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K121Q Polymorphism of the *ENPP1* Gene is Related to Acute Coronary Syndrome in Ukrainian Patients with Normal but not Enhanced Body Mass Index

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Abstract: Ectonucleotide Pyrophosphatase Phosphodiesterase 1 (*ENPP1*) is a class II membrane glycoprotein with two unrelated properties: It can hydrolyze extracellular nucleotides and downregulate insulin receptor signaling. The present study was carried out to investigate whether common single-nucleotide polymorphism K121Q (rs1044498) of the *ENPP1* gene is associated with Acute Coronary Syndrome (ACS) in the representatives of Ukrainian population and to assess if the risk depends on gender and Body Mass Index (BMI). A total 228 DNA samples (118 ACS patients and 110 control subjects) were genotyped for the polymorphism by PCR and restriction fragment length polymorphism method. No associations between the K121Q polymorphisms and ACS were found neither on the whole nor taking into account the gender. However, in the persons with BMI <25 kg/m^2 but not with overweight, genotypes with the minor allele (KQ + QQ) were significantly associated with ACS (OR 3.939, 95% CI 1.148-13.524, P = 0.029). Genotypes with minor allele can be a possible genetic risk factor for ACS in persons with BMI <25 kg/m^2. It is likely that these genotypes affect ACS not by the traditional risk factors (overweight/obesity, insulin resistance and type 2 diabetes) but by direct or undirect influence on pathologic processes in the wall of the coronary vessels (atherosclerosis and arterial calcification).

Keywords: Ectonucleotide Pyrophosphatase Phosphodiesterase 1 (*ENPP1*), Acute Coronary Syndrome, Polymorphism, Body Mass Index

Introduction

Ectonucleotide Pyrophosphatase Phosphodiesterase 1 (*ENPP1*), also known as plasma cell membrane glycoprotein 1 (PC-1), belongs to a class II membrane glycoprotein which is widely expressed in many organs and tissues of the human and animal organisms (liver, skeletal muscle, heart, brain, kidney, lung, adipose tissue, etc.). Nowadays, the biological role of *ENPP1* is not fully understood, but there are two groups of evidences concerning the ENNP1 significance in pathogenesis of some pathological processes and diseases.

The first group is about the ability of *ENPP1* to influence insulin sensitivity by downregulating insulin receptor signaling (Dong *et al*., 2005). It was shown that over-expression of *ENPP1* inhibits tyrosine kinase activity with subsequent diminishing insulin receptor autophosphorylation in various cells (Maddux and Goldfine, 2000). This property of *ENPP1* is considered to be in association with insulin resistance and type 2 diabetes (Goldfine *et al*., 2007).

The second group of data is related to the processes of ectopic calcification. It is known that inorganic Pyrophosphate (PPi) is one of the most important inhibitor of soft tissues mineralization (Abedin *et al*., 2004; Shao *et al*., 2006). The main way of PP_i generating is hydrolysis of extracellular nucleoside

triphosphates, particular ATP, due to *ENPP1* specific enzymatic activity (Johnson *et al*., 2005; Towler, 2005). When the activity of ENNP1 falls off the formation of PP_i is disturbed and calcification of arteries may develop in many cases. The massive arterial calcification is the most impressive feature in genetically knocked out mice (*ENPP1* -/-) and in humans who have defective *ENNP1* gene (Johnson *et al*., 2005).

As a general rule, Acute Coronary Syndrome (ACS) is the consequence of atherosclerotic lesions appearing in arterial walls. It is well known that one of the main risk factors of atherosclerosis is type 2 diabetes which is frequently and tightly associated with insulin resistance. On the other hand, the coronary arteries calcification, as shown in many studies, is an adverse prognostic feature with regard to myocardial infarction in patients with atheromatous plaques (Lehto *et al*., 1996).

Taking into consideration the stated above, a relation of various Single-Nucleotide Polymorphisms (SNPs) of *ENPP1* gene to ACS is of great interest. The most widely investigated *ENPP1* SNP in genotype-phenotype association studies is the polymorphism K121Q. In this SNP located in exon 4, a lysine (K) is substituted by a glutamine (Q) at codon 121 (Pizzuti *et al*., 1999).

There are some studies in which association of the *ENPP1* K121Q polymorphism with early onset of coronary artery disease in Caucasians has been investigated (Endler *et al*., 2002; Bacci *et al*. 2005), but the data obtained in various ethnic groups remain controversial (Chen *et al*., 2006).

The aim of the present study was to perform a case-control study on representatives of the Ukrainian population in order to assess the possible association of the *ENPP1* K121Q polymorphism with ACS in subjects of both genders who had normal and overweight levels of Body Mass Index (BMI).

Materials and Methods

Subjects

The study recruited 118 ACS patients (78% men and 22% women) from 32 to 78 years of age (mean age [± SE] 55.9±0.89) admitted to Sumy Clinical Hospital No.1.

Diagnosis of ACS was established on the basis of clinical, electrocardiography and biochemical examinations according to the recommendations of WHO experts and also according to recommendations of European and American cardiologic societies (Antman *et al*., 2004; Thygesen *et al*., 2007). Patients with hereditary and congenital diseases, severe metabolic pathologies including a severe form of diabetes mellitus, marked renal and liver failures, deficiencies of the haemostatic system, oncology and systemic pathologies, chronic heart failure of IIB-III stage, true cardiogenic shock were excluded from the study group.

The control group consisted of 110 clinically healthy individuals with the absence of cardio- and cerebrovascular pathologies, as confirmed by medical history, ECG and measurement of arterial pressure and biochemical data. The study had been previously approved by the Ethic Committee of the Medical Institute of Sumy State University. Appropriate informed consent was obtained from all patients and control subjects. The participants were unrelated Ukrainian people from the northeastern region of Ukraine.

The subjects of both groups were divided into subgroups by gender and by BMI (BMI<25 kg/m^2 and ≥25 kg/m^2).

Blood sampling for genotyping was performed under sterile conditions into 2.7 ml tubes (SMonovette [Sarstedt, Germany]) containing EDTA potassium salt as an anticoagulant, samples were frozen and stored at -20°C.

Genotyping of SNP

DNA for genotyping was extracted from the venous blood using commercially available kits (Isogene Lab Ltd, Russia) according to the manufacturer's protocol. To identify *ENPPI* K121Q polymorphism (rs1044498) the Polymerase Chain Reaction (PCR) with subsequent Restriction Fragment Length Polymorphism (RFLP) analysis was performed. Specific region of the ENPPI gene was amplified using a pair of specific primers: Upstream (sense)-5' CTGTGTTCACTTTGGACATGTTG 3' and downstream (antisense)-5' GACGCTGGAAGATACCAGGCTG 3'. Primers were provided by Metabion (Germany). PCR was performed for 33cycles in a 25 µL volume containing 50-100 ng of DNA, 5 µL 5X PCR-buffer, 1.5 mM magnesium sulfate, 200 µM of each dNTP, 15pM of each primer and 0.75U of *Taq* DNA polymerase (Thermo Scientific, USA). PCR was carried out in a termocycler GeneAmp PCR System 2700 (Applied Biosystems, USA). Six microlitres (6 µL) of the PCR products (238bp) were subjected to digestion with 5U *Eco47I (AvaII)* (Thermo Scientific, USA) and incubated at 37°C for 18 h. In case of the presence of cytosine at the position 48213 of the exon 4, *Eco47I* restriction enzyme produces two fragments of 148 and 90bp in length. Substitution of cytosine for adenine prevents restriction and the amplified fragment of the exon 4 (238bp) can not be cleaved. The restriction fragments were separated by electrophoresis and analysed on an ethidium bromide-stained 2.5% agarose gel visualized using ultraviolet transillumination.

Statistical Analysis

The normal distribution and homogeneity of variances were tested before further statistical analyses. The comparison of variables between the groups of genotypes was performed using two-tailed Student's *t*-test. The χ2-test was used for comparison of the allele and genotype frequencies between different studied groups and subgroups. Odds ratio was evaluated by using the logistic regression method. The differences were considered statistically significant with a P-value <0.05. All statistical analyses were performed using the Statistical Package for Social Science program (SPSS for Windows, version 17.0, SPSS Inc., Chicago, IL).

Results

The clinical characteristics of 118 patients with ACS and 140 healthy controls are summarized in Table 1. No differences between the groups were noted with respect to sex, age or body mass index. Atherogenic risk factors (including cigarette smoking, hypertension, total cholesterol and glucose concentration) were significantly more prevalent in the ACS patient group.

In Figure 1 the results of RFLP analysis of *ENPP1* K121Q polymorphism are demonstrated. In both groups we studied, the genotype distributions of the *ENPP1* K121Q polymorphism were in Hardy-Weinberg equilibrium. The minor allele frequencies of SNP were not significantly different between the ACS (0.18) and control (0.12) groups. These frequencies were comparable with other studies using the same SNP in populations of European descent (Bottcher *et al*., 2006; Morandi *et al*., 2009).

For genotype case-control analysis we took into consideration two subgroups (KK and KQ+QQ) since there were very few minor allele homozygotes QQ both in ACS (3 individuals) and in control (0 subjects) groups. As shown in Table 2, major allele homozygotes KK and carriers of minor allele KQ+QQ were detected in 66.9 and 33.1% of the ACS group, respectively (control group: 75.5 and 24.5%). The differences in the distribution of allelic variants between the ACS and control groups were not statisticaly significant. Such conclusion was true when subjects of both groups were divided into subgroups by gender but not by BMI.

Using logistic regression analysis (Table 3), it was estimated that in persons with BMI <25 kg/m^2, carrying of minor allele (KQ+QQ) was significantly associated with ACS (OR=3.939; 95% CI, 1.148-13.524, P = 0.029). The same could not be said about individuals with overweight (BMI≥25 kg/m^2).

Discussion

In the present study, we explored associations between genetic variation in the *ENPP1* gene and ACS risk. Analyzing the SNP of this gene we found that K121Q polymorphism in exon 4 was associated with ACS in the representatives of Ukrainian population only with normal (BMI<25 kg/m^2), but not enhanced weight. The risk of ACS in not-overweight persons who were carriers of minor Q-allele was 3.9 fold greater as compared with main K-allele homozygotes.

A functional missense DNA polymorphism in exon 4 causes an amino acid change from lysine to glutamine at codon 121 (K121Q) (Pizzuti *et al*., 1999). Studies in vitro have shown that the Q variant of *ENPP1* binds insulin receptors more strongly than the K variant and reduces insulin receptor autophosphorylation (Costanzo *et al*., 2001). It is therefore a stronger inhibitor of insulin signaling.

In this connection wide range of case-control studies were conducted to asses the possible association between the *ENPP1* gene K121Q polymorphism and (1) insulin resistance, (2) Type 2 Diabetes Mellitus (T2DM) and (3) overweight/obesity in many Caucasian and not-Caucasian populations (Goldfine *et al*., 2007). Although the published results are rather contradictory, it can be concluded that in many cases there are positive associations between Q variant of *ENPP1* gene and aforementioned pathological conditions.

All of these conditions are considered to be tightly pathogenetic connected with atherosclerotic process which is the main cause of ACS. This should be taken into account when studying relationship of the *ENPP1* gene SNPs to cardiovascular diseases.

There is evidence suggesting that the Q allele is associated with an increased risk of earlier onset of myocardial infarction (Endler *et al*., 2002; Bacci *et al*., 2005). This association may be secondary to the effect of the Q allele on insulin resistance, T2DM and overweight/obesity which all predispose to atherosclerosis (Fig. 2).

In our study, we showed that *ENPP1* gene K121Q polymorphism is related to ACS only in nondiabetic patients with normal weight and fasting glucose levels. It suggested the possible impact of this SNP on ACS not only by overweight/obesity and T2DM, but also by processes witch cause coronary insufficiency, i.e., atherosclerosis and arterial calcification.

The influence of *ENPP1* overexpression on arterial wall may be in two different ways (Fig. 3). On the one hand, downregulating insulin receptor signaling by *ENPP1* leads to insulin resistance and contribute to atherosclerotic lesions development. On the other hand, enhanced enzymatic activity of *ENPP1* should increase the formation of PP_i which is one of the most potent anticalcinogenic factor that can prevent arterial wall calcification.

It is yet not known how K121Q polymorphism impacts on enzymatic feature of *ENPP1*. This is a question that needs to be under further investigation.

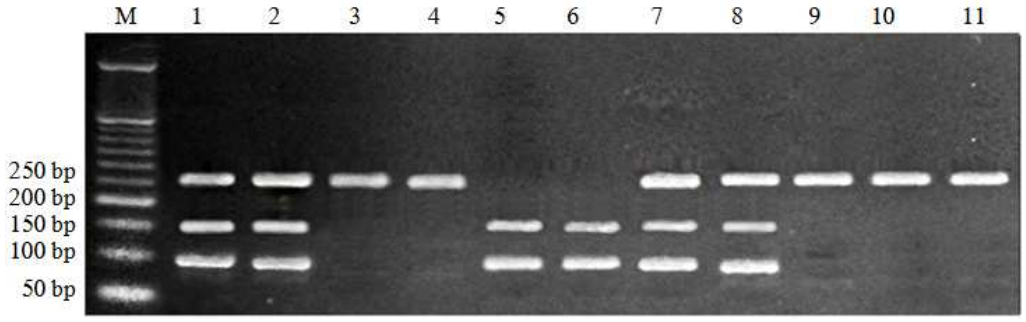

Fig 1. Analysis of *ENPP1* K121Q polymorphism. Results of restriction fragment electrophoresis of polymerase chain reaction amplification products. M-molecular marker (bp-base pairs), lanes 3, 4, 9, 10, 11 (KK genotype); lanes 1, 2, 7, 8 (KQ genotype); lanes 5, 6-(QQ genotype)

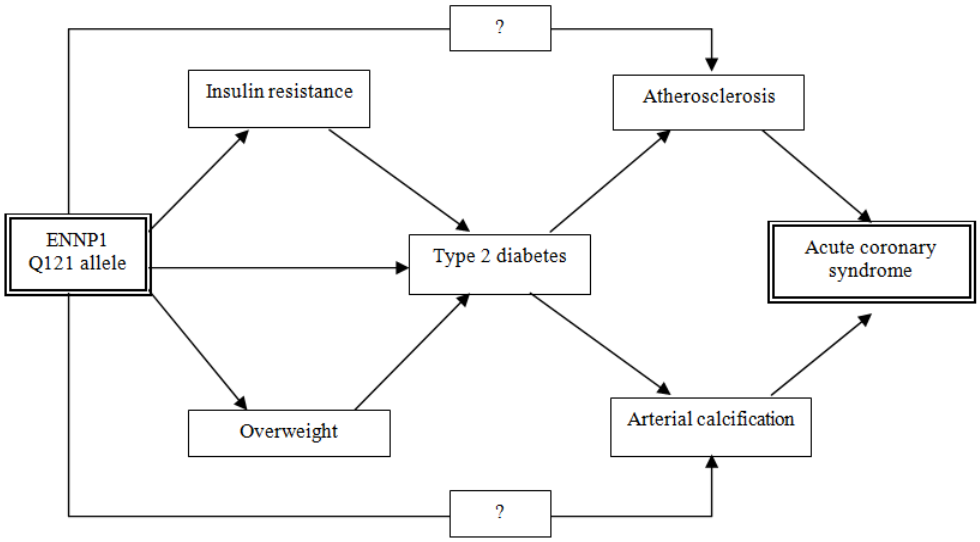

Fig. 2. Possible ways of ENNP1 Q121 allele impact on the pathogenesis of acute coronary syndrome

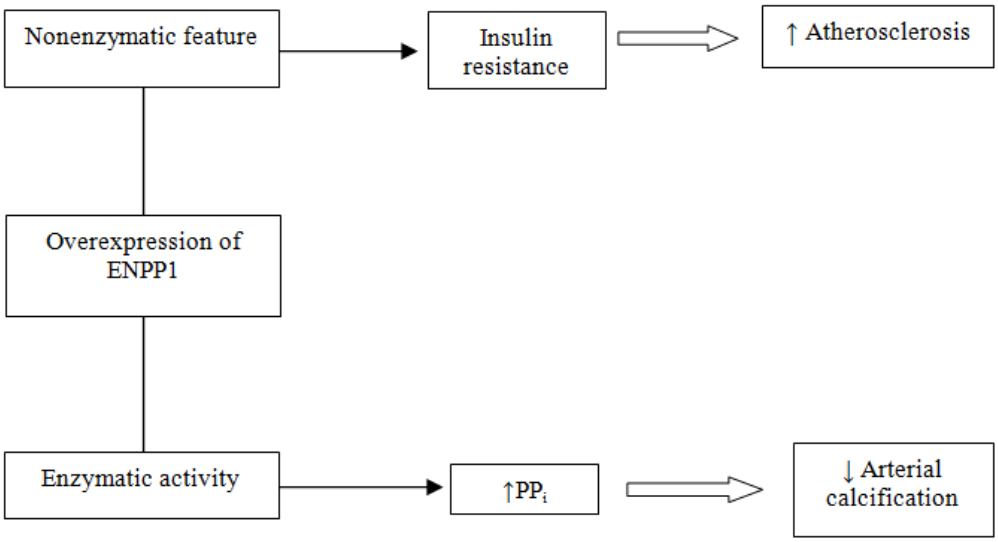

Fig. 3. Two possible opposite effects of *ENPP1* overexpression on the wall of coronary arteries

Table 1. Clinical parameters of Acute Coronary Syndrome (ACS) and healthy (control) subjects

	Subjects		
Parameters	ACS (n = 118)	Control (n = 110)	P-value
Sex, male/female, n/n	92/26	78/32	0.221*
Age, years	55.9±0.89	54.0±0.74	0.105
Current smokers, n (%)	54 (45.8)	29 (26.4)	0.002*
BMI, kg/m^2	28.5±0.41	27.1±0.41	0.022
SBP, mm Hg	140.8±1.7	124.6±1.0	<0.001
DBP, mm Hg	89.5±0.9	80.2±0.7	<0.001
TC, mmol/L	6.4±0.13	5.6±0.25	0.004
HDL-C, mmol/L	1.0±0.12	1.4±0.10	0.012
Fasting glucose, mmol/L	5.8±0.6	4.7±0.7	<0.001

Table 2. Genotypes of *ENPP1* K121Q polymorphism in patients with Acute Coronary Syndrome (ACS) and control subjects

	Total		Men		Women	
Genotype	ACS group (n = 118)	Control group (n = 110)	ACS group (n = 92)	Control group (n = 78)	ACS group (n = 26)	Control group (n = 32)
KK	79 (66.9)	83 (75.5)	62 (67.4)	58 (74.4)	17 (65.4)	25 (78.1)
KQ+QQ	39 (33.1)	27 (24.5)	30 (32.6)	20 (25.6)	9 (34.6)	7 (21.9)
OR (95% CI)	1.518 (0.850-2.709)		1.891 (0.590-6.056)		1.403 (0.718-2.741)	
P-value	0.158		0.284		0.321	

Table 3. Genotypes of *ENPP1* K121Q polymorphism in Acute Coronary Syndrome (ACS) and control subjects with normal (<25 kg/m^2) and enhanced (≥25 kg/m^2) Body Mass Index (BMI)

	BMI<25		BMI≥25	
Genotype	ACS (n = 21)	Control (n = 32)	ACS (n = 97)	Control (n = 78)
KK	11 (52.4)	26 (81.3)	68 (70.1)	57 (73.1)
KK+KQ	10 (47.6)	6 (18.7)	29 (29.9)	21 (26.9)
OR (95% CI)	3.939 (1.148-13.524)		1.158 (0.597-2.246)	
P-value	0.029		0.665	

Conclusion

In the present study, genotypes with minor allele (KQ+QQ) for *ENPP1* K121Q polymorphism were observed as a possible genetic risk factor for ACS only in persons with BMI <25 kg/m^2. It is more likely that these genotypes affect ACS not through the traditional risk factors (overweight/obesity, insulin resistance and type 2 diabetes) but by direct or undirect influence on pathologic processes in the wall of the coronary vessels (atherosclerosis and arterial calcification).

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Author's Contributions

Inna A. Rozumenko: Wrote the manuscript, performed genotyping

Victoria Y. Garbusova: Performed genotyping and biostatistics

Yurij A. Ataman: Performed the clinical research

Alexey V. Polonikov: Coordinated the data-analysis, contributed to writing manuscript

Alexander V. Ataman: Designed the research plan and organized the study

Ethics

The study has been approved by the Ethic Committee of the Medical Institute of Sumy State University.

References

Abedin, M., Y. Tintut and L.L. Demer, 2004. Vascular calcification. Mechanisms and clinical ramifications. Arteriosclerosis, Thrombosis Vascul. Biol., 24: 1161-1170. DOI: 10.1161/01.ATV.0000133194.94939.42

Antman, E.M., D.T. Anbe, P.W. and Armstrong, M.D.R.E. Bates and M.D.L.A. Green *et al*., 2004. ACC/AHA guidelines for the management of patients with ST-elevation myocardial infarction. J. Am. College Cardiol., 44: 671-719. DOI: 10.1016/j.jacc.2004.07.002

Bacci, S., O. Ludovico and S. Prudente, Y.Y. Zhang and R.D. Paola *et al*., 2005. The K121Q polymorphism of the *ENPP1*/PC-1 gene is associated with insulin resistance/atherogenic phenotypes, including earlier onset of type 2 diabetes and myocardial infarction. Diabetes, 54: 3021-3025. DOI: 10.2337/diabetes.54.10.3021

Bottcher, Y., A. Korner and T. Reinehr, B. Enigk and W. Kiess *et al*., 2006. *ENPP1* variants and haplotypes predispose to early onset obesity and impaired glucose and insulin metabolism in German obese children. J. Clin. Endocrinol. Metabol., 91: 4948-4952. DOI: 10.1210/jc.2006-0540

Chen, M.P., F.M. Chung and D.M. Chang, J.C.R. Tsai and H.F. Huang *et al*., 2006. *ENPP1* K121Q polymorphism is not related to type 2 diabetes mellitus, features of metabolic syndrome and diabetic cardiovascular complications in a Chinese population. Rev. Diabetic Stud., 3: 21-30. DOI: 10.1900/RDS.2006.3.21

Costanzo, B.V., V. Trischitta and R. Di Paola, D. Spampinato and A. Pizzuti *et al*., 2001. The Q allele variant (GLN121) of membrane glycoprotein PC-1 interacts with the insulin receptor and inhibits insulin signaling more effectively than the common Kallele variant (LYS121). Diabetes, 50: 831-836. DOI: 10.2337/diabetes.50.4.831

Dong, H., B.A. Maddux and J. Altomonte, M. Meseck and D. Accili *et al*., 2005. Increased hepatic levels of the insulin receptor inhibitor, PC-1/NPP1, induce insulin resistance and glucose intolerance. Diabetes, 54: 367-372. DOI: 10.2337/diabetes.54.2.367

Endler, G., C. Mannhalter and H. Sunder-Plassmann, M. Schillinger and A. Klimesch *et al*., 2002. The K121Q polymorphism in the plasma cell membrane glycoprotein 1 gene predisposes to early myocardial infarction. J. Molecul. Med., 80: 791-795. DOI: 10.1007/s00109-002-0385-8

Goldfine, I.D., B.A. Maddux and J.F. Youngren, G. Reaven and D. Accili *et al*., 2007. The role of membrane glycoprotein plasma cell antigen 1/ectonucleotide pyrophosphatase phosphodiesterase 1 in the pathogenesis of insulin resistance and related abnormalities. Endocrine Rev., 29: 62-75. DOI: 10.1210/er.2007-0004

Johnson, K., M. Polewski, D. van Etten and R. Terkeltaub, 2005. Chondrogenesis mediated by PPi depletion promotes spontaneous aortic calcification in NPP1-/- mice. Arterioscl., Thrombosis Vascul. Biol., 25: 686-691. DOI: 10.1161/01.ATV.0000154774.71187.f0

Lehto, S., L. Niskanen, M. Suhonen, T. Rönnemaa and M. Laasko, 1996. Medial artery calcification. A neglected harbinger of cardiovascular complications in non-insulin-dependent diabetes mellitus. Arterioscl., Thrombosis, Vascul. Biol., 16: 978-983. DOI: 10.1161/01.ATV.16.8.978

Maddux, B.A. and I.D. Goldfine, 2000. Membrane glycoprotein PC-1 inhibition of insulin receptor function occurs via direct interaction with the receptor alpha-subunit. Diabetes, 49: 13-19. DOI: 10.2337/diabetes.49.1.13

Morandi, A., L. Pinelli and A. Petrone, V. Vatin and R. Buzzetti *et al*., 2009. The Q121 variant of *ENPP1* may protect from childhood overweight/obesity in the Italian population. Obesity, 17: 202-206. DOI: 10.1038/oby.2008.470

Pizzuti, A., L. Frittitta and A. Argiolas, R. Baratta and I.D. Goldfine *et al*., 1999. A polymorphism (K121Q) of the human glycoprotein PC-1 gene coding region is strongly associated with insulin resistance. Diabetes, 48: 1881-1884. DOI: 10.2337/diabetes.48.9.1881

Shao, J.S., J. Cai and D.A. Towler, 2006. Molecular mechanisms of vascular calcification: Lessons learned from the aorta. Arterioscl., Thrombosis, Vascul. Biol., 26: 1423-1430. DOI: 10.1161/01.ATV.0000220441.42041.20

Thygesen, K., J. Alpert and H. White, 2007. Universal definition of myocardial infarction. Eur. Heart J., 28: 2525-2538.

Towler, D.A., 2005. Inorganic pyrophosphate. A paracrine regulator of vascular calcification and smooth muscle phenotype. Arterioscl., Thrombosis Vascul. Biol., 25: 651-654. DOI: 10.1161/01.ATV.0000158943.79580.9d