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# Large-scale studies of the functional K variant of the butyrylcholinesterase gene in relation to Type 2 diabetes and insulin secretion

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## Abstract

*Aims/hypothesis.* Polymorphisms of the butyrylcholinesterase gene (*BCHE*) are reported to associate with Alzheimer's disease and a recent study found a significant association of the *BCHE* K variant (*G1615A/Ala539Thr*) with Type 2 diabetes. The objectives of our study were to examine whether the *BCHE* K variant is associated with Type 2 diabetes or estimates of pancreatic beta cell function in large-scale populations of glucose-tolerant Caucasians.
*Methods.* The variant was genotyped in association studies comprising a total of 1408 Type 2 diabetic patients and 4935 glucose-tolerant control subjects. Genotype–phenotype studies were carried out in the 4935 glucose-tolerant control subjects.
*Results.* There was no difference in allele frequency between Type 2 diabetic patients and control subjects (20.3% [95% confidence interval: 18.8–21.8] vs 20.4% [19.6–21.2], non-significant). In the genotype–phenotype studies we found no consistent association with BMI, fasting or post-OGTT plasma glucose, serum insulin or serum C-peptide levels.
*Conclusions/interpretation.* The present study does not support the suggestion that the *BCHE* K polymorphism is associated with Type 2 diabetes or with estimates of pancreatic beta cell function in large-scale Danish Caucasian populations.

**Keywords** Butyrylcholinesterase · Genetic epidemiology · K variant · Mutations · Type 2 diabetes mellitus

## Introduction

Amyloid fibrils formed from pancreatic islet amyloid polypeptide (IAPP) may be present in more than 90% of cases of Type 2 diabetes [1]. Little is known about the molecular mechanisms causing the change of the monomeric, soluble IAPP form to insoluble refolded fibrils. Severe islet amyloidosis is correlated with the need for insulin therapy, loss of islet beta cells and may affect up to 80% of islets of Type 2 diabetic patients [1]. Amyloid fibrils cause excessive production of superoxide radicals, lipid peroxidation and nitric oxide inactivation, all of which may contribute to beta cell apoptosis [1]. The huge variation in pancreatic islet amyloid production and disposition is, however, not explained by variations in *IAPP* [1, 2].

Cytotoxic effects similar to those of pancreatic beta cell amyloid fibrils have been seen with fibrils formed from the amyloid peptide that occurs in Alzheimer's disease, and indeed, deposition of amyloid plaques in the brain of Alzheimer patients is a characteristic histopathological feature [1, 3, 4]. The mechanisms of fibril-induced toxicity, both in Alzheimer's disease and in Type 2 diabetes, involve interaction between the cell membranes and mis-folded insoluble peptides.

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*Abbreviations:* BCHE, butyrylcholinesterase · IAPP, islet amyloid polypeptide · MAF, minor allele frequency · NGT, normal glucose-tolerant · OHA, oral hypoglycaemic agent

It is therefore possible that these molecular processes share common regulatory elements [1].

The *BCHE* gene (3q26) is expressed in many tissues including white adipose tissue, liver, brain and pancreas [5, 6] and may have a direct biological impact on the pathogenesis of the amyloidosis related to Alzheimer's disease [7]. Several variants have been identified in *BCHE*, the most common being a point mutation at nucleotide 1615 (*GCA* to *ACA*) predicting an alanine to threonine substitution at codon 539 [8, 9]. In the pertinent literature, this *BCHE* polymorphism, which has an allelic frequency of about 20% among Caucasians, is often termed the K variant. This variant is associated with a 30% reduction of the catalytic activity of the secreted and circulating BCHE protein [8] and has a high propensity for beta sheet formation, which may be related to amyloidogenesis [10, 11]. It is thus of interest that studies have shown an association between the codon 539 *threonine* allele and late-onset Alzheimer's disease [12, 13, 14].

As reasoned above, *BCHE* is also a plausible biological candidate gene in the pathogenesis of the common and probably polygenic forms of Type 2 diabetes. Moreover, a locus on chromosome 3q27 has been linked to Type 2 diabetes in a French population [15]. The objectives of the present study were to elucidate whether the widespread and functional K polymorphism of *BCHE* is associated in large groups of Danish Caucasians with Type 2 diabetes or altered insulin secretion in response to oral glucose.

## Subjects, materials and methods

*Subjects.* Genotyping of the K variant was performed in two separate studies.

The first study involved a group of unrelated Type 2 diabetic patients recruited at Steno Diabetes Center and a group of unrelated normal glucose-tolerant (NGT) subjects randomly sampled between 1994 and 1997 at Steno Diabetes Center and Research Centre for Prevention and Health [16]. In the group of Type 2 diabetic patients ($n$=809, 496 men, 313 women), the median age was 62 years (interquartile range: 53–69), the age of clinical diagnosis 55 years (48–63), BMI 28.0 kg/m$^2$ (25.4–31.6) and $HbA_1c$ 7.9% (6.8–9.1). These patients were treated with diet alone (27%), with oral hypoglycaemic agents (OHA) (58%), or with insulin alone or in combination with OHA (15%). In the group of NGT participants ($n$=511, 239 men, 272 women) the age was 60 years (52–61) and BMI 25.1 kg/m$^2$ (23.2–27.6).

The second study involved a population-based group of unrelated NGT subjects sampled at Research Centre for Prevention and Health [17] and a group of unrelated Type 2 diabetic patients recruited at Steno Diabetes Center and Research Centre for Prevention and Health [16, 17]. In the group of Type 2 diabetic patients ($n$=599, 338 men, 261 women) the median age was 55 years (46–60), age of diagnosis 53 years (45–60), BMI 29.1 kg/m$^2$ (26.1–32.9), and $HbA_1c$ 6.5% (6.0–7.5). Treatment of these patients was: by diet alone (26%), with OHA (20%), or with insulin alone or in combination with OHA (12%). For 248 (42%) of the patients, diabetes was diagnosed upon examination for inclusion in this study, and they were therefore not receiving any treatment. In the group of NGT participants ($n$=4424, 2052 men, 2372 women) the median age was 45 years (40–50), BMI 25.0 kg/m$^2$ (22.7–27.6) and 14.5% had a family history of Type 2 diabetes.

In both studies we performed case–control studies as well as genotype–phenotype studies in the NGT subjects. Diabetes was diagnosed according to 1999 WHO criteria [18]. All control subjects underwent a standard 75-g OGTT. All participants were Danish Caucasians by self-report. Informed written consent was obtained before participation. The study was approved by the Ethics Committee of Copenhagen and was in accordance with the principles of the Declaration of Helsinki II.

*Biochemical assays.* Blood samples were drawn after a 12-h overnight fast and plasma glucose, serum-specific insulin levels, serum C-peptide, and $HbA_1c$ were measured using Steno Diabetes Center routine methods [17, 19].

*Genotyping.* In the first study, the *BCHE* K variant was genotyped using restriction site generating PCR-RFLP analysis with forward primer 5′-AAG TGG GTC AAG AAA AGA GCA-3′ and reverse primer 5′-TGC TTT CCA CTC CCA TTC AG-3′ (mismatched nucleotide underlined). PCR amplification was carried out in a volume of 25 µl with 2 mmol/l $MgCl_2$ and annealing at 57 °C, followed by digestion with *Alu*I (New England Biolabs, Beverly, Mass., USA). In the second study genotyping was performed using a chip-based matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (DNA MassARRAY) of PCR-generated primer extension products as described [20]. The genotyping success rates of the RFLP-based method and the mass-spectrometry-based method were 97% and 96% respectively. Genotyping 10% of the study participants with any of the two genotyping methodologies resulted in an error rate of less than 0.5%.

*Statistical analysis.* Fisher's exact test was applied to examine differences in allele frequencies and genotype distributions between diabetic and non-diabetic subjects. A general linear model was used to test variables (or transformed variables) for differences between genotype groups in the two samples of unrelated NGT subjects. Genotype and sex were considered as fixed factors and age and BMI as covariates. All phenotype analyses were performed using Statistical Package for Social Science (SPSS, Chicago, Ill., USA) version 11.5. A two-sided $p$ value of less than 0.05 was considered significant. Results of both case-control studies were combined by logistic regression using RGui version 1.7.0 (http://mirrors.sunsite.dk/cran/), which was also used for the power calculation (power.prop.test method).

## Results

In the two separate case-control studies of association of the *BCHE* K variant with Type 2 diabetes, genotypes were in Hardy–Weinberg equilibrium. No difference in genotype distribution or allele frequency was found in either of the studies (Table 1). Combined analysis of 1408 Type 2 diabetic patients and 4935 glucose-tolerant subjects was performed and yielded no difference in genotype distribution ($p$=1.0). In a simple combination of allele frequencies from the two studies there was also no significant difference between Type 2 diabetic patients and glucose-tolerant control subjects (20.3% [95% CI: 18.8–21.8] vs 20.4% [19.6–21.2] respectively, $p$=0.9). When we combined

**Table 1.** Genotype distribution and allele frequencies of the K variant in *BCHE* among Type 2 diabetic patients and glucose-tolerant subjects

| | Type 2 diabetic patients | Glucose-tolerant subjects | *p* for allele frequency | *p* for genotype distribution |
|---|---|---|---|---|
| **Study 1** | 809 | 511 | | |
| *Ala/Ala* | 516 (64) | 336 (66) | | |
| *Ala/Thr* | 254 (31) | 155 (30) | | |
| *Thr/Thr* | 39 (5) | 20 (4) | | 0.7 |
| MAF | 20.5 (18.6–22.5) | 19.1 (16.7–21.5) | 0.4 | |
| **Study 2** | 599 | 4424 | | |
| *Ala/Ala* | 382 (64) | 2781 (63) | | |
| *Ala/Thr* | 195 (33) | 1468 (33) | | |
| *Thr/Thr* | 22 (4) | 175 (4) | | 0.9 |
| MAF | 19.9 (17.7–22.2) | 20.5 (19.7–21.4) | 0.6 | |

Data are numbers of subjects with each genotype (% of each group) and minor allele frequency (MAF) in % (95% CI). All genotype groups obeyed Hardy–Weinberg equilibrium. The *p* values are for comparisons between Type 2 diabetic patients and glucose-tolerant subjects

the oldest control subjects from both studies (1408 sex-matched NGT subjects, median age 55 years [interquartile range: 55–60]) and compared them with the total number of diabetic patients, this yielded a similar non-significant result (data not shown). We also did sub-stratification according to sex, age at diabetes diagnosis, BMI below or equal to and above 30 kg/m$^2$, or insulin requirement. However, this, too, failed to reveal significant associations between subsets of Type 2 diabetes and *BCHE* genotypes (data not shown).

In the initial genotype–phenotype study (study 1) we showed no significant association of the K polymorphism with fasting or post-OGTT plasma glucose, serum insulin or serum C-peptide levels (Table 2). Applying a recessive model of inheritance, we demonstrated that homozygous carriers of the variant had a higher BMI. Thus, in the 20 glucose-tolerant subjects who were homozygous carriers of the *Thr*-allele we found a median BMI of 28.1 kg/m$^2$ (interquartile range: 25.8–29.8) versus 25.1 kg/m$^2$ (23.2–27.6) in 491 glucose-tolerant subjects who were either wild-type or heterozygous for the *Thr*-allele ($p$=0.03). However, in study 2 we failed to replicate an association between the K variant and increased BMI (Table 3). The genotype–phenotype studies were also stratified according to sex and BMI; however, the analyses in the subgroups did not show any significant genotype–phenotype associations (data not shown). Moreover, in a subset of 248 screen-detected Type 2 diabetic patients the *Thr*-allele frequency was 19.2% (95% CI: 15.7–22.6), a value that was similar to the frequency observed in the remaining diabetic patients.

## Discussion

In the present large-scale studies we were unable to show evidence for a significant association between the functional amino acid polymorphism at codon 539 of *BCHE* and Type 2 diabetes in the Danish population. This was also the case when the group of diabetic patients were sub-stratified according to sex, age of clinical diabetes onset, BMI, or need of insulin therapy. The present findings are in contrast to a recent British study involving 276 Type 2 diabetic subjects and 348 non-diabetic subjects; however, the latter subjects were not classified according to their response to a standardised OGTT [21, 22]. In the British study, the allele frequencies were 22.8% (95% CI: 19.3–26.3) and 15.8% (13.1–18.5) among Type 2 diabetic patients and non-diabetic subjects respectively. Assuming a significance level of 0.05 our study has a power of 99.7% for detecting a difference of the same magnitude as that reported in the British study, and a power of 65% to detect a relative diabetes risk of 1.2. Thus, we cannot completely rule out the possibility that the K variant of *BCHE* has a diabetogenic impact. Differences in ethnicity and known glucose tolerance status of the control subjects may account for some of the discrepancy observed. However, the failure to replicate the original finding may be primarily attributable to differences in sample sizes, as larger studies often give a more conservative estimate of the genetic effect [23]. The observation that small studies may yield more favourable outcomes than larger studies suggests either genuine heterogeneity or a bias against publication of small studies with negative results [23]. This underscores the need for large, well-characterised samples in investigations of Type 2 diabetes and other genetically complex diseases.

To show subtle effects of the K variant on pancreatic beta cell function, we measured serum insulin and C-peptide release after an oral glucose load in the population-based samples of normal glucose-tolerant subjects. No consistent impact of the gene variant could be demonstrated.

**Table 2.** Anthropometric and metabolic characteristics of 511 glucose-tolerant Danish Caucasians (study 1) stratified according to *BCHE Ala539Thr* genotype

| | *Ala/Ala* | *Ala/Thr* | *Thr/Thr* | *p* | $p_{rec}$ |
|---|---|---|---|---|---|
| Number (men/women) | 336 (163/173) | 155 (67/88) | 20 (9/11) | | |
| Age (years) | 60 (52–61) | 60 (52–61) | 60 (60–61) | | |
| BMI (kg/m$^2$) | 25.1 (23.3–27.8) | 25.2 (23.1–27.3) | 28.1 (25.8–29.8) | 0.09 | 0.03 |
| Waist (cm) | 87.0 (77.0–94.0) | 86.0 (79.0–93.0) | 88.0 (80.0–98.0) | 0.3 | 0.4 |
| Fasting p-glucose (mmol/l) | 5.1 (4.7–5.4) | 5.1 (4.8–5.4) | 5.2 (4.9–5.6) | 0.5 | 0.4 |
| Post-OGTT AUC p-glucose (min·mmol$^{-1}$·l$^{-1}$) | 183 (104–258) | 196 (99–308) | 185 (120–280) | 0.6 | 0.8 |
| Fasting s-insulin (pmol/l) | 35 (26–48) | 34 (26–48) | 33 (28–48) | 0.6 | 0.3 |
| Post-OGTT AUC s-insulin (min·pmol$^{-1}$·l$^{-1}$) | 19,815 (13,757–26,874) | 20,231 (14,693–30,566) | 17,588 (16,290–24,653) | 0.5 | 0.3 |
| Insulinogenic index s-insulin | 25.5 (16.7–35.8) | 24.6 (17.6–36.3) | 19.1 (15.4–35.0) | 0.8 | 0.5 |
| Fasting s-C-peptide (pmol/l) | 516 (421–640) | 494 (401–632) | 547 (513–673) | 0.8 | 0.7 |
| Post-OGTT AUC s-C-peptide (min·pmol$^{-1}$·l$^{-1}$) | 145,170 (114,540–183,188) | 139,065 (106,748–185,139) | 163,763 (116,543–183,323) | 0.8 | 0.5 |

Data are medians with interquartile ranges in parentheses. Values of insulin or estimates derived from insulin variables were logarithmically transformed before statistical analysis. *p* values were adjusted for age, sex, and BMI where appropriate and were calculated assuming co-dominant (*p*) and recessive ($p_{rec}$) models for the penetrance of the threonine allele at codon 539 of *BCHE*. The insulinogenic index was calculated as fasting serum insulin (s-insulin; pmol/l) subtracted from 30-min post-OGTT serum insulin (pmol/l) and divided by 30-min post-OGTT plasma glucose (p-glucose; mmol/l)

**Table 3.** Anthropometric and metabolic characteristics of 4424 glucose-tolerant Danish Caucasians (study 2) stratified according to *BCHE Ala539Thr* genotype

| | *Ala/Ala* | *Ala/Thr* | *Thr/Thr* | *p* | $p_{rec}$ |
|---|---|---|---|---|---|
| *N* (men/women) | 2781 (1300/1481) | 1468 (675/793) | 175 (77/98) | | |
| Age (years) | 45 (40–50) | 45 (40–50) | 45 (40–50) | | |
| BMI (kg/m$^2$) | 25.0 (22.7–27.7) | 25.1 (22.8–27.6) | 24.5 (22.8–27.5) | 0.4 | 0.3 |
| Waist (cm) | 84.0 (75.0–93.0) | 84.0 (75.0–93.0) | 83.0 (74.0–92.0) | 0.3 | 0.7 |
| Fasting p-glucose (mmol/l) | 5.3 (5.0–5.6) | 5.3 (5.0–5.6) | 5.4 (5.0–5.6) | 0.5 | 0.4 |
| Post-OGTT AUC p-glucose (min·mmol$^{-1}$·l$^{-1}$) | 186 (115–248) | 177 (113–246) | 180 (107–249) | 0.5 | 0.5 |
| Fasting s-insulin (pmol/l) | 31(22–45) | 30 (22–45) | 30 (22–42) | 0.7 | 0.7 |
| Post-OGTT AUC s-insulin (min·pmol$^{-1}$·l$^{-1}$) | 17,745 (12,563–25,185) | 17,655 (12,735–25,253) | 17,940 (13,193–24,630) | 0.4 | 0.6 |
| Insulinogenic index s-insulin | 25.8 (17.9–37.4) | 26.5 (18.7–39.3) | 25.7 (18.0–38.6) | 0.1 | 0.4 |
| Fasting s-C-peptide (pmol/l) | 499 (395–631) | 499 (395–647) | 496 (384–644) | 0.6 | 0.9 |
| Post-OGTT AUC s-C-peptide (min·pmol$^{-1}$·l$^{-1}$) | 150,225 (117,953–184,733) | 147,908 (119,974–185,963) | 155,460 (118,838–183,600) | 0.4 | 0.3 |

Data are medians with interquartile ranges in parentheses. Methods, *p* values etc., see Table 2

In conclusion, our study showed no association between the *BCHE* codon 539 *Thr*-allele and Type 2 diabetes. It was not possible to replicate the initial finding of a higher BMI in homozygous carriers of the *BCHE* polymorphism in 4424 glucose-tolerant subjects, and no association with other pre-diabetic phenotypes was demonstrated.

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