INTRODUCTION

Mutations in the genes for presenilin 1 and 2 (PS1/PS2) are the causative factors for ~50% of early onset familial Alzheimer’s disease (EOFAD) (Sherrington et al., 1995; Rogaev et al., 1995; Levy-Lahad et al., 1995). PS1 and 2 are homologous transmembrane proteins encoded by genes on chromosomes 14 and 1, respectively (Sherrington et al., 1995; Rogaev et al., 1995). Messenger RNAs for both proteins are expressed in the brain and PS1 is present in many non-cerebral tissues, but high levels of PS2 expression are found in muscle and pancreas (Rogaev et al., 1995). The role of PS is unclear. Increased production of the alternative amyloidogenic processing product of Alzheimer’s precursor protein (APP), Aβ1-42, has been demonstrated in subjects with PS mutations (Cruts et al., 1996; Scheuner et al., 1996) and in transfected cells and transgenic mice expressing both human PS and APP (Citron et al., 1997) suggesting that the intracellular PS proteins are involved in protein trafficking in endoplasmic reticulum and Golgi (Walter et al., 1996; Kovacs et al., 1996). PS have been proposed to have a role in apoptosis. Overexpression of PS2 is associated with increased sensitivity to apoptotic stimuli (Deng et al., 1996; Wolozin et al., 1996) although the C-terminal portion (ALG-3) is a putative inhibitor of apoptosis (Vito et al., 1996a,b). These effects may be mediated via intracellular calcium (Guo et al., 1997). The proposed interaction of PS2 with calcium binding proteins (Buxbaum et al., 1998) may be reflected in its distribution since cellular processes in muscle and pancreas are largely calcium dependent. It is therefore possible that PS2 mutations could be associated with pancreatic islet structure and function appear to be unaffected by the Met239Val mutation.

Key words: Presenilin, Diabetes, Islet β-cell, Amyloid, Alzheimer’s Disease

SUMMARY

Localisation of presenilin 2 in human and rodent pancreatic islet β-cells; Met239Val presenilin 2 variant is not associated with diabetes in man

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INTRODUCTION

Mutations in the genes for presenilin 1 and 2 (PS1/PS2) are the causative factors for ~50% of early onset familial Alzheimer’s disease (EOFAD) (Sherrington et al., 1995; Rogaev et al., 1995; Levy-Lahad et al., 1995). PS1 and 2 are homologous transmembrane proteins encoded by genes on chromosomes 14 and 1, respectively (Sherrington et al., 1995; Rogaev et al., 1995). Messenger RNAs for both proteins are expressed in the brain and PS1 is present in many non-cerebral tissues, but high levels of PS2 expression are found in muscle and pancreas (Rogaev et al., 1995). The role of PS is unclear. Increased production of the alternative amyloidogenic processing product of Alzheimer’s precursor protein (APP), Aβ1-42, has been demonstrated in subjects with PS mutations (Cruts et al., 1996; Scheuner et al., 1996) and in transfected cells and transgenic mice expressing both human PS and APP (Citron et al., 1997) suggesting that the intracellular PS proteins are involved in protein trafficking in endoplasmic reticulum and Golgi (Walter et al., 1996; Kovacs et al., 1996). PS have been proposed to have a role in apoptosis. Overexpression of PS2 is associated with increased sensitivity to apoptotic stimuli (Deng et al., 1996; Wolozin et al., 1996) although the C-terminal portion (ALG-3) is a putative inhibitor of apoptosis (Vito et al., 1996a,b). These effects may be mediated via intracellular calcium (Guo et al., 1997). The proposed interaction of PS2 with calcium binding proteins (Buxbaum et al., 1998) may be reflected in its distribution since cellular processes in muscle and pancreas are largely calcium dependent. It is therefore possible that PS2 mutations could be associated with pancreatic disease, trafficking of proteins in endocrine or exocrine cells, cell development and formation of islet amyloid in Type 2 (non-insulin dependent) diabetes.

Type 2 diabetes is a disease of unknown aetiology developing in subjects over the age of 40 as a result of a combination of pancreatic islet cell dysfunction and insulin resistance (Ahmed et al., 1997). There is familial association for susceptibility for Type 2 diabetes (Kaprio et al., 1992) but the disease is heterogeneous and multifactorial in origin. Like Alzheimer’s Disease (AD), clinical diabetes is progressive and the decline of insulin secretion in the course of the diabetic syndrome has been attributed to many features including (a) abnormal nutrient signalling and/or production of β-cell peptides with increased proinsulin secretion being a marker for...
this pathology (Kahn et al., 1996); (b) increased insulin resistance leading to oversecretion and dysfunction of \( \beta \)-cells (Ahmed et al., 1997; de Fronzo et al., 1989); and (c) progressive deposition of islet amyloid associated with \( \beta \)-cell destruction (Westmark and Grimelius, 1973; Clark et al., 1988). Calcium homeostasis is an essential feature for normal exocrine and endocrine function; pancreatitis is associated with duct proliferation and development of insoluble calcium deposits; insulin secretion involves transmembrane calcium currents (Hellman et al., 1992). Islet amyloid deposits are formed by unknown mechanisms from islet amyloid polypeptide (IAPP) or ‘amylin’ (Westmark et al., 1987; Clark et al., 1987); IAPP, like insulin, is synthesised as a larger precursor peptide and cosecreted with insulin (Sanke et al., 1988, 1991). Abnormal post-translational processing of proIAPP could contribute to fibrillogenesis of human IAPP in diabetes (Clark et al., 1993).

Gene linkage for some small subgroups of diabetes has been determined (e.g. maturity onset diabetes of the young, MODY); these groups represent 2-5% of the total Type 2 diabetic population and the affected proteins are expressed largely in pancreatic \( \beta \)-cells (Page et al., 1992; Yamagata et al., 1996; Frayling et al., 1997; Stoffers et al., 1997). Type 2 diabetes and AD are diseases of ageing and are associated with localised amyloid deposits and cellular dysfunction. Therefore, it is possible that, if expressed in islet cells, PS2 could represent a candidate gene for another subgroup of Type 2 diabetes. The cellular localisation (exocrine and/or endocrine cells), and molecular form of pancreatic PS2 in non-diabetic and diabetic tissue has therefore been investigated. Islet structure was examined in a post-mortem pancreatic specimen from a subject with PS2 Met239Val variant (FLO10 pedigree) diagnosed with AD. Unaffected subjects from the same pedigree were tested for evidence of diabetes and \( \beta \)-cell dysfunction.

MATERIALS AND METHODS

Tissue for immunocytochemistry
Post-mortem pancreas was obtained from non-diabetic, and Type 2 diabetic subjects and from one subject from the FLO10 family with the Met239Val PS2 mutation affected by Alzheimer’s disease (Rogaev et al., 1995). Human insulinoma tissue was obtained at surgical intervention. Islet amyloidosis was obtained at surgical intervention. Islet structure was examined in a post-mortem pancreatic specimen from a subject with PS2 Met239Val variant (FLO10 pedigree) diagnosed with AD. Unaffected subjects from the same pedigree were tested for evidence of diabetes and \( \beta \)-cell dysfunction.

Tissue extraction and preparation for western blot analysis
Rat and mouse pancreas was snap frozen in liquid nitrogen and stored at \(-70^\circ C\). Mouse islets were isolated by digestion of pancreas with collagenase (Sigma type XI collagenase for islet separation) in Hanks’ buffered salt solution (HBSS) (Gibco, Paisley, UK), hand picked from the digest into RPMI medium (Gibco) and transferred to lysis buffer. Human islets were obtained by collagenase digestion of a pancreas for a heart-beating organ donor. Islets were hand picked from the collagenase digest and fixed in 0.5% glutaraldehyde, 2.5% parafomaldehyde in 0.1 M phosphate buffer. Islets were washed in 0.01% egg albumen in phosphate buffered saline (PBS, EA) and incubated for 2 hours at R/T with anti-PS2 (1:750) diluted in PBS, EA. Primary antibody binding sites were localised with gold-conjugated Protein A (diluted 1:30) (Utrecht University, The Netherlands) for 1 hour. Contrast of sections was enhanced with uranyl acetate and lead citrate. Sections were viewed with a Jeol JEM 1010 transmission electron microscope with an acceleration voltage of 80KV.

Immunocytochemical methods
Wax sections (5 \( \mu \)m thick) were dewaxed with xylene and rehydrated. Endogenous peroxidases were blocked with 0.3% hydrogen peroxide in methanol and sections were washed with Tris buffered saline (TBS, pH 7.4). On sections and cultured cells, non-specific labelling was blocked with normal swine serum (anti-PS2) or normal rabbit serum (anti-insulin; 1:20 dilution; Dako, High Wycombe, UK). Sections were incubated with primary antibodies overnight at 4°C; antibodies were, polyclonal rabbit anti-PS21-87 (1:750) raised to a GST-fusion protein and shown to be PS2 specific in transfected cells (unpublished data) and polyclonal guinea pig anti-bovine insulin (1:1000; ICN, Thame, UK). Secondary antibodies (1:50 dilution incubation time 30 minutes, R/T) were peroxidase labelled anti-rabbit and anti-guinea pig (Dako). fluoresceine-conjugated anti-rabbit or anti-guinea pig (Dako), rhodamine-conjugated anti-guinea pig (Sigma, Poole, UK) and anti-rabbit IgG (Dako). Peroxidase-conjugated antibodies were visualised using di-aminobenzidine-HCl with hydrogen peroxide (Sigma) and sections were counterstained with haematoxylin. Peroxidase labelled sections were dehydrated and mounted with coverslips while cells labelled with fluorescence were mounted in Citifluor in glycerine (AGAR Scientific, Stanstead, UK).

Electron microscopy
Human islets were obtained by collagenase digestion of a pancreas for a heart-beating organ donor. Islets were hand picked from the collagenase digest and fixed in 0.5% glutaraldehyde, 2.5% parafomaldehyde in 0.1 M phosphate buffer. Islets were washed in 0.1 M acetate buffer, block-stained with 2% uranyl acetate, dehydrated in methanol at \(-20^\circ C\) and embedded in LR gold resin (Taab Laboratories, Reading, UK). Ultrathin sections on nickel grids were washed in 0.01% egg albumen in phosphate buffered saline (PBS, EA) and incubated for 2 hours at R/T with anti-PS2 (1:750) diluted in PBS, EA. Primary antibody binding sites were localised with gold-conjugated Protein A (diluted 1:30) (Utrecht University, The Netherlands) for 1 hour. Contrast of sections was enhanced with uranyl acetate and lead citrate. Sections were viewed with a Jeol JEM 1010 transmission electron microscope with an acceleration voltage of 80KV.
Oral glucose tolerance tests (OGTT) were performed (Table 1) on four subjects with the PS2 Met239Val mutation (subjects 1-4) of the FLO10 pedigree (Rogaev et al., 1995) and 5 first degree relatives without the mutation (subjects 5-9) after an overnight fast. These tests were performed under conditions specified by the Local Ethics Committee and with informed consent from the subjects. Three venous blood samples were taken before (time 0) the subjects received a 75 g glucose drink and three samples were taken 2 hours following the glucose load (time=2 hours). Plasma concentrations of immunoreactive-insulin (IRI) and proinsulin (PI) are expressed as pmol/l. PI/IRI% indicates the proportion of incompletely processed insulin precursor peptides in the circulation. Increased PI/IRI% at 2 hours reflects the persistence of PI due to the longer half life of PI compared to insulin in the circulation following a glucose load. Subject 7 had relatively high concentrations of fasting glucose, insulin and proinsulin which are associated with the increased BMI. No subject had fasting or 2 hour values outside the normal range.

### RESULTS

#### Immunocytochemical localisation of PS2 in pancreatic islets

Antiserum raised to a GST-N-terminal fusion protein of PS2 (PS2 1-87) was used to localise PS2 in specimens of rodent and human pancreas. This antiserum has been shown to be PS2 specific by preabsorption with the peptide antigen, PS21-87. Immunoperoxidase labelling for PS2 was found in pancreatic islets but not in acinar cells of exocrine tissue. In human islets, the cellular density of labelling was heterogeneous (Fig. 1A); labelling was present in both the more centrally situated insulin-containing β-cells and the non-β cells lining the islet capillaries (glucagon-containing α-cells, somatostatin-containing δ-cells and PP cells). Density of labelling in rodent islets was more uniform (Fig. 1B) and was present in β- and non-β-cells in islets of rat and mouse pancreas. In mouse insulin tumour cells (β-TC cells), immunofluorescence for PS2 was localised to the cytoplasmic domain rather than the plasma membrane (Fig. 1C) and was co-localised with insulin. Double immunofluorescent labelling showed that PS2 was predominantly co-localised with insulin in human islets (Fig. 1D,E,F).

Immunoreactivity for PS2 was present in the islet cells of amyloid-containing islets of subjects with Type 2 diabetes but the islet amyloid deposits (formed from IAPP), which were Congo Red positive, were unlabelled (Fig. 1G). PS2 immunoreactivity (PS2-ir) was not detectable on amyloid deposits formed from other peptides; specimens examined were wax embedded surgical or post-mortem human tissue containing deposits formed from β-2 microglobulin in peri-articular material, serum amyloid A in the spleen and Aβ in brain (not illustrated).

PS2-ir was present in insulin-containing cells of human insulinoma but was undetectable in a human pancreatic gastrinoma and pituitary adenoma. PS2-ir was present in human foetal islet cell clusters at 12 weeks gestation (Fig. 1H) and in pluripotent primitive duct cells from which endocrine cells originate. PS2-ir was also present in proliferating duct cells in adult pancreas from a patient with pancreatitis (Fig. 1I). Pancreatic islets in the patient with the Met239Val mutation showed immunoreactivity for insulin; these islets were of

### Table 1. Oral glucose tolerance test: Characteristics of subjects from FLO10 pedigree with (1-4) and without (5-7) the Met239Val mutation that received the 75 g oral glucose tolerance test (OGTT)

<table>
<thead>
<tr>
<th>Patient (M/F) y.o.b.</th>
<th>Mutation</th>
<th>Apo E</th>
<th>BMI</th>
<th>Time (hours)</th>
<th>Glucose (mmol/l ± s.d.)</th>
<th>Insulin (pmol/l ± s.d.)</th>
<th>Proinsulin (pmol/l ± s.d.)</th>
<th>PI/IRI%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 M 1964</td>
<td>M239V</td>
<td>2:3</td>
<td>24.9</td>
<td>0</td>
<td>5.72±0.1</td>
<td>68.42±5.0</td>
<td>8.3±0.0</td>
<td>12.2</td>
</tr>
<tr>
<td>2 F 1959</td>
<td>M239V</td>
<td>3:3</td>
<td>22.1</td>
<td>2</td>
<td>6.01±0.2</td>
<td>142.44±20.2</td>
<td>34.05±0.9</td>
<td>23.9</td>
</tr>
<tr>
<td>3 F 1958</td>
<td>M239V</td>
<td>3:3</td>
<td>20.5</td>
<td>2</td>
<td>3.42±0.7</td>
<td>134.74±6.0</td>
<td>43.99±8.2</td>
<td>32.6</td>
</tr>
<tr>
<td>4 F 1969</td>
<td>M239V</td>
<td>2:3</td>
<td>24.8</td>
<td>2</td>
<td>4.83±0.2</td>
<td>64.61±3.4</td>
<td>8.37±0.3</td>
<td>17.5</td>
</tr>
<tr>
<td>5 M 1945</td>
<td>–</td>
<td>3:3</td>
<td>24.5</td>
<td>2</td>
<td>4.16±0.1</td>
<td>55.45±7.6</td>
<td>5.9±0.2</td>
<td>10.6</td>
</tr>
<tr>
<td>6 F 1952</td>
<td>–</td>
<td>3:3</td>
<td>29.3</td>
<td>2</td>
<td>5.56±0.0</td>
<td>68.73±5.0</td>
<td>5.68±0.1</td>
<td>8.3</td>
</tr>
<tr>
<td>7 M 1940</td>
<td>–</td>
<td>3:4</td>
<td>31.1</td>
<td>2</td>
<td>7.33±0.7</td>
<td>70.92±3.7</td>
<td>13.54±1.2</td>
<td>19.1</td>
</tr>
<tr>
<td>8 F 1948</td>
<td>–</td>
<td>3:3</td>
<td>21.2</td>
<td>2</td>
<td>5.93±0.1</td>
<td>130.58±26.9</td>
<td>46.65±1.6</td>
<td>35.7</td>
</tr>
<tr>
<td>9 M 1950</td>
<td>–</td>
<td>3:3</td>
<td>26.2</td>
<td>2</td>
<td>5.12±0.5</td>
<td>309.87±76.1</td>
<td>71.32±8.5</td>
<td>23</td>
</tr>
</tbody>
</table>

Subjects are listed numerically with gender as M/F. BMI was calculated as (ht) 2 /wt and data is expressed as mean ± s.d. at time zero (time=0) and two hours following the glucose load (time=2 hours); plasma concentrations of immunoreactive-insulin (IRI) and proinsulin (PI) are expressed as pmol/l. PI/IRI% indicates the proportion of incompletely processed insulin precursor peptides in the circulation. Increased PI/IRI% at 2 hours reflects the persistence of PI due to the longer half life of PI compared to insulin in the circulation following a glucose load. Subjects 7 had relatively high concentrations of fasting glucose, insulin and proinsulin which are associated with the increased BMI. No subject had fasting or 2 hour values outside the normal range.

Blood samples were centrifuged and plasma was removed and frozen at −20°C prior to analyses. Plasma glucose was determined by a hexokinase method with Glucoquant kit (Boehringer Mannheim, Lewes, UK; interassay CV, <2%). Immunoreactive insulin was measured by radioimmunoassay using the Pharmacia RIA100 kit (interassay CV 4% at 344 pmol/l; the antibody in this assay cross-reacts 100% with proinsulin). Total proinsulin-like molecules were measured by specific two-site ELISA (Mercodia, Diagenics, Newark, UK; interassay CV, <2%). Immunoreactive insulin was measured by specific two-site ELISA (Mercodia, Diagenics, Newark, UK; interassay CV, <2%).
similar dimensions and pancreatic distribution to those found in non-diabetic subjects without the mutation (Fig. 2A). No islet or perivascular amyloid was detected by immunolabelling for IAPP or with Congo Red staining.

Immunoelectron microscopy demonstrated that PS2 was localised to the insulin granule compartment in normal human pancreatic islet β-cells (Fig. 2B); gold labelling of granules was heterogeneous within the same cell. Although light microscopy indicated labelling for PS2 in non-β cells this was not shown conclusively with electron microscopy. Lysosomes, which are commonly seen in human β-cells, were labelled for PS2 (Fig. 2C). There was no evidence for high concentrations of PS2 in the Golgi or in the endoplasmic reticulum in islet β-cells (Fig. 2B).

**PS2 is expressed as 50 kDa and 30 kDa forms in pancreas**

PS2 is a ~50 kDa protein which is proteolytically cleaved in the cell resulting in a ~30 kDa N-terminal fragment and a ~20 kDa, C-terminal fragment (Kim et al., 1997). Both full length (50 kDa) and cleaved (30 kDa) PS2 were found in extracts of rat pancreas (Fig. 3a). A 30 kDa immunoreactive protein was
Presenilin 2 in pancreatic islet β-cells

identified by western blot analysis of extracts of mouse islets and human insulinoma (Fig. 3b,c). A single band was obtained with mouse islet extracts but a doublet at 28-30 kDa was present in human insulinoma extract. Immunoreactivity was lost by preabsorption of the antiserum with the peptide antigen (Fig. 3d).

**Oral glucose tolerance tests of subjects with Met239Val PS2 mutations and their first degree relatives**

Data from the OGTT on four subjects with the PS2 Met239Val mutation (subjects 1-4) and 5 first degree relatives without the mutation (subjects 5-9) is shown in Table 1. These subjects aged 29-40 years were white Caucasian subjects from the FLO10 pedigree (Rogaev et al., 1995), heterozygous for the mutation and unaffected by EOFAD. Apolipoprotein E (ApoE) genotype is shown in Table 1. Fasting glucose, insulin and proinsulin were in the normal ranges for all subjects (Table 1) and relative increases in these parameters largely corresponded with increased body mass index (BMI). One affected subject and two non-affected subjects had fasting glucose concentrations which were borderline for classification as impaired fasting glucose (IFG) (6.0-7.0 mmol/l) (Gavin et al., 1997). No subject had markedly elevated 2 hour glucose concentrations which would indicate impaired glucose-stimulated insulin secretion or insulin resistance. The proportion of secreted proinsulin (PI) in relation to total immunoreactive-insulin molecules (IRI)

**Fig. 2.** (A) Pancreatic islets from a subject with the PS2 Met239Val mutation affected by EOFAD immunoperoxidase-labelled for insulin. The islet has normal structure and size compared to islets in non-diabetic subjects; the distribution of β-cells (arrows) was normal and there was no evidence of islet amyloid in this slightly autolysed specimen of post-mortem pancreas. (B) Electron micrograph of non-diabetic human islet β-cells immunogold labelled for PS2. Immunolabelling was present in insulin granules containing the characteristic electron dense core of hexameric insulin and clear halo (arrow). There was no labelling in the adjacent endoplasmic reticulum (er). (C) Labelling was also present in β-cell lysosomes (L) which contain lipid and protein material in the process of degradation; PS2 immunoreactivity was largely localised to the protein-containing region (arrowhead). Magnification: (A) ×160. Bars: (B) 0.2 µm; (C) 0.5 µm.

**Fig. 3.** Western blot of non-diabetic rat pancreas, normal mouse islets and of human insulinoma immunolabelled for PS2. Rat pancreas (a) has PS2 protein as both 30 kDa and 50 kDa bands. The mouse islets (b) show PS2 as a single band at 30 kDa. PS2 is present in human insulinoma (c) as a doublet at 30 kDa. One band could represent phosphorylation of PS2 which occurs in the 30 kDa fragment. The presence of only 30 kDa bands for both the mouse islets and the insulinoma could be due to the smaller amount of material available for investigation. Immunoreactivity was lost by preabsorption of the antiserum with the peptide antigen (d).
DISCUSSION

Cellular localisation and functional studies on wild-type and mutant PS2 have been made largely on neuronal and PS2 transfected cells (Scheuner et al., 1996; Citron et al., 1997; Walter et al., 1996; Kovacs et al., 1996). However, the observation that the PS2 mRNA is present in tissues such as skeletal and cardiac muscle and pancreas (Rogaev et al., 1995) indicates that PS2 may have a role in non-neuronal cellular mechanisms. The identification of PS2 protein in pancreas, and particularly in islet cells suggest it has a role in β-cell function.

The most evident clinical and biological effect of PS mutations is on processing of Alzheimer’s protein APP to the amyloidogenic product, Aβ1-42. Increased plasma concentrations of Aβ1-42 were found in patients with the PS2 mutation, N141I (Scheuner et al., 1996). Cells co-transfected with APP and PS1 or PS2 mutants had increased Aβ1-42 secretion (Citron et al., 1997; Xia et al., 1997). These findings associate PS mutations with alternative cleavage of APP and amyloid plaque formation in AD.

PS2 was localised to pancreatic islets in rodent and human tissue and was found primarily associated with secretory granules in the β-cell. Localisation of the gold particles within the insulin granule compartment does not preclude the presence of a membrane associated protein. Membrane integrity is not retained in non-osmicated preparations embedded in LR gold resin and granule membrane components would be distributed within the granule compartment. More than 50 different proteins/peptides have been identified in the granule compartment including proinsulin, insulin, IAPP, betagranin, proteolytic enzymes, ionic pumps and channels (Neophytou et al., 1996). Post-translational cleavage of β-cell secretory propeptides occurs in secretory granules; proinsulin is processed to insulin by two prohormone convertases (PC1 and PC2) and carboxypeptidase H (Hutton, 1994). IAPP is cleaved from proIAPP by PC2 (Badman et al., 1996) and the proteolytic enzymes are themselves derived from larger precursors by autolysis. An increased proportion of incompletely processed proinsulin (and possibly proIAPP) occurs in Type 2 diabetes (Porte and Kahn, 1989) and in insulinomas which are conditions associated with IAPP amyloid formation (Clark, 1992). If PS2 has a role in secretory granules, post-translational modification of granule components could be affected by PS2 mutations in a similar way to that proposed for Aβ in AD. A role for PS2 in controlling calcium flux in the granule would be consistent with localisation of PS2 in nerve, heart and muscle tissues where cytoplasmic calcium plays an important role in cellular function.

PS2 is not a component of islet amyloid formed from IAPP. Other types of systemic and localised amyloid deposits, including amyloid formed from β-2 microglobulin, SAA, and Aβ did not show immunoreactivity for PS2; this suggests that presenilins are not members of the large number of amyloid-associated compounds such as serum amyloid P and heparan sulphate proteoglycans.

PS2 was localised to lysosomes in β-cells. These organelles are prominent in β-cells and contain lipid and protein material which is either in the process of degradation or resistant to lysis. Human IAPP (but not rodent IAPP) is resistant to proteolysis and accumulates in β-cell lysosomes (de Koning et al., 1992). Granule contents are passed to lysosomes by crinophagy (Schnell et al., 1988), but proteins can also come from the Golgi, or by recycling from the plasma membrane. In other cell types, PS2 has been localised by immunofluorescence to the cytoplasmic domain and has been shown to be a component of the endoplasmic reticulum and early Golgi (Walter et al., 1996; Kovacs et al., 1996) where processing of APP to Aβ1-42 occurs (Hartmann et al., 1996). Degradation of PS is thought to occur via the proteosome in transfected cells (Kim et al., 1997). It appears that the localisation of PS2 in β-cells is different from that in transfected cells which could be related to its function in cells with predominantly a regulated secretory pathway.

PS2 is present in islets as a 30 kDa protein and as a doublet in insulinoma tissue with bands at approximately 28-30 kDa. The doublet could represent partly phosphorylated protein; the N-terminal serine residues in PS2 are putative phosphorylation sites (Walter et al., 1996; de Strooper et al., 1997). Protein phosphorylation and increased intracellular calcium concentration are essential processes in insulin secretion including activation of the mechanisms that result in translocation of granules to the plasma membrane (Tian et al., 1996). The ~30 kDa band is the product of cleavage at a site in the hydrophilic loop domain between amino acids 260 and 320 (Thinakaran et al., 1996). The C-terminal fragment of PS2 which contains the cleavage site was not recognised by this antiserum, which was raised to PS2 N-terminal residues 1-87. Intact PS2 (50 kDa) was not apparent in the isolated endocrine cell preparations consistent with other studies performed on untransfected material (Walter et al., 1996; Kovacs et al., 1996). However, a 50 kDa band was present in extracts of whole rat pancreas.

PS2 was found in primitive duct cells in human foetal pancreas and in proliferating cells in adult pancreas with pancreatitis suggesting that PS2 is a component of progenitor ductular cells. PS are homologous to proteins in the notch signalling pathway (Levitan and Greenwald, 1995; Levitan et al., 1996) and therefore could play a role in pancreatic development. β-Catenin, a putative ligand for PS (Yu et al., 1998), is also a component of β-cells where it is essential in pancreatic development for aggregation of β-cells into primitive islets (Dahl et al., 1996). PS mutations have been shown to affect intracellular β-catenin trafficking (Zang et al., 1998; Nishimura et al., 1999) and could therefore interfere with islet development. However, the pancreatic specimen from a member of the FLO10 family who died suffering from Alzheimer’s disease showed no evidence of diabetes or abnormal pancreatic development. Observations on this single case suggest that this mutation does not have a phenotype in pancreatic islet structure but does not exclude a role for PS2 in islet function or development.

The FLO10 subject affected by EOFAD was not receiving therapy for diabetes but her exact diabetic status was unknown. Oral glucose tolerance tests were therefore made on other
family members with and without the PS2 Met239Val mutation to determine if PS2 was associated with abnormal islet function or glucose metabolism. However, these tests demonstrated no evidence of glucose intolerance, diabetes or increased secretion of proinsulin. The age-related penetration of EOFAF in this pedigree is low, and the age of onset of dementia variable (45-88 years; Sherrington et al., 1996). These observations on normal subjects and from EOAFD patients suggest that this PS2 mutation is not associated with overt diabetes or islet development but that PS2 may play a role in pancreatic islet β-cells.

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