Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

Association between SNP rs1800875, serum chymase and immunoglobulin E levels in patients with coronary heart disease*

Chun-na JIN[1,2], Hong MA[1,2], Yan LIN[1,2], Jian-an WANG[1,2], Mei-xiang XIANG[†‡1,2]

([1]*Department of Cardiology, the Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310009, China*)

([2]*Cardiovascular Key Lab of Zhejiang Province, the Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310009, China*)

[†]E-mail: xiangmx@yahoo.com

Received June 30, 2011; Revision accepted July 4, 2011; Crosschecked June 30, 2011

Abstract: Objective: The gene for mast cell chymase (*CMA1*) is an ideal candidate for investigating the genetic predisposition to coronary heart disease (CHD), as activated mast cells have been found to be present in a greater proportion in the shoulder region of atheroma than in normal coronary intimae. Previous studies have indicated that *CMA1* promoter polymorphism rs1800875 may be involved in regulating immunoglobulin E (IgE) levels in patients with eczema, and it is associated with the progression of immunoglobulin A nephropathy. Methods: The association between single nucleotide polymorphism (SNP) rs1800875, serum chymase, and serum IgE levels was examined in 175 CHD subjects and 95 non-CHD subjects. Results: Statistical analysis indicated no significant difference in allele frequency between CHD and non-CHD. However, a significant association was found between *CMA1* genotypes and total IgE levels in CHD subjects. Meanwhile, crossover analysis revealed that, in GG homozygotes, CHD risk was nearly six times higher in those with IgE (U/ml) level <2.58 (natural logarithm conversion), while no association was found with chymase level. Conclusions: Polymorphism rs1800875 of *CMA1* may be associated with serum IgE level in CHD subjects, but not with chymase level in both groups. In GG homozygotes, high IgE level is a protective factor against coronary disease.

Key words: Coronary heart disease, *CMA1*, Serum immunoglobulin E (IgE), Serum chymase
doi:10.1631/jzus.B1101008 **Document code:** A **CLC number:** R543.3

1 Introduction

Mast cells contribute importantly to allergic and innate immune responses by releasing various preformed and newly synthesized mediators (Gurish and Austen, 2001; Galli *et al*., 2005). Previous studies have shown mast cell accumulation in human atherosclerotic lesions (Jeziorska *et al*., 1997). Recently, mast cells were reported to participate in atherosclerosis by releasing pro-inflammatory cytokines, chemokines, and proteases to induce inflammatory cell recruitment, cell apoptosis, angiogenesis, and matrix protein remodeling (Sun *et al*., 2007). Mast cell chymase and tryptase are unique to mast cells and have been directly and indirectly proven to participate in atherosclerosis and abdominal aortic aneurysms (Qin and Shi, 2011).

Mast cell chymase, a glycoprotein with a molecular weight of 29 kDa, is stored in high amounts within the secretary granules of mast cells. It is secreted into the intercellular substance by activated mast cells under strong stimulation (Schwartz and Austen, 1980). The enzyme is one of the important enzymes in generating angiotensin-II (Ang-II) from Ang-I. High levels of Ang-II forming activity and chymase expression have been demonstrated in human atherosclerotic lesions (Ihara *et al*., 1999). Mast cell chymase may contribute to plaque erosion and complications of atherosclerosis by inducing endothelial

‡ Corresponding author

* Project supported by the National Natural Science Foundation of China (No. 30670867) to Mei-xiang XIANG, and the Major Program of Science and Technology Department of Zhejiang Province, China (No. 2007C13058) to Mei-xiang XIANG

cell and smooth muscle cell apoptosis (Heikkila *et al*., 2008). Meanwhile, serum chymase levels have been demonstrated to be higher in patients with acute myocardial infarction or unstable angina pectoris than in patients with stable angina pectoris or those without significant coronary heart disease (CHD) (Xiang *et al*., 2011). The gene encoding mast cell chymase, *CMA1*, has been mapped within a cluster of genes for cellular proteases on chromosome 14q11.2 (Urata *et al*., 1991). This gene, embracing 5 exons and 4 introns, accounts for a total length of approximately 3 kb (Urata *et al*., 1991). Previous studies have focused on rs1800875 in the promoter of *CMA1*. Ortlepp *et al*. (2001) showed that the allele G of rs1800875 is a genetic risk factor for atherosclerosis in venous coronary artery bypass grafts. Meanwhile, in subjects with self-reported eczema or atopic asthma, a significant association was found between rs1800875 and total immunoglobulin E (IgE) levels (Iwanaga *et al*., 2004; Sharma *et al*., 2005). Therefore, the relationship between rs1800875 genotypes and IgE level remains unclear in the coronary patient, although recently increased serum IgE levels have been found in myocardial infarction (Szczeklik *et al*., 1993).

In the present study, we detected the genotype of single nucleotide polymorphism (SNP) rs1800875 in Chinese CHD population, and evaluated the association between this polymorphism and the quantitative traits associated with CHD, such as total serum chymase and IgE levels.

2 Materials and methods

2.1 Subjects

A total of 270 patients admitted to the Department of Cardiology at the Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China, were recruited consecutively from July to December 2008. All patients had no history of inflammation-associated diseases including asthma, history of allergic diseases, rheumatic heart disease, arthritis, cancer, chronic hepatic disease, renal failure, valvular heart disease, and other cardiac diseases. This study was conducted in conformity with the Helsinki Declaration and approved by the Hospital Review Committee. All subjects signed the informed consent and underwent coronary angiography. A total of 175 subjects with at least 50% stenosis of one or more main coronary arteries were selected as substantial CHD. The remaining 95 subjects, with the main coronary artery having less than 50% stenosis or without luminal narrowing, were diagnosed as unsubstantial CHD (non-CHD) controls. We recorded subject age, height, weight, body mass index (BMI), sex, diabetes, history of hypertension, smoking (consuming tobacco for at least three years), and family history of CHD. Arterial blood samples were extracted from the sheath in the radial or femoral artery during the interventional procedure. All serum sample aliquots were stored at −80 °C for routine chymase and IgE measurement.

2.2 PCR amplification and genotyping

Genomic DNA was extracted from the peripheral blood lymphocytes following the previous procedure (Iwanaga *et al*., 2004). The SNP rs1800875 was investigated using primer pairs reported previously: forward primer 5′-TGCCCCACATCAAC ATTCATTC-3′ and reverse primer 5′ TCCGGA GCTGGAGAACTCTTGT-3′ (He *et al*., 2004). Polymerase chain reaction (PCR) amplifications were performed in a total volume of 20 μl containing 0.1 μmol/L of each primer, 1.5 mmol/L $MgCl_2$, 200 μmol/L deoxyribonucleotide triphosphates (dNTPs), 50 ng DNA template, 0.03 U/μl Taq DNA polymerase, and 2× PCR buffer (TaKaRa, China). Reaction conditions used with the thermal cycler (Biometra, Germany) were as follows: an initial incubation at 94 °C for 3 min, 30 cycles of 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 30 s, and a final extension at 72 °C for 5 min (Liang *et al*., 2009). The PCR products were genotyped by restriction digestion with endonuclease *Bst*XI (TaKaRa, China). The digestion products were then separated on a 2% agarose gel stained with gel red for visualization under ultraviolet (UV) light. PCR products from subjects with GG genotypes were refractory to digestion with *Bst*XI while DNA from homozygote for the A allele (AA genotype) was completely digested into two fragments (Fig. 1). The accuracy of the restriction fragment length polymorphism (RFLP) genotyping was confirmed by direct sequencing of the random DNA samples (n=30) for all three respective genotypes.

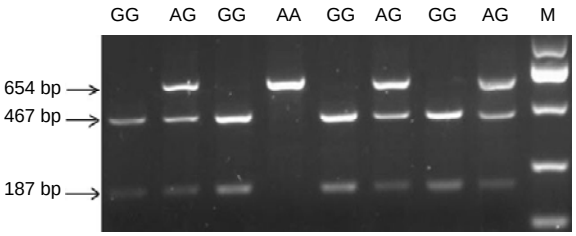

Fig. 1 Agarose gel electrophoresis analysis of restriction endonuclease digestion
DNA bands with sizes at 467 and 187 bp indicated homozygous G alleles, and homozygous A alleles were represented by an uncut fragment of 654 bp, whereas the heterozygous genotype displayed a combination of 654, 467 and 187 bp

2.3 Statistical analysis

Independent sample *t*-test was used for the comparison between two groups for normal distribution and homogeneity variance. For the ranked data, Fisher's exact test was applied for the comparison between two groups. Allele and genotype distributions between non-CHD and CHD were compared by χ^2 test. Odds ratios (ORs) were calculated as a measure of the association of the genotype with the phenotype of CHD using two-by-two table/χ^2 analysis. Crossover analysis was used to discover the interactions of IgE, chymase, and *CMA1* genotypes with CHD. For each OR, two-tailed *P* values and 95% confidence intervals (95% CI) were determined. SAS 9.1 version was used for analysis and $P<0.05$ was considered to be statistically significant.

3 Results

3.1 Baseline for CHD and non-CHD

Independent sample *t*-test showed that CHD subjects were older than non-CHD subjects ((65.09±9.79) vs. (57.93±10.60) years, $P<0.0001$, Table 1). There was no significant difference in BMI between the two groups. Fisher's exact test indicated that both genders ($P=0.0166$) and history of diabetes mellitus ($P=0.0344$), rather than smoking, family history of CHD, or hypertension, affected CHD status (Table 1).

3.2 Genotype and allele frequencies

The genotype and allele frequencies in CHD and non-CHD subjects are listed in Table 2. No significant differences among these genotypes were observed between CHD and non-CHD ($P=0.5326$). The frequencies of alleles were consistent with Hardy-Weinberg expectations in the non-CHD and CHD groups ($\chi^2=1.23$, $P=0.2676$; $\chi^2=0.08$, $P=0.7828$).

Table 1 Clinical data comparisons between subjects with and without CHD

Variables	Value		*P*
	CHD	Non-CHD	
Age (year)	65.09±9.79	57.93±10.60	<0.0001*
BMI (kg/m^2)	23.92±3.14	23.53±3.45	0.3498*
Sex			
Male	121 (67.22%)	59 (32.78%)	0.0166**
Female	47 (52.22%)	43 (47.78%)	
Smoking			
No	109 (61.24%)	69 (38.76%)	0.6420**
Yes	59 (64.13%)	33 (35.87%)	
Family history of CHD			
No	152 (64.14%)	85 (35.86%)	0.0823**
Yes	16 (48.48%)	17 (51.52%)	
Diabetes mellitus			
No	129 (59.17%)	89 (40.83%)	0.0344**
Yes	39 (75.00%)	13 (25.00%)	
Hypertension			
No	48 (55.17%)	39 (44.83%)	0.0995**
Yes	120 (65.57%)	63 (34.43%)	

Total $n=270$. Values are expressed as mean±SD or *n* (%). * Independent sample *t*-test; ** Fisher's exact test. $P<0.05$ was considered to be statistically significant

Table 2 Distribution of *CMA1* genotypes between non-CHD and CHD subjects and exact test for Hardy-Weinberg equilibrium

Group	*n*	Genotype frequency*			*P*	Allele frequency*		Hardy-Weinberg equilibrium	
		AA	AG	GG		A	G	χ^2	*P*
CHD	144	11 (7.64%)	60 (41.67%)	73 (50.69%)	0.5326	82 (28.47%)	206 (71.53%)	0.08	0.7828
Non-CHD	76	9 (11.84%)	28 (36.84%)	39 (51.32%)		46 (30.26%)	106 (69.74%)	1.23	0.2676

Total $n=220$. * Values are expressed as *n* (%)

3.3 Association between serum IgE and chymase levels and genotypes

In CHD subjects, the IgE (U/ml) level is substantially higher in AA/AG genotype than in GG (4.03±1.25 vs. 3.33±1.43 (natural logarithm conversion), P=0.0032). No parallel result was found in the non-CHD subjects (P=0.0745). Meanwhile, no difference of serum chymase levels was found between *CMA1* genotypes (CHD: P=0.3641; non-CHD: P=0.2532; Table 3).

Table 3 Serum chymase and IgE comparisons between *CMA1* genotypes

Group	IgE			Chymase		
	n	lnc*#	*P*	*n*	*c* (μg/ml)*	*P*
CHD	133		0.0032	130		0.3641
GG		3.33±1.43			16.58±4.29	
AA/AG		4.03±1.25			15.90±4.31	
Non-CHD	71		0.0745	70		0.2532
GG		4.10±1.24			18.01±7.37	
AA/AG		3.54±1.37			16.43±3.64	

* Values are expressed as mean±SD; # The natural logarithm conversion of IgE concentration (U/ml)

3.4 Association between CHD risk and genotypes, serum chymase and IgE levels

Stratified analysis was used to explore the interaction of IgE, chymase, and genotypes with CHD. Serum IgE and chymase data were classified into two groups by comparing to the median (Table 4). Crossover analysis was applied to investigate the interaction of chymase, IgE, and genotypes in CHD subjects. No significant difference was found between non-CHD and CHD with respect to the interaction of serum chymase with IgE (Interaction OR=0.684, P=0.51; Table 5). Surprisingly, our data indicated that, in subjects with GG genotype, CHD risk was nearly six times higher in IgE (U/ml) level <2.58 (natural logarithm conversion) group than IgE level ≥2.58 (OR=5.97, P=0.0006; Table 6), while the IgE level had no effect on CHD risk in subjects with AA/AG genotype (OR=3.22 (IgE level ≥2.58) vs. OR=3.33 (IgE level <2.58); Table 6). Therefore, we draw the conclusion that IgE level may have an interaction with GG for CHD risk, but not in AA/AG genotype. The whole interaction OR caused by IgE and

Table 4 Stratified analysis for IgE, chymase and *CMA1* genotypes between non-CHD and CHD subjects

Variables	*n*		OR (95% CI)[c]	*P*
	CHD	Non-CHD		
IgE (*n*=252)[a]				
<2.58	81 (64.29%)	45 (35.71%)	1.00	
≥2.58	75 (59.52%)	51 (40.48%)	0.72 (0.40, 1.27)	0.2546
Chymase (*n*=247)[b]				
<15.81 μg/ml	79 (63.71%)	45 (36.29%)	1.00	
≥15.81 μg/ml	74 (60.16%)	49 (39.84%)	1.10 (0.62, 1.94)	0.7514
Genotypes (*n*=219)				
GG	73 (65.18%)	39 (34.82%)	1.00	
AA/AG	71 (66.36%)	36 (33.64%)	1.27 (0.67, 2.39)	0.4606

[a] Expressed by median after natural logarithm conversion of IgE concentration (U/ml); [b] Expressed by median; [c] Adjusted by age, BMI, sex, diabetic disease, smoking, family history, and hypertension

Table 5 Crossover analysis for the interaction of IgE and chymase between non-CHD and CHD subjects

IgE[a]	Chymase[b]	*n*		OR (95% CI)	*P*
		CHD	Non-CHD		
≥2.58	<15.81 μg/ml	39 (61.90%)	24 (38.10%)	1.00	
≥2.58	≥15.81 μg/ml	33 (55.93%)	26 (44.07%)	0.91 (0.41, 2.02)	0.8133
<2.58	<15.81 μg/ml	40 (65.57%)	21 (34.43%)	1.22 (0.53, 2.79)	0.6447
<2.58	≥15.81 μg/ml	41(64.06%)	23 (35.94%)	1.62 (0.71, 3.67)	0.2523
Interaction				0.68 (0.22, 2.15)	0.5143

Total *n*=247. [a] Expressed by median after natural logarithm conversion of IgE concentration (U/ml); [b] Expressed by median

Table 6 Crossover analysis for IgE and genotypes between non-CHD and CHD subjects

IgE[a]	Genotypes	*n*		OR (95% CI)	*P*
		CHD	Non-CHD		
≥2.58	GG	23 (50.00%)	23 (50.00%)	1.00	
≥2.58	AA/AG	37 (68.52%)	17 (31.48%)	3.22 (1.25, 8.31)	0.0157
<2.58	GG	47 (77.05%)	14 (22.95%)	5.97 (2.16, 16.49)	0.0006
<2.58	AA/AG	26 (61.90%)	16 (38.10%)	3.33 (1.21, 9.19)	0.0201
Interaction				5.77 (1.48, 22.53)	0.0116

Total n=203. [a] Expressed by median after natural logarithm conversion of IgE concentration (U/ml)

genotypes is more notable (OR=5.77, P=0.0116; Table 6) than that calculated for IgE and chymase levels (OR=0.68, P=0.5143; Table 5).

4 Discussion

We examined the association of the SNP rs1800875, serum chymase, and IgE levels in non-CHD and CHD subjects in a Chinese population. The IgE level is substantially higher in AA/AG genotype than in GG homozygote in CHD patients. Meanwhile, in subjects with GG genotype, CHD risk was nearly six times higher in those with IgE level <2.58. Therefore, SNP rs1800875 may be associated with CHD risk and IgE level in CHD patients.

In this study, the allele and genotype distributions of SNP rs1800875 exhibited no significant association between the locus and CHD. Previous studies have shown that rs1800875 was not associated with hypertrophic or dilated cardiomyopathy (Pfeufer *et al*., 1998; Wu *et al*., 2002). Gardemann *et al.* (2000) showed that rs1800875 variation had no significant impact on the risk and extent of CHD after analyzing a population of more than 2000 patients. Our study was consistent with these observations, which may substantiate the hypothesis that this SNP locus is not the crucial locus affecting chymase expression. Considering the important roles of chymase in the formation and progression of atherosclerosis plaques, it is highly possible that other gene variables in *CMA1*, such as SNP, insert and deletion, or repeat polymorphism, affect the serum chymase level. For instance, a novel $(TG)_n(GA)_m$ repeat polymorphism 254-bp downstream of *CMA1* is associated with atopic asthma and total serum IgE levels (Sharma *et al*., 2005).

Interestingly, we observed a significant association between the genotypes of SNP rs1800875 and serum IgE level in CHD patients. Our data suggested that IgE level is substantially higher in AA/AG genotypes than GG only in CHD patients, which is not consistent with two studies involving asthma patients, both of which had reported a higher total IgE level in the GG genotype (Iwanaga *et al*., 2004; Sharma *et al*., 2005). IgE levels are associated with many factors, and the difference in these studies may be attributed to the different subjects used, the ethnicity, and environmental exposure. In addition, it is also possible that some other genes or loci contribute to the high IgE levels. The actions of human chymase may partly contribute to the relationship of IgE responsiveness and rs1800875. On the one hand, in human atherosclerotic lesions, increased IgE levels and enhanced FcεRI expression may have adjuvant activity sufficient to activate mast cells. Interleukin-4 (IL-4) and IL-13, secreted by T helper-2 (Th-2) cells, provide the first signal to B cells to switch the IgE isotopes (Busse and Lemanske, 2001). On the other hand, a previous study demonstrated that IgE synthesis could be promoted by the addition of a rat chymase to a culture of murine spleen cells motivated by IL-4 and lipopolysaccharide (Yoshikawa *et al*., 2001). As well, administration of a synthetic chymase inhibitor (Y-40613) suppressed total IgE levels in a rat model of atopic dermatitis (Imada *et al*., 2002). However, this may not explain the relationship between *CMA1* genotypes and high serum IgE level if one only considers the actions of human chymase. First, genetic susceptibility of IgE responsiveness is likely to be caused by a myriad of polymorphisms in multiple genes regulating immunologic responses (Xu *et al*., 2000). However, it is still unclear whether the association is due to the polymorphism altering

gene expression, or another causal allele in linkage disequilibrium with the rs1800875 or other functional and established loci. Only a few loci could be established consistently and robustly, such as FCER1B, IL-13, and STAT6 (Vercelli, 2008). Meanwhile, it may be effective to investigate the gene-gene interactions in mast cell degranulation and combined effects on atherosclerosis, and gene-gene interactions involved in the biosynthesis of mediators, such as leukotrienes and prostaglandins. Secondly, we know that few B cells were found in human atherosclerotic intima, which appears paradoxical in the light of the mass staining IgE in the same region (Roselaar *et al*., 1996). Moreover, chymase can control the bioavailability of cytokines and growth factors, such as activating IL-1β (Mizutani *et al*., 1991), releasing membrane-bound stem cell factor (Longley *et al*., 1997), and degrading IL-4 (Tunon de Lara *et al*., 1994). However, as a key factor in the generation of IgE, IL-4 is deactivated by human chymase, which appears paradoxical with the view discussed above. Lastly, it is of importance that rs1800875 seems to have little or even no effect on the expression of serum chymase in our study. The relationship between IgE responsiveness and rs1800875 still remains unclear.

Subsequently, it is important to note that in subjects with GG homozygote, CHD risks was nearly six times in IgE level <2.58 group than IgE level ≥2.58, which indicates that a higher IgE level is protective for GG homozygotes. This observation is concordant with the two other studies (Criqui *et al*., 1987; Szczeklik *et al*., 1988). In white populations, IgE levels were found to be significantly higher in the patients with unstable angina and acute myocardial infarction, compared to the patients with stable angina pectoris and controls (Korkmaz *et al*., 1991). Szczeklik *et al*. (1988) suggest that CHD patients with high IgE levels might be protected against complications of infarction. Another study showed that patients with high serum IgE levels might be protected against sudden cardiac death (Szczeklik *et al*., 1993). In our study, the mean IgE level in CHD subjects amounts to the normal serum IgE level range in Chinese population. In subjects with GG homozygote, a corresponding higher IgE level (≥2.58) plays a protective role in terms of the CHD risk. The exact mechanism for this risk reduction remains poorly elucidated thus far. Perhaps, the net effect of the mediators released from activated mast cells is advantageous to the local blood flow environment at the region of necrotic myocardium (Szczeklik *et al*., 1988). Actually, using antiplatelet therapy is effective to reduce the risks associated with percutaneous coronary intervention, such as restenosis and ischemia-reperfusion injury (Ying *et al*., 2010). Besides, impaired platelet aggregability and an extended bleeding time were regularly found in subjects with high IgE (Szczeklik *et al*., 1986). This mild homeostatic imbalance might explain the phenomenon that death from myocardial infarction in patients with atopic bronchial asthma appears to be rare (Szczeklik *et al*., 1977).

Large population-based prospective studies with ethnically diverse populations are needed to further elucidate the relationships of SNP rs1800875 with IgE and chymase levels in CHD patients.

Acknowledgements

We thank all the patients and their families who agreed to participate in this study.

References

Busse, W.W., Lemanske, R.F., 2001. Advances in immunology: asthma. *N. Engl. J. Med.*, **2001**(344):350-362.

Criqui, M.H., Lee, E.R., Hamburger, R.N., Klauber, M.R., Coughlin, S.S., 1987. IgE and cardiovascular disease. Results from a population-based study. *Am. J. Med*., **82**(5):964-968. [doi:10.1016/0002-9343(87)90159-8]

Galli, S.J., Kalesnikoff, J., Grimbaldeston, M.A., Piliponsky, A.M., Williams, C.M.M., Tsai, M., 2005. Mast cells as "tunable" effector and immunoregulatory cells: recent advances. *Annu. Rev. Immunol.*, **23**(1):749-786. [doi:10.1146/annurev.immunol.21.120601.141025]

Gardemann, A., Harnami, M., Katz, N., Tillmann, H., Haberbosch, W., 2000. The chyrnase $A_{(-1903)}G$ gene polymorphism is not associated with the risk and extent of coronary heart disease. *Atherosclerosis*, **150**(2):445-446. [doi:10.1016/S0021-9150(00)00387-7]

Gurish, M.F., Austen, K.F., 2001. The diverse roles of mast cells. *J. Exp. Med.*, **194**(1):F1-F6. [doi:10.1084/jem.194.1.F1]

He, H., Li, L.M., Cao, W.H., Sun, N.L., liu, M.Z., Hu, Y.H., 2004. Association between the I/D polymorphism of the ACE gene, A/B polymorphism of the chymase gene and the left ventricular hypertrophy in essential hypertension. *Chin. J. Hypertens.*, **12**(1):39-43 (in Chinese).

Heikkila, H.M., Latti, S., Leskinen, M.J., Hakala, J.K., Kovanen, P.T., Lindstedt, K.A., 2008. Activated mast cells induce endothelial cell apoptosis by a combined

action of chymase and tumor necrosis factor-α. *Arterioscler. Thromb. Vasc. Biol.*, **28**(2):309-314. [doi:10.1161/ATVBAHA.107.151340]

Ihara, M., Urata, H., Kinoshita, A., Suzumiya, J., Sasaguri, M., Kikuchi, M., Ideishi, M., Arakawa, K., 1999. Increased chymase-dependent angiotensin II formation in human atherosclerotic aorta. *Hypertension*, **33**(6):1399-1405.

Imada, T., Komorita, N., Kobayashi, F., Naito, K., Yoshikawa, T., Miyazaki, M., Nakamura, N., Kondo, T., 2002. Therapeutic potential of a specific chymase inhibitor in atopic dermatitis. *Jpn. J. Pharmacol.*, **90**(3):214-217. [doi:10.1254/jjp.90.214]

Iwanaga, T., McEuen, A., Walls, A.F., Clough, J.B., Keith, T.P., Rorke, S., Barton, S.J., Holgate, S.T., Holloway, J.W., 2004. Polymorphism of the mast cell chymase gene (*CMA1*) promoter region: lack of association with asthma but association with serum total immunoglobulin E levels in adult atopic dermatitis. *Clin. Exp. Allergy*, **34**(7):1037-1042. [doi:10.1111/j.1365-2222.2004.02000.x]

Jeziorska, M., McCollum, C., Woolley, D.E., 1997. Mast cell distribution, activation, and phenotype in atherosclerotic lesions of human carotid arteries. *J. Pathol.*, **182**(1):115-122. [doi:10.1002/(SICI)1096-9896(199705)182:1<115::AID-PATH806>3.0.CO;2-9]

Korkmaz, M.E., Oto, A., Saraclar, Y., Oram, E., Oram, A., Ugurlu, S., Karamehmetoglu, A., Karaagaoglu, E., 1991. Levels of IgE in the serum of patients with coronary arterial disease. *Int. J. Cardiol.*, **31**(2):199-204. [doi:10.1016/0167-5273(91)90216-C]

Liang, Y.H., Chen, X.L., Yu, Z.S., Chen, C.Y., Bi, S., Mao, L.G., Zhou, B.L., Zhang, X.N., 2009. Deletion analysis of *SMN1* and *NAIP* genes in southern Chinese children with spinal muscular atrophy. *J. Zhejiang Univ.-Sci. B*, **10**(1):29-34. [doi:10.1631/jzus.B0820125]

Longley, B.J., Tyrrell, L., Ma, Y., Williams, D.A., Halaban, R., Langley, K., Lu, H.S., Schechter, N.M., 1997. Chymase cleavage of stem cell factor yields a bioactive, soluble product. *PNAS*, **94**(17):9017-9021. [doi:10.1073/pnas.94.17.9017]

Mizutani, H., Schechter, N., Lazarus, G., Black, R.A., Kupper, T.S., 1991. Rapid and specific conversion of precursor interleukin 1 β (IL-1β) to an active IL-1 species by human mast cell chymase. *J. Exp. Med.*, **174**(4):821-825. [doi:10.1084/jem.174.4.821]

Ortlepp, J.R., Janssens, U., Bleckmann, F., Lauscher, J., Merkelbach-Bruse, S., Hanrath, P., Hoffmann, R., 2001. A chymase gene variant is associated with atherosclerosis in venous coronary artery bypass grafts. *Coron. Artery Dis.*, **12**(6):493-497. [doi:10.1097/00019501-200109000-00008]

Pfeufer, A., Busjahn, A., Vergopoulos, A., Knoblauch, H., Urata, H., Osterziel, K.J., Menz, M., Wienker, T.F., Faulhaber, H.D., Steinmetz, A., *et al.*, 1998. Chymase gene locus is not associated with myocardial infarction and is not linked to heart size or blood pressure. *Am. J. Cardiol.*, **82**(8):979-981. [doi:10.1016/S0002-9149(98)00518-9]

Qin, Y., Shi, G.P., 2011. Cysteinyl cathepsins and mast cell proteases in the pathogenesis and therapeutics of cardiovascular diseases. *Pharmacol. Ther.*, **131**(3):338-350. [doi:10.1016/j.pharmthera.2011.04.010]

Roselaar, S.E., Kakkanathu, P.X., Daugherty, A., 1996. Lymphocyte populations in atherosclerotic lesions of apoE –/– and LDL receptor –/– mice. Decreasing density with disease progression. *Arterioscler. Thromb. Vasc. Biol.*, **16**(8):1013-1018. [doi:10.1161/01.ATV.16.8.1013]

Schwartz, L.B., Austen, K.F., 1980. Enzymes of the mast cell granule. *J. Invest. Dermatol.*, **74**(5):349-353. [doi:10.1111/1523-1747.ep12543620]

Sharma, S., Rajan, U.M., Kumar, A., Soni, A., Ghosh, B., 2005. A novel $(TG)_n(GA)_m$ repeat polymorphism 254 bp downstream of the mast cell chymase (*CMA1*) gene is associated with atopic asthma and total serum IgE levels. *J. Hum. Genet.*, **50**(6):276-282. [doi:10.1007/s10038-005-0252-x]

Sun, J., Sukhova, G.K., Wolters, P.J., Yang, M., Kitamoto, S., Libby, P., MacFarlane, L.A., Mallen-St Clair, J., Shi, G.P., 2007. Mast cells promote atherosclerosis by releasing proinflammatory cytokines. *Nat. Med.*, **13**(6):719-724. [doi:10.1038/nm1601]

Szczeklik, A., Nizankowski, R., Mruk, J., 1977. Myocardial infarction in status asthmaticus. *Lancet*, **1**(8012):658-659. [doi:10.1016/S0140-6736(77)92102-X]

Szczeklik, A., Milner, P.C., Birch, J., Watkins, J., Martin, J.F., 1986. Prolonged bleeding time, reduced platelet aggregation, altered PAF-acether sensitivity and increased platelet mass are a trait of asthma and hay fever. *Thromb. Haemost.*, **56**(3):283-287.

Szczeklik, A., Sladek, K., Szczerba, A., Dropinski, J., 1988. Serum immunoglobulin E response to myocardial infarction. *Circulation*, **77**(6):1245-1249. [doi:10.1161/01.CIR.77.6.1245]

Szczeklik, A., Dropinski, J., Gora, P.F., 1993. Serum immunoglobulin E and sudden cardiac arrest during myocardial infarction. *Coron. Artery Dis.*, **4**(11):1029-1032. [doi:10.1097/00019501-199311000-00012]

Tunon de Lara, J.M., Okayama, Y., McEuen, A.R., Heusser, C.H., Church, M.K., Walls, A.F., 1994. Release and inactivation of interleukin-4 by mast cells. *Ann. N. Y. Acad. Sci.*, **725**:50-58. [doi:10.1111/j.1749-6632.1994.tb39789.x]

Urata, H., Kinoshita, A., Perez, D.M., Misono, K.S., Bumpus, F.M., Graham, R.M., Husain, A., 1991. Cloning of the gene and cDNA for human heart chymase. *J. Biol. Chem.*, **266**(26):17173-17179.

Vercelli, D., 2008. Discovering susceptibility genes for asthma and allergy. *Nat. Rev. Immunol.*, **8**(3):169-182. [doi:10.1038/nri2257]

Wu, G.R., Ma, A.Q., Li, Z.H., Geng, T., 2002. Study of the association of the I/D polymorphism of the *ACE* gene, A/G polymorphism of the heart chymase gene and idiopathetic dilated cardiomyopathy. *J. Clin. Cardiol.*, **18**(3):100-103 (in Chinese).

Xiang, M., Sun, J., Lin, Y., Zhang, J., Chen, H., Yang, D., Wang, J., Shi, G.P., 2011. Usefulness of serum tryptase level as an independent biomarker for coronary plaque instability in a Chinese population. *Atherosclerosis*, **215**(2):494-499. [doi:10.1016/j.atherosclerosis.2011.01.006]

Xu, J., Postma, D.S., Howard, T.D., Koppelman, G.H., Zheng, S.L., Stine, O.C., Bleecker, E.R., Meyers, D.A., 2000. Major genes regulating total serum immunoglobulin E levels in families with asthma. *Am. J. Hum. Genet.*, **67**(5): 1163-1173. [doi:10.1086/321190]

Ying, S.Q., Xiang, M.X., Fang, L., Wang, J.A., 2010. Temporal changes in circulating P-selectin, plasminogen activator inhibitor-1, magnesium, and creatine kinase after percutaneous coronary intervention. *J. Zhejiang Univ.-Sci. B*, **11**(8):575-582. [doi:10.1631/jzus.B1001006]

Yoshikawa, T., Imada, T., Nakakubo, H., Nakamura, N., Naito, K., 2001. Rat mast cell protease-I enhances immunoglobulin E production by mouse B cells stimulated with interleukin-4. *Immunology*, **104**(3):333-340. [doi:10.1046/j.1365-2567.2001.01320.x]