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SHORT COMMUNICATION

Evidence for neuroendocrine function of a unique splicing form of *TCF7L2* in human brain, islets and gut

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Abstract
Aims/hypothesis Variants in the *TCF7L2* gene remain the strongest genetic associations with increased risk of type 2 diabetes. Recently, we identified a unique splicing form of *TCF7L2* expressed in pancreatic islets, pancreas and colon and detected by assay 'ex13-13b'. The expression of ex13-13b strongly correlated with proinsulin in glucose-stimulated pancreatic islets, suggesting a potential role for this form in the development of type 2 diabetes. The goal of this study was to further characterise this unique *TCF7L2* splicing form in human tissues.
Methods We used a panel of 34 human tissues and 80 human cell lines to measure the expression of assay ex13-13b with use of quantitative RT-PCR.
Results The highest expression of assay ex13-13b was detected in several areas of the brain (hypothalamus/thalamus, occipital lobe) and in neuronal cell line SHS5Y5. Low expression was confirmed in pancreatic islets, small intestine, pancreas and colon, while no expression was detected in other human tissues and cell lines. The expression of assay ex13-13b correlated with the gene for cocaine- and amphetamine-regulated transcript (*CART*, also known as *CARTPT*) in a panel of human tissues (n=12, r=0.85, p=0.00046), pancreatic islets (n=23, r=0.62, p=0.0016) and colon (n=98, r=0.54, p<0.0001).
Conclusions/interpretation The significant correlation between expression of a unique splicing form of *TCF7L2*, named here *TCF7L2-NE,* and *CART*, the gene for an anorexigenic neurohormone expressed in the central and peripheral nervous system, suggests that these transcripts may share neuroendocrine functions important for brain, gut and pancreatic islets.

Keywords Brain–gut–islets axis · *CART* · Gene expression · Neuroendocrine regulation · *TCF7L2* · Type 2 diabetes

Abbreviations

TCF7L2	Transcription factor 7-like 2
TCF7L2-NE	Neuroendocrine splicing form of TCF7L2

Introduction

Transcription factor 7-like 2 (TCF7L2) (also known as TCF-4) plays a central role in the WNT pathway and is involved in a variety of biological processes. Non-coding genetic variants within *TCF7L2* gene remain the strongest genetic association with increased risk of type 2 diabetes and dysfunction of pancreatic islets [1, 2]. However, the molecular and cellular mechanisms through which *TCF7L2* contributes to these associations are still unclear.

Previously, we described a tissue-specific splicing diversity of the gene resulting from the use of three alternative promoters and six alternative exons [3]. We detected a unique splicing form defined by the presence of an alternative C-terminal exon, 13b, expressed in pancreatic islets, colon

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and pancreas [3]. Expression of this form strongly correlated with insulin expression in glucose-stimulated pancreatic islets and was decreased in islets of carriers of the type 2 diabetes-associated single nucleotide polymorphisms of *TCF7L2*, rs7903146 and rs12255372 [3].

In the current study we aimed to further characterise the *TCF7L2* splicing form containing the alternative exon 13b. We provide evidence for a neuroendocrine pattern of expression of this form, named *TCF7L2-NE*, in human tissues and cell lines. We show that expression of *TCF7L2-NE* correlated with expression of the gene for cocaine- and amphetamine-regulated transcript (*CART*, also known as *CARTPT*), also found in the brain, islets and gut. *CART* has been shown to play a critical role in glucose sensing, stimulation of hormone secretion, cell survival, thermoregulation and appetite suppression. Based on strong correlation between expression of *TCF7L2-NE* and *CART* in a panel of human tissues, islets and colon, these data suggest that *TCF7L2-NE* may have important neuroendocrine functions.

Methods

Samples, cDNA preparation and expression analysis Samples of total RNA from human tissues were purchased from Clontech (Mountain View, CA, USA) and BioChain Institute (Hayward, CA, USA). Samples of human pancreatic islets, monocytes, colon, pancreas and lymphoblastoid cell lines were previously described [3]. RNA from the NCI-60 cell lines was provided by the Molecular Targets Team, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, NCI/NIH. All other cell lines were purchased from ATCC (Manassas, VA, USA) or Asterand (Detroit, MI, USA). Total RNA was prepared with a MirVana kit (Ambion, Austin, TX, USA) and the quality was evaluated with a Bioanalyzer 2100 (Agilent, Santa Clara, CA, USA). cDNA from all samples was prepared from 1 μg total RNA, as previously described [3]. All reactions were run in duplicate in 5 μl volumes in 384-well optical plates on an SDS7900 (Applied Biosystems, Foster City, CA, USA). Negative controls and genomic DNA controls were used for all assays. The expression of endogenous control genes, beta-2 microglobulin (*B2M*) and cyclophilin A (*PPIA*), measured in separate reactions but in the same cDNA samples was used for normalisation of expression. All assays were tested before analysis and showed comparable amplification efficiencies. The list of expression assays is provided in the Electronic supplementary material (ESM) Table 1.

Statistical analysis Average values for technical duplicates were normalised by geometric means of endogenous controls according to the ΔC_t method of relative quantification, as previously described [3]. Pearson's correlation coefficients and two-sided p values were calculated with SPSS 16.0 software (SPSS, Chicago, IL, USA). p values were not adjusted for multiple tests.

Results

Expression of the TCF7L2 splicing form with exon 13b in human tissues and cell lines Initially, the expression of *TCF7L2* assay ex13-13b was detected in pancreatic islets, pancreas and colon [3]. We then tested the expression of this assay in 34 human tissues and 80 cell lines (60 cell lines from the NCI-60 set and 20 additional cell lines; ESM Tables 2 and 3). High expression of assay ex13-13b was detected in several areas of the brain (thalamus, hypothalamus, occipital lobe) and in SHS5Y5 neuroblastoma cell line, but not in other brain-derived cell lines (a human neuronal epithelioma cell line SK-N-MC and glioblastoma and astrocytoma cell lines SF_268, SF_295, SF_539, SNB_19, U251 and SNB_75). Expression was also detected in pancreatic islets, small intestine, colon and pancreas, but not in other human tissues, NCI-60 and additional cell lines (Table 1). We PCR amplified a fragment of *TCF7L2* gene between a constitutive exon 10 and an alternative exon 13b in cDNA from human brain and pancreatic islets (Fig. 1a,b). There were four possible combinations of alternative exons 12, 13, 13a and 13b in splicing forms with exon 13b. The splicing forms that include both exons 13a and 13b have two alternative stop codons separated by 37 bp (Fig. 1a,b). Expression of these forms (assay 13a-13b) was found to be very low in brain and islets and was not studied further (data not shown). However, the expression of assay ex13-13b was up to 750 times higher in the brain than in pancreas (Table 1).

Coexpression of TCF7L2 assay ex13-13b with brain–islet–gut neurohormones The pattern of expression of *TCF7L2* splicing form detected by assay ex13-13b suggested that it might be expressed in neuroendocrine cells of brain, islets and gut. Based on the literature, we selected a number of neurohormones that can be expressed in the brain and enteric nervous system [4]. We tested for correlation in expression of assay ex13-13b with several neurohormone genes, neuropeptide Y (*NPY*) and agouti-related peptide (*AGRP*) expressed in glucose-inhibited neurons, pro-opiomelanocortin (*POMC*) and *CART* expressed in glucose-activated neurons, a marker of endocrine function chromogranin A (*CHRA*, also known as *CHGA*), and several other neuropeptides: ghrelin (*GHRE*, also known as *GHRL*), vasoactive intestinal peptide (*VIP*), gastric inhibitory polypeptide, also known as glucose-dependent insulinotropic peptide (*GIP*) and somatostatin (*SST*).

Table 1 Expression of *TCF7L2* assay ex13-13b and neurohormone genes

Tissue	*TCF7L2* ex13-13b	ex13-13b (fold vs pancreas)	*CART*	*CHRA*	*NPY*	*GHRE*	*VIP*	*AGRP*	*SST*	*POMC*	*GIP*
Thalamus	−4.25	750	−6.13	−4.50	−2.67	−12.83	−8.58	−14.49	−4.55	ND	ND
Occipital lobe	−5.42	333	−5.15	−0.23	−1.00	−10.97	−2.80	−12.75	−0.86	ND	ND
Hypothalamus	−6.49	159	−6.06	−5.57	−3.98	−13.39	−8.58	ND	−2.94	ND	ND
Cerebellum	−7.82	63	−5.13	−6.53	−8.85	−12.74	−8.64	−11.73	−14.85	ND	ND
Pituitary gland	−8.58	37	−9.15	−0.33	ND	−13.76	−14.13	ND	−10.74	ND	ND
SHS5Y5 neuroblastoma cell line	−8.75	33	−10.50	NT	NT	NT	NT	NT	NT	NT	NT
Hippocampus	−8.99	28	−6.57	−3.51	−3.85	−10.85	−6.68	−13.05	−5.54	ND	−14.76
Small intestine	−11.36	5.4	−9.49	−3.83	−7.02	−9.26	−7.01	ND	−5.83	ND	−6.91
Pancreatic islets	−11.84	3.9	−12.05	0.76	−3.14	−5.79	−11.48	−16.50	3.29	−13.18	ND
Colon tumour	−13.24	1.5	−10.90	−9.47	ND	ND	−8.13	ND	−14.09	ND	ND
Colon	−13.84	1.2	−10.77	−6.04	−10.98	ND	−6.80	ND	−8.14	ND	ND
Pancreas	−13.80	1.0	−11.95	−3.31	−7.27	−11.12	−7.62	ND	−2.59	ND	ND
Correlation (r)		–	0.85	0.21	0.66	−0.57	0.10	0.45	0.11	NS	NS
p value			0.00046*	NS	0.06	NS	NS	NS	NS		

Expression values are normalised to endogenous controls *B2M* and *PPIA*. Pearson's correlation coefficients (r) are for expression of *TCF7L2* assay ex13-13b and each neurohormone

Each tissue is represented by one sample or a pool of samples from two or three individuals; expression of assay ex13-13b was not detected in the following tissues: liver, lung, spleen, kidney, skeletal muscle, heart, blood, monocytes, lymphoblasts, breast, parotid, thyroid, ovary, oesophagus, stomach, bladder, prostate, ovarian cancer, lung cancer, kidney cancer, breast cancer, adipose and in NCI-60 cell lines and additional cell lines 293T, HepG2, HeLa, MDA435, MCF10, PANC1, SK-N-MC, prostate cell lines OPCN1, OPCN2, OPCN3, OPCT1, OPCT2, OPCT3, RWPE-1, RWPE-2, PWR1E, bladder cancer cell lines HTB-5, HTB-9, HT-1376 and J-82

*p value will be significant even after adjustment for multiple tests

ND, expression tested but not detected; NS, no significant correlation based on samples with detectable expression for both assays; NT, expression not tested

In a panel of 34 human tissues and 80 cell lines (NCI-60 and 20 additional cell lines) the most similar pattern of tissue distribution and level of expression was observed between *TCF7L2* assay ex13-13b and *CART* (r=0.85, p=0.00046, Table 1). Both these transcripts were expressed highly in the brain and in neuronal cell line SHS5Y5, low in small intestine, islets, colon and pancreas and not expressed in all other tissues and cell lines tested, even though the expression of endogenous controls *B2M* and *PPIA* was equally high in all these samples (data not shown). Based on the observed pattern of expression, we designated the splicing form detected by assay ex13-13b as a neuroendocrine splicing form, *TCF7L2-NE*. Expression of *TCF7L2-NE* also showed correlation with *CART* in human pancreatic islets (n=23, r=0.62, p=0.0016) and colon (n=98, r=0.54, p<0.0001). Expression of both *TCF7L2-NE* and *CART* in pancreas (n=44) was low and not detectable in all samples and was not analysed (data not shown).

The protein encoded by this splicing form is C-terminally truncated and lacks binding sites for the C-terminal binding protein (CtBP) that regulates TCF7L2 post-translationally [5]. Alternative exon 13b contributes only two amino acids before the termination of transcription, making it impossible to distinguish TCF7L2-NE from other TCF7L2 forms at the protein level. We tested cellular localisation of this form by transfecting an expression construct for the full-length *TCF7L2-NE* splicing form (GenBank FJ010169) [3] into human PANC-1 cells. The localisation of this form was clearly nuclear (Fig. 1c), as was expected based on the presence of nuclear localisation signals and the role of *TCF7L2* as a transcription factor.

Discussion

Disturbances in brain–gut–islet regulation of satiety and glucose metabolism are associated with development of obesity and type 2 diabetes [6, 7]. In this study we provide evidence that the unique splicing form of *TCF7L2* that we named a 'neuroendocrine form, *TCF7L2-NE*', might be a part of this regulation. *TCF7L2-NE* exhibits a neuronal pattern of expression, as it is highly expressed in the

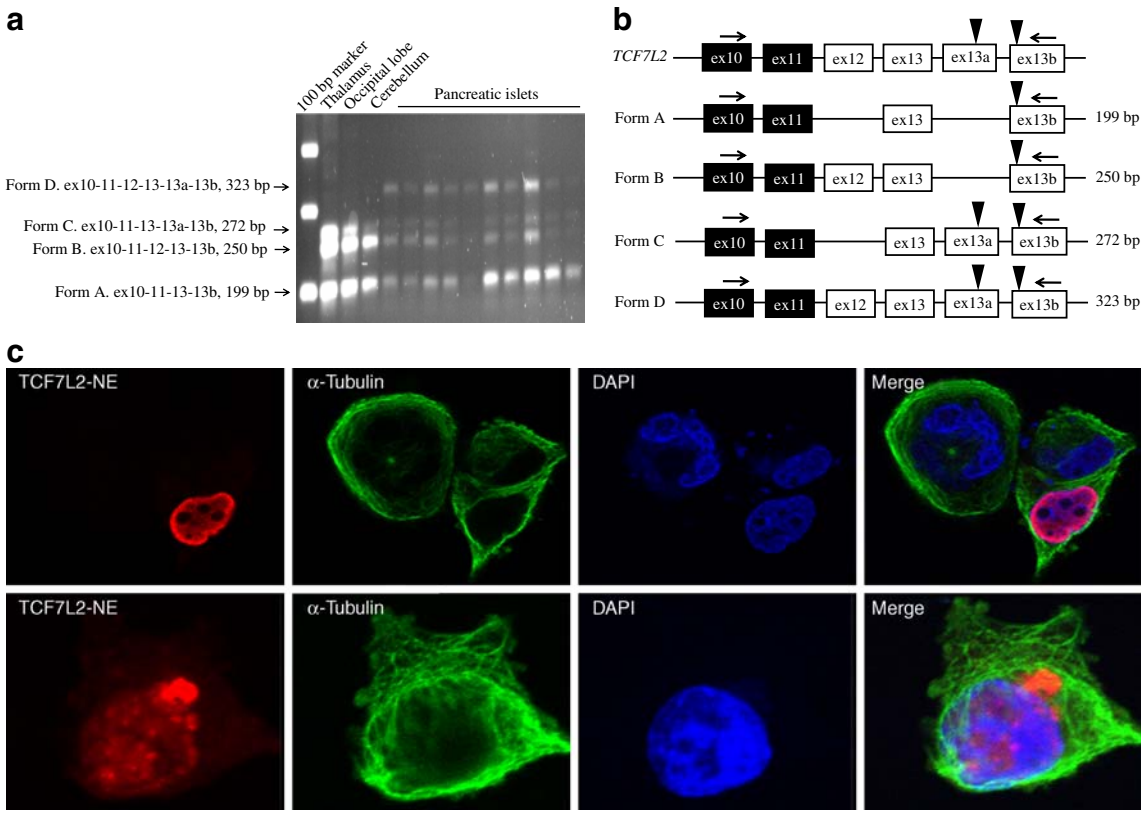

Fig. 1 C-terminal alternative exons of *TCF7L2* in transcripts expressed in total human brain and pancreatic islets and cellular localisation of *TCF7L2* splicing form detected by assay ex13-13b (*TCF7L2-NE*). **a** Results of PCR amplification with primers ex10 Forw and ex13b Rev of *TCF7L2* (ESM Table 1) in cDNA from human brain and pancreatic islets. cDNA prepared from 10 ng total RNA was used for all PCR reactions. The PCRs were performed with Phusion DNA polymerase (New England Biolabs, Ipswich, MA, USA) and the PCR products were resolved on a 2% (wt/vol.) agarose gel. Four types of PCR fragments (forms A–D) were observed based on the combination of alternative exons 12, 13, 13a and 13b. **b** Schematic representation of C-terminal exons of *TCF7L2*. Constitutive exons are represented by black rectangles and alternative exons by white rectangles. Black triangles mark alternative translation stops. Arrows indicate positions of PCR primers. Forms A and B use a stop codon within exon 13b and forms C and D use a stop codon within exon 13a. In the forms with alternative exons 13a and 13b (C and D) two in-frame stop codons are separated only by 37 bp. Expression of these forms (assay ex13a-13b) was very low in brain and islets and was not studied further. **c** Two confocal images showing cellular localisation of the recombinant TCF7L2-NE in human PANC-1 cell line (pancreatic cancer). Panels represent images of PANC-1 cells transfected with the TCF7L2-NE Halo-tag expression construct (GenBank FJ010169) [3]. Non-transfected cells serve as controls for specificity of detection. For the expression construct a full-length cDNA for *TCF7L2-NE* splicing form was cloned into a pFC8A expression vector with a C-terminal Halo-tag (Promega, Madison, WI, USA). The endotoxin-free plasmid was prepared with a EndoFree plasmid Maxi kit (Qiagen, Gaithersburg, MD, USA). For transfection, human PANC-1 (pancreatic cancer) cells were plated on chamber slides (Thermo Fisher Scientific, Rochester, NY, USA) and transfected next day with Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. After 48 h, the cells were fixed and incubated with primary rabbit anti-HaloTag antibodies (Promega) and with mouse anti-α-tubulin antibodies ab7291-100 (Abcam, Cambridge, MA, USA). Secondary donkey anti-rabbit and anti-mouse antibodies labelled with Alexa fluor 594 and 488 were used for imaging (Invitrogen). The anti-fade ProLong Gold mounting media with DAPI (Invitrogen) was used to mount the cover slides. The imaging was performed with a confocal microscope LSM 510 Meta (Carl Zeiss Microimaging, Thornwood, NY, USA) with ×63 magnification

thalamic, hypothalamic and occipital regions of the brain, as well as in a neuronal cell line SHS5Y5. Comparatively lower levels of expression were found in islets and gut (up to 750-fold lower than in brain), while no expression was found in all other human tissues and cell lines tested here. Expression of a similar murine splicing form of *TCF7L2* (designated as a form with exons 12-13-14-17-18) was recently located to postmitotic neurons of mouse midbrain [8].

TCF7L2-NE is highly expressed in the brain areas that contain neurons of the hypothalamic arcuate (ARC) nucleus that regulate energy metabolism and satiety. Glucose-sensing neurons express *CART* and *POMC* [4]. In the panel of human tissues used in this study, expression of *TCF7L2-NE* correlated with *CART* but not with *POMC*, possibly marking a subpopulation of glucose-activated neurons activated by blood glucose, insulin and leptin [9]. Expression of *TCF7L2-NE* did not correlate with *NPY* and *AGRP* (markers of glucose-inhibited neurons activated by ghrelin and inhibited by glucose and leptin [4]). In sum, expression of *TCF7L2-NE* and *CART* strongly correlated in a panel of human tissues

(n=12, r=0.85, p=0.00046), in islets (n=23, r=0.62, p=0.0016) and colon (n=98, r=0.54, p<0.0001) with p values being significant even after adjustment for multiple tests.

CART has been shown to play a critical role in glucose sensing, stimulation of hormone secretion in pancreatic islets, neuronal survival, thermoregulation and appetite suppression [4, 10, 11]. The functional relationship between *TCF7L2-NE* and *CART* is not clear. Based on the strong correlation between expression of *TCF7L2-NE* and *CART*, we suggest that these transcripts may have similar functions related to the brain–islet–gut regulation of metabolism. Further studies will be needed to identify the functional role of *TCF7L2-NE* in different metabolic conditions.

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Duality of interest The authors declare that there is no duality of interests associated with this manuscript.

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