALARCÓN[1], MAITE VALLEJO[2], IRMA ELOISA MONROY-MUÑOZ[1], CARLOS POSADAS-ROMERO[3], ERIC KIMURA-HAYAMA[4], TERESA JUÁREZ-CEDILLO[5], JOSÉ MANUEL FRAGOSO[1] and NONANZIT PÉREZ-HERNÁNDEZ[1]*

[1]*Department of Molecular Biology,* [2]*Sociomedical Department,* [3]*Department of Endocrinology, and* [4]*Department of Tomography, Instituto Nacional de Cardiología Ignacio Chávez, CP 14080, Mexico City, Mexico*
[5]*Epidemiologic and Health Service Research Unit, Again Area, Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social, CP 06725, Mexico City, Mexico*

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Introduction

Previous studies have revealed that tissue inhibitors of metalloproteinases (TIMPs) play a crucial role in atherosclerosis and plaque disruption (Cheng *et al*. 2008). The present study analysed the role of TIMPs gene polymorphisms in the risk of developing myocardial infarction (MI) in a cohort of Mexican Mestizo patients. MI patients were classified into clinical subgroups according to cardiovascular risk factors. Multiple logistic regression models were performed to analyse genetic data. Under different models of heritage, adjusted by age, gender, type 2 diabetes mellitus, and smoking habits, the *TIMP2* rs4789932 *C* allele was associated with decreased risk of hypertension ($P = <0.05$), *TIMP2* rs7501477 *T* allele was associated with increased risk of hypertension ($P = <0.05$). The data suggest that *TIMP2* gene polymorphisms are associated with hypertension in Mexican patients with myocardial infarction.

Coronary artery disease (CAD) and one of its manifestations, MI, is a major cause of death worldwide. Increased expression of several metalloproteinases (MMPs) has been observed in diseased human arteries and in association with arterial morphological changes in experimental models of atherosclerosis (Newby 2005). Studies have shown an association between atherosclerosis and overexpression or underexpression of specific MMPs and TIMPs (Galis *et al*. 1994; Noji *et al*. 2001; Sapienza *et al*. 2005). TIMPs are proteins of 21 to 30 kDa and belong to a family of specific inhibitors that regulate proteolytic activity of all MMPs. TIMPs are involved in several biological activities including cell differentiation, growth, migration, invasion, angiogenesis, and apoptosis. Genes located on chromosome 17q25.3, 22q12.3 and 3p25.2 encode TIMP-2, TIMP-3 and TIMP-4, respectively (Stöhr *et al*. 1995; Hammani *et al*. 1996; Olson *et al*. 1998). In the last decade, several studies have focussed on the role of genetic predisposition in the development of many diseases (Dollery *et al*. 1995; Schaefer *et al*. 1996). Several polymorphisms of genes that encode TIMP molecules have been studied in cardiovascular diseases (Armstrong *et al*. 2007; Horne *et al*. 2007). The results, however, have been inconsistent, with positive and negative associations (Horne *et al*. 2007). The aim of the present study was to establish the role of TIMPs gene polymorphisms in the risk of developing MI in a well-characterized-clinical cohort of Mexican Mestizo patients. We also investigated the relationship between TIMPs gene polymorphisms and cardiovascular risk factors such as obesity, hypertension, dyslipidemia and type 2 diabetes mellitus. The *TIMP1* gene was not studied because it is a poor or deficient inhibitor of MMP19 and MMP17. The studied single nucleotide polymorphisms (SNPs) were selected considering previous association studies on cardiovascular diseases and because they have been validated by HapMap Project. On the other hand, the selected SNPs presented a minor allele

*For correspondence. E-mail: unicanona@yahoo.com.mx
José Manuel Rodríguez-Pérez and Nancy Martínez-Rodríguez contributed equally to this work.

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frequency (MAF) greater than 5% and are located in the promoter region.

Material and methods

Patients and controls

The study included 281 Mexican Mestizo patients with MI (180 with hypertension and 101 without hypertension) referred to the out patient clinic of the National Institute of Cardiology Ignacio Chávez. MI was defined by angina symptoms with ST-segment elevation of 1 mm or more (or development of a new Q wave) in two or more contiguous electrocardiograph leads and/or thrice the upper limit of serum creatine phosphokinase (CPK) MB isoenzyme (normal value = 0.6–6.3 ng/mL) in at least one single sample.

A group of 298 healthy unrelated individuals (159 women and 139 men), with neither symptoms nor previous diagnosis of cardiovascular problems and systemic disease was studied as control group. Calcium score was determined by multidetector computed tomography in all control individuals in order to detect subclinical atherosclerosis. The values were zero in these individuals.

Clinical subgroups

For patients and controls that met the inclusion criteria, anthropometric measures and traditional risk factors were recorded. Individuals were considered to have diabetes mellitus type 2 if they had been previously diagnosed, if they were receiving hypoglycemic treatment and/or insulin, or if they had a fasting glucose level of >120 mg/dL on two or more occasions. Hypertension (HTA) was defined as systolic blood pressure (BP) $\geq$ 140 mmHg, diastolic BP $\geq$ 90 mmHg, or the use of at least one class of antihypertensive drugs. Dyslipidemia was defined as total cholesterol $\geq$ 200 mg/dL and/or low-density lipoprotein cholesterol $\geq$ 130 mg/dL and/or triglycerides $\geq$ 150 mg/dL. Individuals were considered to be active smokers if they smoked five or more cigarettes a day or had suspended this habit for less than a year. Individuals were considered to be active alcohol drinkers if they drank more than 6 g daily. Body mass index was calculated by a standard formula (weight in kg / height in m^2).

All subjects included (patients and controls) in the study were ethnically matched, and we considered as Mexican Mestizos only those individuals who had been born in Mexico for three generations, including their own. A Mexican Mestizo is defined as someone born in Mexico, who is a descendant of the original autochthonous inhabitants of the region and of individuals, mainly Spaniards, of Caucasian and/or African origin, who came to America during the sixteenth century. The study complies with the Declaration of Helsinki. The protocol was approved by the institutional ethics committee, and informed consent was obtained from each participant.

DNA extraction

Genomic DNA from whole blood containing EDTA was isolated by standard techniques (Lahiri and Nurnberger 1991).

Genotyping of TIMP2, TIMP3 and TIMP4

TIMP2-T2803C (rs4789932), *TIMP2-G4804T* (rs7501477), *TIMP3-C109T* (rs9619311), and *TIMP4-C259T* (rs3755724) SNPs were genotyped using 5′ exonuclease TaqMan genotyping assays on an ABI Prism 7900HT Fast Real-Time PCR system, according to manufacturer's instructions (Applied Biosystems, Foster City, USA).

Statistical analysis

Allele and genotype frequencies of the four studied polymorphisms were estimated using direct counting. Hardy–Weinberg equilibrium (HWE) was calculated for each polymorphism using the chi-squared test. Statistical analysis was carried out with Stata10.0 for Windows software. If the exploratory analysis showed that numerical data had a different distribution from normal (Gaussian distribution) (Test of normality Shapiro Wilk's $P > 0.05$), comparison between the study groups was done with Mann Whitney U-test. Data are presented as median and, 25 and 75 percentiles. Categorical variables were analysed with chi-squared or Fisher tests as required and presented as absolute frequencies and proportions. Statistical significance was set at $P < 0.05$. Logistic regression analysis was used to test for associations of polymorphisms with MI and cardiovascular risk factors under inheritance models. Multiple logistic models were constructed to identify the variables that explain better risk of developing hypertension in MI patients. Models were constructed including one variable at a time, and final models included variables with biological relevance or with statistical significance or both. Confounding bias was accepted when changes in estimated odds ratios (ORs) were equal or larger than 10%. When a principal effect model was reached, effect modification was also tested and interaction terms were constructed between the polymorphisms and age, gender, type II diabetes mellitus and smoking habits; the terms were included in the model when the significance of the P value was larger or equal to 0.20. Hosmer–Lemeshow goodness-of-fit test was performed for each multiple logistic model. To address multiple testing, Bonferroni's correction was used considering six independent test and statistical significance was set when $P < 0.008$. Statistical power to detect association exceeded 0.80 as estimated with Quanto software (http://biostats.usc.edu/software). Pairwise linkage disequilibrium (LD, D′, and r^2) estimations between polymorphisms and haplotype reconstruction were performed with Haploview 4.1 (Broad Institute of Massachusetts

Institute of Technology and Harvard University, Cambridge, USA).

Functional prediction analysis

We predicted the potential effect of *TIMP* polymorphisms using bioinformatics tools, including FastSNP (Yuang *et al*. 2006), SNP Function Prediction (http://snpinfo.niehs.nih.gov/snpfunc.htm), Human-transcriptome DataBase for Alternative Splicing (http://www.h-invitational.jp/h-dbas/), SplicePort: an Interactive Splice Site Analysis Tool (http://spliceport.cbcb.umd.edu/) and SNPs3D (http://www.snps3d.org/).

Results

Characteristic of the study sample

The demographic and clinical characteristics are provided in table 1. Similar age distribution was observed between patients and healthy controls; however, as expected, male gender, body mass index, hypertension, type 2 diabetes mellitus and smoking increased in the patients group. There were no significant differences between patients and healthy controls regarding dyslipidemia and alcohol consumption. MI patients were classified into three clinical subgroups according to the presence or absence of hypertension, diabetes mellitus and dyslipidemia. The number of MI patients with hypertension and without hypertension was 101 and 180, respectively.

Allele and genotype frequencies

Observed and expected frequencies of all polymorphic sites were in Hardy–Weinberg equilibrium. A similar distribution of *TIMP2-T2803C* (rs4789932), *TIMP2-G4804T* (rs7501477), *TIMP3-C109T* (rs9619311) and *TIMP4-C259T* (rs3755724) polymorphisms was observed in MI patients and healthy controls under all inheritance models adjusted for age, gender, type 2 diabetes mellitus and smoking habits. Distribution of TIMP polymorphisms was analysed in MI patients grouped according to cardiovascular risk factors. We only detected differences in MI patients with and without hypertension (table 2).

Under a recessive model adjusted by age, gender, type 2 diabetes mellitus and smoking habits, the rs4789932 *C* allele was associated with decreased risk of hypertension in MI patients (OR = 0.47, 95% CI: 0.24–0.96, $P = 0.023$).

Table 1. Cardiovascular risk factors in MI patients and healthy controls.

	Patients ($n = 281$)			Controls ($n = 298$)			
Variable	P25	Median	P75	P25	Median	P75	*P* value
Age (years)	52	59	66	55	59	63	NS
BMI (kg/m^2)	25	27	29	25.5	28	30	$< 10^{-3}$
Gender		(*n*(%))			(*n*(%))		
Male		235 (84)			139 (47)		$< 10^{-3}$
Female		46 (16)			159 (53)		
BMI (kg/m^2)							
Normal range (18.5–24.9)		62 (22)			54 (18)		0.001
Overweight (25–29.9)		164 (59)			140 (47)		
Obese (30–34.9)		51 (18)			101 (34)		
Morbidly obese (35≥40)		4 (1)			3 (1)		
Hypertension							
Yes		180 (64)			88 (30)		$< 10^{-3}$
No		101 (36)			210 (70)		
Dyslipidemia							
Yes		150 (53)			155 (52)		NS
No		131 (47)			143 (48)		
Type 2 diabetes mellitus							
Yes		108 (38)			61 (20)		$< 10^{-3}$
No		173 (62)			237 (80)		
Smoking habits							
Yes		168 (60)			96 (32)		$< 10^{-3}$
No		113 (40)			202 (68)		
Use of alcohol							
Never use		247(88)			261(88)		NS
> 6 g/day use		34(12)			37(12)		

BMI, body mass index

Table 2. Allele and genotypes distribution of polymorphisms in MI patients with and without hypertension.

	Genotype frequency (%)					Adjusted model	
TIMP-2 rs4789932 T-2803C	C/C	T/C	T/T	MAF	Model	OR (95%CI)	*pC
MI with HTA ($n = 180$)	28 (0.16)	98 (0.54)	54 (0.3)	0.43	Codominant 1	1.07 (0.56–2.00)	0.827
					Codominant 2	0.51 (0.24–1.06)	0.070
					Dominant	0.84 (0.47–1.51)	0.569
					Recessive	0.47 (0.24–0.96)	0.023
MI without HTA ($n = 101$)	30 (0.3)	45 (0.45)	26 (0.26)	0.48	Heterozygous advantage	0.70 (0.41–1.17)	0.167
					Additive	0.74 (0.49–1.13)	0.160
TIMP-2 rs7501477 T-4804G	T/T	G/T	G/G				
MI with HTA ($n = 180$)	3 (0.02)	48 (0.27)	129 (0.72)	0.15	Codominant 1	3.39 (1.58–7.24)	0.002
					Codominant 2	2.67 (0.27–26.6)	0.402
					Dominant	3.32 (1.59–6.89)	0.001
MI without HTA ($n = 101$)	1 (0.01)	10 (0.1)	90 (0.89)	0.06	Recessive	0.46 (0.05–4.58)	0.509
					Heterozygous advantage	0.30 (0.14–0.64)	0.002
					Additive	2.88 (1.46–5.67)	0.009
TIMP-3 rs9619311 C-109T	C/C	T/C	T/T				
MI with HTA ($n = 180$)	18 (0.1)	84 (0.47)	78 (0.43)	0.33	Codominant 1	1.16 (0.69–1.95)	0.800
					Codominant 2	0.92 (0.36–2.33)	0.662
					Dominant	1.12 (0.68–1.85)	0.670
MI without HTA ($n = 101$)	8 (0.08)	52 (0.51)	41 (0.41)	0.34	Recessive	0.85 (0.35–2.07)	0.715
					Heterozygous advantage	1.17 (0.71–1.93)	0.531
					Additive	1.03 (0.70–1.53)	0.860
TIMP-4 rs3755724 C-259T	C/C	T/C	T/T				
MI with HTA ($n = 180$)	103 (0.57)	67 (0.37)	10 (0.06)	0.24	Codominant 1	0.84 (0.50–1.43)	0.731
					Codominant 2	1.22 (0.41–3.64)	0.410
					Dominant	0.89 (0.53–1.47)	0.645
					Recessive	1.30 (0.44–3.81)	0.643
MI without HTA ($n = 101$)	61 (0.6)	34 (0.34)	6 (0.06)	0.23	Heterozygous advantage	0.83 (0.49–1.39)	0.480
					Additive	0.96 (0.63–1.45)	0.830

Associations were tested using logistic regression. OR, odds ratio; 95%IC, confidence interval.
*pC, adjusting by age, gender, type 2 diabetes mellitus and smoking habits and Bonferroni's correction was used considering six independent tests and statistical significance was set when $P \leq 0.008$.

On the other hand, under inheritance models: codominant 1, dominant and additive, adjusted for age, gender, type 2 diabetes mellitus and smoking habits, the rs7501477 *T* allele was associated with increased risk of hypertension (OR = 3.39, 95% CI: 1.58–7.24, $P = 0.002$; OR = 3.32, 95% CI: 1.59–6.89, $P = 0.001$; OR = 2.88, 95% CI: 1.46–5.67, $P = 0.009$, respectively). Similar distribution of TIMP3 (rs9619311) and TIMP4 (rs3755724) gene polymorphisms was observed in MI patients with and without hypertension.

Haplotype analysis and SNP function prediction

The studied polymorphisms were not in linkage disequilibrium. Based on SNP functional prediction software, only rs4789932 and rs7501477 polymorphisms seem to be functional. Variation in these polymorphisms affect DNA binding of the transcriptional factors AP4, ATF-6 and AR, ARNT, respectively.

Discussion

The action of MMP enzymes weakens the arterial wall, contributing to the destabilizing and rupture of atheromatous plaque, leading to MI (Newby 2005). Under normal physiological conditions, the activities of MMPs are regulated at the level of transcription, activation of the precursor zymogens, TIMPs, and interaction with specific extracellular matrix (ECM) components and are likely to be reflected in the biomechanical properties of this connective tissue. Polymorphisms of TIMP genes have been studied in several diseases with contradictory results (Ban *et al*. 2009; Chen *et al*. 2009). Association has been observed in stroke (Hansson *et al*. 2011), Kawasaki disease (KD) (Furuno *et al*. 2007), and carotid artery intima-media thickness (IMT) (Armstrong *et al*. 2007). In the present study, we studied two polymorphisms of *TIMP2-T2803C* (rs4789932), *TIMP2-G4804T* (rs7501477), one polymorphism of *TIMP3-C109T*

(rs9619311) and one polymorphism of *TIMP4-C259T* (rs3755724) in Mexican patients with MI and healthy controls. A similar distribution of these polymorphisms was observed in both studied groups. MI patients were classified according to cardiovascular risk factors and the polymorphisms were analysed. In this analysis, the two polymorphisms of *TIMP2* (rs4789932 and rs7501477) were associated with hypertension. Several reports suggest that MMPs and TIMPs lead to the development of hypertension due to their role in endothelial dysfunction and hypertensive heart disease (Park *et al*. 2013; Ahmed *et al*. 2006). These polymorphisms are located in the promoter region of the gene. AP-4, ATF6, AR and ARNT are transcriptional factors that act both as a repressor and an activator for different target genes related with progressive cardiac remodelling. The functional prediction software used here predicted that rs4789932 and rs7501477 polymorphisms have a potential functional effect, and these variants produce binding sites for the transcriptional factors AP-4, ATF-6, AR and ARNT. AP-4 and AR have been shown to act both as a repressor and an activator for different target genes (Mermod *et al*. 1988; Liu *et al*. 2003). On the other hand, ATF-4 directly participates in modulating inflammatory responses in atherosclerosis (Gargalovic *et al*. 2006).

Armstrong *et al*. (2007) studied the association between *MMP* and *TIMP* gene polymorphisms with carotid artery IMT, and just found association of the MMP9 rs175176 polymorphism with IMT. When they analysed the association between genotypes and cardiovascular risk factors, the history of arterial hypertension in these patients was significantly associated with the rs5749511 SNP of the *TIMP3*. These data are in line with our results, because the same gene (but different variant) was associated with increased risk of developing hypertension in our group of patients with MI (Armstrong *et al*. 2007). On the other hand, Furuno *et al*. (2007) evaluated five *TIMP2* polymorphisms (rs8080623, rs8179090, rs8179091, rs8179093, and rs8179096) in KD patients with and without coronary artery lesions (CAL). In this study, no association of the polymorphisms with KD was observed, however, some associations with the presence of CAL were detected. Again, these data are in accordance with our results because the *TIMP2* gene polymorphisms were not associated with MI, but they were associated with hypertension in our patients group.

Ban *et al*. (2009) investigated the relationship between the rs3755724 promoter polymorphism of the *TIMP-4* gene and KD with CALs in a Korean population (Ban *et al*. 2009). In that study, under a recessive model, the rs3755724 *C* allele was associated with susceptibility to CALs. In our study, we analysed this polymorphism; however, we did not detect any association. The different results obtained in that study could be due to ethnic differences. Genetic heterogeneity is a well-recognized reason for the failure to replicate genetic association findings. Study limitations need to be addressed. The findings should also be taken with caution because they are based on a limited simple size. Therefore, the results need to be replicated for confirmation by further studies using larger samples.

In summary, our data suggest that the *TIMP* polymorphisms were not associated with MI, however, the *TIMP2* polymorphisms were associated with hypertension in this group of patients. Additional studies in other populations could help to define the exact genetic role of these polymorphisms in hypertension.

References

Ahmed S. H., Clark L. L., Pennington W. R., Webb C. S., Bonnema D. D., Leonardi A. H. *et al.* 2006 Matrix metalloproteinases/tissue inhibitors of metalloproteinases: relationship between changes in proteolytic determinants of matrix composition and structural, functional, and clinical manifestations of hypertensive heart disease. *Circulation* **113**, 2089–2096.

Armstrong C., Abilleira S., Sitzer M., Markus H. S. and Bevan S. 2007 Polymorphisms in MMP family and TIMP genes and carotid artery intima-media thickness. *Stroke* **38**, 2895–2899.

Ban Y. J., Yoon K. L., Klim S. K., Kang S. and Chung J. H. 2009 Promoter polymorphism (rs3755724, -55C/T) of tissue inhibitor of metalloproteinase 4 (TIMP4) as a risk factor for Kawasaki disease with coronary artery lesions in a Korean population. *Pediatr. Cardiol.* **30**, 331–335.

Chen Q. J., Lu L., Peng W. H., Hu J., Yan X. X., Wang L. J. *et al.* 2009 Polymorphisms of MMP-3 and TIMP-4 genes affect angiographic coronary plaque progression in non-diabetic and type 2 diabetic patients. *Clin. Chim. Acta* **405**, 97–103.

Cheng M., Hashmi S., Mao X. and Zeng Q. T. 2008 Relationships of adiponectin and matrix metalloproteinase-9 to tissue inhibitor of metalloproteinase-1 ratio with coronary plaque morphology in patients with acute coronary syndrome. *Can. J. Cardiol.* **24**, 385–390.

Dollery C. M., McEwan J. R. and Henney A. M. 1995 Matrix metalloproteinases and cardiovascular disease. *Circ. Res.* **77**, 863–868.

Furuno K., Takada H., Yamamoto K., Ikeda K., Ohno T., Khajoee V. *et al.* 2007 Tissue inhibitor of metalloproteinase 2 and coronary artery lesions in Kawasaki disease. *J. Pediatr.* **151**, 155–160.

Galis Z. S., Sukhova G. K., Lark M. W. and Libby P. 1994 Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. *J. Clin. Invest.* **94**, 2493–2503.

Gargalovic P. S., Gharavi N. M., Clark M. J., Pagnon J., Yang W. P., He A. *et al.* 2006 The unfolded protein response is an important regulator of inflammatory genes in endothelial cells. *Arterioscler. Thromb. Vasc. Biol.* **26**, 2490–2496.

Hammani K., Blakis A., Morsette D., Bowcock A. M., Schmutte C., Henriet P. *et al.* 1996 Structure and characterization of the human tissue inhibitor of metalloproteinases-2 gene. *J. Biol. Chem.* **271**, 25498–25505.

Hansson J., Vasan R. S., Ärnlöv J., Ingelsson E., Lind L., Larsson A. *et al.* 2011 Biomarkers of extracellular matrix metabolism (MMP-9 and TIMP-1) and risk of stroke, myocardial infarction, and cause-specific mortality: cohort study. *PLoS One* **19**, e16185.

Horne B. D., Camp N. J., Carlquist J. F., Muhlestein J. B., Kolek M. J., Nicholas Z. P. *et al.* 2007 Multiple-polymorphism associations of 7 matrix metalloproteinase and tissue inhibitor metalloproteinase genes with myocardial infarction and angiographic coronary artery disease. *Am. Heart J.* **154**, 751–758.

Lahiri D. K. and Nurnberger J. I. Jr 1991 A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res.* **343**, 5444.

Liu P. Y., Death A. K. and Handelsman D. J. 2003 Androgens and cardiovascular disease. *Endocr. Rev.* **24**, 313–340.

Mermod N., Williams T. J. and Tjian R. 1988 Enhancer binding factors AP-4 and AP-1 act in concert to activate SV40 late transcriptional *in vitro*. *Nature* **332**, 557–561.

Newby A. C. 2005 Dual role of matrix metalloproteinases (matrixins) in intimal thickening and atherosclerotic plaque ruptura. *Physiol. Rev.* **85**, 1–31.

Noji Y., Kajinami K., Kawashiri M. A., Todo Y., Horita T., Nohara A. *et al.* 2001 Circulating matrix metalloproteinases and their inhibitors in premature coronary atherosclerosis. *Clin. Chem. Lab. Med.* **39**, 380–384.

Olson T. M., Hirohata S., Ye J., Leco K., Seldin M. F. and Apte S. S. 1998 Cloning of the human tissue inhibitor of metalloproteinase-4 gene (TIMP4) and localization of the TIMP4 and Timp4 gene to human chromosome 3p25 and mouse chromosome 6, respectively. *Genomics* **51**, 148–151.

Park H. K., Kim D. H., Yung D. H. and Ban Y. J. 2013 Association between IL10, IL10RA, and ILRB SNPS and ischemic stroke with hypertension in Korean population. *Mol. Biol. Rep.* **40**, 1785–1790.

Sapienza P., di Marzo L., Borrelli V., Sterpetti A. V., Mingoli A., Cresti S. *et al.* 2005 Metalloproteinases and their inhibitors are markers of plaque instability. *Surgery* **137**, 355–363.

Schaefer L., Han X., Gretz N., Häfner C., Meir K., Matzkies F. *et al.* 1996 Tubular gelatinase A (MMP-2) and its tissue inhibitors in polycystic kidney disease in the Han: SPRD rat. *Kidney Int.* **49**, 75–81.

Stöhr H., Roomp K., Felbor U. and Weber B. H. 1995 Genomic organization of the human tissue inhibitor of metalloproteinases-3 (TIMP3). *Genome Res.* **5**, 483–487.

Yuang H. Y., Chiou J. J., Tseng W. H., Liu C. H., Liu C. K., Lin Y. J. *et al.* 2006 FASTSNP: an always up-to-date and extendable service for SNP function analysis and prioritization. *Nucleic Acids Res.* **34**, w635–w641.

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