

Betacellulin variants and type 2 diabetes in the Old Order Amish

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Key words

- betacellulin
- type 2 diabetes
- Old Order Amish
- genetics

Abstract

Betacellulin is a member of the epidermal growth factor (EGF) family and may play a role in islet neogenesis and regeneration. To evaluate whether polymorphisms in this gene are associated with type 2 diabetes (T2DM) and impaired glucose tolerance (IGT), we genotyped 7 previously identified betacellulin variants in Amish subjects with T2DM (n=150), IGT (n=148) and normal glucose tolerance (NGT) (n=361). There were no significant differences in the allele fre-

quencies of the variants among the 3 groups. In an expanded set of 729 nondiabetic Amish subjects, there was no significant association between the betacellulin variants and levels of glucose or insulin either fasting or during a 3 h oral glucose tolerance test, HOMA or insulin secretion index. These results are consistent with previous studies in Caucasian populations and suggest that variants in the betacellulin gene do not play a major role in the development of T2DM in Caucasian populations.

Abbreviations

AFDS	Amish Family Diabetes Study
AUC	area under the curve
bp	base pair
EGF	epidermal growth factor
HOMA	homeostasis model assessment
IGT	impaired glucose tolerance
NGT	normal glucose tolerance
OGTT	oral glucose tolerance test
OOA	Old Order Amish
T2DM	Type 2 diabetes
UHT	Ultra High Throughput

Introduction

Betacellulin is a member of the epidermal growth factor (EGF) family and is encoded by the BTC gene (MIM: 600345) located on chromosome 4q13-21. *In vitro* and *in vivo* studies suggest a role for betacellulin in islet neogenesis and regeneration (Mashima et al., 1996, Li et al., 2003, Li et al., 2001). Therefore, betacellulin may provide one of the signals important for increasing and/or preserving beta cell mass (Mashima et al., 1996, Li et al., 2003, Li et al., 2001). Based on this role, we speculated that betacellulin might be one of the

factors modulating insulin production in the face of insulin resistance, and thus preventing or delaying the onset of T2DM. To assess whether sequence variation in the betacellulin gene was associated with T2DM, we previously identified seven polymorphisms in this gene and found one to be associated with T2DM (Cys7Gly) in African Americans, although not Caucasians (Silver et al., 2005). To expand upon these results, we have now assessed the association of this gene with T2DM and glucose intolerance in another population, the Old Order Amish (OOA) of Lancaster Pennsylvania. Our aim was to determine whether Cys7Gly and/or other betacellulin variants were associated with T2DM, impaired glucose tolerance (IGT), or with glucose and insulin levels. The OOA are descendants of approximately 200 families that immigrated from western Europe (mainly Switzerland) to the U.S. to escape religious persecution during the 1700's (McKusick VA, 1978). While the prevalence of T2DM in the OOA (6.7%) appears to be lower than that in non-Amish Caucasians (14.3%), the prevalence of abnormal glucose tolerance (T2DM+IGT) is similar in the two groups (26.9% vs 29.6%). We have previously speculated that the lower rate of conversion from IGT to T2DM in the Amish might be due to the increased physical activity in this population (Snitker et al., 2003).

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Because of their genetic isolation and homogeneity of lifestyle, the Amish are considered an excellent population for studying genetic susceptibility to common diseases, including T2DM, because many problems arising from population stratification that potentially plague genetic association studies carried out in other populations are minimized. Our previous genetic studies carried out in this population suggest that many findings in the OOA are generalizable to more outbred populations (Hsueh et al., 2003, Hsueh et al., 2000b, Pollin et al., 2004, Pollin et al., 2005, Damcott et al., 2004, Fu et al., 2004, Damcott et al., 2006).

Materials and Methods

The Amish Family Diabetes Study (AFDS) was initiated in 1995 with the goal of identifying susceptibility genes for T2DM. Details of the AFDS design, recruitment, phenotyping, and pedigree structure have been described previously (Hsueh et al., 2000a). Briefly, probands with previously diagnosed T2DM, all willing first- and second-degree relatives of probands and spouses >18 years of age were recruited. Phenotypic characterization of participants included medical and family history, anthropometry, and a 3-hr 75 gram oral glucose tolerance test (OGTT) with insulin levels. Criteria for the diagnosis of T2DM, IGT and normal glucose tolerance (NGT) were adapted from American Diabetes Association 1997 recommendations (Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 1997). Additionally, T2DM was defined by current treatment with insulin or oral hypoglycemic agents, or by confirmed diagnosis by a physician. For the case-control studies, NGT subjects were required to be ≥ 38 years of age. The study was approved by the Institutional Review Board at the University of Maryland Baltimore, and informed consent was obtained from participants prior to enrollment.

DNA was extracted from leukocytes using standard methods. The 5'UTR -233 G>C, 5'UTR -226A>G, and Cys7Gly variants were genotyped by pyrosequencing (Pyrosequencing AB, Uppsala, Sweden). The Leu44Phe, Leu124Met, Intron 2-31T>C, and Intron 4-4C>T variants were genotyped using SNP Stream Ultra High Throughput (UHT) technology (Beckman Coulter; Fullerton, CA). All typing included at least 5% duplicate samples to determine mistyping rates, which were 3% for Pyrosequencing and 0.3% for SNP Stream UHT.

Allele frequencies were estimated for subjects with T2DM (n=150), IGT (n=148) and NGT (n=361). We then compared the distribution of genotypes between T2DM and NGT and IGT and NGT subjects by regressing genotype on disease status, and adjusting for the effects of age, sex, and family structure. Analyses were carried out under a variance component framework, as previously described (Damcott et al., 2004). Additionally, in a larger set of 729 nondiabetic subjects [including the 509 NGT and IGT subjects described above and an additional 220 NGT subjects below 38 years of age], we assessed whether genotype was associated with glucose and insulin levels derived from the OGTT [e.g., insulin and glucose area under the curve (AUC), insulin secretion index [(insulin at 30 min - fasting insulin)/(glucose at 30 min - fasting glucose)] and homeostasis model assessment (HOMA)]. As before, we modeled the continuously distributed traits (e.g., insulin levels) as a function of genotype and covariates (e.g., age and sex). Statistical testing was performed by testing the likelihood of the data given the pedigree structure under a model in which the genotype effect was esti-

mated against the likelihood of a nested model in which the genotype effect was constrained to be zero. Analyses were carried out under a variance components framework using the SOLAR software program (Almasy and Blangero, 1998). Power calculations, conducted prior to analysis using the power calculator program of Skol (Skol et al., 2006), indicated that our sample of 140 T2DM subjects and 361 NGT controls provided 80% power to detect genotype relative risks on the order of 1.40 to 1.69 for disease allele frequencies ranging from 0.10-0.80.

Results

Clinical characteristics of the T2DM, IGT and NGT groups are shown in Table 1. Genotype distributions for all SNPs were consistent with those expected under Hardy-Weinberg equilibrium. Table 2 summarizes the allele frequencies for each of the betacellulin variants in subjects with T2DM, IGT and NGT. None of the seven betacellulin variants were associated with either T2DM or IGT. In the further analysis based on all nondiabetic subjects (NGT and IGT subjects combined), there was no significant association between these variants and levels of glucose or insulin in either the fasting state or during the OGTT. Nor were any associations observed with HOMA, AUC, or insulin secretion index (data not shown).

Discussion

Betacellulin's proposed role in islet neogenesis and regeneration makes it a strong candidate gene for T2DM. In our initial betacellulin studies, we identified 7 variants in the betacellulin gene with the Cys7Gly variant being more common in African Americans with T2DM from Baltimore than in NGT controls (Silver et al., 2005). However, this finding was not confirmed in African Americans from Arkansas (Elbein et al., 2006). Additionally, none of the 7 variants were associated with T2DM in Caucasians from Baltimore (Silver et al., 2005). Our findings in the Amish are consistent with our previous findings and suggest that the negative results in Baltimore Caucasians were not due to stratification bias or other confounders. Furthermore, since the Amish are a genetically homogeneous founder population, population stratification is unlikely to explain the negative results. Similar to our findings in Caucasians, Nakagawa et al. did not find an association of the Cys7Gly, Leu124Met and intron 2-31T>C variants with T2DM in Japanese subjects (Nakagawa et al., 2005). In a subsequent publication, the group screened a number of promoter variants including -233G>C and -226A>G in the same group of Japanese subjects (Nakano et al., 2005). The -226G allele was associated with T2DM and a lower insulin response to glucagon as well as a 50% decrease in promoter activity. The association of

Table 1 Amish Study Population

	T2DM (n=150)	IGT (n=148)	NGT Control (age ≥ 38 yrs) (n=361)
Age (years)	65 \pm 12	52 \pm 15	51 \pm 10
Sex (M/F)	49/101	47/101	189/172
BMI (kg/m ²)	30.1 \pm 4.0	28.8 \pm 5.6	27.4 \pm 4.7
Age of onset of diabetes (yrs)	59.4 \pm 11.4	—	—

Data are mean \pm SD.

Table 2 Allele Frequencies in subjects with T2DM, IGT, and NGT

Polymorphism	Major/minor allele	Minor Allele Frequency			Genotype effect (T2DM vs NGT and IGT vs NGT) P*
		T2DM (n=150)	IGT (n=148)	NGT Control (age ≥38 yrs) (n=361)	
Cys7Gly	T/G	0.21	0.21	0.22	NS
Leu44Phe	C/T	0.0	0.0	0.0	NS
Leu124Met	T/A	0.25	0.30	0.27	NS
5'UT -233G>C	G/C	0.23	0.21	0.21	NS
5'UT -226A>G	A/G	0.32	0.33	0.32	NS
Intron 2-31T>C	C/T	0.51	0.44	0.44	NS
Intron 4-4C>T	C/T	0.25	0.31	0.28	NS

*effect of genotype on disease status (T2DM vs NGT; IGT vs NGT) tested by regression (see text for details)

NS = not significant

the -226A>G variant with T2DM was not found in the Amish population. This difference may be the result of ethnic/genetic background or differences in environmental exposures. While glucagon stimulated insulin secretion was not measured in the Amish, the absence of association between the -226A>G variant and glucose or insulin levels during the OGTT, AUC, and insulin secretion index, makes it less likely that we would find a difference in insulin secretion in the Amish even if more sophisticated measures of insulin secretion had been performed.

In conclusion, variants in the betacellulin gene do not appear to play a significant role in variation in glucose and insulin levels on an OGTT or the development of T2DM in the Old Order Amish. However, based on studies in African Americans and Japanese, betacellulin may play a role in T2DM susceptibility in other ethnic groups. Further studies in other populations will be needed to confirm these findings.

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