Butyrylcholinesterase K variant on chromosome 3q is associated with Type II diabetes in white Caucasian subjects

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Abstract

Aims/hypothesis. To determine the association of three genes associated with Alzheimer's disease – butyrylcholinesterase (*BcHE*) on chromosome 3q, α 2 macroglobulin (α 2M) on chromosome 12p and apolipoprotein E (*ApoE*) on chromosome 19q – with Type II (non-insulin-dependent) diabetes mellitus.

Methods. Frequencies of *BcHE* K variant, α 2M insertion and/or deletion polymorphism, the *ApoE* common polymorphisms and promoter variants at *ApoE*-491 and –291, were examined by fluorescent RFLP in DNA from 276 United Kingdom Prospective Diabetes Study Type II diabetic subjects and 351 non-diabetic subjects from the Diabetes In Families study. Genetic data in diabetic subjects was analysed in relation to clinical characteristics and islet function as assessed by the requirement for insulin therapy 6 years after randomisation.

Results. The *BcHE* K variant allele was more common among Type II diabetic subjects (D) than non-diabetic subjects (ND) (22.8% D vs 15.8% ND; $p = 0.00017$). Subjects homozygous for the variant

were more frequent in the diabetic group (5.8% D vs 2.6% ND; $p = 0.00056$). The K variant allele frequency was not associated with a requirement for insulin therapy (29.0% insulin-requiring vs 21.8% non-insulin-requiring; $p = 0.121$). There were no associations of α 2M and *ApoE* polymorphisms or *ApoE* promoter variants with clinical characteristics or insulin requirement in diabetic subjects. There were differences in total cholesterol ($p = 0.0005$) and LDL-cholesterol ($p = 0.0009$) among non-diabetic subjects in relation to *ApoE*-491 genotypes.

Conclusion/interpretation. The association of the *BcHE* gene (3q26) with Type II diabetes could be related to an identified susceptibility locus on chromosome 3q27 but appears to be independent of islet function. The absence of diabetes-specific associations with α 2M, *ApoE* or *ApoE* promoter variants suggest that these are not important in the onset of hyperglycaemia. [Diabetologia (2001) 44: 2227–2230]

Keywords Butyrylcholinesterase, apolipoprotein E, α 2-macroglobulin, Type II diabetes, Alzheimer's disease, UKPDS, DIF, islet function, lipid, lipoprotein.

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Abbreviations: ICA, Islet cell antibodies; GADA, glutamic acid decarboxylase antibodies; RFLP, restriction fragment length polymorphism; FPG, fasting plasma glucose; *ApoE*, apolipoprotein E; *BcHE*, butyrylcholinesterase; α 2M, α 2-macroglobulin; DIF, Diabetes in Family Study

Type II (non-insulin-dependent) diabetes mellitus and Alzheimer's disease are both age-related diseases with complex multifactorial causes involving both genetic and environmental factors. Both diseases show slow progressive deterioration and are associated with progressive amyloid deposition in the major affected organs: the brain in Alzheimer's disease and pancreatic islets in diabetes [1]. The causal factors for islet dysfunction in Type II diabetes are not known but could be related to genetic susceptibilities associated with age-related disease and amyloid de-

position similar to those identified for Alzheimer's disease. Susceptibility loci for Type II diabetes have been identified on a number of chromosomes including 1q, 2qter, 10q, 12q and 20q. The locus on 3q27 linked to Type II diabetes and impaired glucose tolerance in patients with young onset Type II diabetes (< 45 years) is approximately 25 cM telomeric to the *BcHE* locus (encoded on chromosome 3q26.1–26.2) and variants of both *BcHE* and the serum pan-protease α_2M have been linked to Alzheimer's Disease [2, 3, 5]. Apolipoprotein E (*ApoE*) $\epsilon 4$ allele and the promoter variants at –491 AT and –219 GT are associated with increased risk of early development of more severe Alzheimer's Disease [2]. The lipoprotein isotypes influence lipid metabolism and have been shown to be associated to renal disease in Type I (insulin-dependent) diabetes mellitus [4]. Although the functional significance of *BcHE* and its variants is not clear, α_2M and *ApoE* have been identified as ligands for the large, dense lipoprotein receptor-related protein (LRP) [6] and could therefore have a role in lipoprotein metabolism in diabetes and other conditions.

BcHE occurs in plasma and most tissues and is encoded by a single copy gene on chromosome 3q26.1–26.2. *BcHE* K, G1615A, is the most common variant (homozygous frequency 1–4%) and results in alanine to threonine exchange at position 539 and a 30% reduction in enzyme activity [7]. The common polymorphism of the protease inhibitor, α_2M , is a five base-pair insertion/deletion occurring in the 5' splice site of exon 18 [8]. This study aimed to determine the frequency of genetic variants for *BcHE*, α_2M and the *ApoE* gene common polymorphisms and promoter mutations in Type II diabetic subjects and in a non-diabetic control population and to identify any characteristic phenotype associated with the genetic variants.

Subjects and methods

DNA was extracted from peripheral blood leucocytes from 276 white Caucasian subjects with newly diagnosed Type II diabetes included in the United Kingdom Prospective Diabetes Study (UKPDS) [9], 351 subjects without diabetes from the Diabetes in Families study (DIF), randomly chosen from general practitioner lists, but unrelated to the probands. Approval of our ethical committee was obtained, and all individuals gave their informed consent for DNA analyses.

A subset of UKPDS subjects were chosen at random [9] from patients who either had a family history of diabetes and FPG of 8.0 mmol/l or more ($n = 59$); or were 50 years of age or under at diagnosis with FPG between 6.0 and 7.8 mmol/l ($n = 25$); or were 50 years of age or more at diagnosis with FPG between 6.0 and 15.0 mmol/l ($n = 192$).

UK Prospective Diabetes Study (UKPD) subjects were GADA and ICA negative, 60% male, age (mean (SD)) 51 [9] years, BMI 25.8 (5.3) kg m⁻², fasting plasma glucose (median (IQR)) 7.8 (6.3 to 11.7) mmol/l, fasting plasma insulin (geometric mean (1SD interval)) 81(46 to 142) pmol/l, total cholesterol 5.3 (1.1) mmol/l, LDL-cholesterol 3.4 (1.0) mmol/l, HDL-

cholesterol 1.08 (0.22) mmol/l and plasma triglyceride (geometric mean (1SD interval)) 1.4 (0.9 to 2.2) mmol/l.

To assess islet dysfunction, UKPDS subjects were classified by requirement for insulin therapy 6 years after randomisation into the study. Subjects not randomised to insulin were deemed insulin-requiring when maximal doses of oral hypoglycaemic agents failed to maintain FPG at under 15.0 mmol/l. Those randomised to insulin therapy were deemed insulin requiring if their insulin dose was over 0.7 U/kg. In this situation requirement for insulin can be used as an indicator of islet dysfunction because endogenous insulin supply is insufficient to maintain glycaemia within acceptable levels.

Non-diabetic control subjects from DIF were 43% male but of similar age (means $\pm$ SEM 55 (18) years) and BMI (25.3 (4.0) kg/m²) to the UKPDS subjects.

All gene variants were detected using fluorescently tagged primers and RFLPs which were identified against standards (Perkin Elmer, Foster City, Calif., USA) run on an automated sequencer (ABI 377, Applied Biosystems, Foster City, Calif., USA) except the *ApoE* 112 C-T which required RFLP analysis on agarose gels.

Statistical analyses were carried out with SAS statistical software package (SAS, Carey, N.C., USA). The significance of differences between genotypic and allelic frequency were assessed by chi square (χ^2) or Fisher's exact test. Odds ratios (OR (95%-CI)) were calculated from frequency tables. Comparisons of demographic and biochemical characteristics were done using Kruskal-Wallis and Mann-Whitney U tests or chi square (χ^2) or Fisher's exact tests as appropriate. We considered a p value of less than 0.05 to be statistically significant.

Results

All genotypes examined were in Hardy-Weinberg equilibrium. An increased frequency ($p = 0.00079$) of subjects homozygous for the *BcHE* K variant (AA) was observed in newly diagnosed Type II diabetic subjects ($n = 276$) compared with the non-diabetic control group ($n = 348$) (Table 1). The frequency of the K-variant allele (A) was increased in the diabetic group compared with the control group ($p = 0.00017$). The odds ratio (OR) of 1.44 (95%-CI, 1.15 to 1.82) represents a 44% increased risk of having diabetes associated with the presence of the K variant. There were no significant associations of genotypes or alleles with sex, age, BMI, FPG, plasma insulin, total, LDL-cholesterol or HDL-cholesterol fractions, or plasma triglyceride in these newly diagnosed Type II diabetic subjects. A similar distribution of genotype and allele frequencies was found in the DIF Type II diabetic subjects.

To determine whether there was an association with islet dysfunction, genotype and allele, frequencies were compared in relation to insulin requirement at 6 years after entry into the UKPDS (Table 1). There were no significant differences in either genotype ($p = 0.296$) or allele ($p = 0.121$) frequencies between the insulin requiring ($n = 50$) and non-insulin requiring ($n = 225$) groups. These data indicate that although there is an association of the *BcHE* K variant with Type II diabetes, it is not related to islet dys-

Table 1. Frequencies (%) of *BcHE* genotypes and alleles in subjects with newly-diagnosed Type II diabetes and non-diabetic (control) subjects

	Genotype frequencies			<i>p</i> value	Allele frequencies		<i>p</i> value
	<i>GG</i>	<i>GA</i>	<i>AA</i>		<i>G</i>	<i>A</i>	
Newly-diagnosed Type II diabetes <i>n</i> = 276	166 (60.1)	94 (34.1)	16 (5.8)	0.00079 ^b	426 (77.2)	126 (22.8)	0.00017 ^a
Control subjects <i>n</i> = 348	247 (71.0)	92 (26.4)	9 (2.6)		586 (84.2)	110 (15.8)	OR (95 %-CI) 1.46 (1.16 to 1.84)
Insulin requiring ^a <i>n</i> = 50	25 (50.0)	21 (42.0)	4 (8.0)	0.296 ^a	71 (71.0)	29 (29.0)	0.121 ^a
Not insulin requiring <i>n</i> = 225	139 (61.8)	74 (32.9)	12 (5.3)		352 (78.2)	98 (21.8)	OR (95 %-CI) 1.36 (0.92 to 2.00)

K variant = A

^aInsulin requiring 6 years after randomisation to the UKPDS;
 χ^2 test^bFisher's exact test**Table 2.** Frequencies of *α2M* and *ApoE* gene and promoter genotypes and alleles in subjects with newly diagnosed Type II diabetes and non-diabetic (control) subjects

	Genotype frequencies			<i>p</i> value ^a	Allele frequencies		<i>p</i> value ^a
	<i>II</i>	<i>ID</i>	<i>DD</i>		<i>I</i>	<i>D</i>	
<i>α2M</i>							
Newly-diagnosed Type II diabetes <i>n</i> = 274	192 (70.1)	69 (25.2)	13 (4.7)	0.816 ^b	453 (82.7)	95 (17.3)	0.606
Control subjects <i>n</i> = 348	248 (71.3)	87 (25.0)	13 (3.7)		583 (83.8)	113 (16.2)	
<i>ApoE</i> – 491 promoter	<i>AA</i>	<i>AT</i>	<i>TT</i>		<i>A</i>	<i>T</i>	
Newly-diagnosed Type II diabetes <i>n</i> = 272	193 (70.1)	73 (26.7)	6 (2.2)	0.691 ^b	459 (84.4)	85 (15.6)	0.516
Control subjects <i>n</i> = 347	236 (68.0)	104 (30.0)	7 (2.0)		576 (83.0)	118 (17.0)	
<i>ApoE</i> – 219 promoter	<i>GG</i>	<i>GT</i>	<i>TT</i>		<i>G</i>	<i>T</i>	
Newly-diagnosed Type II diabetes <i>n</i> = 276	70 (25.4)	136 (49.2)	70 (25.4)	0.652 ^b	276 (50.0)	276 (50.0)	0.960
Control subjects <i>n</i> = 351	83 (23.6)	186 (53.0)	82 (23.4)		352 (50.1)	350 (49.9)	
<i>ApoE</i>	Genotype frequencies				Allele frequencies		<i>p</i> value
	$\epsilon 2/\epsilon 2$	$\epsilon 2/\epsilon 3$	$\epsilon 2/\epsilon 4$	$\epsilon 3/\epsilon 3$	$\epsilon 3/\epsilon 4$	$\epsilon 4/\epsilon 4$	
Newly-diagnosed Type II diabetes <i>n</i> = 276	2 (0.7)	39 (14.1)	4 (1.5)	170 (61.6)	57 (20.7)	4 (1.5)	0.372 ^b
Control subjects <i>n</i> = 349	3 (0.9)	37 (10.6)	13 (3.7)	208 (59.6)	81 (23.2)	7 (2.0)	
	Allele frequencies			<i>p</i> value ^a			
	$\epsilon 2$	$\epsilon 3$	$\epsilon 4$				
Newly-diagnosed Type II diabetes <i>n</i> = 276	47 (8.5)	436 (79.0)	69 (12.5)	0.323			
Control subjects <i>n</i> = 349	56 (8.0)	534 (76.5)	108 (15.5)				

^a χ^2 test except^b Fisher's exact test

function as defined by the requirement for insulin therapy.

There was no association with diabetes of the genotype and allele frequencies for the *α2M* ins/deletion polymorphism (Table 2). Neither was there any association of the *α2M* variant with any of the demo-

graphic or biochemical characteristics of the UKPDS subjects. The allele frequencies were similar to those occurring in the general population.

The frequencies of *ApoE* polymorphisms in UKPDS diabetic subjects (Table 2) were similar to those in the normal population as reported previously

[10]. The allele and genotype frequency distribution of the *ApoE* -491 and -219 promoter variants also reflected those in the general population [11] and no significant association was found for either variant with Type II diabetes. There were no significant associations of -491 or -219 promoter genotypes with any of the demographic or biochemical characteristics of the UKPDS subjects.

In control subjects, there were significant differences in both total and LDL-cholesterol concentrations in relation to the *ApoE* -491 promoter genotypes. Total cholesterol concentration (means $\pm$ SEM was 4.7 (0.8) mmol/L (TT, $n = 7$) compared with 5.1 (1.) mmol/l (AT, $n = 104$) and 5.5 (1.1) mmol/l (AA, $n = 236$) ($p = 0.0005$). LDL-cholesterol concentration was 2.8 (0.7) mmol/l (TT) compared with 3.0 (0.8) mmol/l (AT) and 3.5 (1.0) mmol/l (AA) ($p = 0.0009$). Tests for trend across the genotypes did not reach statistical significance. There were no significant differences in HDL-cholesterol or plasma triglyceride concentrations in these groups.

Discussion

These data indicate an association between the variant for *BcHE* (encoded on chromosome 3q26) and diabetes. Diabetes susceptibility loci have been identified in 3q27-qter close to *BcHE* and quantitative trait loci for diabetes-related complications have also been reported in this region [3, 5], suggesting that the K variant could be in linkage disequilibrium with an unidentified gene or genes. However this association is not related to the requirement for insulin treatment as might be expected if it were an important factor in islet dysfunction and amyloid deposition. No associations of *ApoE* common polymorphisms or the promoter variants with Type II diabetes were detected suggesting that activity of this gene does not play a primary role in causing hyperglycaemia.

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References

1. Clark A (1992) Islet amyloid: an enigma of Type 2 diabetes. *Diabetes Metab Rev* 8: 117–132
2. Tilley L, Morgan K, Kalsheker N (1998) Genetic risk factors in Alzheimer's disease. *J Clin Pathol* 51: 293–304
3. Vionnet N, Hani E-H, Dupont S et al. (2001) Genome-wide search for Type 2 susceptibility genes in French Whites: evidence for a novel susceptibility locus for early-onset diabetes on chromosome 3q27-qter and independent replication of a Type 2 diabetes locus on chromosome 1q21q24. *Am J Hum Genet* 67: 1470–1480
4. Chowdhury TA, Dyer PH, Kumar S et al. (1998) Association of apolipoprotein $\epsilon 2$ allele with diabetic nephropathy in Caucasian subjects with IDDM. *Diabetologia* 47: 278–280
5. Rainwater DL, Almasy L, Blangero JA et al. (1999) Genome search identifies major quantitative trait loci on human chromosomes 3 and 4 that influence cholesterol concentrations in small LDL particles. *Arterioscler Thromb Vasc Biol* 19: 777–783
6. Kang DE, Pietrzik CU, Baum L et al. (2000) Modulation of amyloid β -protein clearance and Alzheimer's disease susceptibility by the LDL receptor-related protein pathway. *J Clin Invest* 106: 1159–1166
7. Bartels CF, Jensen FS, Lockridge O et al. (1992) DNA mutation associated with the human butyrylcholinesterase K-variant and its linkage to the atypical variant mutation and other polymorphic sites. *Am J Hum Genet* 50: 1086–1103
8. Blacker D, Wilcox MA, Laird NM et al. (1998) Alpha-2-macroglobulin is genetically associated with Alzheimer's disease. *Nat Genet* 19: 357–360
9. UKPDS Group (1991) UK Prospective Diabetes Study VIII: Study design, progress and performance. *Diabetologia* 34: 877–890
10. Powell DS, Maksoud H, Hattersley AT et al. (1999) Apolipoprotein E genotype is not associated with islet amyloid as assessed by disease severity in Type 2 diabetes *Diabetologia* 42 [Suppl 1]: A146 (Abstract)
11. Lambert J-C, Brousseau T, Defosse V et al. (2000) Independent association of an ApoE gene promoter polymorphism with increased risk of myocardial infarction and decreased ApoE plasma concentrations – the ECTIM Study. *Hum Mol Genet* 9: 57–61