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Molecular scanning of the betacellulin gene for mutations in type 2 diabetic patients

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Abstract

Betacellulin (BTC), a member of the epidermal growth factor (EGF) family, is an important factor in the growth and/or differentiation of pancreatic β cells. In this point of view, we determined the transcriptional start site of the human *BTC* gene and screened the protein-coding region for mutations. The transcriptional start site was located 347 bp upstream from the translational initiation codon. After screening the protein coding exons (exons 1–5), we identified two novel missense mutations, Cys (TGC) to Gly (GGC) at codon 7 (*C7G*) and Leu (TTG) to Met (ATG) at codon 124 (*L124M*), and a single nucleotide substitution (*−31c/t*) in the intron 2. The *C7G* was located in the signal peptide and the *L124M* in the transmembrane domain and this Leu at codon 124 was conserved among human, bovine, rat, and mouse. The frequencies of these variants, however, were similar between type 2 diabetic patients ($n = 228$) and non-diabetic control subjects ($n = 170$). These data suggest that genetic variations in the protein-coding region of the human *BTC* gene are unlikely to be a major contributor to development of type 2 diabetes.

Keywords: Human betacellulin; Transcriptional start site; Gene polymorphisms; Type 2 diabetes mellitus

1. Introduction

Betacellulin (BTC), a member of the epidermal growth factor (EGF) family, was purified from the conditioned medium of a cell line derived from mouse pancreatic β cell tumors [1]. Its primary translational product is composed of 178 amino acid residues, which contains a signal sequence, transmembrane and cytoplasmic domains in addition to the EGF-like domain. It is known that membrane-anchored BTC often remains uncleaved on the cell surface and communicates with adjacent cells through juxtacrine

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stimulation such as transforming growth factor-α (TGF-α) and heparin-binding EGF-like growth factor (HB-EGF) [2]. Mature BTC is composed of 80 amino acid residues with extensive glycosylation and has a molecular weight of about 32 kDa [3]. BTC expresses in α, β, and duct cells in normal adult pancreas [4,5], and converts rat pancreatic amylase-secreting cells (AR42J cells) to insulin expressing cells together with activin A [6]. In addition, PDX-1, one of the transcriptional factors for β cells, induces insulin gene expression in α TC1 cells in the presence of BTC [7]. BTC also has the potential for the growth of a rat insulinoma cell line, INS-1 cells [8], and the recombinant human BTC accelerates the improvement of glucose tolerance in mice with diabetes induced by selective alloxan perfusion [9]. According to these observations, BTC is thought to be necessary for differentiation and/or growth of the pancreatic β cells.

Mutations in the human *BTC* gene may have some relationship with the development of diabetes. However, there have been no reports of scanning mutations in the human *BTC* gene. We report here the transcriptional start site of the human *BTC* gene and the result of scanning mutations in the protein-coding region.

2. Materials and methods

2.1. Identification of the transcriptional start site

To determine the transcriptional start site, we performed the 5′-rapid amplification of cDNA ends (5′-RACE) using human pancreas marathon ready cDNA (Clontech, CA, USA) according to the manufacture's instruction. We amplified human pancreas marathon ready cDNA with a human *BTC* gene specific primer (5′-CAGGCACCCTCT-CAGGCGCCCGA-3′) and a marathon ready cDNA adaptor primer (AP1 primer). 5′-RACE products were confirmed with southern blot analysis with an internal gene-specific primer (5′-AAATAGGAGGCTCCC-TCCTCCCGGC-3′) using Gene Images 3′-oligolabelling module and Gene Images CDP-Star detection module (Amersham Pharmacia Biotech UK Limited, UK). After confirmation, the 5′-RACE fragment was subcloned into pGEM-T easy vector (Promega, WI, USA) and was sequenced in both directions.

2.2. Screening of mutations

Two hundred and twenty-eight unrelated Japanese type 2 diabetic patients were recruited from patients attending the outpatient clinic of Wakayama University of Medical Science Hospital. Subjects, who were GAD antibody positive and/or were started insulin therapy within 3 years after diagnosis of diabetes, were excluded from this study. Diabetes was diagnosed according to the criteria of the World Health Organization. As non-diabetic control, 170 subjects were chosen using the following criteria: age >60 years old, HbA1c <5.6%, fasting plasma glucose of <6.1 mmol/l, and no family history of diabetes mellitus. A positive family history was defined as diabetes diagnosed in siblings, parents, grandparents, aunts, and uncles. The clinical characteristics of non-diabetic and diabetic subjects are shown in Table 1. All participants gave their written informed consent before participating in the study. The study was approved by Ethical Committee of Wakayama University of Medical Science and was accordance with the principle of the Declaration of Helsinki.

Table 1
Clinical characteristics of the subjects enrolled in the present study

	Non-diabetic subjects	Type 2 diabetic subjects
n (male/female)	170 (51/119)	220 (133/87)
Age (years)	76.6 ± 9.6	63.0 ± 9.3
BMI (kg/m^2)	22.4 ± 3.7	22.7 ± 2.8
HbAlc (%)	4.94 ± 0.43	7.02 ± 3.55
Age at diagnosis (years)	–	43.2 ± 7.5
Treatment (D/OHA/Ins) (%)	–	15/40/45

Data are means ± S.D.; D, diet; OHA, oral hypoglycemic agent; Ins, insulin.