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# Identification of clinical factors related to antibody-mediated immune response to the subfornical organ

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**Abstract**

**Objective:** We recently reported cases of adipsic hypernatremia caused by autoantibodies against the subfornical organ in patients with hypothalamic-pituitary lesions. This study aimed to clarify the clinical features of newly identified patients with adipsic hypernatremia whose sera displayed immunoreactivity to the mouse subfornical organ.

**Design:** Observational cohort study of patients diagnosed with adipsic hypernatremia in Japan, United States, and Europe.

**Methods:** The study included 22 patients with adipsic hypernatremia but without overt structural changes in the hypothalamic-pituitary region and congenital disease. Antibody response to the mouse subfornical organ was determined using immunohistochemistry. The clinical characteristics were compared between the patients with positive and negative antibody responses.

**Results:** Antibody response to the mouse subfornical organ was detected in the sera of 16 patients (72.7%, female/male ratio, 1:1, 12 pediatric and 4 adult patients). The prolactin levels at the time of diagnosis were significantly higher in patients with positive subfornical organ (SFO) immunoreactivity than in those with negative SFO immunoreactivity ( $58.9 \pm 33.5$  vs.  $22.9 \pm 13.9$  ng/ml,  $p < .05$ ). Hypothalamic disorders were found in 37.5% of the patients with positive SFO immunoreactivity. Moreover, six patients were diagnosed with rapid-onset obesity with hypothalamic dysfunction, hypoventilation, and autonomic dysregulation/neural tumor syndrome after the diagnosis of adipsic hypernatremia. Plasma renin activity levels were significantly higher in patients with serum immunoreactivity to the  $\text{Na}_x$  channel.

**Conclusions:** The patients with serum immunoreactivity to the SFO had higher prolactin levels and hypothalamic disorders compared to those without the immunoreactivity. The clinical characteristics of patients with serum immunoreactivity to the subfornical organ included higher prolactin levels and hypothalamic disorders, which were frequently associated with central hypothyroidism and the presence of retroperitoneal tumors.

**KEYWORDS**

adipsic hypernatremia, dysnatremia, hypothalamus dysfunction,  $\text{Na}_x$  channel, ROHHAD syndrome, sensory circumventricular organ, subfornical organ

## 1 | INTRODUCTION

Adipsic hypernatremia is clinically characterized by an increase in both the osmotic set point for vasopressin release and the threshold for thirst perception, leading to persistent hypernatremia with a euvolemic state.<sup>1-3</sup> Since the 1970s, cases of patients with adipsic hypernatremia in the absence of structural changes in the hypothalamic-pituitary area have been reported in several studies.<sup>4-9</sup> These patients exhibit rapid-onset obesity, autonomic failure, and hypopituitarism, including growth hormone deficiency (GHD). The underlying mechanism of adipsic hypernatremia has been recently

revealed in a series of case reports. We reported a patient with a ganglioneuroma who presented with adipsic hypernatremia caused by autoantibodies to  $\text{Na}_x$ , which is a sodium sensor channel specifically expressed in the circumventricular organs including the subfornical organ (SFO).<sup>10</sup> In a second study, we reported three patients with adipsic hypernatremia whose sera contained immunoglobulin G (IgG) which bound to the mouse SFO in the absence of an anti- $\text{Na}_x$ -antibody response.<sup>11</sup> Intravenous injection of the patients' immunoglobulin fractions to mice induced hypernatremia accompanied by inflammation and apoptosis in the SFO. Accordingly, we proposed autoimmune inflammatory response in the SFO as a major

mechanism for adipic hypernatremia without demonstrable hypothalamic-pituitary lesions.<sup>10,11</sup>

Several of the clinical features of these previously reported four cases were different from those of other diseases manifesting with dysnatremia. For example, patients with adipic hypernatremia secondary to congenital abnormalities, such as septo-optic dysplasia, often present with neurodevelopmental delay, seizures, thermal dysregulation, and anterior pituitary dysfunction, which may result in the dysregulation of growth hormone (GH), thyroid-stimulating hormone (TSH), and adrenocorticotrophic hormone (ACTH).<sup>12</sup> Patients with rapid-onset obesity, hypothalamic dysfunction, hypoventilation, and autonomic dysregulation (ROHHAD) and ROHHAD with neural tumor (ROHHAD/NET) syndromes develop alveolar hypoventilation, ophthalmologic manifestations, and thermal dysregulation in addition to dysnatremia.<sup>12</sup> On the other hand, the four patients we previously reported did not exhibit hypoventilation or thermal dysregulation, suggesting that different mechanisms might underlie the pathogenesis in patients with anti-SFO autoimmune antibodies compared to those with congenital abnormalities, tumors, or inflammation within the hypothalamus and pituitary gland.<sup>10,11</sup> In this multicenter cohort study, we analyzed the sera of patients with adipic hypernatremia from across the globe to delineate the detailed clinical features of adipic hypernatremia with anti-SFO antibodies.

## 2 | METHODS AND SUBJECTS

### 2.1 | Participants and study design

This observational cohort study included patients diagnosed with adipic hypernatremia in Japan, United States, and Europe. First, a questionnaire-based survey that targeted all members of the Japanese Society for Pediatric Endocrinology (JSPE) was conducted to identify patients with adipic hypernatremia who developed antibodies to the mouse SFO of these patients. Responses to the survey were received from 122 of a total of 1340 members (response rate: 9.1%) and 10 patients were enrolled based on the questionnaire-based survey. After the first survey of the JSPE members, eight additional Japanese patients were enrolled based on personal communication with treating physicians. Moreover, five patients from Europe and one patient from the United States were included in the current study. One of the first 10 enrolled patients was removed from further analysis because of the presence of congenital disease (Kabuki syndrome) and previously reported case with anti-Nax antibody. Finally, a total of 22 patients from 14 institutions from June 2017 to January 2021 were included in the analysis of specific antibody responses to the mouse SFO (Figure 1).

Written informed consent was obtained from all patients or their legal guardians after enrollment was approved by the Ethics Committee of JSPE, participating institutions, and Hiroshima University. The study patients were categorized into SFO-immunopositive and immunonegative groups based on the antibody response of their serum samples to the mouse SFO.

### 2.2 | Immunohistochemistry of the mouse SFO incubated with patient sera

All experiments with animals were performed according to the guidelines of Hiroshima University (number A17-157). The antibody response to the mouse SFO was evaluated by immunohistochemistry as previously described.<sup>10,11</sup> Briefly, 8–16 week-old wild-type male mice (C57BL/6J; CLEA Japan, Tokyo, Japan) under deep anesthesia were transcardially perfused with phosphate-buffered saline, followed by perfusion with 20% neutralized formalin. Brains were coronally dissected, fixed overnight at 4°C, and cryoprotected by immersion in a graded sucrose series. Next, the brains were embedded in OCT compound (Sakura, Tokyo, Japan) and sectioned at 30–40 μm using a cryostat (CM3050S; Leica Microsystems). The sections were incubated with 25 mM glycine in phosphate-buffered saline for 30 min and pretreated with a blocking buffer, containing 5% FBS, 0.1% TX100, overnight at 4°C. Next, the sections were incubated with specific patient or control serum samples (1:250 dilution in blocking buffer) for 2 days at 4°C, as described previously.<sup>10,11</sup> Antibody binding to the brain sections were determined by overnight incubation with cross-adsorbed goat anti-human IgG (H + L) secondary antibody conjugated to Alexa Fluor 488 (1:500 dilution; Thermo Fisher Scientific). The sections containing the SFO were analyzed by confocal fluorescence microscopy (FV-1000D; Olympus; Tokyo, Japan). All analyses were performed with negative and positive controls and repeated three times with each patient serum sample. A previously reported patient sample<sup>11</sup> was used as positive control. Quantification of immunostaining was performed

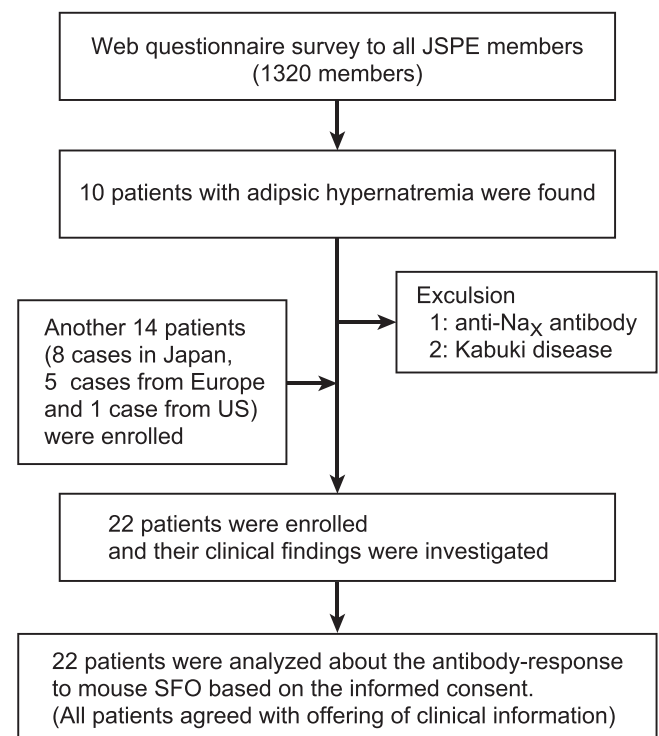


FIGURE 1 Flow diagram of the study

using the ImageJ software (National Institutes of Health, Bethesda, MD, USA). The sections with intensity levels of >10.0 arbitrary units were defined to exhibit anti-SFO antibody positivity. The average intensity levels for positive and negative controls were 18.6 and 6.8, respectively.

## 2.3 | Detection of anti-Na<sub>x</sub> antibodies

The presence of anti-Na<sub>x</sub> antibodies was determined using a custom-made enzyme-linked immunosorbent assay (ELISA) as previously reported.<sup>13</sup> Briefly, microtiter plates were treated with a solution including 50 mM glutathione, 1 mM ethylenediaminetetraacetic acid, 10 mM NaH<sub>2</sub>PO<sub>4</sub>, and 150 mM NaCl. The plates were incubated with the N-terminal GST-tagged recombinant human SCN7A protein (HQ258196.1; 88.2 ng/well), which was expressed using a wheat germ cell-free system, for 1 h at 25°C. Human IgG, which was expressed using a wheat germ cell-free system, was used as the positive control, and mock (H<sub>2</sub>O instead of messenger RNA) was used as the negative control. The prepared ELISA plates were stored at -80°C until use and thawed at 25°C before the assay. The plates were incubated with serum samples for 1 h at 25°C. Next, the wells were incubated with goat anti-human IgG (1:10,000 dilution; cross-adsorbed secondary horseradish peroxidase-conjugated antibody [H + L]; A18811, Thermo Fisher Scientific) for 1 h at 25°C, followed

by incubation with 100 μl tetramethylbenzidine-H<sub>2</sub>O<sub>2</sub> (Pierce TMB substrate kit). After 30 min of incubation at 25°C, the reaction was stopped by the addition of 100 μl of 2 M H<sub>2</sub>SO<sub>4</sub> and intensity of the yellow dye was measured at 450 nm. The threshold value (average of 2 mock wells + 1.5× the difference of 2 mock wells) was calculated for each serum sample.

## 2.4 | Clinical data

Clinical data was collected from the medical records at the time of diagnosis and at the time of analysis based on the retrospective survey of the participants. We analyzed patient characteristics in the SFO-immunopositive and immunonegative groups. The clinical data included the following: white blood cell count, red blood cell count, hemoglobin, platelet count, blood Na, blood K, blood Cl, blood urea nitrogen, plasma osmolality, arginine vasopressin (AVP) or copeptin, free triiodothyronine, free thyroxine, thyroid-stimulating hormone, prolactin, cortisol, adrenocorticotrophic hormone, plasma renin activity, plasma aldosterone, insulin-like growth factor-1, luteinizing hormone, and follicle stimulating hormone (Table 1, Tables S1A and S1B). Additionally, data on the presence of GHD, other pituitary dysfunction, and tumors were collected. Other related clinical evaluations are described in Tables S1A and S1B.

**TABLE 1** Summary of average clinical data in all and pediatric cases

	All cases (22)		p value	Pediatric case (16)		p value
	Positive (16)	Negative (6)		Positive (12)	Negative (4)	
Age at analysis	18.3 ± 22	24 ± 13	.31	7.4 ± 4.9	12.5 ± 0.7	.06
White blood cells (/μl)	7827 ± 2452	5882 ± 889	.11	8502 ± 2226	6065 ± 911	.06
Hemoglobin (g/dl)	12.2 ± 2.09	13.8 ± 1.11	.05	12.0 ± 2.06	14.1 ± 1.11	.08
Platelets (×10 <sup>4</sup> /μl)	21.6 ± 8.69	22.7 ± 7.82	.82	21.0 ± 9.24	25.5 ± 5.33	.38
Sodium (mM)	156.4 ± 14.5	158.8 ± 18.1	.95	158.8 ± 16.1	153.5 ± 14.3	.57
Plasma osmolality (mOsm/L)	345.8 ± 45.5	334.4 ± 56.7	.95	357.2 ± 50.8	314.0 ± 39.0	.38
Plasma renin activity (ng/ml/h)	29.4 ± 35.7	5.1 ± 4.29	.08	33.0 ± 36.4	5.87 ± 4.5	.17
Adrenocorticotrophic hormone (ACTH) (pg/ml)	21.5 ± 10.8	21.5 ± 10.8	.05	20.3 ± 9.78	51.2 ± 50.3	.08
Cortisol (μg/dl)	10.6 ± 7.2	10.9 ± 5.1	.93	9.2 ± 7.2	10.9 ± 5.8	.68
Prolactin (PRL) (ng/ml)	58.9 ± 33.5	22.9 ± 13.9	<.01	67.8 ± 30.2	19.8 ± 13.9	.01
TSH (μIU/ml)	1.76 ± 1.38	1.73 ± 1.23	.96	1.90 ± 1.44	1.93 ± 1.33	.97
Free thyroxine (fT4) (ng/dl)	1.10 ± 0.40	1.03 ± 0.28	.72	1.10 ± 0.41	1.13 ± 0.20	.88
Insulin-like growth factor I (IGF-I) (ng/ml)	98.5 ± 103	116.2 ± 142.4	.77	92.9 ± 112.0	141.3 ± 151.2	.52
Vasopressin (pg/ml)	3.07 ± 3.68	1.92 ± 1.89	.51	3.35 ± 3.83	1.10 ± 0.55	.31
Growth hormone deficiency (%)	43.8	66.7	.14	50	75	.43
Central hypothyroidism (%)	50	33.3	.65	43.8	25	.57
Hypothalamic disorders (%)	37.5	0	<.01	58.3	0	<.01
Retropituitary tumor (%)	25	0	<.01	25	0	<.01

## 2.5 | Statistical analysis

All data were described as means  $\pm$  standard deviation. The SPSS statistical software (version 19.0; SPSS) was used to compare the clinical characteristics of the patients in the immunopositive and immunonegative groups. Statistical significance of the data was determined using unpaired two-group *t* test. A *p* value of  $< .05$  was considered to indicate statistical significance for all analyses.

## 3 | RESULTS

### 3.1 | Overall patient characteristics

Table 1 and Tables S1A and S1B summarize the clinical characteristics of the study patients and their antibody response to the mouse SFO. The study cohort includes 16 pediatric and 6 adult patients. In the pediatric patients, the average ages were  $6.8 \pm 4.1$  and  $8.3 \pm 4.8$  at first diagnosis and at the time of blood sampling for the analyses using sera, respectively. The average age of the adult patients was  $45.5 \pm 17.8$  at the time of blood sampling for the analyses using sera. Most of the patients had hypernatremia ( $157.1 \pm 15.2$  mM). In addition, hyperosmolality ( $335.7 \pm 47.6$  mOsm/kgH<sub>2</sub>O), higher serum prolactin levels ( $47.7 \pm 33.1$  ng/ml), and relatively low AVP levels ( $2.56 \pm 3.04$  pmol/L) were commonly observed in these patients. Of note, in all patients urine osmolality was above 300 mOsm/kg, which was higher than that observed in central diabetes insipidus and was comparable to that observed in complete diabetes insipidus (average urine osmolality:  $571 \pm 189$  mOsm/L). Six patients were clinically diagnosed with suspicious ROHHAD/NET syndrome (patients 1, 2, 3, 4, 14, and 16). Sixteen patients, including 12 pediatric and 4 adult patients (72.7%, patients 1–16, Table S1A) exhibited positive antibody response to the mouse SFO as determined by immunofluorescence staining.

### 3.2 | Comparison of the clinical findings between the immunopositive and immunonegative groups

In the 16 patients with anti-mouse SFO antibodies, the average age at the time of analysis was 18.3 years ( $7.4 \pm 4.9$  and  $50.7 \pm 20.3$  years in pediatric and adult patients, respectively). Moreover, the sera obtained from 9 patients (56.3%) in the group of 16 patients in the immunopositive group exhibited immunoreactivity to Na<sub>x</sub> antibody based on ELISA (Figure 2 and Table S1A). Of note, none of the patients had a history of toluene exposure or were treated for their symptoms. The following complications were more frequently observed in the immunopositive group compared with the immunonegative group: hypothalamic dysfunction including thermal dysfunction and hyperactivity (37.5% vs. 0%), and retroperitoneal tumors (25% vs. 0%). A summary of the clinical characteristics at the time of analysis is presented in Table 1 and Tables S1A and S1B. Of note, the patients in the immunopositive group exhibited significantly higher

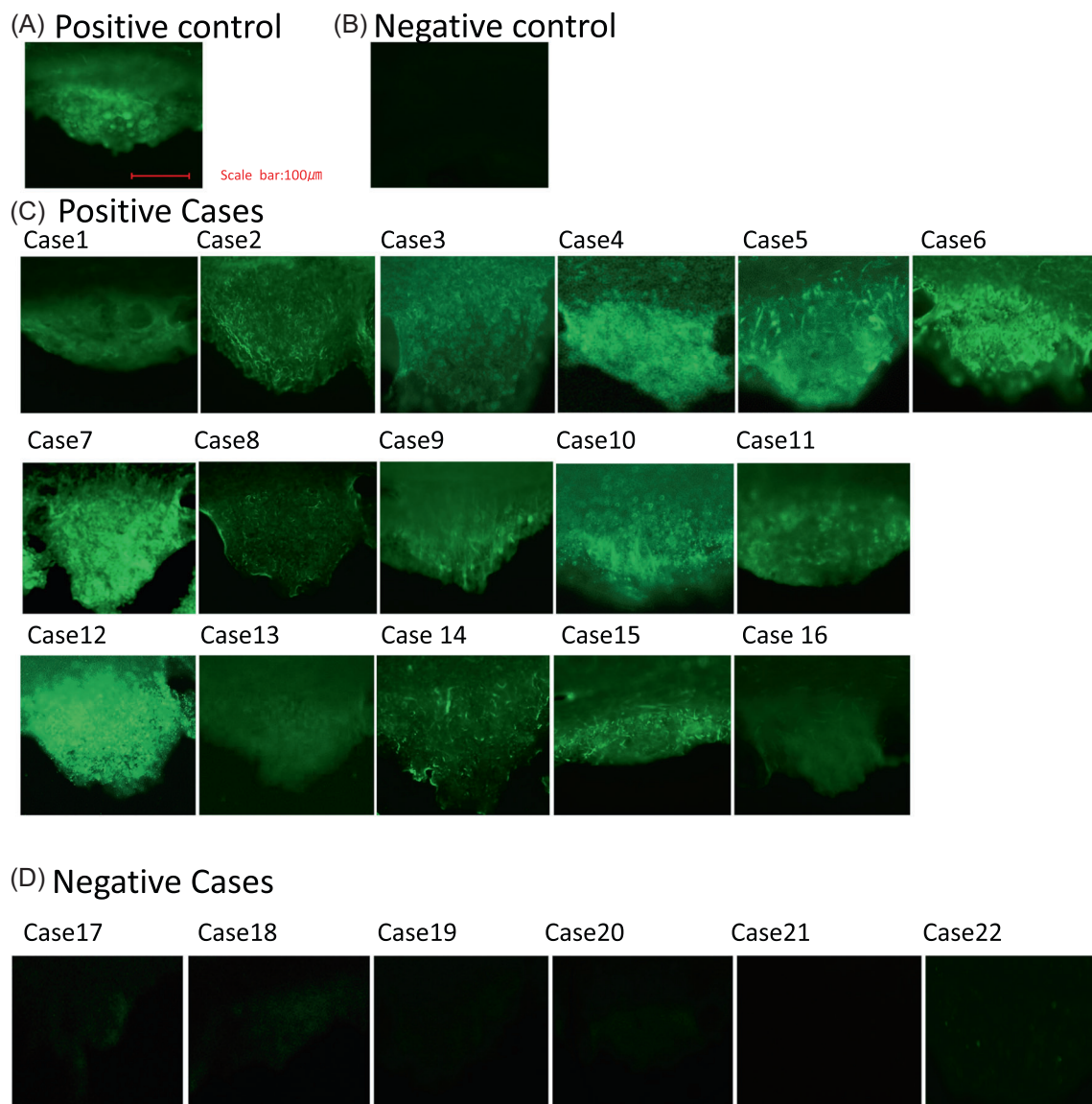
prolactin levels compared to those in the immunonegative group at the time of diagnosis (all patients,  $58.9 \pm 33.5$  vs.  $22.9 \pm 13.9$  ng/ml; pediatric patients,  $67.8 \pm 30.2$  vs.  $19.8 \pm 13.9$  ng/ml,  $p < .05$ ; Table 1 and Figure 3). A correlation between Na<sub>x</sub>-antibody-positive patients and plasma renin activity level was observed (relative ratio, 0.79,  $p < .05$ , Figure 4). The AVP levels were relatively low compared to the plasma osmolality irrespective of the immunoreactivity status, suggesting that the patients in both the immunopositive and immunonegative groups exhibited some of the features of partial diabetes insipidus (Table 1 and Figure 5).

Hypothalamic disorders, such as thermal dysfunction, obesity, and sleep apnea syndrome, were also observed in some of the patients in the immunopositive group (6/16 patients). Among these, the serum of one patient, who experienced relapse 3 years after the disease onset, exhibited central apnea in addition to sleep apnea syndrome because of obesity (patient 1). Thermal dysfunction and hyperactivity symptoms were often observed in the immunopositive group. In contrast, hypothalamic disorders were not observed in any of the patients in the immunonegative group. Ophthalmologic disorders, including ptosis and strabismus, were observed in two patients in the immunopositive group (Table S1).

Some of the patients in the immunopositive and immunonegative groups developed tumors. Specifically, retroperitoneal tumors, including ganglioneuroma, were found in three patients in the immunopositive group. In contrast, no patients in the immunonegative group had tumors originated from neural crest cells. Magnetic resonance imaging, which was performed in all patients, revealed no structural anomalies in hypothalamus or the pituitary gland (data not shown). In addition, two patients in the immunopositive group had other autoimmune disorders, including juvenile idiopathic arthritis and multiple sclerosis. One patient in the immunopositive group (patient 3) died despite intensive treatment with steroids. The patient was admitted for treatment of heart and pulmonary failure and severe anemia and succumbed to sudden cardiac death.

## 4 | DISCUSSION

In this first global cohort study of patients with adipsic hypernatremia in the absence of structural disorders in the hypothalamus–pituitary axis, we focused on the immunoreactivity of patient sera to the mouse SFO and Na<sub>x</sub>. Immunofluorescence analyses using sera from these patients applied to the mouse brain sections led to the identification of 16 newly diagnosed patients with a positive antibody response to the mouse SFO. Some of these SFO-immunoreactive patients exhibited several clinical abnormalities, including higher prolactin levels, hypothalamic disorders. We also found that plasma renin activity levels were higher in Na<sub>x</sub>-positive patients than in Na<sub>x</sub>-negative patients among the SFO-immunoreactive patients, suggesting that dysfunction of the Na<sub>x</sub> channel, which is a sodium sensor, might lead to a compensatory increase in plasma renin activity. This is also the first report of adult patients whose sera exhibited immunoreactivity to the mouse SFO, indicating that adipsic

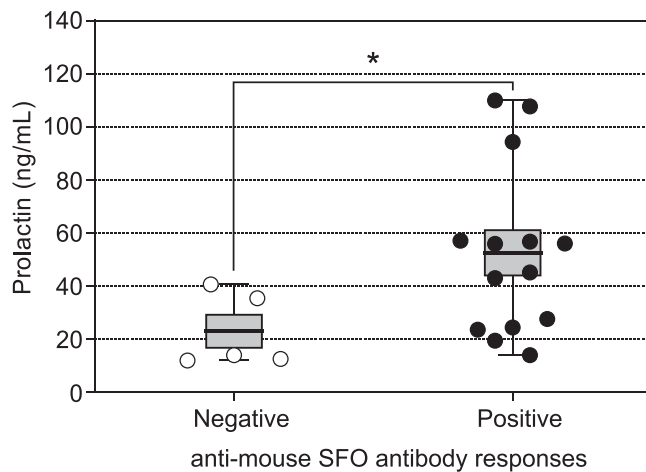


**FIGURE 2** Representative Immunostaining figures showing anti-mouse SFO antibody responses. (A) positive control, (B) negative control, (C) all patients with positive response. SFO, subfornical organ, (D) all patients with negative response

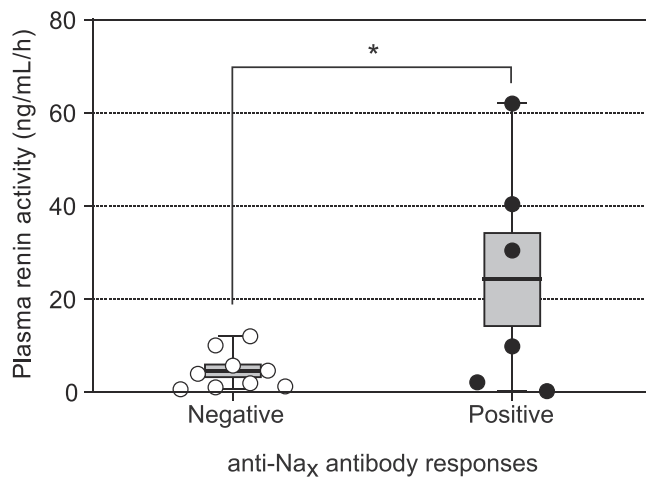
hypernatremia may not always manifest during childhood or may remain undiagnosed until adulthood. Importantly, the etiology of adipsic hypernatremia in adult patients remains unclear and we propose that the evaluation of anti-mouse SFO antibody response should be considered in these patients.

In our previous reports of four pediatric patients,<sup>10,11</sup> we described the clinical characteristics of adipsic hypernatremia with anti-mouse SFO antibody response as follows: (1) hypernatremia without thirst sensation, (2) impaired vasopressin release, (3) lack of structural lesions in the hypothalamus–pituitary axis, (4) childhood onset, (5) obesity, (6) increased serum prolactin level, (7) impaired growth hormone release, (8) increased plasma renin activity, and (9) intact urine-concentrating capacity.<sup>14</sup> Overall, the results of the current study are consistent with our previous findings. Interestingly,

among the patients evaluated in the current study, clinically diagnosed hypothalamic disorders was present only in the anti-SFO immunopositive group, suggesting a relationship between hypothalamic disorders and the anti-SFO antibodies. It is also noteworthy that no structural anomalies were detected in hypothalamus based on magnetic resonance imaging. The SFO has extensive efferent neural projections to hypothalamus including supraoptic nuclei, where vasopressin is produced.<sup>15,16</sup> Complementary reactions triggered by autoantibodies might cause apoptosis and inflammation in the SFO, leading to overall SFO dysfunction, including altered neuronal projections and dysregulation of several hypothalamic nuclei such as supraoptic nuclei. These events might underlie the pathogenesis of hypothalamic disorders observed in these patients. A tendency for GHD was also observed in the anti-SFO immunopositive group,



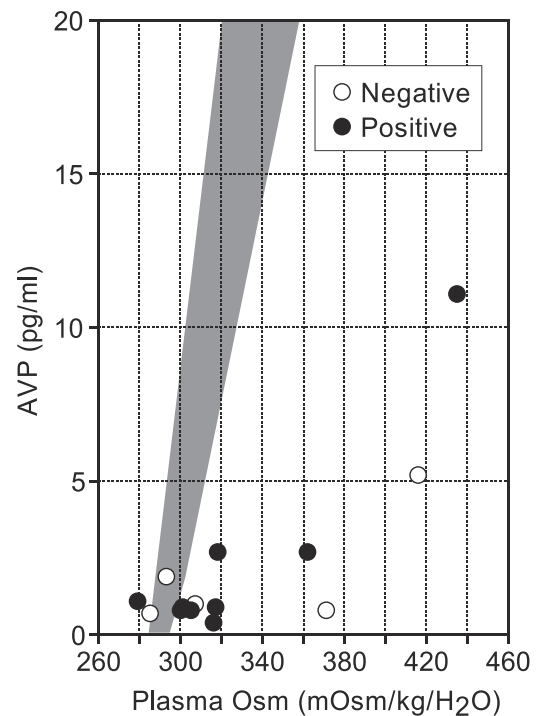
**FIGURE 3** Clinical findings with significant differences between pediatric patients with positive and negative anti-SFO antibody response. \* $p < .05$ . SFO, subfornical organ



**FIGURE 4** Comparison of plasma renin activity between the patients with and without anti- $\text{Na}_x$  antibodies among the patients with positive anti-SFO antibody response. \* $p < .05$ . SFO, subfornical organ

although GHD might not be specific to adipsic hypernatremia, considering the pathophysiology of adipsic hypernatremia.

Organum vasculosum lamina terminalis and lamina terminalis are also suggested to play important roles in the pathogenesis of adipsia and AVP regulation. Several studies using experimental animals indicated that lesions in the anterior hypothalamus, including not only the SFO but also the organum vasculosum lamina terminalis and lamina terminalis (the so-called AV3V lesions), were necessary to impair osmotically stimulated thirst and AVP secretion.<sup>17,18</sup> However, we did not examine immunoreactivity in these regions of the mouse brain specimens because the primary aim of our immunohistochemical analyses was to diagnose and distinguish the patients. Future studies are warranted to examine the immunoreactivity of patient sera in these regions, which should reveal the mechanisms underlying AVP deficits.



**FIGURE 5** Relationship between patient serum osmolality and plasma arginine vasopressin levels. Gray area indicates the normal area

The pathogenesis of adipsic hypernatremia has been explained by the upward resetting of both the osmotic set point for vasopressin release and the threshold for thirst sensation. In our latest case report, we followed the relationships between plasma vasopressin and serum sodium concentrations in a 5-year-old patient with positive anti-SFO antibody response.<sup>19</sup> We observed that the plasma vasopressin levels were maintained at steady levels and did not increase in response to hyperosmolality. We found that vasopressin secretion was normal at times when serum sodium level was within the normal range, an observation that was compatible with the lack of polyuria in our patient. This finding suggested that water and sodium imbalance in adipsic hypernatremia might be explained by the dysregulation of vasopressin release due to the lack of response to hyperosmolality rather than the upward resetting of the osmotic set point. Further analyses are required to uncover the precise mechanism.

We detected positive immunoreactivity to the mouse SFO in some patients who were previously diagnosed with ROHHAD or ROHHAD/NET syndrome. ROHHAD syndrome is characterized by early and rapid-onset of obesity accompanied with hypoventilation, autonomic dysregulation, and endocrine abnormalities,<sup>20</sup> whereas ROHHAD/NET syndrome also includes the presence of tumors of neural crest origin.<sup>20</sup> In some SFO-immunopositive patients also harbors tumors, suggesting some common paraneoplastic mechanisms with the ROHHAD/NET syndrome. A recent report suggested that some patients with ROHHAD/NET syndrome harbored rare variants of several genes including *PIK3R3*, *SPTBN5*, *PCF11*, *SRMS*,

ZNF83, and KMT2B.<sup>20</sup> However, no report to date has described common gene variants or specific candidate genes underlying the pathology in these patients. An autoimmune mechanism has been considered based on autopsy findings of hypothalamic inflammation and the presence of oligoclonal bands in the cerebrospinal fluid.<sup>21,22</sup> Patients with ROHHAD/NET syndrome frequently develop dysnatremia and hyperprolactinemia,<sup>23,24</sup> which were also detected in the current study patients with anti-mouse SFO antibody response, which suggests a clinical overlap between patients with positive anti-SFO antibody response and those with ROHHAD/NET syndrome. Further studies including larger cohorts of patients with ROHHAD/NET are required to clarify the pathophysiology that might be shared between ROHHAD/NET syndrome and adipsic hypernatremia with immunoreactivity to the SFO.

Hyperprolactinemia is one of the most common endocrinological disorders of the hypothalamic-pituitary axis.<sup>25</sup> The estimated incidence of hyperprolactinemia (serum prolactin level >25 ng/ml) is low (0.4%) in the healthy population.<sup>26</sup> Prolactin-secreting pituitary adenomas are the most common cause of serum prolactin levels >100 ng/ml, and serum prolactin levels >200 ng/ml are usually caused by macroadenomas.<sup>27–29</sup> In the current study, no patient with a positive antibody response had a serum prolactin level >120 ng/ml. Prolactin release is controlled by prolactin-inhibiting factors, such as dopamine, which is produced in the arcuate nucleus.<sup>30</sup> As the arcuate nucleus also receives neural inputs from the SFO, autoimmune damage in the SFO might cause dysfunction in the arcuate nucleus and dysregulation of prolactin release. Taken together, in patients with adipsic hypernatremia and prolactin levels <120 ng/ml, potential autoimmunity to the SFO should be taken into consideration. In pediatric patients, central hypothyroidism may develop from hyperprolactinemia based on the tendency for central hypothyroidism observed in the immunopositive group in the current study. However, the patients with hyperprolactinemia in the immunopositive group were not symptomatic. Therefore, it is likely that the observed difference in prolactin levels between the immunopositive and immunonegative groups in the current study did not reflect a difference in the incidence of hyperprolactinemia.

A number of limitations of this study should be acknowledged. First, adipsic hypernatremia is a rare disorder and the number of patients was modest, which limited the statistical power of our study. We plan to enroll more patients from Japan and other countries and to include patients diagnosed with ROHHAD/NET syndrome. Second, the method for immunofluorescence staining was limited to semiquantitative analysis and we only analyzed the antibody response to the mouse SFO. Future studies are needed to further elucidate the mechanisms underlying the adipsic hypernatremia without demonstrable hypothalamic structural lesions.

Among positive-response patients, one patient had a poor prognosis and died. As long as the SFO antibody continues to be produced by a patient, the disorder will progress, which may result in various complications and life-threatening conditions. Establishing an effective management and a strategy to reduce the damage caused

by anti-SFO antibodies is required to improve patient prognosis and develop a diagnostic approach. In the future, the identification of patients by early diagnosis and development of precise management may result in a better prognosis. Moreover, detection of specific antigen in patients with anti-SFO antibody response will clarify further details about this mechanism.

In conclusion, in this study we reported a global cohort of patients with adipsic hypernatremia who lacked structural lesions in the hypothalamus and pituitary gland. In this unique cohort, we identified 16 patients who exhibited a positive anti-mouse SFO antibody response; our analyses revealed that the clinical characteristics of these patients included higher prolactin levels and hypothalamic disorders, which were frequently associated with the presence of retroperitoneal tumors. Some of the patients in the SFO immunopositive group were clinically diagnosed with ROHHAD/NET syndrome. Our results also suggest that some patients with autoimmune-related adipsic hypernatremia may have features of ROHHAD/NET, a paraneoplastic neurological syndrome associated with peripheral nervous system tumors. These findings may provide insights for considering the pathogenesis of adipsic hypernatremia.

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#### CONFLICTS OF INTEREST

The authors declare no conflicts of interests.

#### DATA AVAILABILITY STATEMENT

Data are available from the corresponding author upon request.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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