Association between the Low Density Lipoprotein Receptor (A370T) Gene Polymorphism with Lipid Profile in Type 2 Diabetes Mellitus

E-Mail: phmrwa@yahoo.com ; f_altoma_56@yahoo.com

Marwa Ali Zghair[1] ; Fadhil Jawad Al-Tu'ma[1] and Hassan Mahmoud Abu Al-Maali[2]

[1]College of Medicine, University of Kerbala / Kerbala-Iraq.

[2]College of Pharmacy, University of Kerbala / Kerbala – Iraq.

Abstract:

Background: determining the genetic profile of diabetic patients with dyslipidemia is considered important in terms of reducing the risk of possible vascular complications. Recent studies have shown that the low density lipoprotein receptor gene may have a direct effect on lipids level and one of its important genetic variants is the common single nucleotide polymorphism (A370T) that's produced by a guanine to adenine substitution in exon 8 in the epidermal growth factor precursor homology region.

Aim: To evaluate the association between the low density lipoprotein receptor (A370T) single nucleotide polymorphism with lipid Profile in type 2 diabetic patients with dyslipidemia.

Method: A case control study for total 180 subjects. 120 patients with type 2 diabetes mellitus (80 males and 40 females) and 60 patients of them were diagnosed previously with dyslipidemia, these diabetic patients were compared with 60 (40 male and 20 female) healthy control subjects. The blood sample collection were done at the outpatient department of Al-Imam Al-Hussein (as) Medical City in holy Karbala/Iraq during the period from January to June 2017.

Results: The results obtained demonstrate that there is no relationship between the low density lipoprotein receptor (A370T) single nucleotide polymorphism and dyslipidemia in patients with type 2diabetes mellitus with no significant association between this polymorphism and the lipid profile in these patients .

Conclusion: In the current study, the genotyping analysis results agreed with much of studies which revealed that there is no relationship between the low density lipoprotein receptor (A370T) single nucleotide polymorphism and lipid levels in patients with type 2diabetes mellitus with and without dyslipidemia.

Keywords: low density lipoprotein receptor, single nucleotide polymorphism , lipid profile .

الخلاصة:

الخلفية:

ان تحديد الشكل الجيني للمرضى المصابين بالسكري واضطراب شحوم الدم يعتبر من المعايير المهمة في تقليل خطر الاصابة بمضاعفات الاوعية الدموية لدى هؤلاء المرضى. وقد اظهرت الدراسات الجينية الحديثة بأن جين مستقبلات الدهون منخفضة الكثافة له تأثير مباشر على مستويات الدهون في الجسم وأحد اهم اشكاله الجينية هي تعدد الشكل النيوكليوتيدي (A370T) والذي ينتج من استبدال القاعدة النتروجينية الكوانين بالادينين في الموقع 8 من منطقة التماثل للعوامل البادئة .

الهدف

تقييم الارتباط بين تعدد الشكل النيوكليوتيدي لمستقبلات الدهون منخفضة الكثافة مع مستويات الدهون للمرضى المصابين بالنوع الثاني لداء السكري واضطراب شحوم الدم.

الطريقة

اجريت الدراسة على 180 شخص, منهم 120 مريض بالنوع الثاني لداء السكري و60 مريض منهم مصاب باضطراب شحوم الدم بالاضافة للسكري , هؤلاء المرضى تمت مقارنتهم مع 60 شخص سليم. تم جمع عينات الدم في مستشفى الامام الحسين عليه السلام التعليمي في محافظه كربلاء المقدسة للفتره من شهر كانون الثاني الى حزيران لسنة 2017 .

النتائج

تتضمن النتائج عدم وجود علاقة بين تعدد الشكل النيوكليوتيدي لمستقبلات الدهون منخفضة الكثافة ومستويات الدهون في الاشخاص المصابين بالنوع الثاني من داء السكري واضطراب شحوم الدم.

الاستنتاج

في الدراسة الحالية ,نتائج التحليل الجيني تتفق مع اغلبية الدراسات السابقة والتي تنص على عدم وجود علاقه بين تعدد الشكل النيوكليوتيدي لمستقبلات الدهون منخفضة الكثافة ومستويات الدهون في الاشخاص المصابين بالنوع الثاني من داء السكري مع او بدون اضطراب شحوم الدم.

Introduction

Type 2 diabetes mellitus (T2DM) is associated with a two to fourfold excess risk of coronary heart disease and the usual risk factors for coronary artery disease account for only 25–50% of increased atherosclerotic risk in diabetes mellitus. Other obvious risk factor is dyslipidemia which contribute substantially to the increased risk of macro-vascular disease in diabetic patients (1).

Some studies have shown in summary that the polymorphisms or mutations of genes that encode proteins and enzymes involved in lipoprotein metabolism could play a significant part in the growth of diabetic dyslipidemia (2). Therefore, determining the genetic profile of diabetic patients with dyslipidemia is considered important in terms of reducing the risk of possible micro-vascular and macro-vascular complications. Recent studies of genetic association recognized also more than one hundred genes that may have a direct effect on lipids level and one of them are the low density lipoprotein receptor gene which have over 800 genetic variants like the common single nucleotide polymorphism (A370T) (3).

The Low-Density Lipoprotein Receptor(LDLR) gene consisting of 18 exons of 45 kb in length located on chromosome 19p13 and it provides instructions for making a protein called LDLR (4) which is a mosaic protein of 839 amino acids sit on the outer surface of many types of cells

particularly in the " liver" the organ responsible for removing most excess cholesterol from the body. The LDLR is the prototype of a large family of structurally homologous cell surface receptors, which fold in the endoplasmic reticulum and function as endocytic and signaling receptors in a wide variety of cellular processes (5).

Furthermore, the LDLR play a critical role in regulating the amount of circulating cholesterol in the blood by picking up LDL and transport them into the cell ending up in lysosomes where the protein is degraded and the cholesterol is made available for repression of microsoml enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase, the rate-limiting step in cholesterol synthesis, the cholesterol is then used by the cell, stored, or removed from the body. At the same time, a reciprocal stimulation of cholesterol ester synthesis takes place (6)

The LDL Receptor mutations/polymorphisms change individuals' LDLR protein structures and functions and lead to the creation of diverse clinical phenotypes (7) Studies performed to date have recognized more than 800 large deletions, small deletions, insertions, point mutations, splice site mutations and polymorphisms of LDLR gene (8).   Mutations in the LDLR gene have been categorized into 5 functional groups based on the features of the mutant protein formed and studied in patients' fibroblasts.

The single nucleotide polymorphism (A370T) of the LDLR was first reported in a South African white population (9,10) and is produced by a Guanine to Adenine substitution in exon 8 of the LDLR gene in the epidermal growth factor precursor homology region, repeat B. This region of numerous copies of a 40-amino acid, cysteine-rich structure is involved in acid-dependent separation of ligand and receptor in the endosomes preceding the recycling of the receptor to the cell surface. The majority of infrequent mutations in this part of the gene result biochemically in type V defects, that is, the manufacture of receptors that bind and internalize LDL, but be failed to reprocess to the cell surface (recycling-defective alleles)(11). Its known that the frequency of the 370T allele has been reported worldwide to be between 0.022 and 0.170 , with no clear association with high cholesterol levels or risk for coronary heart disease and stroke (12).

Subjects, Materials and Methods

The blood samples have been collected during the period from June

to January 2017.nearly 5 mL venous blood was drawn from each candidate after being fasting for approximately 12 hours.

The subjects in the study were divided into two broad groups:

A-Patients group: One hundred and twenty candidates subdivided into two subgroups:-

1-Sixty T2DM patients without dyslipidemia:(forty males and twenty females).

2-Sixty T2DM patients with dyslipidemia: (forty males and twenty females).

B-Control group: Sixty participants (forty males and twenty females).

The mean value of the BMI for the study subjects is 25.4906 (Kg/m²) and the subjects in the study were in range of 45-75 years .

Patients chosen to have T2DM of varies duration based on physicians' diagnosis prior to the current visit to the laboratory department of Al-Imam Al-Hussein (as) Medical City in holy Karbala/Iraq, T2DM diagnosis was based on a fasting blood glucose (FBG) level > 126 mg/dL and/or postprandial glucose level > 200 mg/dL and HbA1c level > 6.5%. Subjects with triglyceride levels > 200 mg/dL and/or HDL-C levels < 45 mg/dL were diagnosed with dyslipidemia (13). The exclusion criteria include: patients diagnosed with T1DM, chronic renal failure, chronic liver diseases, congestive heart failure, malignancy or patients who had inflammatory diseases such as rheumatoid arthritis and systemic lupus erythematous.

Serum total cholesterol (TC) was measured by a MR ,laboratory kit (Linear, Spain) and according to Burits ,1994 principle (14). Triglycerides are enzymatically hydrolyzed to glycerol and fatty acids and measured according to Fossati equation by (BioMrieux®sa. France) Triglyceride kit (15).

The HDL-C fraction was determined according to Wilson principle by (Linear, Spain) HDL - cholesterol kit (16).

LDL-C was determined mathematically from the total cholesterol, triglycerides; and the HDL-C concentration according to Ana Vujovic principle (17), VLDL-C was calculated by using Friedwald's formula (18). In the FBS measurement glucose is oxidized by glucose oxidase to gluconate and hydrogen peroxide according to Huggett and Nixon equation using (Plasmatic , France) Glucose kit (19).

Genomic DNA extraction was made by laboratory kit . the concentration and the purity of the extracted DNA was determined by nano drop device.the The process of the (A370T) polymorphism genotyping was done by the amplification of 150 bp region of LDLR gene by Polymerase Chain Reaction (PCR) using the primers (20):

P1: 5'-GAG TGT CAG GAT CCC GAC ACC TGC GCC-3'

P2: 5 '-AAG TCG ACC CAC CCG CCT GCC TCC CGT-3'

The PCR mixture is prepared in lyophilize PCR premix formula by adding:- 1µl of 10 pmol/µl of   each primers and 5 µl of extracted DNA and The volume was completed to 20 µl by distilled water.

The thermal program comprise of 35 cycles as mention in Table (1).

Table (1): The Thermal Cycles for LDLR Gene Amplification Reaction

Step	Temperature	Duration	Number of cycle
1-Initial denaturation	95°C	7 minutes	1 cycles
2-Denaturation	95°C	60 seconds	40 cycles
3-Annealing	68°C	60 seconds	40 cycles
4-Extension	72°C	120 second	40 cycles
5-Final extension	72°C	7 minutes	1 cycles

The PCR products were digested with the enzyme *Hae*III, and fragments were separated on 3.5% agarose gel and visualized by ethidium bromide staining. SPSS 22 software was used for the statistical analysis of the study numbers.

Results

The product yielded from the PCR was 150 base pair in size and as confirmed by electrophoresis with 25 bp ladder In the digestion process by *HAE III* enzyme ,The fragments representing A allele were 77, 47 and 26 bp (Figure 5)., and those representing the T allele were 124 and 26 bp (25) as show in Figure 5.
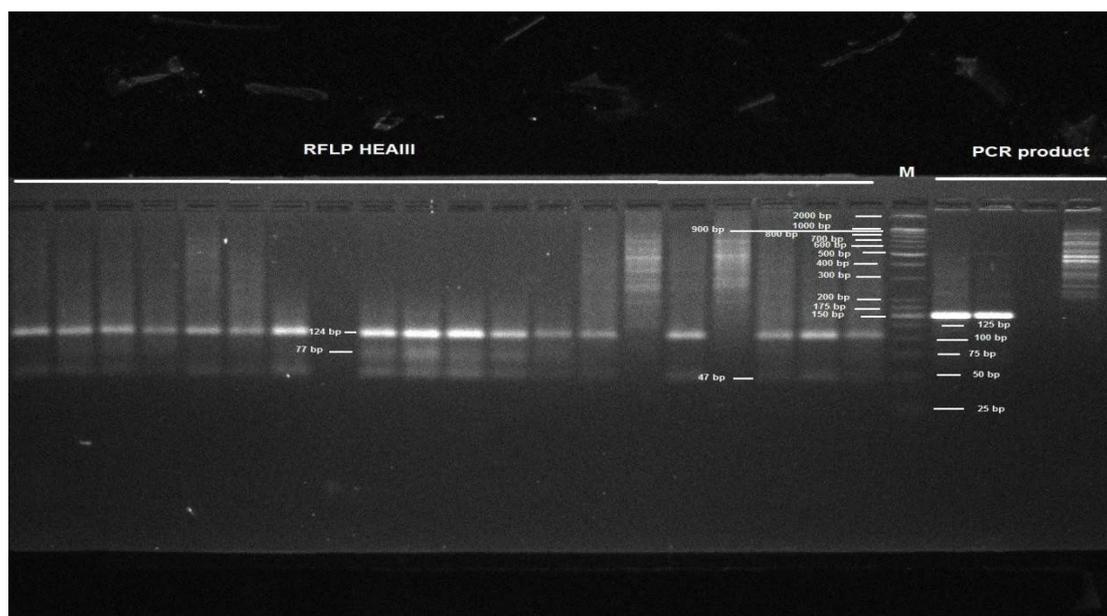
Figure(5): Electrophoresis Bands for PCR and RFLP Products Digested by HAE III Enzyme .(PCR product:-150 bp, ladder:-25 bp in size, RFLP products:-(AA polymorphism:- 77, 47 and 26 bp fragments, TT polymorphism :- 124 and 26 bp; AT polymorphism:-124,77,47,26 bp fragments)

The results obtained from genotyping illustrate the distribution of the LDLR genotypes among the study groups (Table 1).

Table (1): LDLR genotypes distribution among study groups

LDLR Genotype	Control	DM patients without dyslipidemia	DM patients with dyslipidemia	Total number of allele (n)	Percentage n/180	P value
AA	46	51	48	145	80.5%	0.643
AT	12	5	7	25	13.4%	
TT	2	4	5	11	6.1%	
Total	60	60	60	180	100%	
P value derived from ANOVA test, Significant: p<0.05, Highly significant: p<0.001, No significant: p>0.05., DM=diabetes mellitus						

However, the outcomes demonstrate that there is no relationship between the low density lipoprotein receptor gene (A370T) single nucleotide polymorphism and lipid profile in each of the control and diabetic patients group (Table 3).

Table (3): Association Between LDLR Genotypes and Lipid Profile

Parameter	allele	Control	DM patients without dyslipidemia	DM patients with dyslipidemia
Total cholesterol (mg/dl)	AA	153.332 ± 21.8173	161.392 ± 29.3352	262.905 ± 117.934
	AT	156.3250± 23.482	171.96 ± 29.05724	226.3875 ±148.253
	TT	163.80 ± 24.18305	158.00 ± 32.76207	177.260 ± 51.366
	P.value	N.S	N.S	N.S
HDL-C (mg/dl)	AA	55.7130 ±10.0612	51.8020 ±9.52383	38.8149 ± 9.66790
	AT	54.2250 ±11.0734	50.660 ±11.83841	39.7625 ±7.87907
	TT	60.9000 ± 7.21249	47.3250 ±1.81911	38.4400 ± 6.965
	P.value	N.S	N.S	N.S
Triglyceride (mg/dl)	AA	109.8848 ± 24.317	185.375 ± 50.2307	311.4404 ±108.3613
	AT	112.7917± 26.233	152.9400 ± 38.336	317.3625 ±109.3620
	TT	149.2000 ±1.9799	190.6500 ± 3.1932	308.820 ± 94.92148
	P.value	N.S	N.S	N.S
LDL-C (mg/dl)	AA	116.2696 ± 21.829	133.8651± 26.3733	171.4787 ± 58.1098
	AT	129.220 ± 24.4954	112.1600 ±18.0174	154.7000 ± 81.1357
	TT	96.4500 ± 2.05061	132.1500 ±12.2693	139.540 ± 54.71013
	P.value	N.S	N.S	N.S
VLDL-C (mg/dl)	AA	27.4712 ± 6.07939	46.3436 ±12.55770	62.2881± 21.67226
	AT	28.1979 ± 6.55916	38.235 ± 9.55842	63.4725 ± 21.87241
	TT	37.3000 ± .49497	47.6625 ± .79831	61.7640 ± 18.98430
	P.value	N.S	N.S	N.S

p value derived from ANOVA test, Significant: p<0.05, Highly significant: p<0.001, No significant: p>0.05.,values expressed as mean± standard deviation (SD) DM=diabetes mellitus, BMI=body mass index , FBS=fasting blood sugar HDL=high density lipoprotein, LDL =low density lipoprotein, VLDL=very low density lipoprotein,

Discussion

Dyslipidemia is a common feature of diabetes and it is known that diabetic dyslipidemia happens not only as a disruption of lipoprotein metabolism resultant from changes in the quantitative and qualitative features of lipoproteins, but may also progress due to genetic and environmental factors (21). Dyslipidemia in diabetes commonly manifests as raised low-density lipoprotein cholesterol (LDL-C), decreased high-density lipoprotein cholesterol (HDL-C) levels, or elevated triglyceride (TG) levels (22) .

Its known that there is an association between atherosclerotic cardiovascular disease and serum cholesterol and triglyceride levels in both type 1 and type 2 diabetes (23) and The risk of Chronic heart disease is greater at any given level of serum cholesterol in patients with diabetes and its association with hypertriglyceridemia is stronger than in the general population (24) (25).

Its well-known that in genetics, there are several gene polymorphisms and mutations that have been identified and associated with dyslipidemia. Consequently, the identification of genetic markers involved with dyslipidemia may lead to the recognition of patients with genetic susceptibility to abnormal lipid metabolism and levels and guide interventions to correct these abnormalities.

Based on that aims, genotypes were obtained for 180 individuals from a general population samples, of which 120 patients with type 2 diabetes mellitus and 60 control subjects to investigate the distribution pattern of the (A370T) polymorphism of the LDLR gene in local patients and to identify their association with dyslipidemia phenotype in type 2 diabetic patients.

The LDL receptor gene consists of 18 exons spanning 45 kb on chromosome 19p13.The 5.3 kb mRNA encodes a mature protein of 839 amino acids (9) . Overall, more than 800 mutations, including gross deletions, minor deletions, insertions, point mutations, and Single nucleotide polymorphism scattered over the LDL receptor gene have been reported . These mutations affect the synthesis (class 1), posttranslational processing (class 2), ligand binding activity (class 3), internalization (class 4), or recycling (class 5) of the LDL receptor. Among the different polymorphisms found in the LDLR gene, the A370T has been investigated for its association with increased lipid levels and risk of diabetic dyslipidemia (26) .

There were many studies that have looked systematically at the relationship between the A370T genotype and plasma lipid levels in population-based samples. The first, from Iceland (27) reported that men with the homozygous 370T (N = 18) had approximately 8.3% higher cholesterol, 11.8% higher LDL-cholesterol, and 10.3% higher ApoB levels than AA men (N = 134) (28), whilst women carrier of homozygous 370T (N = 12) had 7.4%, 13.3% and 10.1% lower levels for cholesterol, LDL-cholesterol and ApoB respectively, than AA women (N = 154). The largest study to date was in canada which showed no significant difference in cholesterol, LDL-cholesterol, or ApoB levels in more than 9,000 subjects (29). Two other small studies, one from Singapore (N = 539) with a very low frequency of the TT polymorphism subjects (N = 3), the other from Germany (N = 101) with 12 AT subjects and only one TT subject, and reported no difference by genotype. also one more study from Denmark reported that individuals homozygous for the 370T allele had 80% higher risk of ischaemic heart disease (not statistically significant), but a statistically significant 3.6-fold higher risk of ischaemic stroke (30) The present study results for the LDLR genotypes agreed with these studies which states no association between the LDLR gene polymorphism(A370T) and lipid profile in the study groups and the (A370T) polymorphism doesn't considered as a risk factor for dyslipidemia development in patients with type 2 diabetes mellitus.

References

1. Chou, Roger (March 2015). "Cardiac Screening with Electrocardiography, Stress Echocardiography, or Myocardial Perfusion Imaging: Advice for High-Value Care From the American College of Physicians". Annals of Internal Medicine. 162 (6): 438.

2. Moran, AE; Forouzanfar, MH; Roth, GA; Mensah, GA; Ezzati, M; Murray, CJ; Naghavi, M (April 2014). "Temporal trends in ischemic heart disease mortality in 21 world regions, 1980 to 2010: the Global Burden of Disease 2010 study". Circulation. 129 (14):1483-92.

3. Leren TP (November 2014). "Sorting an LDL receptor with bound PCSK9 to intracellular degradation". Atherosclerosis. 237 (1): 76–81.

4. Milasan A, Dallaire F, Mayer G, Martel C (2016). "Effects of LDL Receptor Modulation on Lymphatic Function". Scientific Reports. 6: 27862.

5. Jamaldini SH, Babanejad M, Mozaffari R, Nikzat N, Jalalvand K, Badiei A, Sanati H. et al. (2014) Association of polymorphisms at LDLR locus with coronary artery disease independently from lipid profile. Acta Med Iran. 52(5):352–359

6. Ma RC and Chan JC. (2013) Type 2 diabetes in East Asians: similarities and differences with populations in Europe and the United States. Ann NY Acad Sci. 1281: 64–91.

7. Rye, K.A. and P.J. Barter (2014) Cardioprotective functions of HDLs. J Lipid Res, 55(2): 168-79.

8. Wang B, Zhao H, Zhou L, Dai X, Wang D, Cao J, Niu W. (2009) Association of genetic variation in apolipoprotein E and low density lipoprotein receptor with ischemic stroke in Northern Han Chinese. J Neurol Sci. 276 (1-2):118–122.

9. Oguma Y, Sesso HD, Paffenbarger RS, Lee IM. (2005) Weight change and risk of developing type 2 diabetes. Obes Res. 13:945–951.

10. West KM, Ahuja MM, Bennett PH, et al. (1983) The role of circulating glucose and triglyceride concentrations and their interactions with other "risk factors" as determinants of arterial disease in nine diabetic population samples from the WHO multinational study. Diabetes Care. 6:361–369.

11. Stamler J, Vaccaro O, Neaton JD, Wentworth D. (1993) Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. Diabetes Care. 16:434–444.

12. Baigent C, Keech A, Kearney PM, et al. (2005) nEfficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in randomised trials of statins. Lancet. 366:1267–1278.

13. Ma RC and Chan JC. (2013) Type 2 diabetes in East Asians: similarities and differences with populations in Europe and the United States. Ann NY Acad Sci. 1281:64–91.

14. Burits CA; Ashwood ER. (1994). Tietz Clinical Guide to Laboratory Tests. 3rd Edition. 8: 266-273.

15. Fossati P; Prencip L. (1982). Serum triacylglycerols determined calorimetrically with an enzyme that produces hydrogen peroxide Clin. Chem. 28: 2077–80.

16. Wilson, P.W. and Abbott, R.D., (1988) High density lipoprotein cholesterol and mortality. The Framingham Heart Study. Arteriosclerosis, 8, P. 737-741.

17. Ana Vujovic, Jelena Kotur-Stevuljevic, Slavica Spasic, Nada Bujisic, Jelena Martinovic, Dragoljub Pajic (2010) Evaluation of different formulas for LDL-C calculation Lipids Health Dis. 9: 27.

18. Friedewald WT; Levy RI; Fredrickson DS. (June 1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem;18(6):499-502

19. Huggett C; Nixon D. (1997). Use of glucose oxidase peroxide and odianisine in the determination of blood and urine glucose. Lancet 2: 368-72

20. Chapman MJ, Ginsberg HN, Amarenco P, et al. (2011) Triglyceride-rich lipoproteins and high-density lipoprotein cholesterol in patients at high risk of cardiovascular disease: evidence and

guidance for management. Eur Heart J. 32:1345–1361.

21. Cirillo,A., Pandit,S., Ni,Y.G., Hubbard,B., Sitlani,A. and Carfi, (2011) Mechanistic implications for LDL receptor degradation from the PCSK9/LDLR structure at neutral pH EMBO Rep. 12 (12).

22. Dixit AK, Dey R, Suresh A, Chaudhuri S, Panda AK, Mitra A, Hazra J. (2014) The prevalence of dyslipidemia in patients with diabetes mellitus of ayurveda Hospital. J Diabetes Metab Disord. 2014;13:58.

23. Mooradian AD. (2009) Dyslipidemia in type 2 diabetes mellitus. Nat Clin Pract Endocrinol Metab. 5(3):150–159.

24. Miller M, Zhan M. Genetic determinants of low high-density lipoprotein cholesterol. Curr Opin Cardiol. 2004;19(4):380–384.

25. Diego García-Giustiniani and Ricardo Stein (2016) Genetics of Dyslipidemia Arq Bras Cardiol. 106(5): 434–438.

26. Gudnason, V., Patel, D., Sun, X. M., Humphries, S., Soutar, A. K. & Knight, B. L. (1995) Effect of the StuI polymorphism in the LDL receptor gene (Ala 370 to Thr) on lipid levels in healthy individuals. Clin Genet 47(2), 68–74.,

27. Frikke-Schmidt, R., Nordestgaard, B. G., Schnohr, P., Tybjaerg-Hansen, A. & Schmidt, R. (2004) Single nucleotide polymorphism in the low-density lipoprotein receptor is associated with a threefold risk of stroke. A case-control and prospective study. Eur Heart J 25(11), 943–951.

28. Choong (, M. L., Sethi, S. K. & Koay, E. S. (1998) Asian cohort. Ann Acad Med Singapore 27< 503–506. Dedoussis, G. V) 55-Weiss, N., Binder, G. & Keller, C.

29. Weiss, N., Binder, G. & Keller, C. (1998) Heterozygosity for the missense mutation Ala370→Thr in exon 8 of the low density lipoprotein receptor gene does not cause hypercholesterolemia. Eur J Med Res 3(1–2), 20– Clustal.

30. Wang JC, Bennett M Circ Res. 2012 Jul 6; 111(2):245-59 Aging and atherosclerosis: mechanisms, functional consequences for cellular senescence.