

Incidence of pituitary autoantibodies in idiopathic diabetes insipidus

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Abstract

Diabetes insipidus is a disorder resulting from insufficient action of vasopressin (ADH) characterized by excretion of highly diluted urine in large amounts. Idiopathic diabetes insipidus is associated with the presence of both autoantibodies against ADH-secreting neurons and pituitary autoantibodies. The aim of the present study was to evaluate the occurrence of autoantibodies against the pituitary microsomal fraction. The study included 33 sera of diabetes insipidus patients and 10 control sera obtained from 10 healthy persons. In all patients the secretion of pituitary hormones and thyroid autoantibodies was assessed. Human pituitaries were obtained during autopsy and homogenized in 0.01 mol/l pH 7.4 phosphate buffer. In addition, for the autoantibody evaluation, the electrophoretic method of separation in polyacrylamide gel and western blot were employed. Among the 33 subjects, in 23 patients the presence of autoantibodies against the pituitary was shown. Sera of 15 patients reacted with the pituitary microsomal fraction protein of 55 kDa. In other cases, 10 sera reacted with the pituitary antigen of 67 kDa. In addition, 5 sera reacted with the 60 kDa antigen, 5 sera with 52 kDa protein, 3 sera with 105 kDa protein, 3 sera with the 97 kDa antigen and 2 sera with pituitary antigen of 92 kDa weight. In our study, based on the immunoblotting method, we observed that pituitary autoantibodies against 55, 60 and 67 kDa antigens occurred frequently.

Key words: diabetes insipidus, pituitary autoantibodies, immunoblotting.

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Introduction

Diabetes insipidus is a disorder resulting from insufficient action of vasopressin (ADH) characterized by excretion of highly diluted urine in large amounts. The disorder must be differentiated from other states of excessive urine, such as primary polydipsia and osmotic diuresis. In fact, central (neurogenic) diabetes insipidus is caused by failure of the posterior part of the pituitary, whereas renal diabetes insipidus occurs when the kidneys do not respond to ADH. The causes of central diabetes insipidus include surgery of pituitary tumors, tumors of the hypothalamus, such as craniopharyngioma, granulomatous diseases or histiocytosis. What is more, familial central diabetes insipidus, which is a recessive inherited disorder, is rare and manifests in infancy. Additionally, the autosomal dominant form occurs in association with diabetes, optic nerve atrophy, and deafness (DIDMOAD – Wolfram syndrome). The idiopathic form of diabetes insipidus is associated with the presence of autoantibodies against ADH-secreting neurons, as well as of pituitary autoantibodies. Furthermore, this form is often accompanied by other autoimmune diseases. Previ-

ous studies have shown that pituitary autoantibodies are present in the sera of most pituitary disease patients [1-3], as well as in patients with other autoimmune diseases of the endocrine glands, such as Graves' disease or Addison's disease [4, 5]. To demonstrate the presence of serum autoantibodies, immunofluorescence involving frozen sections of human pituitary gland [6], or pituitary cell lines, such as rat GH3 or mouse AtT20, were typically used [7, 8]. The presence of pituitary autoantibodies was first identified in 1975 by Bottazzo *et al.* [9, 10] in patients with autoimmune polyendocrine syndrome. They also studied 287 patients with various autoimmune endocrine diseases and in 19 cases antibodies against lactotrophic pituitary cells were identified using immunofluorescence. Subsequent studies based on the immunoblotting method involving antigen isolated from human pituitary gland indicated the presence of antibodies against antigenic proteins of the pituitary in the range of 14 to 98 kDa in sera of patients with various autoimmune endocrine diseases. On the other hand, some sera react with several antigenic proteins varying in weight, and some only with a single protein of a specific gravity [11]. Bando *et al.* described the presence of IgG4

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antibodies in the hypothalamic-pituitary inflammation area in 19 out of 32 cases [12]. Similar results were presented by Nakasone *et al.*, who described the rapid conversion of hypothalamus inflammation towards the empty sella syndrome [13]. Moreover, Scherbaum *et al.* presented data concerning the prevalence of antibodies against vasopressin-secreting cells in 11 patients suffering from idiopathic diabetes insipidus [14]. Similar reports were presented by Pivonello *et al.*, confirming the presence of hypothalamic autoantibodies in 53% of patients with central diabetes insipidus [15]. Additionally, Maghnia *et al.* demonstrated the co-existence of pituitary autoantibodies (anti-glutamic acid decarboxylase – GAD) and anti-islet (tyrosine phosphatase – IA2) in patients with central diabetes insipidus [16]. De Bellis *et al.* presented a significant correlation in the co-existence of both pituitary and hypothalamic antibodies [17].

Chilorio *et al.* pointed out the coexistence of pituitary autoantibodies and anti-nuclear antibodies (anti-ANA) [18]. The aim of the present study was to evaluate the occurrence of autoantibodies against the pituitary microsomal fraction, as well as molecular weight characterization of autoantigens by immunoblotting in patients with the idiopathic form of diabetes insipidus.

Material and methods

The study included 33 sera of patients diagnosed with idiopathic central diabetes insipidus. The studied group involved 20 women aged 33 to 65 years (average 47.3 ± 8.4), and 13 men aged 39 to 59 years (average 44.3 ± 7.1). In all patients the diagnosis had been confirmed on the basis of biochemical tests: plasma and urine osmolality, dehydration test and the vasopressin test. Furthermore, in all patients the pituitary hormone secretion in the circadian rhythm and in stimulation tests was evaluated. As a result, panhypopituitarism was observed in 23 cases, partial hypopituitarism (ACTH deficiency) in 5 cases, and in 5 cases the pituitary function was normal. Additionally, all patients underwent thyroid antibody assays (a-TPO, a-TG, TRAb). Commercial ELISA kits were employed in order to determine thyroid antibodies. Pituitary gland MRI was performed in all cases to exclude an organic cause of the disease. However, the study did not include patients with hypopituitarism and diabetes insipidus following neurosurgery of adenoma and craniopharyngioma. Additionally, the study also excluded patients with Sheehan and empty sella syndrome, as well as subjects following radiotherapy. Physiologically, anterior and posterior parts of the pituitary are distinct in MRI. The anterior part is isointense on both T1 and T2 weighted images. However, the posterior pituitary has an intrinsic high T1 signal (bright spot) and is hypointense on T2 weighted images. In the studied group symmetric enlargement of the pituitary gland, a thickened pituitary stalk and an intact sellar floor with absence of a posterior pituitary bright spot were

found in 27 cases with partial and complete hypopituitarism. In 5 patients presenting normal function of pituitary and diabetes insipidus only the lack of a posterior pituitary bright spot was revealed. The control sera were obtained from 10 healthy persons including 7 women and 3 men aged 21 to 45 years (average 30.6 ± 7.1). Human pituitaries were taken during autopsy and homogenized in 0.01 mol/l pH 7.4 phosphate buffer. After connective tissue removal, homogenate was centrifuged for 20 minutes at 4°C at $900 \times g$ in a refrigerated centrifuge. The precipitate was removed, and subsequently centrifugation at $27\,000 \times g$ for 30 minutes was performed. The supernatant was centrifuged at $105\,000 \times g$ for one hour and the resultant precipitate was suspended in phosphate-hydrochloric acid buffer with ultracentrifugation repeated four times. The protein content of the obtained sediment was determined by spectrophotometry. The obtained microsomal fraction of human pituitary glands was then solubilized in 1% sodium deoxycholate. For the evaluation of the autoantibodies, the electrophoretic method of separation in polyacrylamide gel (SDS-PAGE) and western blot (immunoblotting) were employed. For the purpose of the study, 12.5% separation gel and a 6% thickening gel were prepared. The microsomal fraction was denatured in a solution composed of 0.3 mol/l Tris-HCl, pH 6.8, 6% SDS, 30% glycerol, 6% 2-mercaptoethanol and 0.1% bromophenol blue in proportions allowing for obtaining protein concentration of 1 mg/ml. Preparations of pituitary microsomal fraction were subsequently applied in an amount of 40 ml per each gel pocket. A similar procedure was performed with model proteins (Pharmacia). After separation completion by SDS-PAGE, proteins were electrophoretically transferred to a nitrocellulose membrane (Bio-Rad) in the presence of buffer (25 mM Tris, 190 mM glycine, 20% methanol) at pH 8.3. Incubation with tested sera was carried out at $+4^\circ\text{C}$ in 1 : 200 dilution for 16 hours. Incubation with the second antibody (anti-human IgG labeled with horseradish peroxidase) was carried out at room temperature for 1 hour and chemiluminescence reaction was subsequently performed followed by autoradiography (ECL kit – Amersham).

Results

Among the 33 studied patients with central diabetes insipidus the presence of autoantibodies against pituitary was revealed in 23 cases. Sera of 15 patients reacted with the pituitary microsomal fraction protein of 55 kDa specific gravity. In other cases, 10 sera reacted with the pituitary antigen of specific gravity of 67 kDa. In addition, 5 sera reacted with the 60 kDa antigen, 5 sera with the 52 kDa protein, 3 sera with the 105 kDa protein, 3 sera with the 97 kDa antigen and 2 sera with pituitary antigen of 92 kDa weight. Furthermore, it should be noted that 6 sera reacted with both the microsomal fraction protein of the human pituitary gland of a specific gravity of 67 and 55 kDa,

Table 1. Results of SDS-PAGE and Western blot from diabetes insipidus patients' sera with pituitary autoantigens

No.	Patient	Sex (F/M)	Age (years)	Molecular weight of pituitary antigens (kDa)
1	M.C.	F	54	20, 55, 67, 97
2	S.U.	F	42	20, 55, 60, 67, 97
3	P.H.	F	61	55
4	Z.K.	F	49	55
5	M.G.	F	52	55
6	W.L.	F	63	20, 55, 60, 67
7	W.D.	F	57	20, 55, 60, 67, 97, 105
8	K.A.	F	47	36, 55
9	K.K.	F	49	36, 55
10	R.K.	M	58	55
11	N.Z.	F	59	55, 105
12	R.A.	M	41	67
13	N.J.	F	66	44, 67
14	S.B.	F	58	20, 36, 52, 55
15	S.M.	F	42	52, 55, 67, 92
16	M.P.	F	37	67
17	D.M.	F	52	52
18	S.T.	F	41	18, 52, 92, 105
19	P.G.	F	33	67
20	S.V.	F	56	20, 55, 67
21	K.G.	F	37	36, 55
22	L.A.	F	62	36, 52
23	O.B.	M	48	36, 60

and 4 sera reacted with the proteins of gravity 55, 60 and 67 kDa. In the group of patients with diabetes insipidus, 4 sera reacted with a single pituitary antigen of 55 kDa weight and 3 sera reacted only with a single protein of 67 kDa weight. Other sera of this patient group reacted with several pituitary microsomal proteins, up to six with different weights from 18 to 105 kDa.

Detailed results of diabetes insipidus patients' sera following immunoblotting using pituitary microsomal proteins are shown in Table 1, as well as in Figures 1 and 2. However, in the control group 1 of 10 tested sera gave a weak reaction with only one pituitary microsomal fraction protein of a specific gravity of 55 kDa.

For the determination of antibodies against thyroid peroxidase, thyroglobulin and thyroid stimulating hormone (TSH) receptor, commercial ELISA kits were used. The serum was considered positive for thyroid autoimmunity in the concentration greater than 60 IU/ml in both a-TPO and a-TG, and greater than 2 IU/ml in TRAb. In the study of a-TPO antibody prevalence, elevated concentrations of

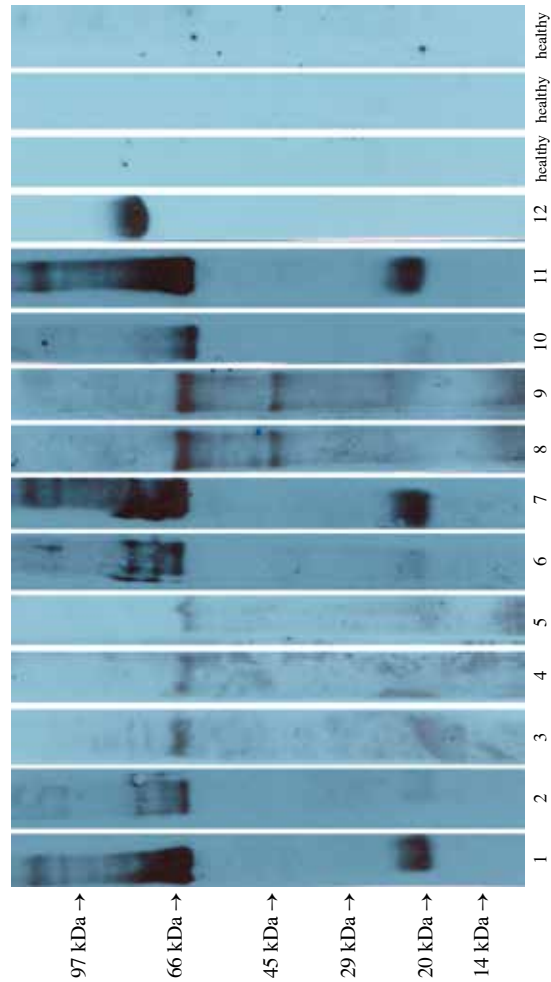


Fig. 1. Immunoblotting of pituitary microsomal proteins with sera from diabetes insipidus patients (1-12) and from control subjects (healthy)

antibodies were found in 20 of 33 tested patients. The antibody concentration ranged from 89 to 987 IU/ml with an average 406 IU/ml. In the study of a-TG antibody prevalence, a positive reaction was found in 15 of 33 patients, where the antibody concentration ranged from 69 to 342 IU/ml with an average 119 IU/ml. In the case of TRAb antibodies only 3 patients presented an elevated concentration, ranging from 2.1 to 3.2 IU/ml, with an average 2.7 IU/ml (Table 2).

Discussion

The clinical presentation of lymphocytic hypophysitis can vary depending on the part of the pituitary affected, as well as on the size of the lesion. Lymphocytic hypophysitis can be classified as adenohypophysitis (anterior pituitary), lymphocytic infundibular neurohypophysitis (posterior pituitary) or lymphocytic infundibular panhypophysitis

(affecting both the anterior and posterior pituitary). In the studied group partial or complete hypopituitarism was observed in 28 cases, whereas in 5 cases the pituitary function was normal. In our study based on the immunoblotting method, we frequently observed the presence of autoantibodies directed mainly against the microsomal fraction of human pituitary autoantigens of specific gravities (namely 55, 60 and 67 kDa) in the sera of patients with diabetes insipidus. Furthermore, 10 patients revealed no pituitary antibodies. This may be due to low antibody titers or the need to use more sensitive methods. It is worth mentioning that thyroid antibodies coexist (a-TPO positive in 20 cases, a-TG positive in 15 cases and TRAb positive in 3 cases) in many diabetes insipidus patients. These results may suggest the complexity of autoimmunity, as in patients with diabetes insipidus there is a plurality of autoantibodies against a number of microsomal pituitary antigens. Therefore in order to determine their nature, there is a need for further studies including characterization of their specificity. Furthermore, it is also possible that a more diverse autoimmune process is involved in the study, where antigens may come from different tissues. Such a wide range of pituitary autoantibodies present in autoimmune diseases may relate to the existence of five different types of cells originating from the anterior part of the pituitary gland secreting six different hormones. In fact, Falorni *et al.* describe the presence of autoantibodies in patients with lymphocytic inflammation of the pituitary gland. Moreover, they observed the presence of antibodies against alpha-enolase, gamma-enolase and anti-PGSF1 and anti-PGSF2 (pituitary gland specific factor) in 45% of patients [19]. Ziemnicka *et al.*, using the immunoblotting method, described pituitary autoantibodies against antigens of weights of 67, 60, 50 and 36 kDa in congenital hypopituitarism patients [20]. Bensing and Kasperlik-Zaluska and Czarnocka [21, 22] described the presence of pituitary antibodies in an isolated ACTH deficiency. In the study antigenic proteins isolated from cytosolic fractions of pituitaries were used, where in 18.5% of cases the presence of antibodies against 36 kDa antigens and in 21.5% of the cases the presence of 49 kDa antigen were revealed. Crock *et al.* [23, 24] studied the prevalence of pituitary antibodies involving immunoblotting in different endocrinopathies, where pituitary autoantigens isolated from cytosolic fractions of pituitaries were used. The above-mentioned studies demonstrated the presence of antibodies against the human pituitary of 40 and 49 kDa weights in 70% of patients with lymphocytic inflammation of the pituitary gland. Nishiki *et al.* [25] observed the presence of antibodies against membrane proteins of the pituitaries of 68, 49 and 43 kDa weights in patients with lymphocytic inflammation of the pituitary gland as well as in patients with autoimmune thyroid diseases. Lim *et al.* [26], using immunoenzymatic methods, demonstrated that pituitary antibodies may be present in 36% of Hashimoto's thyroiditis patients. In fact, similar observa-

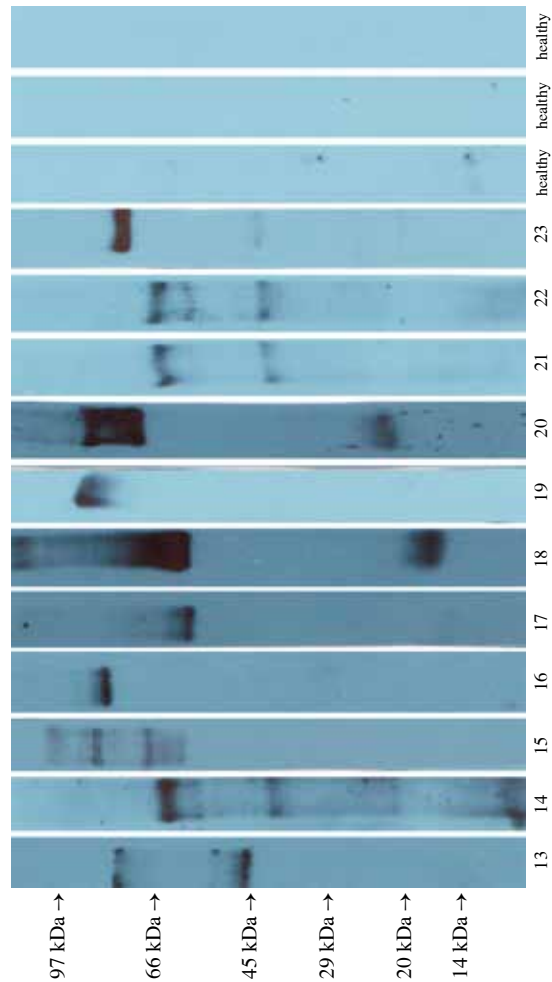


Fig. 2. Immunoblotting of pituitary microsomal proteins with sera from diabetes insipidus patients (13-23) and from control subjects (healthy)

tions were described by Manetti, where the occurrence of pituitary antibodies in 18% of patients with Hashimoto's thyroiditis and Graves' disease [27] was observed. Additionally, Garovic *et al.* described the presence of pituitary antibodies in patients with Wegener disease, while Kajiyama observed the presence of antibodies against vasopressin in systemic lupus erythematosus and dermatomyositis [28, 29]. According to previous studies, the incidence of antibodies directed against pituitary autoantigens of 65 and 67 kDa weight suggested that the pituitary autoantigen might be glutamic acid decarboxylase (GAD). Two isoforms of glutamic acid decarboxylase, with weights of 65 and 67 kDa (GAD65 and GAD67), are known so far. In fact, GAD65 is encoded by a gene located on chromosome 10, and it is found mainly in the pancreatic cells. In contrast, GAD67 is encoded by a gene located on chromosome 2 and is found predominantly in the brain nerve cells, responsible for the synthesis of gamma-aminobutyric acid (GABA),

Table 2. Assessment of pituitary hormones and thyroid antibodies in patients with diabetes insipidus

No.	Patient	Age years	Sex F/M	TSH [mIU/l]	LH [mIU/ml]	FSH [mIU/ml]	ACTH [mIU/ml]	HGH [ng/ml]	PRL [μIU/ml]	a-TPO [IU/ml]	a-TG [IU/ml]	TRAb [IU/ml]
1	A.J.	34	F	0.2	0.1	0.1	0.2	0.1	89	256	89	0.7
2	R.K.	37	F	0.1	0.0	0.2	0.3	0.1	132	786	12	0.3
3	P.G.	40	F	0.0	0.2	0.3	0.2	0.0	54	43	123	1.2
4	J.K.	44	F	2.3	4.5	7.5	0.1	1.9	234	458	8	0.5
5	R.J.	65	F	0.2	0.1	0.1	0.0	0.3	43	23	342	0.9
6	A.O.	39	M	0.3	0.2	0.1	0.5	0.0	76	345	21	1.1
7	R.O.	59	M	0.1	0.0	0.2	0.1	0.1	35	34	91	0.6
8	M.R.	59	F	1.9	34	43	24	1.5	267	95	34	1.7
9	M.G.	45	F	0.3	0.1	0.2	0.1	0.1	41	723	26	1.9
10	W.K.	57	F	0.0	0.1	0.2	0.0	0.1	25	645	77	2.1
11	M.B.	49	F	2.2	12.6	17.9	41	1.1	324	54	14	0.2
12	A.K.	55	F	0.1	0.2	0.1	0.0	0.1	76	567	7	0.6
13	M.G.	50	F	1.5	10.4	16.5	39	2.1	214	112	167	0.4
14	M.W.	52	F	0.1	0.0	0.1	0.0	0.2	23	12	6	0.6
15	B.L.	54	F	0.0	0.2	0.1	0.1	0.1	34	129	201	0.2
16	E.H.	41	M	4.1	5.4	6.7	0.1	1.2	132	9	75	0.9
17	T.L.	38	M	0.1	0.2	0.1	0.0	0.1	45	14	5	1.2
18	P.E.	47	F	0.0	0.2	0.1	0.2	0.1	51	89	41	0.5
19	J.F.	62	F	2.8	45	62	23	1.5	278	256	82	0.4
20	F.G.	42	M	0.0	0.1	0.2	0.0	0.1	65	11	34	1.1
21	J.S.	57	M	0.1	0.0	0.2	0.2	0.1	45	987	21	1.5
22	R.D.	46	F	0.0	0.1	0.1	0.2	0.0	21	34	92	0.4
23	U.W.	41	M	3.9	4.3	5.2	0.0	1.1	141	287	15	0.2
24	M.W.	42	F	0.1	0.2	0.1	0.0	0.1	36	51	8	0.3
25	W.W.	43	M	2.9	5.4	7.2	0.1	2.3	253	6	69	1.8
26	T.D.	39	F	0.1	0.0	0.2	0.0	0.1	38	523	10	1.6
27	H.N.	44	M	0.0	0.1	0.2	0.1	0.0	28	8	134	0.4
28	K.P.	45	M	2.3	5.6	6.5	34	2.1	245	455	7	2.8
29	A.P.	36	F	0.1	0.0	0.2	0.1	0.1	25	399	92	0.4
30	B.R.	46	M	0.0	0.1	0.1	0.2	0.0	37	188	8	3.2
31	Z.M.	42	M	0.1	0.2	0.1	0.0	0.1	87	10	87	0.5
32	D.S.	33	F	0.0	0.1	0.1	0.0	0.1	74	543	15	0.9
33	S.L.	39	M	3.7	5.6	7.2	0.0	1.8	126	277	73	0.4

Reference values: TSH (thyroid stimulating hormone) – 0.27-4.2 mIU/l, LH (luteinizing hormone) – 1.7-8.6 mIU/ml, FSH (follicle-stimulating hormone) – 1.5-12.4 mIU/ml, ACTH (adrenocorticotrophic hormone) – 10-65 mIU/ml, HGH (human growth hormone) – 1.9-29 ng/ml, PRL (prolactin) – 85-390 μIU/ml, a-TPO (thyroid peroxidase autoantibody) – 0-60 IU/ml, a-TG (thyroglobulin autoantibodies) – 0-60 IU/ml, TRAb (TSH receptor antibodies) – 0-2 IU/ml

i.e. one of the major inhibitory transmitters [30]. Finally, Sawicka *et al.* [31-33] observed that the incidence of pituitary antibodies in autoimmune endocrine diseases was 41% and that these antibodies react mostly with an autoantigen weighing 68 kDa. Despite the number of studies conducted so far, only a few pituitary autoantigens responsible for the

autoimmune processes have been identified. These include the growth hormone, neurospecific α-enolase and the recently described PGSF1a (pituitary gland specific factor 1a) with a molecular weight of 16 kDa, as well as PGSF2 with a molecular weight of 27 kDa. Therefore isolation and characterization of other described pituitary autoantigens will al-

low for a better understanding of the pituitary autoantibody formation process, and provide deeper insight into how they affect the pituitary gland hormonal function.

The authors declare no conflict of interest.

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