



# *The Effect of Intercellular Adhesion Molecule-1 Gene Polymorphism on Atherosclerosis in Patients with Glycogen Storage Disease Type 1*

Glikojen Depo Tip 1 Hastalarında Intersellüler Adezyon Molekülü Tip 1 Gen Polimorfizmin Ateroskleroza Etkisi

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## **ABSTRACT**

**Aim:** Glycogen storage disease type 1 is characterized with hypertriglyceridemia which does not lead to atherosclerosis. The human intercellular adhesion molecule-1 gene has been shown to be affected in atherosclerosis and postprandial hypertriglyceridemia.

**Materials and Methods:** In the present paper, the authors genotyped the Glu241Arg polymorphism in the intercellular adhesion molecule-1 gene in 35 patients with Glycogen storage disease type 1 and 108 healthy controls.

**Results:** The results indicated a significant decrease in the frequency of the GG genotype in Glycogen storage disease type I patients compared to healthy controls. In addition, a significant correlation between the GG genotype and triglyceride levels was seen in Glycogen storage disease type 1 patients.

**Conclusion:** It is thus concluded that the GG genotype could protect Glycogen storage disease type I patients from the development of atherosclerosis.

*The Journal of Pediatric Research 2015;2(1):7-10*

**Key Words:** Glycogen storage disease type 1, intercellular adhesion molecule-1

**Conflicts of Interest:** The authors reported no conflict of interest related to this article.

## **ÖZET**

**Amaç:** Glikojen depo hastalığı tip 1'de görülen hipertrigliserideminin ateroskleroza yol açmadığı gösterilmiştir. İnsan intersellüler adezyon molekülü tip 1'in ateroskleroz ve postprandial hipertrigliseridemi ile bağlantısı belgelenmiştir.

**Gereç ve Yöntem:** Sunulan çalışmada 35 glikojen depo hastalığı tip 1 hastanın ve 108 sağlıklı kontrol bireyin insan intersellüler adezyon molekülü tip 1 geni için Glu241Arg polimorfizm çalışması yapılmıştır.

**Bulgular:** Bulgularda GG genotipinde glikojen depo hastalığı tip 1 hastalarında sağlıklı kontrollere göre anlamlı bir yükseklik saptanmıştır. Ayrıca, GG genotipinin glikojen depo hastalığı tip 1 hastaların trigliserid düzeyleri ile arasında anlamlı korelasyon gözlenmiştir.

**Sonuç:** Bu veriler doğrultusunda glikojen depo hastalığı tip 1 hastalarında GG genotipin ateroskleroz açısından koruyucu olabileceği düşünülmüştür. *The Journal of Pediatric Research 2015;2(1):7-10*

**Anahtar Kelimeler:** Glikojen depo hastalığı tip 1, intersellüler adezyon molekülü tip 1

**Çıkar Çatışması:** Yazarlar bu makale ile ilgili olarak herhangi bir çıkar çatışması bildirmemiştir.

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Received/Geliş tarihi: 13.04.2014 Accepted/Kabul tarihi: 09.10.2014

## Introduction

Glycogen storage disease GSD type 1 (GSDI, von Gierke disease; OMIM  $\beta$  232.200) is the most common type and is characterized by a deficiency of glucose-6-phosphatase/transporter enzyme complex, which catalyzes the terminal step in both glycogenolysis and gluconeogenesis in the liver, kidney and small intestine (1). The main metabolic features of this disease are severe fasting hypoglycemia and marked hyperlipidemia, characterized by high triglycerides and total cholesterol with a low level of high-density lipoprotein. Despite the existence of longstanding dyslipidemia, it has been documented that GSDI is not associated with premature atherosclerosis (2-5). On the other hand, hyperlipemia has also been reported to be associated with increased levels of cellular adhesion molecules, particularly intercellular adhesion molecule-1 (ICAM-1) (6), and evidence also suggests that postprandial hyperlipemia may condition an increase of these molecules (7). Intercellular adhesion molecule-1 (ICAM-1, CD54) is a member of the immunoglobulin superfamily of adhesion molecules. It plays an important role in the adhesion of circulating leukocytes to the blood vessel wall and transendothelial migration to the vascular intima (8). Porsch-Oezçueruemez et al. (9) found that circulating ICAM could serve as a marker for atherosclerotic changes in the coronary system. Hajilooi et al. (10) showed that ICAM-1 values were significantly higher in patients with CAD than in control participants. Furthermore, Poston et al. (11) reported that increased expression of ICAM-1 was detected in all subtypes of atherosclerotic lesions. Therefore, ICAM-1 might play an important role in the pathogenesis of atherosclerosis presented as coronary artery disease (12). The human ICAM-1 gene is a single-copy gene on chromosome 19 and contains two polymorphic sites in codons 241 (G241R; Glu/Arg; exon 4) and 469 (K469E; Lys/Glu; exon 6) (13). ICAM-1 gene polymorphisms have been implicated in the susceptibility to a range of inflammatory diseases, including atherosclerosis and postprandial hypertriglyceridemia (14).

We hypothesized that genetic predisposition for up regulation of the expression of pro-inflammatory gene of ICAM-1 does not exist in patients with GSDI. Therefore, we investigated the Glu241Arg polymorphism in the ICAM-1 gene in patients with GSDI.

## Materials and Methods

A total of 35 patients with GSDI and 108 healthy control subjects were enrolled into the study. GSDI diagnosed via enzymatic and genetic analysis, received a diet consisting of high carbohydrate intake, moderate fat and protein restriction (10). Exclusion criteria included endocrine, cardiovascular disorders, familial hyperlipidemia and use of medication. All GSD patients from our outpatient clinic were approached for participation and age-matched controls were taken after the selection of individual patients. The subjects of control group were recruited randomly from the department of Pediatrics.

Examinations were performed 3 to 8 hours after meals for GSD type 1, and after 8 to 10 hours fasting for controls. Written consent was obtained from the patients or parents. The study was approved by the local ethics committee and conducted in accordance with the guidelines in the Declaration of Helsinki.

Lipid profiles were determined by colorimetric test using standardized procedures. Germline DNA was extracted from peripheral blood by Qiagen MiniBlood Purification System kits. Amplification was carried out on a GeneAmp PCR System 9700. Controls of known genotype were included for every set of digestions carried out. Genotype was determined using appropriate restriction enzymes [BSrGI (Biolab)]. The G-A transition polymorphism in exon 4 was determined by PCR-RFLP method using the following primers: forward 5-CCGTGGTCTGTTCCCTGTAC-3'; reverse 5'-GAA GGAGTCGTTGCCATAGG-3' (Figure1).

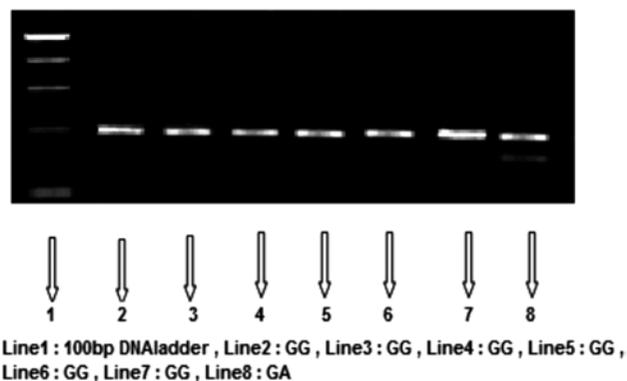
## Statistical Analysis

Statistical analysis was performed using the SPSS statistical package. The frequencies of the alleles and genotypes among the patient and control groups were determined and were then compared by  $\chi^2$  test. A p value of <0.05 was accepted for statistical significance. Odds ratios (ORs) were calculated, together with their 95% confidence intervals (CIs).

## Results

The basic characteristics and the fasting lipid profile of the patients enrolled into the study are summarized in Table I.

For the point mutation polymorphism located in ICAM-1 exon 4, which contains the information for codon 241, two alleles (i.e. allele G and allele A) and different genotypes (i.e. homozygotes GG/AA or heterozygote GA) were present (Figure 1). Table II shows the allele and genotype frequencies of the G241R ICAM-1 gene polymorphism in 35 cases of GSDI and 108 controls. In both groups, there was a strong predominance of allele G. Results have shown a significant decrease in A allele frequency (OR=0.8, 95CI% 0.7-0.9,  $p=0.03$ ) and a significant increase in GG genotype (OR=8.7,



**Figure 1.** G/A genotyping by PCR-RFLP method using BSrGI (Biolab) restriction enzymes

Parameters	Numbers (%) / Mean $\pm$ SD
Number	35
Gender (male/female)	16/18
Age (years)	7.2 $\pm$ 5.6
Consanguinity	31 (88%)
Hepatomegaly	35 (100%)
Hypoglycemia (blood level below 40 mg/dl)	30 (85%)
Liver biopsy (Glycogen deposition)	19 (54%)
Low enzyme activity (Glucose 6-phosphatase)	18 (51%)
High biotinidase activity	35 (100%)
Homozygous/compound heterozygous (G6PC, SLC37A4 gene) mutations	19(54 %)
Total Cholesterol (mg/dl)	204.5 $\pm$ 61.5
Triglyceride (mg/dl)	345.3 $\pm$ 36.6
HDL-Cholesterol (mg/dl)	43.0 $\pm$ 18.3
LDL-Cholesterol (mg/dl)	101.1 $\pm$ 54

Variable	GSD		Controls		p
<b>Allele</b>					
A	1/70	1.5%	27/216	12.5%	0.03
G	69/70	98.5%	189/216	87.5%	
<b>Genotype</b>					
AA	0/35	0%	0/108	0 %	0.01
GA	1/35	2.9%	27/108	25%	
GG	34/35	97.1%	81/108	75%	

95CI %1.2-62.1,  $p=0.01$ ) in GSDI patients compared to healthy controls. There was significant negative correlation between the ICAM genotype GG frequency in CSD group and triglyceride levels ( $r= -3.36$ ,  $p=0.00$ ).

## Discussion

Activation of the endothelium is a key event in the recruitment of cells of the immune system to the vessel wall. This requires the expression of adhesion molecules that play a key role in the development of atherosclerosis (15). Postprandial hypertriglyceridemia may represent an independent predictor of cardiovascular disease (1). Moreover, it has been shown that hypertriglyceridemia is associated with significant increase in ICAM levels (17). On the other hand, a considerable body of research undertaken in recent years has identified polymorphisms in many cytokine genes, especially within the upstream promoter sequences, which may be functionally relevant, because they may influence the levels of expression of these genes (18). Because inflammation is a consistent

feature of atherosclerosis and it does not present in patients with GSDI despite their dyslipidemic status, we speculated that up regulation of ICAM-1, one of the important molecules in inflammation cascade does not occur. ICAM-1 is a protein with a core of 55 kDa with five extracellular Ig-like domains (8,19) coded by the second to the sixth exons. The human ICAM-1 gene is a single copy gene located on chromosome 19p13 and is known to contain at least two polymorphic sites, situated in codons 241 (G241R) and 469 (K469E) (20,21). These two polymorphisms are single base mutations. At position 241, located in exon 4, the mutation GGG-AGG results in the exchange of glycine for arginine in the protein Ig-like domain 3. Similarly, at position 469, located in exon 6, the mutation AAG-GAG also leads to an amino acid change (lysine $\pm$ glutamine) in the Ig-like domain 5 (22). In this preliminary study we found positive association between ICAM-1 G241R GG genotype frequency and patients with GSD. The G allele frequency was increased significantly in GSD groups compared to control group. This study suggests that absence of alterations in the amino acid sequences of the immunoglobulin like domains of ICAM-1 may prevent an influence of interactions with the respective ligands and, thus, the duration and/or intensity of the inflammatory reaction. The functional role of these point mutations might be related to the altered amino acid coding sequence. In this regard, it is noteworthy that the exon 4 mutation is located in the domain which has been shown to be of importance in binding to the Mac-1 form of the leukocyte integrin (19). The interaction between Mac-1 and ICAM-1 makes an important contribution to leukocyte adhesion in the execution of immunological and inflammatory functions and may play a role in regulating localization of leukocytes (23). The mutation of glycine with arginine in the third domain of ICAM-1 might affect the adhesive function of ICAM-1. Indeed, the common allele G, which encodes for glycine, is maintained in the analogous position in different species thus supporting the importance of this site for the control of ICAM-1-mediated interactions (24). Therefore, it may contribute to the escape of hypertriglyceridemic patients with GSD I from atherosclerosis. Our study has several limitations. First of all, we did not measure serum ICAM-1 level. Secondly, the number of the patients is relatively small for a study aiming polymorphism analysis. Thirdly, we did not investigate the association between the ICAM-1 K469E polymorphism and atherosclerosis which might have been more informative.

In conclusion, this is the first report on the genetic reasons involved in the atherosclerotic escape of the dyslipidemic patients with GSDI. Further studies are required to investigate whether genetic factors determining the endothelial function in patients with GSDI are definitely responsible for the diminished pro-inflammatory and pro-atherogenic profiles.

## References

1. Lei KJ, Shelly LL, Pan CJ, Sidbury JB, Chou JY. Mutations in the glucose-6-phosphatase gene that cause glycogen storage disease type 1a. *Science* 1993; 262: 580-3.

2. Ubels FL, Rake JP, Slaets JPJ, Smit GPA, Smit AJ. Is glycogen storage disease 1a associated with atherosclerosis? *Eur J Pediatr* 2002; 161 Suppl 1: S62-4.
3. Wittenstein B, Klein M, Finckh B, Ullrich K, Kohlschütter A. Plasma antioxidants in pediatric patients with glycogen storage disease, diabetes mellitus, and hypercholesterolemia. *Free Radic Biol Med* 2002; 33: 103-10.
4. den Hollander NC, Mulder DJ, Graaff R, Thorpe SR, Baynes JW, Smit GP, Smit AJ. Advanced glycation end products and the absence of premature atherosclerosis in glycogen storage disease Ia. *J Inher Metab Dis* 2007; 30: 916-23.
5. Kalkan Uçar S, Coker M, Sözmen E, Goksen Simsek D, Darcan S. "A monocentric pilot study of an antioxidative defense and hsCRP in pediatric patients with glycogen storage disease type IA and III." *Nutr Metab Cardiovasc Dis* 2009; 19: 383-90.
6. Hackman A, Abe Y, Insull W Jr, Pownall H, Smith L, Dunn K, Gotto AM Jr, Ballantyne CM. Levels of soluble cell adhesion molecules in patients with dyslipidemia. *Circulation* 1996; 93: 1334-8.
7. Nappo F, Esposito K, Cioffi M, Giugliano G, Molinari AM, Paolisso G, Marfella R, Giugliano D. Postprandial endothelial activation in healthy subjects and in type 2 diabetic patients: role of fat and carbohydrate meals. *J Am Coll Cardiol* 2002; 39: 1145-50.
8. Hayflick JS, Kilgannon P, Gallatin WM. The intercellular adhesion molecule (ICAM) family of proteins. New members and novel functions. *Immunol Res* 1998; 17: 313-27.
9. Porsch-Oezçueruemez M, Kunz D, Kloer HU, Luley C. Evaluation of serum levels of solubilized adhesion molecules and cytokine receptors in coronary heart disease. *J Am Coll Cardiol* 1999; 34: 1995-2001.
10. Hajilooi M, Sanati A, Ahmadi A, Ghofraniha A, Massoud A. Circulating ICAM-1, VCAM-1, E-selectin, P-selectin, and TNF $\alpha$  in patients with coronary artery disease. *Immunol Invest* 2003; 32: 245-57.
11. Poston RN, Haskard DO, Coucher JR, Gall NP, Johnson-Tidey RR. Expression of intercellular adhesion molecule-1 in atherosclerotic plaques. *Am J Pathol* 1992; 140: 665-73.
12. Li D, Qu C, Dong P. The ICAM-1 K469E polymorphism is associated with the risk of coronary artery disease: a meta-analysis. *Coron Artery Dis* 2014; 25: 665-70.
13. Wawryk SO, Cockerill PN, Wicks IP, Boyd AW. Isolation and characterization of the promoter region of the human intercellular adhesion molecule-1 gene. *Int Immunol* 1991; 3: 83-93.
14. Norata GD, Grigore L, Raselli S, Redaelli L, Hamsten A, Maggi F, Eriksson P, Catapano AL. Post-prandial endothelial dysfunction in hypertriglyceridemic subjects: molecular mechanisms and gene expression studies. *Atherosclerosis* 2007; 193: 321-7.
15. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med* 2005; 352: 1685-95.
16. Ebenbichler CF, Kirchmair R, Egger C, Patsch JR. Postprandial state and atherosclerosis. *Curr Opin Lipidol* 1995; 6: 286-90.
17. Benítez MB, Cuniberti L, Fornari MC, Gomez Rosso L, Berardi V, Elikir G, Stutzbach P, Schreier L, Wikinski R, Brites F. Endothelial and leukocyte adhesion molecules in primary hypertriglyceridemia. *Atherosclerosis* 2008; 197: 679-87.
18. Bidwell J, Keen L, Gallagher G. Cytokine gene polymorphism in human disease: on-line databases. *Genes Immun* 1999; 1: 3-19.
19. Diamond MS, Staunton DE, Marlin SD, Springer TA. Binding of the integrin Mac-1 (CD11b/CD18) to the third immunoglobulin-like domain of ICAM-1 (CD54) and its regulation by glycosylation. *Cell* 1991; 65: 961-71.
20. Vora DK, Rosenbloom CL, Beaudet AL, Cottingham RW. Polymorphisms and linkage analysis for ICAM-1 and the selectin gene cluster. *Genomics* 1994; 21: 473-477.
21. Wenzel K, Ernst M, Rohde K, Baumann G, Speer A. DNA polymorphisms in adhesion molecule genes a new risk factor for early atherosclerosis. *Hum Genet* 1996; 97: 15-20.
22. Macchioni P, Boiardi L, Casali B, Nicoli D, Farnetti E, Salvarani C. Intercellular adhesion molecule 1 (ICAM-1) gene polymorphisms in Italian patients with rheumatoid arthritis. *Clin Exp Rheumatol* 2000; 18: 553-8.
23. van de Stolpe A, van der Saag PT. Intercellular adhesion molecule-1. *J Mol Med (Berl)* 1996; 74: 13-33.
24. Yang H. Analysis of ICAM-1 gene polymorphism in immunologic subsets of inflammatory bowel disease. *Exp Clin Immunogenet* 1997; 14: 214-25.