ORIGINAL ARTICLE
Iran J Allergy Asthma Immunol
June 2007; 6(2): 49-57

E-Selectin S128R Polymorphism Leads to Severe Asthma

Ebrahim Nadi [1], Mehrdad Hajilooi[2], Fatemeh Zeraati[3], Mostafa Ansari[1], Sasan Tavana[1], Sayed Hamid Hashemi[1], and Alireza Rafiei[4]

[1] Division of Pulmonary Sciences and Critical Care Medicine, Hamadan Research Group of Tuberculosis and Lung Disease, Ekbatan Hospital, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran

[2] Department of Immunology, Hamadan Research Group of Tuberculosis and Lung Disease, Hamadan University of Medical Sciences, Hamadan, Iran

[3] Department of Pharmacology, Hamadan Research Group of Tuberculosis and Lung Disease, Ekbatan Hospital, Hamadan University of Medical Sciences, Hamadan, Iran

[4] Department of Immunology and Microbiology, Sari Medical School, Mazandaran University of Medical Sciences, Sari, Iran

Received: 1 November 2006; Received in revised form: 9 January 2007; Accepted: 21 January 2007

ABSTRACT

The E-selectin mediates the interaction of activated endothelial cells with leukocytes and plays a fundamental role in the pathogenesis of asthma. It has been suggested that an S/R (Serine128Arginine) polymorphism of E-selectin alters ligand binding function. Our purpose in this study was to determine whether this Serine128Arginine polymorphism influences the risk of asthma and also to analyze the possible correlation of disease severity in Iranian patients with polymorphism of E-selection.

We studied human E-selectin gene polymorphism in 172 asthmatic patients and 173 healthy volunteers by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). To determine the severity of the asthma's situation, a questionnaire was prepared requesting the following information: age, sex, clinical signs and symptoms and past medical history. After the participants filled in the questionnaire, all active or ex-smoker patients were excluded. A trained observer assessed airway reversibility, peak flowmetry and spirometry in asthmatic patients.

We found increased serum levels of soluble E-selectin (sE-selectin) in asthmatic patients compared with healthy subjects (P<0. 0001). Frequencies of the SS, SR, and RR genotypes were found as 66.3%, 31.4%, and 2.3% in the patients and 91.9%, 8.1%, and 0.0% in control subjects, respectively. The 128Arg allele was more prevalent in patients than controls (OR 5.78; 95% CI, 3.07-10.86, P<0.0001). However, in this study the polymorphism was not associated with circulating sE-selectin levels. We found a direct correlation between the level of sE-selectin and the severity of asthma (P=0.001). On the other hand, there was a close relation between 128Arginine carriage and disease severity (P<0.0001).

These results suggest that the Ser128Arg polymorphism of the E-selectin gene is a genetic factor that may be associated with the severity of asthma.

Keyword: Asthma; E-selectin; Gene polymorphism

Corresponding Author: Ebrahim Nadi, MD;
Division of Pulmonary Sciences and Critical Care Medicine, Hamadan Research Group of Tuberculosis and Lung Disease, Ekbatan Hospital, Hamadan University of Medical Sciences, Hamadan, Iran.Tel: (+98 918) 1116 518, Fax: (+98 918) 8226 035, E-mail: Ebrahim_nadi@yahoo.com

INTRODUCTION

Adhesion molecules such as members of the selectin family facilitate the recruitment and migration of inflammatory cells from the blood to the airway walls and therefore play an important role in the pathogenesis of asthma. On one hand, early primate studies had shown that blockade of E-selectin reduced allergic pulmonary inflammation. [1] Thus, increased expression of the E-selectin was subsequently observed in asthmatic patients, [2-4] suggesting that these molecules were up-regulated in human disease and may contribute to the alteration of pathophysiology. However, the migration of leukocytes into an asthmatic lung is dependent upon multiple mechanisms that are initiated by the binding of leukocytes at the endothelial border to selectins. Blockade of the initial selectin-mediated adhesion event should inhibit subsequent migration into the lung during an allergen challenge.

E-selectin is exclusively expressed on endothelial cells (ECs), mainly after their activation. [5] It is expressed and proteolytically cleaved from the surface of ECs upon activation by different stimuli[6, 7] including interleukin 1(IL-1), tumor necrosis factor (TNF). Such a shedding leads to an increase of plasma levels of soluble(s)E-selectin *in vivo*, [8]. Furthermore, sE-selectin levels correlate with its surface expression on ECs *in vitro*. [9]

Thus, sE-selectin serves as an excellent marker for endothelial activation in numerous cardiovascular and inflammatory diseases. [10]

The common Ser128Arg polymorphism has been reported to regulate plasma levels of sE-selectin. [11, 12]. This polymorphism is functional in that, it alters ligand affinity. [13] E-selectin has an aminoterminal C-type lection domain that is amino terminal the carbohydrate-binding site which binds the sialylated Lewis-x antigen (sLex or CD15s) (Neu5Acalpha2-3Galbeta1-4 (Fucalpha1-3) GlcNAc). Substitution of E-selectin endothelial growth factor (EGF) domain residue Ser128 with an arginine results in E-selectin proteins that have lost the requirement for alpha1-3–linked fucose and are thus able to bind to sialyllactosamine. [13] Probably as a consequence, this SNP enhances tethering of myeloid cells[14] and extends the range of lymphocytes recruited by E-selectin. [15] In addition, Ser128Arg transduced ECs support significantly more rolling and adhesion of neutrophils and mononuclear cells compared with ECs transduced with wild-type E-selectin. These Ser128Arg transduced ECs also exhibit significantly greater levels of phosphorylation of extracellular signal–regulated kinase 1, 2 and p38 mitogen activated protein kinase. This suggests that an altered endothelial signaling pathway is associated with this polymorphism. [16] Clinically, the polymorphism has been associated with atherosclerosis, [17] myocardial infarction, [16] and restenosis after angioplasty, [12] but the underlying mechanism is unclear. Recent advances in the pathophysiological understanding have underpinned the frequent involvement of the protein family of selectins in the progression of serious illnesses, including cancer, cancer metastasis, and immunological diseases such as asthma, allergy and autoimmune reactions. [18, 19] Our purpose in this study was to determine whether this Serine128Arginine polymorphism influences the risk of asthma and also to analyze the possible correlation of disease severity in Iranian patients with polymorphism of E-selectin.

MATERIAL AND METHODS

Study Population

Our case-control study was carried out in pulmonology clinics of Ekbatan Hospital of Hamadan University of Medical Sciences from 2004 to 2005. Ethical approval was obtained from the Ethics Committee of Hamden University of Medical Sciences.

One hundred seventy-two patients with asthma (Table 1), before treatment, were recruited from the out-patient clinics.

Table 1. Patients' characteristics.

Characteristic	Asthmatic patients (n=172)
Age (year)	56. 56±12. 69
Gender (F/M)- %	57. 6/42. 4
FEV1 %	54. 3±22. 4
PEF %	49. 6±20. 75
PEF Varibility %	26. 42±8. 1
Reversibility %	19±6. 24
Severity-n (%)	
Mild intermittent (Step1)	27 (15. 7%)
Mild persistent (Step2)	31 (18%)
Moderate persistent (Step3)	46 (26. 8)
Severe persistent (Step4)	68 (39. 5)

Table 2. Stepwise asthma severity classification.

Step	Symptoms		PEFR orFEV1 (PEFR Variability)
	Day	Night	
Step1: Mild intermittent	≤2days/week	≤2nights/months	≥80% (<20%)
Step2: Mild persistent	>2days/week but<1 per day	>2nights/month	≥80% (20-30%)
Step3: Moderate persistent	Daily	>1night/weak	<60%-<80% (>30%)
Step4: Sever persistent	Continual	Frequent	≤60% (>30%)

According to National Asthma Education and Prevention program method (The NAEPP EXPERT PANEL. 2002; Kasper. DL. et al. 2005)

The inclusion criterion for all cases was bronchial asthma, where the diagnosis was established through demonstrating reversible airway obstruction. The participants were requested to fill in a questionnaire for identifying their demographic characteristics such as age, sex, asthma history, past medical history and details related to current asthma exacerbation, nocturnal and diurnal clinical signs and symptoms. In order to identify the severity of asthma, a trained observer assessed air-way reversibility, peak flowmetry and spirometry in the asthmatic patients. At least three acceptable maneuvers meeting American College of Chest Physicians standards were required, with at least two reproducible forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) maneuvers within 5% of best required for each test. [20] The airway responsiveness was performed in a standardized fashion[21] and the airway reversibility was evaluated by spirometry before and 15 minutes after inhalation of two puffs of a βeta-adrenergic agonist (albuterol) as metered dose inhaler and equal or more than 15% increase in FEV1 was meant diagnostic for asthma. [21] Peak expiratory flow (PEF) was also used to assess acute asthma severity and was expressed as percentage of the value based on age, sex, race and height. Changes in PEF are expressed as the relative change in percentage of predicted value. According to National Asthma Education and Prevention program method, asthmatic patients were categorized in 4 steps. [22, 23] The exclusion criteria were the presence of any inflammatory diseases and past history of recurrent infections, viral hepatitis, known collagen vascular diseases, autoimmune diseases, chronic obstructive lung disease(other than bronchial asthma), myocardial infarction/unstable angina and having undergone any surgical procedures during the previous month, as well as positive drug history for lipid lowering agents (Table 2). Patients who previously used inhaled steroid or systemic steroid within the past four weeks and who were active and ex-smokers were excluded from the study. One hundred seventy-three healthy, non asthmatic adults unrelated control subjects with no personal or family history of asthma were recruited from the same geographical area through blood donor clinics.

Table 3. Ser128Arg allele and genotype frequencies in asthmatic patients and healthy controls.

E-selectin *polymorphism*	Asthmatics (n= 172)	Controls (n=173)	P-value
Allele -n/total (%)			
128 Ser (S)	282 (82%)	332 (96%)	NS
128 Arg (R)	62 (18%)	14 (4%)	<0. 0001
Genotype-n/total (%)			
Ser/Ser	114 (66. 3%)	159 (91. 9%)	NS
Ser/Arg	54 (31. 4%)	14 (8. 1%)	0. 000
Arg/Arg	4 (2. 3%)	0	NS

NS=Not significant

E-selectin Genotype

Venous blood from each subject was collected in tubes containing 50 mmol of EDTA per liter. The genomic DNA was isolated from anti-coagulated peripheral blood buffy coat using Miller's salting out method. [24] A polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to detect the substitution responsible for E-selectin polymorphism.

Amplification was carried out using a PCR Techne Flexigene apparatus (Roche, Germany) in a total volume of 50µl containing 0.2 ng of genomic DNA. The primers were used and their specificities are shown (Table 3).

The internal control primers were used in all reactions to amplify a 927 bases segment of human growth hormone gene to check for successful PCR amplification. Each pair of primer consisted of 10 pmol of each allele-specific primer, 200mol/l each dNTP; 10 mol/l Tris-HCl (PH 8.3); 50 nmol/l KCl, 1.5 mol/l MgCl2 and 0.5 IU Taq DNA polymerase. PCR performed without DNA template represented the negative controls.

The reaction was carried out under following conditions: initial denaturation was at 94 ^{o}C for 2 min, followed by 30 cycles of amplification at 94 ^{o}C for 15 sec and annealing at 60 ^{o}C for 45 sec, with final extension for 2 min at 72 ^{o}C. The PCR product of 186 bp was then digested by the restriction enzyme *Pst I* (10-µl PCR product and 5 U of *Pst I* were digested for 3 h at 37 °C), and separated in 3% agarose gel electrophoresis and visualized by ultra-violet tran illumination. Genotype SS yielded two fragments of 123 and 63 bp; genotype RR yielded one fragment of 186 bp, SR yielded three digestion fragments of 186, 123, and 63 bp. [25] To prevent observer's bias, the investigator was unaware of sample origin and a different individual crosschecked all gels.

Soulbe E-selectin Measurement

Whole blood was drawn in tubes and allowed to coagulate at room temperature for 1h. The serum was then separated by centrifugation and stored at -80 ^{o}C before being analyzed. Serum levels of sE-selectin were measured by commercially available ELISA kits (sE-Selectin; Bender Med Systems, BMS 205, Vienna, Austria) following manufacturers instructions. The lower limit of detection sensitivity was 1.6ng/mL for sE-selectin.

Statistical Analysis

The consistency of genotype frequencies were checked with Hardy-Weinberg equilibrium. Results of the gene polymorphism studies were analyzed by the comparison of allele frequencies (ratio of test allele to total alleles). Frequencies of allele and genotype distribution were analyzed using Fisher's exact test or χ^2 test as appropriate. Multiple linear regression analysis was applied to assess the association between E-selectin genotype (independent variable) and plasma levels (dependent variable).

E-selectin plasma levels were log-transformed before being entered into the model. Because of skewed data, E-selectin plasma was log-transformed and differences between groups were confirmed by Mann-Whitney U Test. Odds ratios (OR) were calculated for disease susceptibility or severity in carriers of specific alleles. The 95% confidence intervals (CI) for the OR were also calculated. P-values of less than 0.05 (two-tailed) were considered significant.

RESULTS

Patients Data

The characteristics of the population are presented in Table1. Patients and control subjects were in the age range of 43.87 to 69.25 (mean age: 56.56±12.69) years and 44.32 to 64.54 (mean age: 54.43±10.11) years respectively, and 99 (57.6%) patients and 114 (65.9%) of control subjects were male. There were no age and sex significant differences between the patients and control subjects (P=0.08 and P=0.12, respectively).

E-selectin Genotypes and Plasma Levels

DNA samples from 172 subjects with asthma and 173 healthy individuals were analyzed for Ser128Arg polymorphism of E-selectin gene. The frequencies of E-selectin genotypes in the patients and control individuals were found in accordance with those expected by the Hardy–Weinberg equilibrium (P=0. 41 and P=0.57, respectively). Homozygous Arg128Arg genotype was found in 4 (2. 3%) patients, heterozygous Ser128Arg genotype was found in 54 (31. 4%) patients, and 114 (66.3%) asthmatic patients were wild type (Ser128Ser) (Table 3). The allele and genotype frequencies of the polymorphism were significantly differed between patients and controls.

Table 4. Comparison of demographic data and lung function of asthmatic patients and controls according to E-selectin Ser128Arg genotype.

Variables	Asthmatic (n=172)			Healthy controls (n=173)		
	Arg/Arg+Ser/Arg (n=58)	Ser/Ser (n=114)	P-value	Arg/Arg+Ser/Arg (n=159)	Ser/Ser (n=14)	P value
Age (year)	55.6±9.76	57.05±13.95	0.48	46.07±3.56	55.16±10.16	0.001
Sex (M/F) (%)	28.3/41.1	71.7/58.9	0.103	0/23.7	71.7/28.3	0.0001
deselecting	110.84±71.36	101.6±47.84	0.31	30.0	49.06±17.98	0.0001
FEV1	48.88±21.22	57.05±22.95	0.02			ND
PEF	45.53±20.91	51.74±20.44	0.06			ND
PEF variability	27.67±7.93	25.76±8.12	0.156			ND
Reversibility	19.12±7.55	18.95±5.49	0.86			ND

ND: Not done

The Arg allele was more prevalent in patients than controls (OR 5. 78; 95% CI, 3.07-10. 86, P<0. 0001). No homozygous mutant (Ser128Ser) genotype was found in the control subjects. Since the mutant Arg128Arg homozygous genotype was too low in the patients for assessing any statistical analysis, further analysis proved dominance for the Arginin allele, i.e. Arg/Arg +Ser/Arg vs. Ser/Ser. (Table 4)

The levels of sE-selectin was significantly higher in asthmatic patients than controls (P<0.0001). The geometric mean of sE-selectin was 104.71±56.83 for the patients with asthma and 47.51±18.01 for the control subjects (Table 5).

DISCUSSION

In this case-control study of asthmatics of varying severity, on one hand we found that the levels of serum E-selectin were elevated in severity status of asthma. On the other hand, stepwise increases of sE-selectin levels were observed from intermittent to severe persistent asthma.

Table 5. sE-selectin levels, genotypes and lung function activity according to severity of asthma.

Variables	Mild intermittent (n=27)	Mild persistent (n=31)	Moderate persistent (n=46)	Severe persistent (n=68)	P-value
Ser128Arg polymorphism-n (%)					
Arg/Arg + Ser/Arg	5 (18.5)	3 (9.7)	16 (34.8)	34 (50)	0.000
Arg/Arg	22 (81.5)	28 (90.3)	30 (65.2)	34 (50)	
sE-selectin	72.46±32.79	93.78±44.58	107.87±54.07	125.1±65.8	0.001
Age(year)	47.58±12.97	47.84±13.54	60.80±9.73	61.69±9.81	0.001
Lung function					
FEV1	78.08±9.24	73.04±9.2	52.33±15.64	36.87±17.53	0.0001
PEF	76.17±5.34	62.76±7.91	47.52±13.14	35.42±19.78	0.0001
PEF variability	17.92±3.56	20.52±4.58	27.56±6.66	31.09±7.41	0.001
Reversibility	14.92±4.23	17.12±5.43	19.0±5.13	21.25±7.23	0.0001

In addition our results have shown that there is an association between clinical expression of asthma and E-selectin gene Ser 128 Arg polymorphism. 128 R was over-represented in asthmatic patients in comparison to controls.

Thus, 128R variant might confer susceptibility against the development of asthma. This finding is in agreement with a study which has shown that asthma is characterized by airway hyper-responsiveness and inflammation of the lungs, and is accompanied by the accumulation of lymphocytes and eosinophils. [26]

On the basis of the strong association of S128R with asthma, we were interested to determine whether a correlation existed between the concentration of sE-selectin in serum and Ser128Arg genotypes. We found that Arg allele accounted for 18% (Table 3) in the asthmatic group, which was significantly higher than in normal controls (4%, $p<0.05$). It indicates that Arg allele is associated with early onset asthma and may be a risk factor for asthma. The role of adhesion molecules in asthma is supported by several studies showing an increased expression of adhesion molecules in bronchial epithelial and endothelial cells from asthmatics. [27,28] The association of the E-selectin genotype and phenotype (sE-selectin levels), and analysis of the association with a clinical outcome is the novel aspect of this study.

Increased expression of soluble E-selectin is associated with a number of inflammatory conditions including atherosclerosis. [29] In addition, the increased expression was also observed in non-insulin-dependent diabetes. [11] Recent advances in our pathophysiological understanding have underpinned the frequent involvement of the protein family of selectins in the progression of serious illnesses, including cancer, cancer metastasis and immunological diseases such as asthma, allergy and autoimmune reactions. [18]

Changes in the concentration of soluble selectins in plasma usually reflect altered cell surface turnover and proteolytic cleavage, therefore these changes are often used as markers of a role for the selectins in asthma and allergy. [30] There are some reports that plasma levels of soluble E-selectin in homozygous Arg128Arg subjects and heterozygous Ser128Arg subjects were significantly higher than in wild-type Ser128Ser[12] and the Ser128Arg allele enhanced thrombin generation and fibrin formation significantly. [16]

In the study of asthmatics of varying severity, the levels of sE-selectin were related to clinical asthma severity. Thus, levels of serum E-selectin were elevated in severity status of asthma. This finding is in accordance with accumulation of inflammatory cells in the airways of asthmatics. [31]

The S128R polymorphism lies in the EGF domain of E-selectin that is conserved among all selectins. Although the principal ligand contact points of the selectins lie within the lection domain. [32] Domain swaps between E-selectin have suggested that the EGF-domain can modulate the binding properties of the lection domain to surface-immobilized ligand without affecting the equilibrium binding properties toward soluble ligand. [33] The nucleotide substitution of E-selectin EGF domain has been shown to profoundly affect the ligand binding affinity. The polymorphism studied, is located within a gene, which is crucial for the recruitment of leukocyte into airway endothelium, thus an association of the Arg allele of Ser128Arg single nucleotide polymorphism with asthma could be reasonable.

The relationship between the E-selectin Ser128Arg polymorphism and asthma may reflect an amplified inflammatory response resulting from the action of altered selectin molecules containing the serine 128 arginine mutation. The substitution of arginine for serine has been shown to dramatically decrease binding specificity while increasing affinity for additional ligands, resulting in two to threefold increase in cellular adhesion. The E-selectin 128R allele may thus increase leukocyte adherence to activated airway endothelium thereby contributing to the progression of bronchial asthma. Up regulation of endothelial adhesion molecules facilitates the interaction with leukocytes and platelets. [34] E-selectin is of particular interest in this context, because it is expressed on activated endothelial cells and therefore can induce localized inflammation at the airway tract. When E-selectin is shed from the endothelial surface it can be detected as soluble sE-selectin in the plasma. [35] The level of sE-selectin increases in the severe asthmatic patients compared with mild/moderate asthmaic patients. [36]

It has been hypothesized that frequent genetic variants may contribute significantly to the genetic risk for common and complex phenotypes, [37] and therefore high prevalence of the Arg128Arg allele also provides

a strong biological rationale for the involvement of this polymorphism in the regulation of secretion of serum sE-selection levels in asthmatics. A transition from 561A to 561C at the coding region of the E-selectin gene causes a conservative change of a serine with an arginine at codon 128 (Ser128Arg). [25] This mutation alters selectin binding specifically, [38] leading to a gain of function under flow conditions, possibly amplifying the number of leukocytes that roll and subsequently arrest on endothelium.[39]

This tethering mechanism could theoretically amplify the number of leukocytes interacting with mutated airway endothelial cells during bronchial asthma.

It is noteworthy that while the wild type E-selection recruits specifically activated Th-1 lymphocytes, [40] which produces proinflammatory cytokines and chemokines,[41] the presence of the 8128R polymerphism extends the range of lymphocytes recruited by E-selection, including Th2 and B lymphocytes. [42, 43] This subset of T cells produces interleukin 4 (IL-4), IL-5 and IL-13, leading to recruitment of eosinophils and enhancing production of immunoglobulin (particularly IgG, and IgE) from activated B cells, and increases recruitment of eosinophils and other leukocytes to inflammed airway endothelium. Furthermore, children were shown to have elevated serum levels of E-selectin during acute asthma exacerbation.[44] For the reason of the prevelance of severe persistant asthma in the subjects in this study, it seems that contrary to different studies conducted in other countries, most of the subjects because of not showing up in time for recieving the appropriate treatment and moreover experiencing recurrent episodes of asthmatic attacks, showed up with more severity of the disease at the hospital clinics, and so participated in our study. However this finding can be carried out simultanaously in different hospitals applying a different research design with appropriate samples.

Finally, in this study the polymorphism was not associated with high circulating sE-selectin levels, we found a direct correlation between the level of sE-selectin and severity of asthma (P=0.001). Moreover, there is a close relation between 128Arginine carriage and disease severity (P<0.0001). These results suggest that the Ser128Arg polymorphism of the E-selectin gene is a genetic factor that may be associated with the severity of asthma.

ACKNOWLEDGMENTS

We thank Miss. Marzieh Naderi Shahab for editing the manuscript.

REFERENCES

1. Gundel RH, Wegner CD, Torcellini CA, Clarke CC, Haynes N, Rothlein R, et al. Endothelial leukocyte adhesion molecule-1 mediates antigen-induced acute airway inflammation and late-phase airway obstruction in monkeys. J Clin Invest 1991; 88(4):1407-11.
2. Yamashita N KS, Kouro O, Furue M, Yamamoto S, Sakane T. Soluble E-selectin as a marker of disease activity in atopic dermatitis. J Allergy Clin Immunol 1997; 99(3):410-6.
3. Kobayashi T Hashimoto S, Imai K, Amemiya E, Yamaguchi M, Yachi A, Horie T. Elevation of serum soluble intercellular adhesion molecule-1 (sICAM-1) and sE-selectin levels in bronchial asthma. Clin Exp Immunol 1994; 96(1):110-5.
4. Symon FA, McNulty CA, Wardlaw AJ. P- and L-selectin mediate binding of T cells to chronically inflamed human airway endothelium. Eur J Immunol 1999; 29(4):1324-33.
5 Dong ZM, Wagner DD. Leukocyte-endothelium adhesion molecules in atherosclerosis. J Lab Clin Med 1998; 132(5):369-75.
6. Drake TA, Cheng J, Chang A, Taylor FB Jr. Expression of tissue facto, 6- thrombomodulin, and E-selectin in baboons with lethal Escherichia coli sepsis. Am J Pathol 1993; 142(5):1458-70.
7. Wyble CW, Hynes KL, Kuchibhotla J, Marcus BC, Hallahan D, Gewertz BL. TNF-alpha and IL-1 upregulate membrane-bound and soluble E-selectin through a common pathway. J Surg Res 1997; 73(2):107-12.
8. Kulander L, Pauksens K, Venge P. Soluble adhesion molecules, cytokines and cellular markers in serum in patients with acute infections. Scand J Infect Dis 2001; 33(4):290-300.
9 Leeuwenberg JF, Smeets EF, Neefjes JJ, Shaffer MA, Cinek T, Jeunhomme TM, Ahern TJ, Buurman WA. E-selectin and intercellular adhesion molecule-1 are released by activated human endothelial cells in vitro. Immunology 1992; 77(4):543-9.
10. Roldan V, Marin F, Lip GY, Blann AD. Soluble E-selectin in cardiovascular disease and its risk factors. A review of the literature. Thromb Haemost 2003; 90(6):1007-20.
11 Bannan S, Mansfield MW, Grant PJ. Soluble vascular cell adhesion molecule-1 and E-selectin levels in relation to vascular risk factors and to E-selectin genotype in the first degree relatives of NIDDM patients and in NIDDM patients. Diabetologia 1998; 41(4):460-6.

12. Mlekusch W, Exner M, Schillinger M, Sabeti S, Mannhalter , Minar E, et al. E-Selectin and restenosis after femoropopliteal angioplasty: prognostic impact of the Ser128Arg genotype and plasma levels. Thromb Haemost 2004; 91(1):171-9.
13. Revelle BM, Scott D, Beck PJ. Single amino acid residues in the E- and P-selectin epidermal growth factor domains can determine carbohydrate binding specificity. J Biol Chem 1996; 271(27):16160-70.
14. Rao RM, Clarke JL, Ortlepp S, Robinson MK, Landis RC, Haskard DO. The S128R polymorphism of E-selectin mediates neuraminidase-resistant tethering of myeloid cells under shear flow. Eur J Immunol 2002; 32(1):251-60.
15. Rao RM, Haskard DO, Landis RC. Enhanced recruitment of Th2 and CLA-negative lymphocytes by the S128R polymorphism of E-selectin. J Immunol 2002; 169(10):5860-5.
16. Yoshida M, Takano Y, Sasaoka T, Izumi T, Kimura A. E-selectin polymorphism associated with myocardial infarction causes enhanced leukocyteendothelial interactions under flow conditions. Arterioscler Thromb Vasc Biol 2003; 23(5):783-8.
17. Ghilardi G BM, Turri O, Guagnellini E, Scorza R. Ser128Arg gene polymorphism for Eselectin and severity of atherosclerotic arterial disease. J Cardiovasc Surg (Torino) 2004; 45(2):143-7.
18.Kneuer C, Ehrhardt C, Radomski MW, Bakowsky U. Selectins--potential pharmacological targets? Drug Discov Today 2006; 11(21-22):1034-40.
19. Rosen SD, Tsay D, Singer MS, Hemmerich S, Abraham WM. Therapeutic targeting of endothelial ligands for L-selectin(PNAd)in asheep model of asthma. Am J Pathol 2005; 166(3):935-44.
20. Snider GL, Woolf CR, Kory RC. Criteria for the assessment of reversibility in airway obstruction: Report of the committee on Emphysema, American College of Chest Physicians. Chest 1974; 65(5):552-3.
21. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometery. Eur Respir J 2005; 26(2):319-38.
22. Snider GL, Woolf CR, Kory RC, et al: Criteria for the assessment of reversibility in airway obstruction: Report of the committee on Emphysema, American College of Chest Physicians. Chest 1974; 65(5):552-3.
23. Scheffer AL. Global strategy for asthma management and prevention. NHLB/WHO workshop Report. National Institute of Health, Betesda MD, 2002, Publication no.92,3659.
24. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988; 16(3):1215.
25 Wenzel K, Hanke R, Speer A. Polymorphism in the human E-selectin gene detected by PCR-SSCP. Hum Genet 1994; 94(4):452-3.
26. Hakansson L, Bjornsson E, Janson C, Schmekel B. Increased adhesion to vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 of eosinophils from patients with asthma. J Allergy Clin Immunol 1995; 96(6 Pt 1):941-50.
27. Gosset P, Tillie-Leblond I, Janin A, Marquette CH, Copin MC, Wallaert B, et al. Expression of E-selectin, ICAM-1 and VCAM-1 on bronchial biopsies from allergic and non-allergic asthmatic patients. Int Arch Allergy Immunol 1995; 106(1):69-77.
28. Vignola AM, Bonsignore G, Siena L, Melis M, Chiappara G, Gagliardo R, et al. ICAM-1 and alpha3beta1 expression by bronchial epithelial cells and their in vitro modulation by inflammatory and anti-inflammatory mediators. Allergy 2000; 55(10):931-9.
29. Li Y, Wei YS, Wang M, Zhang PA, Jiang XJ, Huang CX. Association between the Ser128Arg variant of the E-selectin and risk of coronary artery disease in the central China. Int J Cardiol 2005; 103(1):33-6.
30. Dogu F, Ikinciogullari A, Egin Y, Babacan E. Circulating adhesion molecule levels in childhood asthma. Indian Pediatr 2002; 39(11):1017-21.
31. Amin K, Ludviksdottir D, Janson C, Nettelbladt O, Bjornsson E, Roomans GM, et al. Inflammation and structural changes in the airways of patients with atopic and nonatopic asthma. BHR Group. Am J Respir Crit Care Med 2000; 162(6):2295-301.
32. Somers WS, Tang J, Shaw GD, Camphausen RT. Insights into the molecular basis of leukocyte tethering and rolling revealed by structures of P- and E-selectin bound to SLe(X) and PSGL-1. Cell 2000; 103(3):467-79.
33. Kansas GS, Saunders KB, Ley K, Zakrzewicz A, Gibson RM, Furie BC, et al. A role for the epidermal growth factor-like domain of P-selectin in ligand recognition and cell adhesion. J Cell Biol 1994; 124(4):609-18.
34. Tsakiris DA, Tschopl M, Jager K, Haefeli WE, Wolf F, and Marbet GA. Circulating cell adhesion molecules and endothelial markers before and after transluminal angioplasty in peripheral arterial occlusive disease Atherosclerosis 1999; 142(1):193-200.
35. Rauchhaus M, Gross M, Schulz S, Francis DP, Greiser P, Norwig A, et al. The E-selectin SER128ARG gene polymorphism and restenosis after successful coronary angioplasty. Int J Cardiol 2002; 83(3):249-57.
36. Hamzaoui A, Ammar J, El Mekki F, Borgi O, Ghrairi H, Ben Brahim M, et al. Elevation of serum soluble E-

selectin and VCAM-1 in severe asthma. Mediators Inflamm 2001; 10(6):339-42.
37. Risch N, Merikangas K. The future of genetic studies of complex human diseases. Science 1996; 273(5281):1516-7.
38. Revelle BM, Scott D, Beck PJ. Single amino acid residues in the E- and P-selectin epidermal growth factor domains can determine carbohydrate binding specificity. J Biol Chem 1996; 271(27):16160-70.
39. Yoshida M, Takano Y, Sasaoka T, Izumi T, Kimura A. E-selectin polymorphism associated with myocardial infarction causes enhanced leukocyte-endothelial interactions under flow conditions. Arterioscler Thromb Vasc Biol 2003; 23(5):783-8.
40. Austrup F, Vestweber D, Borges E, Lohning M, Brauer R, Herz U, et al. P- and E-selectin mediate recruitment of T-helper-1 but not T-helper-2 cells into inflammed tissues. Nature 1997; 385(6611):81-3.
41. Galimberti D, Bresolin N, Scarpini E. Chemokine network in multiple sclerosis: role in pathogenesis and targeting for future treatments. Expert Rev Neurother 2004; 4(3):439-53.
42. Rao RM, Haskard DO, Landis RC. Enhanced recruitment of Th2 and CLA-negative lymphocytes by the S128R polymorphism of E-selectin. J Immunol 2002; 169(10):5860-5.
43. Armerding D, Fuhlbrigge RC, Kieffer JD, Kupper TS. Tonsillar B cells do not express PSGL-1, but a significant fraction displays the cutaneous lymphocyte antigen and exhibits effective E- and P-selectin ligand activity. Int Arch Allergy Immunol 2001; 126(1):78-90.
44. Tang RB, Chen SJ, Soong WJ, Chung RL. Circulating adhesion molecules in sera of asthmatic children. Pediatr Pulmonol 2002; 33(4):249–54.