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Research report

Differential expression of the neuroendocrine polypeptide 7B2 in hypothalami of Prader-(Labhart)-Willi syndrome patients

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Abstract

Prader-(Labhart)-Willi syndrome (PWS) is characterized by infantile hypotonia, early childhood obesity, mental deficiency, short stature, small hands and feet and hypogonadism. In 70% of the cases this syndrome is associated with a defect of chromosome 15 at 15q11–q13, close to the location of the 7B2 gene (15q13–q14). The majority of the remaining PWS patients display maternal uniparental disomy on chromosome 15. Since the 7B2 gene products are expressed in neuroendocrine cells that are probably affected in PWS, e.g. by a pleiotrophic influence of the neighboring deletion, the presence of 7B2 was studied in the supraoptic and paraventricular nucleus of the hypothalamus of five subjects clinically diagnosed as PWS patients using five antibodies against various parts of the 7B2 precursor polypeptide. Three of the five PWS patients studied showed no reaction to the 7B2 antibody MON-102, whereas all 30 control patients did. However, one of the three MON-102 non-reacting PWS patients reacted to other 7B2 antibodies. In conclusion, the vanishing of 7B2 gene products is not obligatory for PWS, possibly due to the variable genetic background of PWS patients. However, in most patients there is a clear modification of 7B2 expression, pointing to altered neuroendocrine functions.

Key words: Prader-(Labhart)-Willi; 7B2; Immunocytochemistry; Hypothalamus; Chromosome 15; Vasopressin-associated glycopeptide

1. Introduction

The Prader-(Labhart)-Willi syndrome (PWS) was described in 1956 on the basis of the following symptoms: grossly diminished fetal activity, severe infant hypotonia, feeding problems in infancy, hypogonadism and hypogenitalism, retarded bone age and short stature, small hands and feet, delayed mental and psychomotor development, characteristic face, mental retardation, onset of gross obesity due to insatiable hunger in early childhood, behaviour problems and a tendency to develop diabetes mellitus in adolescence

[27]. However, these features may vary widely among individual PWS patients [2]. Major components of this syndrome are the result of disturbances in the hypothalamus [1]. PWS is the most common syndromal cause of human obesity with an incidence of approximately 0.5 per 10⁴ births [4]. An association between a cytogenetic deletion of part of chromosome 15 and PWS was found by Ledbetter in 1981 [13]. He reported that approximately 60% of all PWS patients have an interstitial deletion of chromosome 15q11–q13, 37% apparently normal chromosomes, and 3% a variety of other abnormalities involving chromosome 15 [14]. Using DNA markers specific for the 15q11–q13 subregion, Nicholls suggested that in cytogenetically normal PWS patients the paternal gene contribution to this specific region is absent and that both copies of chromosome 15 are inherited from the mother. This phenomenon of

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uniparental disomy demonstrated that the loss of the paternally expressed allele of imprinted gene(s) can cause PWS [23,24]. Using molecular techniques it has recently been established that 60% of the patients

without cytogenetic deletion has maternal uniparental disomy, 27% large molecular deletions and 13% normal biparental inheritance for chromosome 15. With the combined use of cytogenetic and molecular tech-

Table 1
Control brain material

Patient number	Sex	Age	Brain weight (g)	Post-mortem delay (h)	Fixation (d)	Clinical diagnosis	Medicines used	MON ₁₀₂ immunoreactivity in SON/PVN	
								researcher 1	researcher 2
87.280	m	2 d	400	24	33	hypoplastic left heart syndrome; anoxia		+++	+++
88.259	f	4 d	350	48	56	perinatal asphyxia		++++	+++
85.263	m	3 mo	635	24	n.d.	supravalvular aortastenose, aorta descendens hypoplastic		++++	++++
84.217	m	3 mo	710	n.d.	792	sudden infant death syndrome		++	+
85.002	f	3 mo	610	24	33	bronchopneumonia; myocarditis; small subarachnoidal haemorrhagia		++	++
86.14276	m	4 mo	640	20	326	sudden infant death syndrome		++	++
84.1002	m	5 mo	700	66	240	asphyxia		++++	++++
86.311	f	5 mo	735	10	40	sudden infant death syndrome		++++	++++
86.411	m	6 mo	800	14	14	sudden infant death syndrome; brain oedema		++++	++++
84.191	m	5 y	1565	n.d.	108	meningococcal sepsis		++++	++++
80.280	f	15 y	1480	20	29	cerebellar haematoma		++++	++++
87.326	m	19 y	1310	88	44	multitraumata		+++	++
84.186	m	29 y	1400	13	41	congenital heart disease; cardiac failure		++++	++++
85.124	f	29 y	1150	24	60	alcoholic induced hepatitis		+++	+++
81.255	f	30 y	1460	24	39	acute heart arrest		++++	++++
86.354	f	33 y	1035	24	20	low differentiated adeno-carcinoma with metastases	nicomorphine hydrochloride	++++	++++
81.012	f	38 y	1360	3	47	cervix carcinoma	antibiotics	+++	+++
87.345	m	41 y	1440	120	44	contusio cerebri, lung embolism		+++	+++
82.165	f	52 y	1370	5	33	bronchopneumonia	5-FU, adriamycine, cyclophosphamide, vincristine, MTX, nicomorphine-hydrochloride	+++	+++
82.161	f	57 y	1220	45	35	polymyalgia rheumatica; endocarditis; mitralis- and aortastenose; lung embolism and haemorrhagic infarction	anticoagulants prednison, digoxine	+++	+++
84.049	m	63 y	1420	32	35	myocardial infarction; cardiac failure		++++	++++
81.014	f	64 y	1090	8	44	haemorrhagic peptic ulcer; hypovolemic shock; renal insufficiency	Prednison cimetidine, antacida, diuretics	++++	++++
80.278	f	72 y	1200	8	59	lung infarction bronchopneumonia, highly differentiated squamous carcinoma	antibiotics	++++	+++
81.032	m	74 y	1410	13	48	cardiac failure; bronchopeumonia	digoxine, diuretics, antibiotics, theophylline, alimemazine-tartrate	++++	++++
81.064	m	83 y	1280	22	42	diverticulitis	antibiotics	++++	++++
81.097	m	88 y	1370	47	60	decompensatio cordis, with asthma cardiale	digoxine	++	++
81.100	f	88 y	1030	11	35	myocard infarction	lasix		
81.033	f	90 y	1110	13	48	basalioma of ear; cardiac failure; amnestic syndrome; anaemia	antibiotics	+++	+++
85.38	f	90 y	1300	6	33	femur fracture	antibiotics	++	+++
83.179	f	91 y	1060	48	28	bronchopneumonia			
						non-toxic goiter		++	+++
						respiratory insufficiency		+	+

Abbreviations: f, female; m, male; d, days; mo, months; y, years; g, grams; h, hours; n.d., not determined.

Stainability: + faint; ++ moderate; +++ intense; ++++ very intense.

Table 2
Clinical data and staining intensity in the SON and PVN of Prader–Willi patients

Patient number	Sex	Age (y)	Brain weight (g)	Postmortem delay (h)	Fixation (days)	7B2 antibodies				Vasopressin-associated glycopeptide antibody			
						AG-7 (23–39)		RB-7 (23–39)		MON ₁₄₄ (64–94)		MON ₁₀₂ (128–143)	
						R1	R2	R1	R2	R1	R2	R1	R2
44282	m	28	1415	±30	7–14	–	–	–	–	–	–	–	–
43830	f	30	1310	4.5	ND	+	+	+	+	+	+	+	+
91–252	f	33	1223	5	33	–	–	–	–	+	+	–*	–
93–056	m	38	1540	45	385	–	–	–	–	–	–	–	–
317–90	f	64	1150	20	14	–	–	–	–	+	+	+	+

Abbreviations: f, female; m, male; y, years; g, grams; h, hours; ND, not determined; R, researcher.

Stainability: – no staining; + faint; ++ moderate; +++ intense; ++++ very intense; *: one large solitary cell.

Table 3
Pituitaries

Patient number	Sex	Age (y)	Brain weight (g)	Postmortem delay (h)	Fixation (days)	7B2 antibody		Vasopressin-associated glycopeptide antibody							
						MON ₁₀₂ (128–143)		IZ		AL		IZ		NL	
						R1	R2	R1	R2	R1	R2	R1	R2	R1	R2
92.001 (control)	m	83	1300	6½	41	+	+	+	+	+	+	–	–	+	+
91–252 (PWS)	f	33	1223	5	33	+	+	+	+	+	+	–	–	+	+

Abbreviations: f, female; m, male; y, years; g, grams; h, hours; AL, anterior lobe of pituitary; IZ, intermediate zone of pituitary; NL, neural lobe of pituitary; R, researcher.

Stainability: + faint; ++ moderate; +++ intense; ++++ very intense.

niques, the genetic basis of PWS can now be identified in up to 95% of all patients [18].

The present study was initiated to examine whether the neuroendocrine polypeptide 7B2 is expressed in the hypothalamus of PWS patients. 7B2, originally isolated from human and porcine pituitaries [9,29], is present in both neurons and endocrine cells [15,17]. It has been shown to be highly conserved during evolution [17,20]. 7B2 is synthesized in humans as a precursor consisting of 185 amino acids [16], which is enzymatically processed to a 150 amino acid form at specific pairs of basic residues studied in pigs [12]. The distribution of 7B2 mRNA in the central nervous system of the mouse has been described in the literature [21] and recently the 7B2 gene of the rat was cloned [35]. 7B2 was tentatively classified as a member of the group of acidic secretory proteins called the granin family and it was named Secretogranin V [10]. In situ hybridization analysis of metaphase chromosomes with a 7B2 cDNA probe of human pituitary cells enabled the regional localization of the 7B2 gene to chromosome 15 at q13–q14 [19,28]. The function of 7B2 is still unknown.

7B2 may be involved in PWS for two reasons [32]. First, 7B2 gene products are expressed in cells that are probably affected in PWS, i.e. in the neuroendocrine cells of the hypothalamus. Secondly, the genomic PWS defect (15q11–q13), which may exert a pleiotrophic influence, is located near the gene for 7B2 (15q13–q14).

In order to investigate whether the 7B2 gene expression is affected in PWS, we studied the presence of 7B2 immunoreactive products in the supraoptic (SON) and paraventricular nucleus (PVN), where the highest 7B2 immunoreactivity has been detected in the rat [15], of 35 hypothalami, i.e. of five PWS patients and 30 controls. In addition, we determined the immunoreactivity in the pituitary of one PWS patient and one control. We also examined vasopressin-associated glycopeptide [3], immunoreactivity in the SON and PVN of five PWS patients, as VP is one of the major neuropeptides produced by these nuclei [5].

2. Materials and methods

For the present study the brains of 35 subjects, including five clinically diagnosed PWS patients, were investigated (for clinical and pathological details see Tables 1, 2 and 3). The required, separate permission for brain autopsy was obtained either from the patients themselves or from partners or relatives. The reference group consisted of 14 male and 16 female subjects, without any primary neurological or psychiatric disease, ranging in age from 2 days up to

91 years. A clinical description of the five PWS patients is given below.

2.1. Case #1. 44282 (Johns Hopkins Hospital, Baltimore, USA)

The patient was a 28-year-old male with a history of morbid obesity, hyperphagia, hypogonadism and mental retardation. In order to control his weight he underwent a gastric stapling procedure and gastrostomy. He was admitted to hospital for the evaluation of a personality disorder characterized by immature demanding and manipulative behavior, including self-mutilation. His IQ at that time was 77. Some days before his death a non-contrast CT scan revealed hydrocephalus without focal lesions, ventricular calcifications and slight prominence of the sulcal pattern. Despite aggressive management, the patient died of septic shock in dehydrated condition. There were no specific neuropathological features.

2.2. Case #2. 43830 (Massachusetts General Hospital, Boston, USA)

The patient was a 30-year-old woman who was the first child of a full-term gestation weighing 3260 g at birth. In retrospect, the mother felt that the fetus had not been as active as her siblings in utero. The first couple of months she was very hypotonic and she could not suck the breast or bottle effectively, so she was drip-fed. In adulthood she suffered from massive obesity, gonadotropin deficiency [LH = 1.6 IE/l (normal levels 3–120 IE/l depending on the stage of the menstrual cycle), FSH = 0.5 IE/l (normal levels 2–30 IE/l)], strabism, short distal extremities and mental retardation (full scale IQ = 87; verbal IQ = 97; performance IQ = 76). Before her death she was frequently operated because of her obesity and she died of renal failure and generalized sepsis following one of these operations. The general pathology showed ovarian atrophy with markedly underdeveloped secondary sexual characteristics. At autopsy her length was 155 cm and her weight 125.5 kg. There were no special neuropathological features.

2.3. Case #3. 91-252 (Onze Lieve Vrouwe Gasthuis, Amsterdam, The Netherlands)

The patient was a 33-year-old woman. In the few years after she had been diagnosed as a Prader–Willi patient, she was frequently admitted to various hospitals for her congestive heart failure as a complication of her obesity. She died of a pneumonia. At autopsy she had severe dysmorphisms: a length of 150 cm, an approximate weight of 160 kg with enormous fat accumulation in the abdomen and upper legs, narrow bifrontal diameter, hypopigmentation (hair, eyes, skin), pectus excavatus, short extremities, atrophic ovaries and hydrosalpinx of the left tuba.

2.4. Case #4. 93-056 (Cambridge Brain Bank Laboratory, Cambridge, UK)

The patient was a 38-year-old male with a history of hypotonia, obesity, small hands and feet, hypogonadism, an excessive sleeping pattern and mental retardation. At the age of 3 years he was investigated for gross obesity (50% above average). No specific endocrine causes were found. From the age of 9 to 11 he received anabolic steroids to boost growth and development. At the age of 15 he underwent a bilateral orchidopexy and an IQ test (verbal score 79,

Fig. 1. MON-102 immunoreactivity in SON (A,B) and PVN (C) of control patient 86354 (very intense). MON-102 immunoreactivity in SON (D) of control patient 83179 (faint). Note that MON-102 immunoreactivity is present throughout the cytoplasm. *, the same location in A and B. CO, chiasma opticum; III, third ventricle. Bar in A: 100 μ m, in B, C and D: 50 μ m.

performance score 70). His behavior became more and more problematic: he was caught stealing and therefore lost his job in an engineering firm. At that time hospital assessment diagnosed a small, chubby, sexually underdeveloped man showing stubborn, demanding, manipulative and self-opinionated behavior. Some years later he was operated for recurrent inguinal hernia. The patient died of ketoacidosis, known to occur with diabetes mellitus. Karyotyping did not provide any evidence for a deletion on chromosome 15.

2.5. Case #5. 317-90 (Mental Retardation Institute, Zwammerdam, The Netherlands)

The patient was a 64-year-old woman. From childhood she had been slow and inactive with fits of anger. At the age of 34 she was referred to a psychiatric clinic because of her behavioral problems. From the age of 40 she was given low doses of neuroleptics. She had periods of somnolence which became more frequent with age. She had many dysmorphisms: obesity (length 165 cm, weight 84 kg), small hands and feet, narrow bifrontal diameter, ptosis on the left side, scoliosis and rotatory nystagmus. She was mentally retarded and had primary amenorrhea. She died of respiratory insufficiency. General pathology revealed a small uterus and small ovaries with a smooth surface (estrogen insufficiency).

Following autopsy, the brains were fixed in 4% formaldehyde at room temperature. In general, after one month the hypothalamic area, containing the PVN and SON, and the pituitary of two patients were dissected, dehydrated in graded ethanol and embedded in paraffin via toluene (for details see [31]).

Serial 6- μ m sections were cut frontally on a Leitz microtome, mounted on chrome-alum-coated object slides, and stored at room temperature. Before use, the sections were deparaffinized in xylene and hydrated via graded ethanol series. For orientation, every 50th section was stained with thionin (0.1% thionin in acetate buffer, pH = 4) for 15 min. From the caudal region of the PVN and SON and from the pituitary several sections were selected for immunocytochemistry.

2.6. Immunocytochemistry

For immunocytochemistry the hydrated sections were rinsed in TBS (0.05 M Tris/0.9% saline, pH = 7.6) for 10 min and subsequently incubated with one of the following first antisera (all antisera were diluted in 0.05 M Tris, 0.5 M NaCl, 0.5% Triton-X-100, pH = 7.6):

- polyclonal rabbit anti-7B2, against a synthetic fragment of this protein representing the residues 23–39 of the 7B2 sequence, #AG-7, diluted 1:500 (kindly provided by S.R. Bloom, Department of Medicine, Royal Postgraduate Medical School, Hammsmith Hospital, London, UK; for details see [34]);
- polyclonal rabbit anti-7B2 against the same synthetic fragment (residues 23–39) of this protein, #RB-7, 27-10-1988, diluted 1:500 (for details see [7,29]);
- monoclonal mouse anti-7B2, with the 7B2 region sequence 64–94 involved in epitope recognition, designated MON-144 (supernatant), diluted 1:10 (for details see [33]);
- monoclonal mouse anti-7B2, with the 7B2 region sequence 128–143 involved in epitope recognition, designated MON-102 (ascites), diluted 1:1000 (for details see [33]);
- polyclonal rabbit anti-7B2, against a mouse 7B2 fragment representing the residues 156–186 of the sequence and recognizing

residues 156–171 of the C-terminus of 7B2, # CT 7B2, Rb-4, 14-2-1991, diluted 1:250 (for details see [25]);

- polyclonal rabbit anti-glycopeptide (#K.1.7) directed against the C-terminus of the vasopressin precursor of guinea pig, diluted 1:500 (kindly provided by J.C.A.F. Robinson, National Institute for Medical Research, Mill Hill, London, UK; for details see [3]).
- All the above-described antibodies were incubated for 1 h at room temperature and subsequently overnight at 4°C in a humid chamber. After rinsing in TBS goat-anti-rabbit IgG (Betsie, diluted 1:100) was added to the section initially incubated with the polyclonal antibodies for 1 h at room temperature followed by peroxidase-anti-peroxidase complex diluted 1:1000 for 1 h at room temperature. Sheep-anti-mouse IgG biotinylated (RPN: 1021, Batch: 24, Amersham, UK, diluted 1:100) was added to the sections initially incubated with the monoclonal antibodies for 1 h at room temperature followed by Elite Vectastain ABC kit (Vector Laboratories, Inc., diluted A 1:100 and B 1:100, prepared 30 min before incubation at room temperature) for 1 h at room temperature. In between these steps the sections were washed twice for 15 min in TBS. Peroxidase activity was detected with 0.05% 3,3' diaminobenzidine tetrahydrochloride in TBS and 5 μ l of 30% H₂O₂ to which 0.2% nickel ammonium sulphate was added as intensifying agent for 15 min. Subsequently they were washed in TBS, dehydrated via graded ethanol to xylene and mounted in Entellan. Preliminary sets of experiments were performed in three control patients (85124, 86354 and 81014) in order to investigate which of the five different antibodies against 7B2 gave the best immunoreactivity, especially in the PVN and SON. Subsequently we tested the five PWS patients for MON-102 and compared their immunoreactivity with that of 30 control patients. We also tested all other antibodies against 7B2 in the SON and PVN of PWS patients. The pituitary of a control (92.001) and a PWS patient (91-252) were tested for MON-102 and vasopressin-associated glycopeptide. In addition, we assessed vasopressin-associated glycopeptide immunoreactivity in the PVN and SON of all PWS patients.

The intensity of the immunoreactivity was estimated semi-quantitatively by two researchers.

2.7. Controls

The antibodies raised against different parts of 7B2 (see Table 2) were adsorbed with recombinant GST-7B2 hybrid protein (prepared by D.W. Eib; for details see [33]). After adsorption no reaction whatsoever was seen. When the first antibodies were omitted from the procedure the reaction was also absent.

3. Results

In pilot experiments MON-102 appeared to give the highest immunoreactivity of all 7B2 antibodies used in SON and PVN neurons. The PVN and SON of 30 control patients showed a MON-102 staining ranging from faint to very intense (Fig. 1; Table 1). No influence of sex differences or postmortem delay on the stainability was observed. However, a lower immunoreactivity was found in some controls immediately after birth up to about four months postnatally and in old

Fig. 2. MON-102 immunoreactivity in the SON (A,B) and PVN (C,D) of PWS patient 43830 (very intense). Note that MON-102 immunoreactivity is present throughout the cytoplasm as in the control patients. *, the same location in A and B and in C and D. CO, chiasma opticum; III, third ventricle. Bar in A and C: 100 μ m, in B and D: 50 μ m.

Fig. 4. With MON-102 PWS patient 91-252 shows immunoreactivity in one solitary cell in the SON. *, the same location in A and B; CO, chiasma opticum. Bar in A: 100 μ m, in B: 50 μ m.

subjects from 88 years onwards. MON-102 stained the PVN and SON in two of the five PWS patients (43830 and 317-;90) (Fig. 2; Table 2). In the hypothalamus of the other three patients (44282, 91-252 and 93-056) there was no immunoreactivity whatsoever to MON-102 (Fig. 3A,C), with the exception of one large solitary cell in the SON of PWS patient 91-252 (Fig. 4). In contrast, the anterior lobe and intermediate zone of the pituitary of this patient stained well with MON-102 (Fig. 5B,D), whereas the neural lobe of the pituitary hardly showed any staining (Fig. 5F; Table 3). The distribution and intensity of the immunoreactivity obtained with MON-102 was similar with regard to the anterior lobe and intermediate zone except the neural lobe which showed a slightly more intense staining in control patients 92001 (Fig. 5A,C,E). In both pituitaries

the vasopressin-associated glycopeptide immunoreactivity was similar and restricted to the neural lobe (Fig. 5G,H).

In order to elucidate whether the entire 7B2 precursor was absent in the three PWS patients without any immunoreactivity to MON-102 in the PVN and SON, with the exception of the single large positive cell in patient 91-252, all other available antibodies against 7B2 were applied to the five PWS patients. One patient (91-252) that had not shown any reaction to MON-102 showed a moderate immunoreactivity to CT 7B2 (Fig. 6) and a faint to moderate immunoreactivity towards MON-144 (Table 2). Patients 44282 and 93-056 showed no reaction to any of the 7B2 antibodies. All PWS patients showed a very intense reaction to vasopressin-associated glycopeptide (Fig. 3B,D).

←

Fig. 3. No MON-102 immunoreactivity in SON (A) and PVN (C) of PWS patient 44282 while there is a very intense staining for vasopressin-associated glycopeptide in the SON (B) and PVN (D) of the same patient. Note that the absence of any 7B2 immunoreactivity is not due to inconsistent fixation or embedding. CO, chiasma opticum; III, third ventricle. Bar: 100 μ m.

Fig. 6. CT-7B2 immunoreactivity in the SON of PWS patient 91-252. Most cells in the SON react with CT-7B2, whereas there is no MON-102 reaction (Fig. 4). Note the peripheral cytoplasmic immunoreactivity of CT-7B2 compared with the overall immunoreactivity of MON-102 (Fig. 1,2,4). *, the same location in A and B. CO, chiasma opticum. Bar in A: 100 μ m, in B: 50 μ m.

4. Discussion

The present study was undertaken in order to investigate whether expression of the neuroendocrine polypeptide 7B2 is affected in PWS patients since the 7B2 gene is located in close proximity to the chromosome 15 defect in this syndrome. If 7B2 expression were affected in PWS patients, this could reveal essential information on the possible function of 7B2 in neuroendocrine cells. For this purpose we stained hypothalamic sections, obtained by autopsy, of five PWS patients with five antibodies against 7B2 recognizing different parts of the 7B2 molecule. At least one of these antibodies reacted in the PVN and SON of three

PWS patients. Two PWS patients (44282 and 93-056) showed no reaction with any of the 7B2 antibodies. From these results we drew the conclusion that the absence of 7B2 immunoreactivity is not a specific feature for PWS patients, which is in agreement with observations of the normal plasma 7B2 levels of PWS patients compared to those of controls [8].

There were, however, great differences between the PWS patients concerning their stainability with the various 7B2 antibodies. Two of the five patients displayed a reaction to MON-102 in the PVN and SON. However, the anterior lobe and the intermediate zone of the pituitary of patient 91-252, which showed no staining in the SON and PVN, displayed a MON-102

Fig. 5. The MON-102 immunoreactivity in the anterior lobe of the pituitary of control patient 92.001 (A,C) and that in PWS patient 91-252 (B,D) are the same. C and D are higher magnifications of the anterior lobe. The MON-102 immunoreactivity was more prominent in the neural lobe of control patient 92.001 (E) than in that of PWS patient 91-252 (F). The vasopressin-associated glycopeptide immunoreactivity in the neural lobe of control patient 92.001 (G) and in PWS patient 91-252 (H) is the same. Note the absence of vasopressin-associated glycopeptide immunoreactivity in the anterior lobe (G). AL: anterior lobe of pituitary; IZ, intermediate zone of pituitary; NL: neural lobe of pituitary. Bar in A, B and G: 100 μ m, in E, F and H: 50 μ m, in C and D 20 μ m.

immunoreactivity similar to that of a control patient. A possible cause of the tissue-specific difference in MON-102 reactivity in PWS patient 91-252 is tissue-specific alternative splicing or a tissue-specific defect in posttranslational enzymatic processing leading to a modification of the epitope of MON-102. Alternative splicing has been described for 7B2, although so far not for the 7B2 part recognized by MON-102 [26]. The presence of normal vasopressin-associated glycopeptide immunoreactivity in patients 44282 and 93-056 indicated that the absence of any 7B2 immunoreactivity is not due to a deviated fixation or embedding procedure. It also made clear that the absence of 7B2 did not influence the expression of the VP precursor. We must consider the possibility that such a large deletion is present in these patients that not only the gene or genes responsible for the PWS are deleted, but also the 7B2 gene which is located in close proximity to the PWS region. Additional information on whether a normal 7B2 mRNA is present in the SON and PVN of PWS patients 44282 and 93-056 may be obtained by *in situ* hybridization using oligonucleotide probes reactive towards a number of different regions within the 7B2 mRNA. The faint MON-102 immunoreactivity in the neural lobe of patient 91-252, as compared with a control patient, is in agreement with the near absence of MON-102 immunoreactivity in the SON and PVN of this patient. There is no likely explanation for the strong immunoreactivity to MON-102 in one large cell located in the SON of patient 91-252.

The low MON-102 immunoreactivity in the perikarya of the PVN and SON cells of some of the control patients between birth and 4 months postnatally may either be explained by a lower production rate or by an increased turnover of 7B2 in the hypothalamo-neurohypophyseal system. Since literature indicates that around birth the plasma 7B2 levels of the neonate are much higher than those of the mother [11], one might expect the latter phenomenon to take place. However, many endocrine organs have the ability to produce 7B2 and might thus contribute to plasma levels of 7B2. That 7B2 immunoreactivity in the SON and PVN diminishes from the age of 88 years onwards seems to contradict the slow increase of 7B2 plasma levels with aging reported by Natori [22], and rather indicates that other organs than the brain contribute to the 7B2 blood levels. From the present study the conclusion can be drawn that the vanishing of 7B2 gene products is not obligatory for PWS. The variable reaction with regard to 7B2 may be due to its heterogeneous genetic background [5]. Unfortunately, it appeared to be impossible to assess this genetic background (Smeets, personal communication) in formalin-fixed tissue from these PWS patients obtained a long time before. In order to ensure an unequivocal diagnosis of PWS in

future, the clinical diagnosis must be accompanied by molecular genetic analysis [30].

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