References

1. Anderson SI, Hotchin NA, Nash GB (2000) Role of the cytoskeleton in rapid activation of CD11b/CD18 function and its subsequent downregulation in neutrophils. *J Cell Sci* 113:2737–2745
2. Advani A, Marshall SM, Thomas TH (2002) Impaired neutrophil actin assembly causes persistent CD11b expression and reduced primary granule exocytosis in Type 2 diabetes. *Diabetologia* 45:719–727
3. Davey PC, Zuzel M, Kamiguti AS, Hunt JA, Aziz KA (2000) Activation-dependent proteolytic degradation of polymorphonuclear CD11b. *Br J Haemat* 111:934–942
4. Alexiewicz JM, Kumar D, Smogorzewski M, Klin M, Massry SG (1995) Polymorphonuclear leukocytes in non-insulin-dependent diabetes mellitus: abnormalities in metabolism and function. *Ann Intern Med* 123:919–924
5. Noble JM, Ford GA, Thomas TH (1999) Effect of aging on CD11b and CD69 surface expression by vesicular insertion in human polymorphonuclear leucocytes. *Clin Sci (Lond)* 97:323–329
6. Balletshofer BM, Rittig K, Enderle MD et al. (2000) Endothelial dysfunction is detectable in young normotensive first-degree relatives of subjects with Type 2 diabetes in association with insulin resistance. *Circulation* 101:1780–1784
7. Janssen JA, Lamberts SW (2002) The role of IGF-I in the development of cardiovascular disease in Type 2 diabetes mellitus: is prevention possible? *Eur J Endocrinol* 146:467–477

A. Advani (✉)

School of Clinical Medical Sciences, Floor 4,
William Leech Building, Medical School,
Newcastle University, 1 Framlington Place,
Newcastle upon Tyne, NE2 4HH, United Kingdom
E-mail: Andrew.Advani@ncl.ac.uk

Abbreviations: PMA, Phorbol 12 myristate 13 acetate · NC, normal control subjects · FDR, first-degree relatives of patients with Type 2 diabetes

SNP3 polymorphism in apo A-V gene is associated with small dense LDL particles in Type 2 diabetes

Keywords Apo A-V · SNP3 · Diabetes · LDL subfractions · Cardiovascular risk factors

To the Editor: The –1131 T>C (SNP3) polymorphism of apo A-V gene has been associated in some studies to an increase in triglycerides and in few cases to a decrease in HDL-cholesterol. Its frequency is 12% in Afro-Americans, 16% in Hispanics, 6 to 16% in Caucasians and 7% in a Spanish control cohort [1, 2, 3, 4]. The heterozygote subjects for rare SNP3 allele (C/T) are related to an increase in triglycerides in Hispanic and Caucasian men, although these data were not observed in another study where the increase in triglycerides was only statistically significant in homozygotes for the mutated allele [3]. Hypertriglyceridaemia is the main factor in determining the size of the LDL. There are many causes that influence the metabolism of triglycerides, some are primary, such as the apo E genotype, and others secondary. Type 2 diabetes mellitus is linked to different lipid disorders such as hypertriglyceridaemia, a decrease in HDL-cholesterol and an increase in small, dense LDL.

The aim of this study was to assess the frequency and the influence of SNP3 polymorphism on different lipid parameters, LPL activity and LDL subfractions in a diabetic population.

The study was approved by the Ethics Committee of our hospital. We selected 127 patients with Type 2 diabetes mellitus who had given their written consent.

Venous blood samples were taken after a 12-h fast. Cholesterol and triglyceride concentrations were measured by enzymatic techniques. LDL-cholesterol was calculated with direct measuring.

Using density gradient centrifugation according to the method described by Sanchez Quesada [5], six LDL subfractions were isolated [1, 2, 3, 4, 5, 6] from lesser to greater density: LDL1 (1.020–1.026 g/ml), LDL2 (1.026–1.030 g/ml), LDL3 (1.030–1.036 g/ml), LDL4 (1.036–1.043 g/ml), LDL5 (1.043–1.051 g/ml), LDL6 (1.051–1.056 g/ml). Plasma lipase activity was measured according to a previous study [6].

SNP 3 genotyping was done according to methodology used previously [1]. We following the Hixon's method for Apo E restriction isotyping [7].

To check the restriction technique, all samples with the mutated allele and part of those with the common allele were sequenced.

Statistical analysis was done with StatView (5.0.1 version) for Windows. Statistical significance was taken as a *p* value of less than 0.05. The only non-nominal variables were compared using Mann-Whitney U test. Fisher's exact test was applied to examine differences between rare and common allele groups.

We found 14 patients with the mutated allele in the SNP3 polymorphism, which gave us a mutated allele frequency of 11% in the group of 127 diabetic patients. Of the 127 patients, 16% had apo E genotype $E_3 E_4$, 13% were $E_3 E_2$ and 71% were $E_3 E_3$. Among the diabetic patients with $E_3 E_3$ genotype, there were no differences between the groups with the mutated allele and with the common one in SNP3 in the concentrations of total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol and apo B. The mean lipase lipoprotein activity was similar in the group of patients with the common allele and with the mutated one (230 ± 103 pkat/ml vs 243 ± 80 pkat/ml). The figures for the smaller, denser subfractions were higher in patients with the mutated allele in SNP3 polymorphism LDL5 (33.1 ± 15.2 mg/dl vs 24.7 ± 11.6 mg/dl, $p < 0.05$) and LDL6 (18.9 ± 11.0 mg/dl vs 13.2 ± 5.8 mg/dl, $p < 0.05$), whereas no differences were noted among the rest of the LDL subfractions LDL1, LDL2, LDL3 and LDL4.

The frequency of the mutated allele of the SNP3 polymorphism is approximately 11% in our cohort [4], whereas a frequency of 7% was found in another Spanish diabetic cohort for the mutated allele, which appears to be similar.

The effects of the SNP3 polymorphism of the apo A-V gene on different lipid parameters have been studied in several articles. An increase in triglyceride concentrations has been observed in heterozygote subjects for the mutated allele [1, 2]. However, another study found no significant increase in triglycerides in heterozygote subjects, but found one among homozygotes for the mutated allele [3]. In our group, we found no significant differences in triglyceride concentrations.

A significant increase in the smaller, denser LDL subfractions (LDL5 and LDL6) was observed in the subjects with the same apo E genotype that had the mutated SNP3 allele as compared to the subjects that had the common allele.

Triglyceride concentrations predict by 60% the size of LDL [8]. Other parameters related to LDL size were tested in our work. In our study the groups with the common and mutated allele with apo E genotype $E_3 E_3$, showed no differences in the HbA_{1c}, BMI, waist to hip index, LPL activity or in the distribution by sexes. Taking into account the negative correlation between triglyceride concentrations and LDL size and the results obtained in the triglyceride figures [1, 2], the results obtained in LDL subfractions by our group must be considered to be logical. As to why, in our study, no differences were found in triglycerides, this could be due to the individual variability in the triglyceride figures or to the fewer mean triglycerides of our entire group.

In conclusion, the prevalence of the mutated allele in the SNP3 polymorphism in the diabetic population is 11%. The LPL activity was no different among the diabetic patients with the common or mutated allele. However, in the subjects with the mutated allele the figures for the smaller, denser LDL subfractions were higher.

E. Esteve · E. Faure · F. Calvo · E. Aguillo · C. Blasco ·
M. J. Roche
University Clinical Hospital "Lozano Blesa", Zaragoza, Spain

P. Mozas · M. Pocovi
Department of Biochemistry, Molecular and Cell Biology,
University of Zaragoza, Zaragoza, Spain

References

1. Pennacchio LA, Olivier M, Hubacek JA et al. (2001) An apolipoprotein influencing triglycerides in humans and mice revealed by comparative sequencing. *Science* 294:169–173
2. Pennacchio LA, Olivier M, Hubacek JA, Krauss RM, Rubin EM, Cohen JC (2002) Two independent apolipoprotein A5 haplotypes influence human plasma triglyceride levels. *Hum Mol Genet* 11:3031–3038
3. Talmud PJ, Hawe E, Martin S et al. (2002) Relative contribution of variation within the APO C3/A4/A5 gene cluster in determining plasma triglycerides. *Hum Mol Genet* 11:3039–3046
4. Ribalta J, Figuera L, Fernández-Ballart J et al. (2002) Newly identified apolipoprotein AV gene predisposes to high plasma triglycerides in familial combined hyperlipidemia. *Clin Chem* 48:1597–1600
5. Sánchez-Quesada JL, Pérez A, Caixas A, Ordóñez-Llanos J, Carreras G, Payes A (1996) Electronegative low density lipoprotein subform is increased in patients with short-duration IDDM and is closely related to glycemic control. *Diabetologia* 39:1469–1476
6. Iglesias A, Contreras JA, Martínez-Pardo M, Entrala A, Herrera E, Lasuncion MA (1993) Cholesteryl ester transfer activity in lipoprotein lipase deficiency and other primary hypertriglyceridemias. *Clin Chim Acta* 221:73–89
7. Hixson JE, Vernier DT (1990) Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. *J Lipid Res* 31:545–548
8. Haffner SM, Mykkanen L, Valdez RA, Paidi M, Stern MP, Howard BV (1993) LDL size and subclass pattern in a biethnic population. *Arterioscler Thromb* 13:1623–1630

E. Faure (✉)
University Clinical Hospital "Lozano Blesa",
San Juan Bosco, 15, 50009 Zaragoza, Spain
E-mail: endh@hcu-lblesa.es
Tel.: +34-976-556400 ext. 2464

Abbreviations: LPL, lipoprotein lipase