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Severe hypertriglyceridaemia in Type II diabetes: involvement of *apoC-III Sst-I* polymorphism, *LPL* mutations and *apo E3* deficiency

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Abstract

Aims/hypothesis. Hypertriglyceridaemia is common in Type II (non-insulin-dependent) diabetes mellitus. Only subgroups of patient however have type V hyperlipidaemia. To investigate the coordination between genetic factors in the modulation of hypertriglyceridaemia in Type II diabetes, we studied three major modifier loci: *apoC-III* (both Sst-I and insulin-responsive element polymorphisms), apolipoprotein E genotypes and lipoprotein-lipase mutations.
Methods. We studied *apoCIII* gene polymorphisms, apolipoprotein E genotypes and lipoprotein-lipase gene mutations in 176 patients with Type II (non-insulin-dependent) diabetes mellitus, either normolipaemic (group N, $n = 116$), mildly hypertriglyceridaemic (group T, $n = 28$) or with a history of severe hypertriglyceridaemia (triglyceride > 15 g/l) (group H, $n = 32$).
Results. Mild hypertriglyceridaemia in Type II diabetes did not associate with any gene variants in this study. Severe hypertriglyceridaemia was, however, associated with the presence of the *apoC-III S2* allele (50 % of the patients in group H compared with 15.5 % in group N, $p < 0.0001$). Additionally this particular phenotype was associated with a low prevalence of the *apo E3* allele (35.9 % in group H vs 18.1 % in group N, $p < 0.005$) and a statistically significant over-representation of the *E2E4* genotypes. Inactivating lipoprotein-lipase mutations were found in four patients (three heterozygotes, one homozygote), none was found in group N or T. Thus 68.7 % of group H patients (22/32) (vs 21.4 % in group T, $p < 0.0005$) were carriers of either *S2* allele, lipoprotein-lipase mutants or *E2E4* genotype with most lipoprotein-lipase mutants or *E2E4* genotypes or both in the non-carriers for the *S2* allele (6/7).
Conclusion/interpretation. Our results strongly support the hypothesis that severe hyperlipaemia in Type II diabetes crucially depends on genetic factors which impair the clearance of triglyceride-rich lipoproteins.
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Keywords Type II diabetes, dyslipaemia, triglyceride, type V hyperlipidaemia, apolipoprotein C-III, apolipoprotein E, lipoprotein lipase, polymorphism, mutation, genetics.

Hypertriglyceridaemia and reduced HDL-cholesterol concentrations are the prominent features of the atherogenic dyslipaemia commonly observed in insulin-resistance states [1, 2] and Type II (non-insulin-dependent) diabetes mellitus [3–6]. Severe hypertriglyceridaemia is not uncommon in Type II diabetes [7, 8] but its prevalence and mechanism are not clearly established. Mild hypertriglyceridaemia in Type II diabetes is dependent upon glucose control and nutritional factors such as intake of carbohydrates and alcohol. Genetic factors might, however, determine individual susceptibility to major hypertriglyceridaemia. Although VLDL hepatic overproduction is well documented in Type II diabetes, genetic defect in the clearance of the triglyceride-rich

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Abbreviations: apo, Apolipoprotein; LPL, lipoprotein lipase; HDLc, high-density lipoprotein cholesterol; LDLc , low density lipoprotein cholesterol; IRE, insulin responsive element; SSCP, single strand conformational polymorphism

Table 1. Biometric and plasma lipid characteristics of groups N, T and H

	Group N	Group T	*p* value	Group H	*p* values	
	n = 116	*n* = 28	T vs N [e]	*n* = 32	H vs N [e]	H vs T [e]
Sex ratio (men/women)	2.52 (*83/33*)	0.75 (*12/16*)	< 0.01	2.5 (*23/9*)	NS	< 0.05
Age (year)	54.3 ± 0.7 [a]	55.0 ± 1.6	NS	48.9 ± 1.6	< 0.01	< 0.01
BMI	28.0 ± 0.4	30.2 ± 1.0	< 0.05	29.3 ± 0.9	NS	NS
HbA_{1c} (%)	8.8 ± 0.2	8.8 ± 0.4	NS	10.1 ± 0.5	< 0.01	< 0.05
Triglyceride (mmol/l)	1.3 ± 0.1	3.1 ± 0.1	< 0.0001	12.7 ± 2.3	< 0.0001	< 0.0001
Total cholesterol (mmol/l)	4.8 ± 0.7	5.80 ± 0.2	< 0.0001	10.2 ± 1.0	< 0.0001	< 0.0001
HDLc (mmol/l)	1.04 ± 0.02	0.91 ± 0.05	< 0.05	0.62 ± 0.06	< 0.0001	< 0.0001
LDLc (mmol/l)	3.22 ± 0.06	3.53 ± 0.14	NS	4.28 ± 0.42	< 0.0001	< 0.05
apo B (g/l)	1.06 ± 0.20	1.39 ± 0.07	< 0.0001	1.60 ± 0.09	< 0.0001	< 0.05

[a]: ± (standard error of mean)

lipoproteins could further modulate the expression of dyslipaemia.

As individual factors, heterozygote lipoprotein lipase (LPL) deficiency and carrier status for *apo E2* and/or *E4* variants have been suggested to be determinants of genetic susceptibility to dyslipaemia in Type II diabetes [7, 8, 9]. Additionnally apoC-III is a major modulator of triglyceride concentrations through a down-regulation of LPL activity and triglyceride-rich lipoprotein clearance, as shown by studies in transgenic and knockout models [10, 11]. The gene cluster polymorphisms *apo AI-CIII-AIV* were associated with hypertriglyceridaemia and coronary heart disease in population studies [12]. The most consistent associations were reported for the *S2* variant (RFLP-Sst-I +) in *apoC-III* exon 4 (*C3238G*) [13–18]. Moreover this polymorphism has a modifier effect on familial combined hyperlipidaemia [19, 20] and type III hyperlipoproteinaemia [21]. No study has, however, been conducted in diabetic dyslipaemia. Additionnally two *apoC-III* promotor polymorphisms (*T-455C and C-482T*) were shown to inactivate a negative insulin-responsive element located between nucleotide –449 and –490 [22] and are in linkage-disequilibrium with the *apoC-III* Sst-I polymorphism [17].

We therefore undertook an association study in Type II diabetes hypertriglyceridaemia to investigate gene alterations at *apoC-III*, *apo E* and *LPL* loci either in combination or as alternative risk factors. We decided to study a particular subgroup of diabetic patients with a history of severe hypertriglyceridaemia considering that this particular phenotype might depend on genetic predisposition factors.

Subjects and methods

Patient assessment. The normotriglyceridaemic and mildly hypertriglyceridaemic patients were 144 consecutive Type II diabetic patients (35–73 years old, 49 women, 95 men) with at least one additional risk factor included in the DIACOR study (Table 1) aimed to test the predictive value of carotid intima thickness for cardiovascular events. Exclusion criteria were: ketonuria, fasting glycaemia above 15 mmol/l, insulin therapy, concomitant illness (hepatic or renal insufficiency, neoplasm) history of severe hypertriglyceridaemia and heavy alcohol consumption (> 40 g/day). The 119 patients of group N were normolipaemic at the moment of inclusion with triglyceride or total cholesterol plasma concentrations below the 90th centile after adjustment for age and sex without hypolipaemic medication. The 28 patients of group T had triglyceride plasma concentrations above the 90th centile after adjustment for age and sex, the highest triglyceride concentration in this group was 4.9 mmol/l and none of the patients from group T were receiving hypolipaemic agents. The 32 group H patients (Table 1) all fulfilled the criteria of Type II diabetes without ketosis, were obese (BMI > 25) and over 40 years old. Patients were included in group H when they had a documented history of transient episodes of severe fasting hypertriglyceridaemia with plasma triglyceride concentrations over 15 mmol/l and with a triglyceride to total cholesterol ratio above 2.5 (in g/l). They were consecutive patients referred to our university hospital lipid clinics for uncontrolled severe hypertriglyceridaemia and therefore did not originate from the DIACOR cohort. A plasma triglyceride concentration cut-off of 15 mmol/l was selected because it was established that this concentration corresponds with the occurrence of hyperchylomicronaemia [23] which was confirmed in the subjects who still had triglyceride concentrations above 15 mmol/l when referred to our lipid clinic (11/32). Due to the severity of hypertriglyceridaemia, some of the group H patients were treated with insulin (15/32) or fibrates (18/32) to restore plasma triglyceride concentrations to normal. This group was therefore excluded from the analysis of genotype effects on lipid variables. A registered dietetician recorded the diet of all the patients. The study protocol accorded with the requirements of the local ethics committees and written informed consent was obtained from all the patients included in the study.

Biological variables. Blood samples were obtained in the fasting state and drawn on EDTA. Triglyceride and total cholesterol plasma concentrations were measured enzymatically. Apolipoprotein B was measured by ELISA. High density lipoprotein cholesterol (HDLc) and low density lipoprotein cholesterol (LDLc) were measured after ultracentrifugation of triglyceride-rich lipoproteins when plasma triglyceride concentrations were over 4.5 mmol/l. The HbA_{1c} values were measured by HPLC analysis.

Analysis of apoC-III Sst-I and apoC-III IRE DNA polymorphism. We analysed the Sst-I RFLP at nucleotide 3238 of

Table 2. *ApoC-III* allele and genotype distribution of Sst1 (nt-3238) and IRE polymorphisms in Type II diabetic patients

Group	N		T		*p* value	H		*p* value	*p* value
apoC-III	*n*	%	*n*	%	(vs N)	*n*	%	(vs N)	(vs T)
Sst-I alleles	232		56			64			
S1 (3238C)	213	91.8	51	91.1		44	68.7		
S2 (3238G)	19	8.2	5	8.9	NS	20	31.3	< 0.0001	< 0.005
Sst-I genotypes	116		28			32			
S1S1	98	84.4	23	82.1	NS	16	50.0	< 0.0001	< 0.01
S1S2	17	14.7	5	17.0	NS	12	37.5	0.005	NS
S2S2	1	0.9	0	0.0	NS	4	12.5	0.005	NS
nt-455 alleles	232		56			64			
T	136	58.6	32	57.1	NS	37	57.8	NS	NS
C	96	41.4	24	42.9	NS	27	42.2	NS	NS
nt-455 genotypes	116		28			32			
TT	38	32.8	10	35.7	NS	12	37.5	NS	NS
TC	60	51.7	12	42.9	NS	13	40.6	NS	NS
CC	18	15.5	6	21.4	NS	7	21.9	NS	NS
nt-482 alleles	232		56			64			
C	154	66.4	32	57.1	NS	37	57.8	NS	NS
T	78	33.6	24	42.9	NS	27	42.2	NS	NS
nt-482 genotypes	116		28			32			
CC	51	44.0	10	35.7	NS	12	37.5	NS	NS
CT	52	44.8	12	42.9	NS	13	40.6	NS	NS
TT	13	11.2	6	21.4	NS	7	21.9	NS	NS

apoC-III locus in all patients as reported previously [24]. The alleles for the *apoC-III –455T/C* and *–482C/T* polymorphisms were determined in all patients. For this purpose, a 146 bp PCR products (from nucleotide –421 to –525) encompassing the apoC-III IRE were prepared with the following primers: AC3P1 (5'-GAGGTGCTGGAAGGGGCTGTGA-3') and AC3P2 (5'-GAGGGGTGAGGGACTTCTTC-3'). Polymerase chain reaction mix was done with *Taq* polymerase and buffer (Appligene, Oncor, Illkirch, France), deoxyribonucleotide triphosphate (dNTP) (100 µmol/l), primers (100 nmol/l each), DMSO (3%). After 5 min of initial denaturation at 94 °C, 35 cycles of 40 s at 94 °C, 1 min at 58 °C, 40 s at 72 °C were carried out followed by 10 min of primer extention at 72 °C. Two methods were systematically carried out. The nucleotides at position –455 and –482 positions were determined using an allele-specific oligonucleotide hybridization as described previously [17]. A single strand conformational polymorphism (SSCP) analysis was done to investigate the presence of unreported *apoC-III IRE* sequence variations in Type II diabetes. For SSCP analysis, PCR of the *apoC-III IRE* sequence was modified as follows: dNTP were 5 µmol/l and 10 µCi dATPα^{33}P was added (> 93 TBq/mmol). The SSCP analysis of ^{33}P labelled PCR products was carried out using non-denaturing electrophoresis in 12% polyacrylamide (37:1) gels under two conditions: at 4 °C with 10% glycerol and at 20 °C without glycerol.

LPL gene mutation screening and apo E genotyping. In all patients of groups T and H, PCR products for SSCP analysis of *LPL* were prepared as reported previously for exon 1 to 9 [25, 26] and for the proximal promoter sequences (NT –103 to + 45) [27]. The SSCP analysis was carried out according to two methods of electrophoresis as described for the *apoC-III IRE*. The DNA sequencing was done with both forward and reverse primers using the Thermosequenase kit (USB, Amersham Life science, Cleveland, Ohio, USA) according to the manufacturer's instructions. Apolipoprotein E genotypes were determined as reported previously [28].

Statistical methods. Differences between groups of patients for allele and genotype frequencies were tested using either the chi-squared or the Fisher's exact test when appropriate. Differences between groups for the mean values of quantitative measurements were tested using ANOVA or non-parametric analysis (Mann-Whitney or Kruskall-Wallis tests) when appropriate. Differences between groups and/or genotypes were considered as significant when the *p* value was less than 0.05.

Results

In all patients (group N, T and H), only the four reported haplotypes corresponding to a combination of NT *–455C/T* and *–482T/C* polymorphisms were found by systematic SSCP screening for mutations of *apoC-III* proximal promoter (NT –421 to –525). In the Type II diabetic patients without a history of severe hypertriglyceridaemia, the prevalence of alleles or genotypes for *C3Sst-I* RFLP, *apoC-III IRE* polymorphisms and *apo E* were similar in the normolipaemic (group N) or mildly hypertriglyceridaemic (group T) patients (Table 2 and 3). In these subjects (N and/or T) none of the genotypes at *C3Sst-I*, *apoC-III IRE* or *apo E* loci showed a statistically significant association with fasting concentrations of triglyceride, total cholesterol, HDLc, LDLc or apoB (data not shown). The genotype distributions for *apoC-III IRE*, *Sst-I* and *apo E* polymorphisms were in the Hardy-Weinberg equilibrium.

Among the patients with Type II diabetes and a history of severe hypertriglyceridaemia (group H) the *C3Sst-I S2* allele was over threefold as frequent as in group N ($p < 0.0001$) or group T ($p < 0.005$). In

Table 3. *apo E* allele and genotype in Type II diabetes patients

Group	N		T			H			
apo E	*n*	%	*n*	%	*p* (T vs N)	*n*	%	*p* (vs N)	*p* (vs T)
Alleles	232		56				64		
E3	190	81.9	44	78.6	NS	41	64.1	< 0.005	NS
E4	27	11.6	9	16.1	NS	15	23.4	< 0.05	NS
E2	15	6.5	3	5.3	NS	8	12.5	NS	NS
Genotypes	116		28				32		
E3E3	75	64.7	18	64.3	NS	15	46.9	NS	NS
E3E4	26	22.3	6	21.4	NS	8	25.0	NS	NS
E2E3	14	12.1	2	7.1	NS	3	9.4	NS	NS
E4E4	0		1	3.6	NS	1	3.1	NS	NS
E2E4	1	0.9	1	3.6	NS	5	15.6	< 0.005	NS

Table 4. *LPL* alleles in hypertriglyceridaemic Type II diabetic patients

LPL		Group T		Group H	
Alleles (total)		*n* = 56	%	*n* = 64	%
Mutants alleles		–		5	7.8
LPL polymorphisms	D9N/c-93g	2	3.6	2	3.7
	N291S	–		2	3.7
	S447ter	2	3.6	3	4.7
Mutant genotypes		0/28	%	4/32	%
Homozygotes		–		1	3.1
Heterozygotes		–		3	9.4

group H, 50 % of the patients were carriers of the *S2* allele in contrast with only 15.5 % in group N ($p < 0.0001$) and 17.9 % in group T ($p < 0.01$). Both heterozygotes and homozygotes carriers of *S2* allele were statistically significantly over-represented compared with subjects without a history of severe hypertriglyceridaemia (Table 2). In contrast, for the –455 and –482 nucleotide polymorphisms, the allele and genotype frequencies were similar in the three groups of patients (Table 2).

In group H compared with either group N or N + T, the frequency of the common *apo E3* allele was reduced ($p < 0.005$) and the *E2E4* genotype was over-represented ($p < 0.005$) (Table 3). Because *apoC-III S2* polymorphism was highly over-represented in group H, we considered the status of *apo E* locus separately in *S2* carriers and non-carriers. The statistically significant decrease in *apo E3* allele frequency and the increase in *apo E2E4* genotype frequency were found only among the subjects with the *S1S1* genotype (allele *E3*: 59.4 % in group H vs 81.0 % in group N + T, $p = 0.005$ and *E2E4* genotype: 25.0 % in group H vs 1.0 % in group N + T, $p < 0.005$). In contrast the *E2E4* genotype was uncommon among the *S2* carriers of both group H (1/16 patients) and N + T (1/121 patients).

Results of *LPL* gene mutation screening in the 60 hypertriglyceridaemic patients with mild hypertriglyceridaemia or history of severe hypertriglyceridaemia are summarized in Table 4. Inactivating nonsense or missense *LPL* mutations were observed only in group H. One patient was homozygote for the *CGA192TGA* (R192X) mutant. This patient had the apo *E3E3* and *apoC-III Sst-I S1S1* genotypes. Three out of 32 patients were heterozygotes (9.3 %), two for the *GAG188GGG* mutant (G188E) and one for the *AAG147TAG* mutant (K147X). In contrast, common *LPL* gene coding polymorphisms at codon 9, 291 and 447 were found at similar frequencies in group T as in group H (Table 4). In group H, patients with either *LPL* mutants or *E2E4* genotypes or both tended to cluster in the subgroup with the *S1S1* genotype (6/7). Overall a genetic alteration in either loci was found in the majority of the diabetic patients with a history of severe hypertriglyceridaemia: 22 out of 32 patients (68.7 %) were carriers of either *apoC-III S2* allele, *LPL* mutants or *E2E4* genotype, in contrast to only 6 out of 28 in group T (21.4 %, $p < 0.0005$) (Fig. 1).

Discussion

Our study of a large group of Type II diabetic patients with a history of severe hypertriglyceridaemia led us to uncover a critical influence of three loci involved in the regulation of triglyceride-rich lipoprotein clearance: *apoC-III* (*S2* polymorphism), *apo E* (*E3* allele deficiency) and LPL gene mutations. Among Type II diabetic subjects without a history of type-V hyperlipidaemia, the influence of these genetic variants on plasma lipid parameters was, however, insufficient to be detected in our study. Patients with a history of severe hypertriglyceridaemia had a 1.3 % increase in HbA_{1c} value compared with the two other groups. Such a mild impairment in glycaemic control is expected to account for only a moderate increase of triglyceride plasma concentration [29]. It was reported that severe hypertriglyceridaemia in Type II diabetes occurs in only a minority of patients with an additional familial predisposition to dyslipaemia [9, 30].

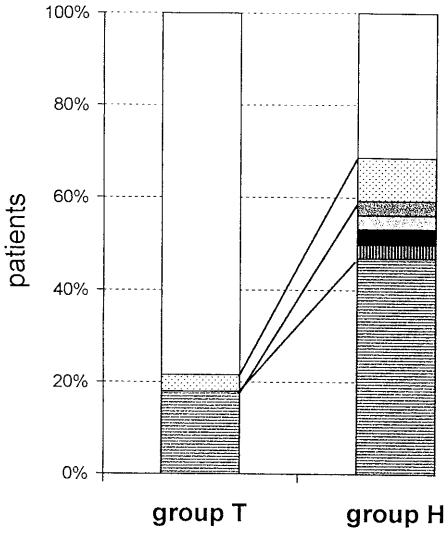

Fig. 1. Distribution of genotypes *apoC-III* and *apo E* polymorphisms, and *LPL* gene mutations in Type II diabetic patients from group T and H. LPL mut: *LPL* deficiency; Het lipoprotein lipase: heterozygous *LPL* deficiency; Ho LPL: homozygous *LPL* deficiency. □ E2E4 genotype, ▒ Het LPL and E2E4, ▒ LPL Het LPL, ■ mut Ho LPL, ▒ Het LPL, S2 and E2E4, ▒ S2 carriers, □ Others

We found a strong association between the occurrence of severe hypertriglyceridaemia and the carrier status for *apoC-III Sst-I* polymorphism (*S2* allele) in Type II diabetes. In non-selected population studies the *S2* variant was consistently associated with hypertriglyceridaemia [12] and with higher plasma concentrations of apoC-III [18, 31], a key modulator of plasma triglyceride clearance which can cause hypertriglyceridaemia when over-expressed [10, 11]. Additionally, the presence of an *S2*-associated *apoC-III* allele is a dominant risk factor for familial forms of dyslipaemia such as familial combined hyperlipidaemia [20, 32] or type III hyperlipoproteinaemia [21]. Furthermore among *apo E2* homozygotes the presence of the *S2* allele has recently been reported to be strongly associated with severe hypertriglyceridaemia exclusively in the subjects with hyperinsulinaemia although not in the overall study [21]. Our results from type II diabetic patients indicate that an *S2*-linked genetic alteration could have a modifier effect on diabetic dyslipaemia and thus provides further confirmation of the hypothesis of an interaction between insulin resistance and the *apoC-III S2* allele.

Although discovered in 1983 [13, 14], the association between the S2 allele and hypertriglyceridaemia has not been explained. The *S2* mutation locates in a non-coding region of *apoC-III* exon 4 and thus does not alter a known functional domain. It was recently suggested that the association of *S2* with increased triglyceride concentrations is related to an enhanced allele-specific expression of the *apoC-III* transcripts bearing the *S2* variant compared with the *S1*-linked allele [33]. This could result from a linkage to a mutation in promotor sequences regulating *apoC-III* transcription. The *T-455C* and *C-482T* variants in the *apoC-III* promoter insulin-responsive element (*IRE*) were shown to associate with hypertriglyceridaemia in non-diabetic subjects [25] but subsequent studies clearly established this to be related to linkage-disequilibrium with the *S2* allele [12]. An involvement of *IRE* polymorphisms in diabetic dyslipaemia was, however, not ruled out because both *IRE* variants induce an insulin-responsiveness defect [22] and insulin was shown to down-regulate the transcription of *apoC-III* gene both in the human hepatoma hepG2 cell line and in a rodent diabetes model [34]. Our mutation-screening of *apoC-III IRE* only found the previously reported *T-455C* and *C-482T* polymorphisms and neither statistically significantly influenced plasma triglyceride concentration nor were they associated with the occurrence of mild or severe dyslipaemia. Our data, overall, indicates that a contribution of genetic alterations in the *apoC-III IRE* to mild or to severe hypertriglyceridaemia in Type II diabetes is, in contrast to *Sst1* polymorphism, unlikely.

Heterozygote *LPL* deficiency has been shown to be a risk factor for severe dyslipaemia in non-diabetic subjects [25, 35, 36] and could, together with age-related influences, account for the occurrence of a familial form of hypertriglyceridaemia [37–39]. Furthermore, insulin resistance was suggested to be a determinant of the occurrence of hypertriglyceridaemia in heterozygous *LPL* deficiency [9, 40, 41]. We did not, however, detect *LPL* gene alterations in patients with mild hypertriglyceridaemia, in agreement with a previous study of diabetic and hypertriglyceridaemic members of Type II diabetic families [42]. In Type II diabetes with severe hypertriglyceridaemia, *LPL* gene mutations were reported either in isolated cases [9] or in a small group of patients [43]. Lipoprotein lipase activity in plasma after heparin treatment and in adipose tissue is decreased in hypertriglyceridaemic Type II diabetic patients and in insulin-resistant obese subjects [30, 44]. Therefore, heterozygote genetic impairment could further reduce the functional lipoprotein lipase activity and lead to severe hypertriglyceridaemia. We found, however, only 4 patients with *LPL* gene mutations in our larger group of 32 subjects with severe hypertriglyceridaemia. The mutant alleles detected in our study were either previously documented as defective (G188E) [26] or were nonsense (K147X and R192X) predicting severe lipoprotein lipase function defects [45]. Deficiency of LPL is likely to account, however, only for a minority of the severe hypertriglyceridaemic states in Type II diabetic patients (only 12.5 % in our study).

Both *E2* and *E4* alleles are over-represented in non-diabetic patients with severe hypertriglyceridaemia [46, 47]. In Type II diabetes, only two studies of small groups ($n < 10$) of patients with severe hyper-

triglyceridaemia evidenced a low frequency of the *apo E3* allele compared with normolipaemic diabetic patients [7, 8]. We confirm and extend these observations: we report an over-representation of the *E2E4* genotype in severe hypertriglyceridaemia. This rare *apo E* genotype could therefore determine a particular risk for severe dyslipaemia, at least in Type II diabetes. It was established in 1989 that *E2* allele is a determinant of triglyceride concentrations in Type II diabetes [48] as in non-diabetic subjects [49]. The *E4* allele was also associated with increased fasting triglyceride concentrations [49, 50] and considered as a statistically significant determinant of the postprandial response of triglycerides in population studies [51, 52]. Furthermore in Type II diabetic patients without fasting hyperlipaemia the carrier status for either *E2* or *E4* or both was a determinant of delayed and increased postprandial lipaemia [53]. An enhanced risk for hyperlipoproteinaemia when *E2* or *E4* variants or both are expressed could therefore account for the low prevalence of the *E3* allele that we observed in our group of Type II diabetic patients with severe hypertriglyceridaemia.

Our data, overall, suggests that *S2* or *IRE*-linked *apoC-III* gene alteration, *LPL* mutations or *apo E3* deficiency do not play a major part in the occurrence of mild hypertriglyceridaemia in Type II diabetes when subjects with a history of severe hypertriglyceridaemia are excluded. It was earlier suggested that most Type II diabetic patients with severe hyperlipaemia have an independent familial form of hypertriglyceridaemia [30]. We found an association between severe hypertriglyceridaemia in Type II diabetes and variants at three loci crucial for the control of triglyceride-rich lipoprotein clearance, in nearly 70 % of the patients. In these subjects, the most frequent variant was the *apoC-III S2* polymorphism, present in half of the patients. Notably the *E2E4* and *LPL* mutation carriers tended to cluster in the subgroup of patients without the *S2* allele. This finding needs to be considered with caution because of the small number of patients in the subgroups. Our results strongly suggest that the predisposition to severe hypertriglyceridaemia in Type II diabetes frequently requires, at least as co-factors, alterations at genetic loci crucially involved in the control of triglyceride-rich lipoprotein clearance and associated with plasma triglycerides variability in population studies. No relevant association was observed in about one third of these patients; thus other alterations at *apoC-III*, *LPL*, or *apo E* loci as well as at additional genetic loci could be involved.

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