PLASMINOGEN ACTIVATOR INHIBITOR-1 (PAI-1) GENE (-675 4G/5G)
POLYMORPHISM ASSOCIATED WITH OBESITY AND VASCULAR RISK IN CHILDREN
PLASMINOGEN ACTIVATOR INHIBITOR-1 (PAI-1) GENE POLYMORPHISM ASSOCIATED WITH OBESITY AND VASCULAR RISK IN CHILDREN

Berberoğlu M,
Professor in Pediatric Endocrinology Department

Evlıyaoğlu O,
Pediatric Endocrinologist in Pediatric Endocrinology Department

Adıyaman P,
Associated Professor in Pediatric Endocrinology Department

Öcal G,
Professor in Pediatric Endocrinology Department

Ulukol B,
Associated Professor in Social Pediatrics Department

Şimşek F,
Pediatrician in Social Pediatrics Department

Şıklar Z,
Fellow in Pediatric Endocrinology Department

Törel A,
Fellow in Pediatric Endocrinology Department

Özel D,
PhD in Pediatric Molecular Genetics Department

Akar N.
Professor in Pediatric Molecular Genetics Department
Ankara University Faculty of Medicine, Department of Pediatric Endocrinology, Department of Pediatric Molecular Genetics, Department of Social Pediatrics.

**Corresponding Author:** Merih Berberoğlu, MD

**Address:** Ankara Universitesi Tıp Fakültesi
Çocuk Sağlığı ve Hastalıkları Ana Bilim Dalı 06100
Cebeci Ankara Turkey

e-mail : merihbtr@yahoo.com
Fax : +90-312-362 0581
Tel : +90-312 362 3030 / 6434

**Key words:** PAI-1 gene polymorphism, childhood obesity, vascular disease
ABSTRACT
Atherothrombotic complications in insulin resistance are partly attributed to impaired fibrinolysis caused by increased PAI-1 plasma levels. In addition 4G/5G promotor polymorphism of the PAI-1 gene may modulate PAI-1 transcription.

Objective: In this study PAI-1-675 4G/5G allele gene polymorphism and its relation with obesity in children were investigated.

Material and Method: The study participants were 133 apparently healthy nonobese subjects and 24 probable exogene obese without family history (Group I), 66 probable familial obese (Group II), 44 obese children who refered to the pediatric endocrinology department with any complication of obesity (Group III). Group I and Group II obese subjects were gathered with school based epidemiologic study.

Results: High level socioeconomic school had 19% obesity, whereas low level socioeconomic school had only 4% obesity. Frequencies of 4G/4G were 24.81%, 37.50%, 64.80% and 61.11% in the control, group I, II, and III respectively. 4G/4G gene polymorphism was more common among group II and group III and 5G/5G was more common among nonobese control subjects.
Carrying the 4G allele either in heterozygous or homozygous state increases the vascular disease risk (odds ratio [OR]: 6.10, confidence interval [CI] 95% 1.64-22.90) in the presence of family history for obesity and metabolic syndrome (OR: 4.48, CL: 95% 1.26-15.82) in obese subjects.
In acanthosis nigricans, elevated IRHOMA, hypertriglyceridemia and elevated atherogenic index had been shown to have more remarkable 4G/4G genotype frequencies compared to other features of the metabolic syndrome.
In conclusion, the high increasing prevalence of childhood obesity in high socioeconomic status is associated with health risks. Carrying 4G/4G genotype in the PAI-1 gene is more common than the 4G/5G and 5G/5G genotype in obese children with family history of obesity and cardiovascular disease or type 2 diabetes and the patients who had any metabolic syndrome finding. These patients can be at an increased risk of developing vascular disease. Acanthosis nigricans, high IRHOMA, hypertriglyceridemia and high aterogenic index can also reflect the high risk of vascular disease in metabolic syndrome.
INTRODUCTION

Obesity is a chronic condition of complex aetiology and rapidly growing health problem in the westernised countries. Obesity is clearly not one disease but is multifactorial, involving genetic, metabolic and behavioral factors. Obese patients are at risk for the development of vascular disease (1,2). Different studies have investigated the association between fibrinolytic parameters and the insulin resistance syndrome (IRS) in obese subjects (3,4,5). Central obesity, hypertension, glucose intolerance, hyperinsulinemia, dyslipidemia with elevated triglyceridemia lowered high lipoprotein cholesterol (HDL) concentration and an increased proportion of small dense lipoproteins are markers of insulin resistance. These IRS patients have higher values of fibrinogen, factor VII, VIII, Von Willebrand factor and Plasminogen Activator Inhibitor (PAI) compared to nonobese subjects (3).

Recently it has been demonstrated that the adipocyte itself is able to produce PAI-1 possibly explaining the high levels found in obesity (5). Plasminogen Activator Inhibitor-1 (PAI-1) is the primary inhibitor of plasminogen activating that limits the fibrinolytic process. Atherothrombotic complications in insulin resistance syndrome are partly attributed to impaired fibrinolysis caused by increased PAI-1 plasma levels. Disruption of the PAI-1 gene reduces the adiposity of the obese ob/ob mice suggested that the PAI-1 gene can control fat mass (6). However the mechanism of action is not yet known.

A functional polymorphism in the promotor region of the PAI-1 gene (-675 4G/5G) affects the binding of nuclear proteins regulating the transcription of the gene (7). The 4G/5G polymorphism is associated with feature of the metabolic syndrome in some, but not all populations (8). Genetic and enviromental factors both regulate the
plasma PAI-1 levels. Elevated plasma PAI-1 levels are associated with the 4G allele of a 4G/5G insertion/deletion polymorphism located in the promoter region 675 bp upstream from the transcription start sequence of the PAI-1 gene (9,10). Vascular risk of carrying 4G allele of PAI-1 gene was found to be controversial in the previous studies (8-11).

We aimed to investigate to the role of the 4G/5G polymorphism in determining susceptibility, outcome and complications of obesity and metabolic syndrome.

**MATERIAL AND METHOD**

**Patients and Controls:**

The study participants were 133 apparently healthy non-obese subjects and 24 probable exogene obese without family history (Group I), 66 probable familial obese (Group II), 44 obese children who refered to the pediatric endocrinology department with any complication of obesity (Group III) (Figure 1). Group I and group II obese subjects were gathered with school based epidemiologic study screening total of 3024 children. Exogenous obese subjects (Group I) were gathered from a high level socioeconomic school who had not any familial history of obesity and vascular disease. Familial obese (Group II) subjects were gathered from a low socioeconomic school who had familial history of obesity and vascular disease. In high level socioeconomic school 757 children and in low socioeconomic school 2267 children were included in the screening program. The questionnaire form, which included the assessment of nutrition behavior, daily life style and presence of family histories of hypertension, obesity, diabetes mellitus, were obtained in all school based obese subjects.
Obesity was defined as a body mass index (BMI) percentile greater than 95 (12). The control groups’ BMI percentile was less than 80.

Controls were (age range 34.50 ± 8.50 years) consecutively selected among unrelated healthy subjects without personal and familial history of vascular disease. Mean ages of the group I, II, III were 13.54 ± 3.02, 11.75 ± 3.18 and 11.22 ± 2.66 respectively.

The clinical characteristics of the groups were given at Table I. Metabolic disturbances such as hyperlipidemia, insulin resistance, polycystic over syndrome (PCOS), hypertension, acantosis nigricans were evaluated in group III subjects.

**Laboratory Methods:**

The plasma glucose concentration was determined with a sigma glucose kit (SIGMA, USA) and plasma insulin levels were measured by radioimmunoassay (RIA) with a DSL kit (Diagnostic System Laboratories, Webster, Texas,USA). Plasma lipid concentrations were assayed after a 12h fasting period colorimetrically using a Tecnico RA-XT auto analyzer.

Evaluation of the children’s hyperinsulinemia included fasting plasma insulin and the 120. minute oral glucose tolerance test result (1.75g glucose/kg body weight, maximum 75g). Blood samples were obtained before and 30, 60, 120, 180 minutes after the glucose load. Fasting insulin levels above 20µIU/ml or 120 minute (post OGGT) insulin levels above 150µIU/ml were accepted as hyperinsulinemia (13).

Useful parameters as homeostasis model assessment of insulin resistance (HOMA-IR) and fasting glucose/fasting insulin ratio (G/I), in assessing insulin resistance (14) were calculated using the following formulae:

HOMA-IR: \[ \frac{\text{fasting insulin (µ IU/ml)} \times \text{fasting glucose (nmol/L)}}{22.5}. \]

G/I: fasting glucose (mg/dl) / fasting insulin (µIU/ml).
Hypertension was defined as a blood pressure percentile greater than 90 (15). Triglyceride above 120mg/dl and aterogenic index above 4 were accepted as hyperlipidemia. Hirsutism, menstruel disturbances, hyperandrogenemia and specifically an augmented 17-OHP response to gonadotropin releasing hormon agonist and typical apperance at overian ultrasonography revealed PCOS. The local ethics committee of Ankara University Faculty of Medicine approved the study and written informed consent was obtained from all participants.

DNA was extracted by conventional methods and polymerase chain reaction of the PAI-1 4G/5G polymorphism was performed according to previously described method by using primers 5’-CACAGAGAGAGTCTGGCCACGTC3’ and 5’-CCAACAGAGGACTCTTGTCCTCTTTGTC3’. Amplification was performed for 35 cycles with anrealing temprature of 60°C (Ericomp, USA). Amplified 98/99 bp product was digested with Bse I (Fermentas, Vilnius, Lithuania) at 55°C and subjected to 6% polyacrylamide gel electroforesis (16).

**Statistical Analysis:**

Odds ratios were determined by logistic regression analysis. Values were expressed as mean ± SD and were compared by analysis of variance.

**RESULTS**

High level socioeconomic school had 19% obesity. Whereas low level socioeconomic school had only 4% obesity (Figure 1).

The distrubution of the metabolic syndrome findings and clinical characteristics of group III were given at Figure 2 and Table II.
Frequencies of 4G/4G were 24.81%, 37.50%, 64.80% and 61.11% in the control, group I, II, and III respectively. Predicted gene polymorphism distribution in all groups were given at Table III.

4G/4G gene polymorphism was more common among group II and group III and 5G/5G was more common among non-obese control subjects.

The odds ratio and confidence limits of the group’s were given at Table IV. The patients who had a positive family history for obesity (Group II) showed high risk compared to exogeneous obese patients (Group I). By contrast, group II patients had similar genotype compared to the patients who had clinical findings of metabolic syndrome (Group III).

Our data indicated that carrying the 4G allele either in heterozygous or homozygous state increases the vascular disease risk (odds ratio [OR]: 6.10, confidence interval [CI] 95% 1.64-22.90) in the presence of family history for obesity and metabolic syndrome (OR: 4.48, Cl 95% 1.26-15.82) in obese subjects.

Group III had more vascular risk compared to exogeneous obese subjects (Group I) by carrying 4G/4G genotype like as group II.

Acanthosis nigricans, elevated IRHOMA, hypertriglyceridemia and elevated atherogenic index had been shown to have more remarkable 4G/4G genotype frequencies compared to other features of the metabolic syndrome (Table V).

**DISCUSSION**

Focusing on the mediterranean part of Europe, according to the largest study performed in Spain by Moreno et all obesity rates has increased from 6.4 to 14.4 % in male children and from 10 to 17.7 % in females (17). In France, during the 90’s the
same rates have increased to 10-12 % (18). In Italy in a study among adolescents obesity was at 9 % in males and 10% in females (19).

There is no nation-wide data available in our country. The regional but representative data of the present study showed that high socioeconomical status contributed largely to the increase of obesity prevalance.

Obesity has major risk factors for chronic diseases such as type 2 diabetes, cardiovascular disease, hypertension, stroke. The combination of obesity, hyperinsulinemia, hyperlipidemia and hypertension is referred to as syndrome X, or metabolic syndrome. Our data showed that the group III patients who had metabolic syndrome are at major risk to develop disease in the future. This selected group of patients does not reflect the general population but its obvious that the high prevalence of insulin resistance and hyperinsulinemia indicated much more focus and early intervention. For this purpose more exact methods are needed to determine whether the individual child is healthy or at any risk. Besides, preventive strategies are urgently needed to stop the increasing obesity in our region.

As it is well known that PAI-1 4G allele carriers have potentially decreased fibrinolytic activity constituting an additional prothrombotic factor (9,10,16).

Our data indicated that carrying PAI-1 4G allele either in heterozygous or homozygous state create a vascular thrombotic risk in the presence of obesity either with metabolic syndrome or history of familial obesity compared to control nonobese subjects and exogeneous obese subjects. As previous data indicated that, this study also found the PAI-1 gene polymorphism is strongly associated with obesity (8). Whereas the 5G allele was more common among the control group. Furthermore this study showed that the risk of having a vascular disease in IR syndrome and familial obesity instead of exogeneous obesity for those having 4G allele was four or six fold
increased. Recently Hoffstedt et al also showed that the 4G allele had a strong association with obesity and the risk of being obese was increased by two fold in carriers of the 4G allele (8). However this study didn’t allow an analysis of the interaction between PAI-1 genotype and metabolic syndrome.

Festa’s study recently demonstrated that in a large cohort of healthy nondiabetic subjects, patients who developed an incident diabetes within 5 years presented higher levels of PAI-1 as well as other acute phase proteins at baseline than converters (5,20) Our findings together with this study suggest that PAI-1 genotyping contribution to this phenomenon may play a role in the developing vascular disease and in type 2 diabetes. Juhan-Vague suggested that PAI-1 inhibitors could be a potential target for therapeutic intervention to decrease the risk of vascular disease (5).

Moreover, a recent study suggested that PAI-1 might not merely increase in response to obesity and insulin resistance, but might have a direct causal role in obesity and insulin resistance, inhibition of PAI-1 might provide a novel anti-obesity and anti-insulin resistance treatment (21).

Interestingly, the obesity is associated with feature of the metabolic syndrome in some but not all populations. Genetic and environmental factors both regulate the plasma PAI-1 levels. Elevated PAI-1 levels associated with the 4G allele is probably, the important finding to detect risk of metabolic syndrome in obese children before the existing findings of metabolic syndrome. Our results suggest that exogeneous obese patients do not have a high risk of vascular disease if they don’t carry 4G allele.

Carrying the 4G/4G genotype in the obese patients with the family history of obesity and cardiovascular disease or type 2 diabetes mellitus and the obese patients who
had any metabolic syndrome finding was more common than the 4G/5G and 5G/5G genotyped patients. Carrying this genotype can increase the risk of vascular disease. These two groups had similar genotype and ORS. Owing a positive family history is as important as having a clinical sign of metabolic syndrome. During the evaluation of an obese child, detailed family history must be obtained and could gather the candidate obese subjects to evaluate for metabolic syndrome and vascular disease. Obesity is clearly not one disease, but is multifactorial, involving genetic, metabolic and behavioral factors. Actually the therapeutic interventions such as dietary management and exercise programs are more effective prior the signs of metabolic syndrome.

In this study 4G/4G PAI-1 gene polymorphism frequencies were found to be high in the cases who had acanthosis nigricans, high IRHOMA values, hypertriglyceridemia and the high atherogenic index. Evaluation of 4G/4G polymorphism in obese children with metabolic syndrome has given clues to highlight whether which finding reflect the high risk of vascular disease. However the number of subjects were too few to allow a reliable analysis of the differences between groups for genotype and allele frequencies. Studies with large series are needed to delineate the contribution of PAI-1 genotype to metabolic syndrome and vascular disease.

**In conclusion**, the high and fast increasing prevalence of childhood obesity in high socioeconomic status is associated with health risks. Carrying 4G/4G genotype in the PAI-1 gene is more common than the 4G/5G and 5G/5G genotype in obese children with family history of obesity and cardiovascular disease or type 2 diabetes and the patients who had any metabolic syndrome finding. These patients can be at
an increased risk of developing vascular disease. Acanthosis nigricans, high IRHOMA, hypertriglyceridemia and high aterogenic index can also reflect the high risk of vascular disease in metabolic syndrome.

Acknowledgement

This study was supported by the “Ankara University Research Found”. 
Table I: The clinical characteristics of the groups

<table>
<thead>
<tr>
<th></th>
<th>GROUP I</th>
<th>GROUP II</th>
<th>GROUP III</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (year)</strong></td>
<td>13.54 ± 3.02</td>
<td>11.75 ± 3.18</td>
<td>11.22 ± 2.66</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td>42 %G</td>
<td>55%G</td>
<td>39%G</td>
</tr>
<tr>
<td><strong>RBMI</strong></td>
<td>140.58 ± 17.21</td>
<td>129.9 ± 8.36</td>
<td>147.61 ± 16.09</td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td>28.30 ± 4.66</td>
<td>24.52 ± 3.16</td>
<td>27.78 ± 4.18</td>
</tr>
<tr>
<td><strong>Metabolic Syndrome</strong></td>
<td>Not evaluated</td>
<td>Not evaluated</td>
<td>89.48%</td>
</tr>
</tbody>
</table>
Table II: Clinical characteristics of the Group III obese patients

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>44</td>
</tr>
<tr>
<td>Age (decimal year)</td>
<td>11.22±2.66</td>
</tr>
<tr>
<td>Gender</td>
<td>15 G</td>
</tr>
<tr>
<td>Fasting Glucose (mg/dl)</td>
<td>91.75±12.53</td>
</tr>
<tr>
<td>OGTT 120 min glucose (mg/dl)</td>
<td>101.67±18.53</td>
</tr>
<tr>
<td>Frequency of fasting glucose intolerance</td>
<td>7.89 %</td>
</tr>
<tr>
<td>Frequency of glucose intolerance</td>
<td>2.63 %</td>
</tr>
<tr>
<td>Fasting insulin (µU/ml)</td>
<td>18.98±10.87</td>
</tr>
<tr>
<td>OGTT 120 min insulin (µU/ml)</td>
<td>62.40±49.6</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>4.48±3.11</td>
</tr>
<tr>
<td>Frequency of high HOMA-IR (&gt;3,5)</td>
<td>50 %</td>
</tr>
<tr>
<td>Atherogenic index (Col/HDL)</td>
<td>3.77±0.67</td>
</tr>
<tr>
<td>Frequency of high atherogenic index (&gt;4)</td>
<td>26.31 %</td>
</tr>
<tr>
<td>Frequency of achantosis nigricans</td>
<td>67.56 %</td>
</tr>
<tr>
<td>G/l</td>
<td>6.76±4.8</td>
</tr>
<tr>
<td>Frequency of G/l &lt;6</td>
<td>59 %</td>
</tr>
<tr>
<td>Trygliceridemia (mg/dl)</td>
<td>119.2±49.16</td>
</tr>
<tr>
<td>Hypertriglyceridemia</td>
<td>42.10 %</td>
</tr>
<tr>
<td>Hypertension</td>
<td>13.15 %</td>
</tr>
<tr>
<td>Hepatosteatosis</td>
<td>10.52 %</td>
</tr>
<tr>
<td>Frequency of PCOS in girls</td>
<td>6.6 %</td>
</tr>
<tr>
<td>Frequency of premature pubarche</td>
<td>7.89 %</td>
</tr>
<tr>
<td>Family history of obesity</td>
<td>76.31 %</td>
</tr>
<tr>
<td>Family history of diabetes mellitus</td>
<td>47.36 %</td>
</tr>
<tr>
<td>Family history of atherosclerosis</td>
<td>21.05 %</td>
</tr>
</tbody>
</table>
Table III: The predicted gene polymorphism distribution in all groups

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>GROUP I</th>
<th>GROUP II</th>
<th>GROUP III</th>
</tr>
</thead>
<tbody>
<tr>
<td>5G/5G</td>
<td>33</td>
<td>11</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td>5G/4G</td>
<td>67</td>
<td>4</td>
<td>29</td>
<td>8</td>
</tr>
<tr>
<td>4G/4G</td>
<td>33</td>
<td>9</td>
<td>24</td>
<td>22</td>
</tr>
<tr>
<td>n</td>
<td>133</td>
<td>24</td>
<td>66</td>
<td>36</td>
</tr>
<tr>
<td>Frequency of 4G/4G (%)</td>
<td>24.81</td>
<td>37.5</td>
<td>64.80</td>
<td>61.11</td>
</tr>
<tr>
<td>Frequency of 4G allele (%)</td>
<td>50</td>
<td>45.8</td>
<td>58.34</td>
<td>72.23</td>
</tr>
</tbody>
</table>
Table IV: The odds ratio and confidence limits of the group’s

<table>
<thead>
<tr>
<th>PAI-I</th>
<th>Group I</th>
<th>Group II</th>
<th>Odd's Ratio</th>
<th>Confidency Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>5G/5G</td>
<td>11</td>
<td>13</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>4G/5G</td>
<td>4</td>
<td>29</td>
<td>6,13</td>
<td>(1,64-22,9)</td>
</tr>
<tr>
<td>4G/4G</td>
<td>9</td>
<td>24</td>
<td>2,25</td>
<td>(0,74-6,84)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PAI-I</th>
<th>Group II</th>
<th>Group III</th>
<th>Odd's Ratio</th>
<th>Confidency Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>5G/5G</td>
<td>13</td>
<td>6</td>
<td>1</td>
<td>(0,17-17,42)</td>
</tr>
<tr>
<td>4G/5G</td>
<td>29</td>
<td>8</td>
<td>0,59</td>
<td>(0,64-6,13)</td>
</tr>
<tr>
<td>4G/4G</td>
<td>24</td>
<td>22</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PAI-I</th>
<th>Group I</th>
<th>Group III</th>
<th>Odd's Ratio</th>
<th>Confidency Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>5G/5G</td>
<td>11</td>
<td>6</td>
<td>1</td>
<td>(0,77-17,42)</td>
</tr>
<tr>
<td>4G/5G</td>
<td>4</td>
<td>8</td>
<td>3,66</td>
<td>(1,26-15,82)</td>
</tr>
<tr>
<td>4G/4G</td>
<td>9</td>
<td>22</td>
<td>4,48</td>
<td></td>
</tr>
</tbody>
</table>
Table V: 4G/4G allele frequencies (%) according to clinical findings of the metabolic syndrome

<table>
<thead>
<tr>
<th></th>
<th>4G/4G(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acanthosis Nigricans</td>
<td>52.17</td>
</tr>
<tr>
<td>IR homa &gt;3.5</td>
<td>57.14</td>
</tr>
<tr>
<td>Triglyceride&gt;120mg/dl</td>
<td>56.25</td>
</tr>
<tr>
<td>Atherogenic Index &gt;4</td>
<td>75.00</td>
</tr>
</tbody>
</table>
757 subjects were scanned
High Socioeconomical school

2267 subjects were scanned
Low Socioeconomical school

GROUP I (n:24)
Obese (19%)
Normal (81%)

GROUP II (n:66)
Obese (4%)
Normal (96%)

GROUP III (n:44)

TOTAL 266 SUBJECT

GROUP I (ns24)
GROUP II (ns66)
GROUP III (ns44)
Obese (19%) Normal (81%)
Obese (4%) Normal (96%)

p < 0.01

757 subjects were scanned
High Socioeconomical school

2267 subjects were scanned
Low Socioeconomical school
Fasting intolerance
Premature pubarche
Hypertension
PCOS

Glucose/insulin <6
HOMAIR >3.5
Achontosis nigricans
Atherogenic index >4

Hypertryglycemia
Glucose intolerance

67.56%
26.31%
42.1%
59.4%
7.89%
2.63%
7.89%
6.6%
LEGENDS

Figure 1: The scheme of the draft of the study groups.

Figure 2: The distribution of metabolic syndrome findings in Group III
REFERENCES

1. Reaven G. Syndrome X, 10 years after Drugs 1999; 58:19-20

2. Isomaa B; Almgren P; Tuomi T; Forsen B; Lahti K; Nissen M; Taskinen MR; Groop L. Cardiovascular morbidity and mortality associated with the metabolic syndrome. Diabetes Care 2001; 24(4) : 683-689.


