

## Investigation of TSH receptor blocking antibodies in childhood-onset atrophic autoimmune thyroiditis

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**Abstract.** Atrophic autoimmune thyroiditis (AAT) is a type of autoimmune hypothyroidism without goiter. TSH receptor-blocking antibodies (TSBAb) are involved in its etiology in adults. Reportedly, this disease is extremely rare in children. In this study, we aimed to investigate the prevalence of TSBAb during AAT onset in children using a commercially available cell-based bioassay TSAb kit. We conducted a multicenter retrospective observational study. We collected data of patients with AAT who were < 15 yr old, enrolled in a collaborative research group, and diagnosed since July 2003. AAT was defined as acquired autoimmune hypothyroidism without thyroid enlargement. Eighteen patients (including 15 females) whose TSH receptor antibody (TRAb) or TSBAb levels were measured within a year from the initial visit were included. The median age at diagnosis was 9.3 years, and the estimated time between onset and diagnosis was 2.6 yr. The positive rate for either TSBAb or TRAb was 38.8% (95% confidence interval: 18.3–59.5%). There were no significant differences in age, the estimated time between onset and diagnosis, and FT4 levels at diagnosis between the TSBAb-positive and -negative groups. Unlike previous reports, we showed that the prevalence of TSBAb-positivity in childhood-onset AATs is not rare, as in adults.

**Key words:** atrophic autoimmune thyroiditis, autoimmune hypothyroidism, TSH receptor-blocking antibody, TSH receptor antibody, children

### Introduction

Atrophic autoimmune thyroiditis (AAT) causes hypothyroidism without thyroid enlargement via an autoimmune mechanism. The incidence of AAT in children is rare. It is more common at a young age

before puberty and is characterized by severe primary hypothyroidism (1). In adults, approximately 40–50% of the patients with AAT test positive for TSH receptor-blocking antibodies (TSBAb), suggesting the involvement of these antibodies in the pathogenesis of this disease (2, 3). Conversely, TSBAb-positivity has been reported

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to be extremely rare in children. Therefore, it has been speculated that an alternate mechanism might be responsible for affecting the thyroid gland in children than in adults (1). Subsequently, several cases have been reported wherein TSBAb were detected in patients with AAT and severe hypothyroidism before puberty (4–6).

Most patients with the onset of AAT during childhood were diagnosed due to reduced growth rate and short stature; however, in many cases, the diagnosis took a long time (1, 4–7). Since 2016, anthropometric assessment of children using a growth curve has been conducted during physical examinations in schools in Japan. Therefore, it became possible to detect the reduced growth rate before resulting in short stature, which led to early diagnosis of the disease.

In the past, bioassays to assess TSBAb were laborious and time-consuming, and each laboratory established in-house assays that were proprietary. In Japan, TSBAb has been measured since 2003 using a commercially available cell-based bioassay TSA kit (Yamasa, Corp., Choshi, Chiba, Japan). It measures cAMP production in thyroid cells using the RIA method. In July 2014, a new bioassay, enzyme immunoassay (EIA), was introduced with improved sensitivity and specificity.

A previously reported study on the involvement of TSBAb in childhood-onset AATs was also based on a laboratory-based bioassay measurement (1). In this study, we aimed to investigate the prevalence of TSBAb-positive cases during the onset of AAT in children using a commercially available cell-based bioassay TSA kit.

## Subjects and Methods

We conducted a multicenter retrospective observational study. Data were collected from 21 different hospitals in Japan from September 2018 to April 2019. These hospitals belonged to “the next generation research meeting of east Japan pediatric endocrinology” group which was formed in 2016 by volunteers to promote the clinical research involving pediatric endocrinologists working mainly at university hospitals in eastern Japan. The present study was approved by the Ethics Committee of Niigata University (Approval no. 2018–0161). We have published the information related to the content of research on each hospital’s homepage. The patients and/or their parents were informed of their right to refuse the access to their medical records from being used in the study.

Patients < 15 yr old who were diagnosed with AAT after July 2003 from 13 hospitals were included. The diagnosis of AAT was based on the following criteria: 1) patient presented any one or more of the symptoms, including reduced growth rate, lethargy, fatigability, periorbital edema, cold intolerance, weight gain, slow movements, impaired memory, constipation, or hoarseness; 2) low FT4 or T4 levels, and high TSH levels; 3) positive for antibodies against thyroid peroxidase (TPOAb), thyroglobulin (TgAb), or TSH receptor (TRAb); 4) thyroid ultrasonography performed

at each institution revealed no evidence of thyroid enlargement. The exclusion criteria comprised a history of head and neck, spinal cord, or total body irradiation. Patients undergoing any pharmacological treatment for comorbidities other than AAT and congenital anomaly syndromes, such as Down’s syndrome, were also excluded from the auxological evaluation. Clinical data were collected retrospectively, including sex, major complaints, age, height, weight, past medical history, family history of thyroid disease, and the estimated time between onset and diagnosis, based on the point of reduction in growth rate on the growth curve as an onset point. Data on TSH, FT3, and FT4 levels at the time of diagnosis, thyroid size on ultrasonography, and thyroid-associated autoantibodies (TgAb, TPOAb, TRAb, TSH receptor-stimulating antibody (TSA), and TSBAb) measured within the first year after diagnosis were also collected. The SD value of the sum of the transverse diameters of both lobes was calculated using the height-specific reference values (8). Thyroid function tests (TSH, FT4, and FT3) were evaluated using the electrochemiluminescent immunoassay (Roche, *n* = 12), fluorometric enzyme immunoassay (TOSOH, *n* = 3), and chemiluminescent immunoassay (Architect, *n* = 3) methods routinely in each hospital laboratory. There was no standardization between the kits, and we used the data available without any modification.

Since July 2003, the TSA kit “Yamasa Bioassay RIA” method has been used to measure TSBAb levels as described previously (9, 10), with a reference value < 45.6%. The TSA kit “Yamasa Bioassay EIA” has been used since July 2014, with a reference value < 31.7%, which was considered to be more than positive. It has been reported that TSA levels can be false-positive when markedly high TSH levels are present during initial diagnosis, reflecting the thyroid-stimulating effect of TSH, and TSBAb levels are elevated in patients with strongly positive TSA results (11). Therefore, we adopted TSA and TSBAb levels with a TSH decline below 100  $\mu$ IU/ml after treatment.

The primary endpoint was to determine the prevalence of either TSBAb- or TRAb-positive results within a year after the diagnosis among patients with AAT who were < 15 years old. The secondary endpoint was to identify the differences in clinical characteristics (e.g., age at the time of onset, the estimated time between onset and diagnosis, sex, severity of hypothyroidism, and comorbidities) between the TSBAb- or TRAb-positive and -negative groups.

Statistical analyses were performed using GraphPad Prism ver8. Background data of patients with AAT are presented as median (quartile range). The frequency of TSBAb- or TRAb-positive cases among those who met the eligibility criteria was calculated with a 95% confidence interval (CI). Differences in age at the time of diagnosis, the estimated time between onset and diagnosis, sex, severity of hypothyroidism, and comorbidities between the TSBAb-positive and -negative groups were assessed using the Mann–Whitney U test

or Wilcoxon signed-rank test. The statistical significance was set at 5%.

## Results

A total of 21 patients with AAT from 13 institutions were selected, of which 3 patients were excluded as they lacked both TSBAb and TRAb measurements. Therefore, 18 patients were enrolled to participate in this study. Clinical data of 18 patients with AAT is shown in **Table 1**. Among 18 patients, 15 (83.3%) were female, with a median (interquartile range) age at diagnosis of 9.3 (range, 6.9–13.6) yr, a median estimated duration of disease of 2.6 (range, 1.8–4.0) yr, and a median estimated age at the time of onset of 6.5 (range 4.9–9.5) yr. Only two patients had autoimmune diseases other than AAT. A family history of autoimmune thyroid diseases was found in one-third of the patients. Two-thirds of the patients exhibited reduced growth rate or short stature, and the others were under investigation for either hypercholesterolemia or obesity. Thyroid function tests at the time of diagnosis showed marked hypothyroidism, as reported previously (1, 7).

### Prevalence of TRAb or TSBAb positivity (Table 2)

The antibody titer of patients with a positive result

for TRAb or TSBAb are shown in **Table 2**. The positive rate for TRAb or TSBAb was 38.8% (CI 95%: 18.3–59.5%). One patient was assessed as TSBAb-positive because she exhibited a strong positive titer for TRAb, although her TSBAb was not measured. TSBAb level in all patients was higher than 80%.

### Differences in clinical characteristics between TSBAb-positive and -negative groups (Table 3, Fig. 1)

There were no significant differences in age, height SD score, indices of overweight, FT4 levels at the time of diagnosis, and the estimated time between onset and diagnosis between the TSBAb-positive and -negative groups. There was no association between the estimated age at the time of onset or between onset and diagnosis and the prevalence of TSBAb positivity (**Fig. 1**).

## Discussion

In the present study, a retrospective analysis of 18 patients with childhood-onset AAT revealed that 38.8% of them were either TSBAb- or TRAb-positive. Although the prevalence of the onset of AAT during childhood is considered to be extremely rare (1), this result indicates that TSBAb positivity is not unusual in children, as in adults.

**Table 1.** Background of 18 patients with autoimmune atrophic thyroiditis (AAT)

Sex	Male 3, Female 15
Height SD score at diagnosis*†	-2.5 (-2.9– -1.2)
% overweight at diagnosis (%)*†	14.3 (5.2–32.0)
Estimated time between onset and diagnosis (yr)*	2.6 (1.8–4.0)
Comorbidity	Sjogren’s syndrome 1, developmental delay and baldness 1, autoimmune hepatitis 1, Down’s syndrome 1
Family history of thyroid diseases	Hashimoto’s disease or Grave’s disease 6
Chief complaints (with overlap)	Reduced growth rate or short stature 12, hypercholesterolemia 3, obesity 3, loss of appetite 2, etc.
TSH level at diagnosis (μIU/ml)*	471.5 (292–1,107)
FT4 level at diagnosis (ng/dl)*	0.145 (0.07–0.21)
FT3 level at diagnosis (pg/ml)*	0.81 (0.44–1.23)
Thyroid width (SD)*, ††	-0.75 (-1.2– -0.33)

\*, median (quartile range). † Case of Down’s syndrome was excluded; ††, data available for 13 patients.

**Table 2.** Results of antibody titers in TSH receptor blocking antibody (TSBAb)- or TRAb-positive patients

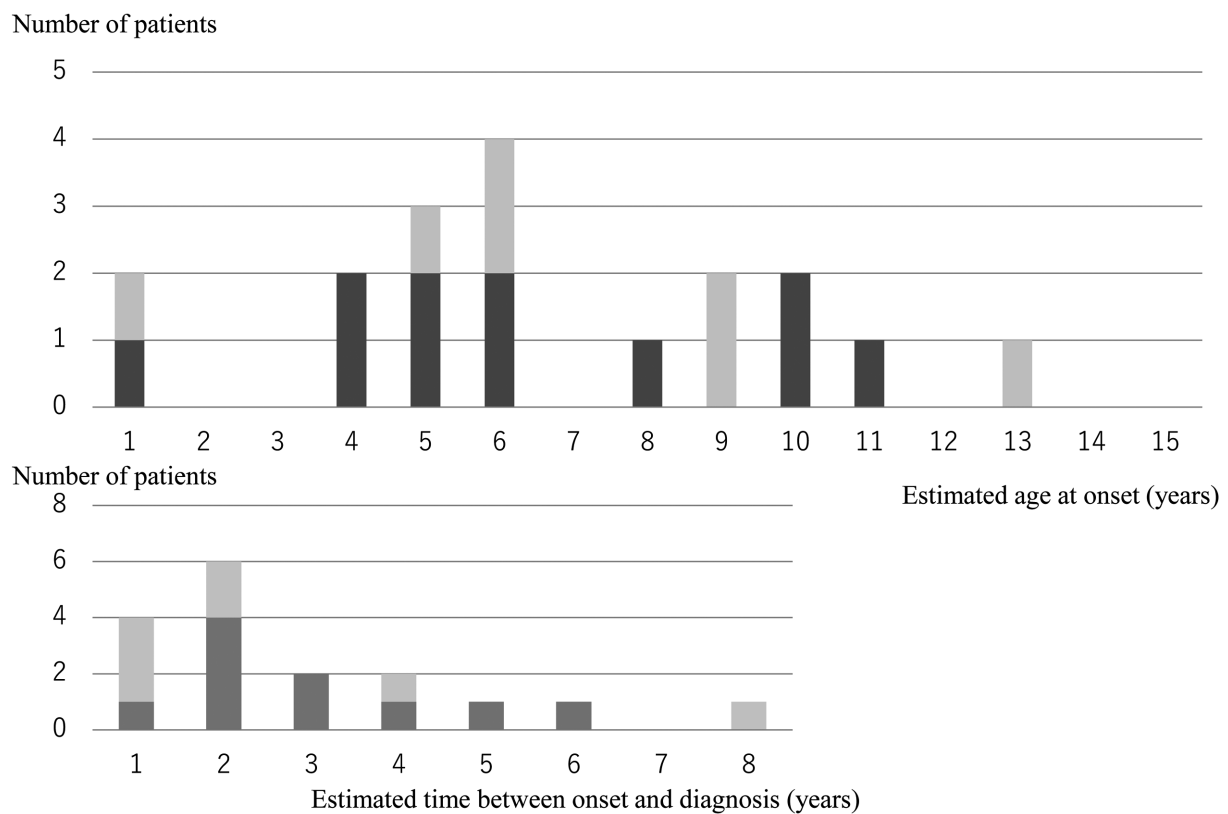
Patient	1	2	3	4	5	6	7	Reference range
TRAb (3rd) IU/l	31.3	4.5*	132.5	> 40	NA	85.1%*	40	< 2 IU/L
TSBAb% (bioassay EIA)	89.3	90.8†	99.1	99.1†	100	84†	NA	< 31.7%

TRAb (3rd), third generation TSH receptor antibody. \*, Second-generation TRAb measurements (reference range < 15% or < 1 IU/L); † TSAb kit “Yamasa Bioassay RIA” method (reference range < 45.6%).

**Table 3.** Differences in clinical characteristics between TSH receptor blocking antibody (TSBAb)-positive and -negative groups

	TSBAb- or TRAb-positive n = 7	TSBAb-negative n = 11	Statistical analysis <sup>†</sup>
Sex	Female 6, Male 1	Female 9, Male 2	NS
Age at diagnosis (yr)*	9.1 (6.8–14.2)	9.4 (6.3–14.0)	NS
Estimated time between onset and diagnosis (yr)*	2.15 (1.5–3.6)	2.8 (1.8–4.3)	NS
Height SD score at diagnosis*	–2.5 (–2.7––1.2)**	–2.5 (–3.0––1.5)	NS
% overweight at diagnosis (%)*	13.7 (9.2–21.1)**	14.4 (5.1–40.7)	NS
FT4 level at diagnosis (ng/dl)*	0.19 (0.09–0.25)	0.10 (0.02–0.21)	NS
Comorbidities	Down’s syndrome 1, autoimmune hepatitis 1	Sjogren’s syndrome 1, developmental delay and baldness 1	

\*, Median (quartile range); <sup>†</sup> Wilcoxon rank test was used for sex and Mann-Whitney U test was used for others; \*\*, Case of Down’s syndrome was excluded. NS, not significant.



**Fig. 1.** TSH receptor-blocking antibodies (TSBAb) positivity in terms of estimated age at onset, and the estimated time between onset and diagnosis. Light gray indicates TSBAb-positive patients and dark gray indicates TSBAb-negative patients.

This discrepancy in results may be partly due to the differences in TSBAb testing methods. Matsuura *et al.* assessed basal and TSH-induced cAMP levels through bioassays using cultured thyroid adenoma cells or porcine thyroid cells to determine TSBAb (1, 12). They assessed TSBAb level in 19 patients with childhood-onset AAT using this method, and reported that all cases were negative. Feingold *et al.* used a bioassay based on cAMP-inducible luciferase expression using Chinese hamster ovary (CHO) cells to determine TSBAb level (13). This

CHO cell-based cAMP and luciferase bioassay was considered as a second-generation TSBAb assessment assay, with simplified assay procedure and improved reproducibility (13–15). They reported that 8 out of 45 patients with childhood-onset chronic thyroiditis (including goiter) with TSH  $\geq 20$   $\mu$ IU/ml were TSBAb-positive. Only 2 of the 8 patients had a goiter; therefore, 6 patients with AAT were TSBAb-positive. In our study, TSBAb measurement using the bioassay method based on a commercially available TSBAb kit was conducted.

The current TSBAb assessment kit uses the dextran coated charcoal method for serum pre-measurement. It exhibits improved reproducibility and operability, and the addition of anti-hTSH antibodies reduces the effect of endogenous TSH (11). Therefore, improvements in these kits enabled us to measure TSBAb levels more accurately.

In adult patients with AAT, it has been reported that TSBAb disappears during treatment and there is improvement in hypothyroidism (10, 16). Takasu N *et al.* reported 34 TSBAb-positive patients with hypothyroidism (24 atrophic and 10 goitrous) over 10 yr (10). TSBAb disappeared in 7 of 24 TSBAb-positive patients with AAT, 5 of whom showed recovery from hypothyroidism. Conversely, all 10 patients with TSBAb-positive goitrous hypothyroidism exhibited recovery from hypothyroidism with the disappearance of TSBAb (10). It is unknown whether TSBAb also disappears over time in childhood-onset AAT, but we should also consider that TSBAb test may be negative depending on the timing of the test.

There are no reports on the difference in clinical features between the TSBAb-positive and -negative groups in patients with childhood-onset AAT. In a previous study on the onset of chronic thyroiditis during childhood, Feingold *et al.* reported that TSBAb-positive group had high TSH levels, low FT4 levels, and high frequency of positivity in Down's syndrome (13). Additionally, TSBAb-positive patients were young, exhibited high incidence of concomitant autoimmune diseases, and a slightly high prevalence of family history of autoimmune thyroid disease (13). The present study did not establish any associations between the estimated time between onset and diagnosis, age at onset, or severity of hypothyroidism. This result might be attributed to the homogeneous population of patients with childhood-onset AAT, which was the subject of our

study.

The present study is the first to show that the frequency of TSBAb positivity in patients with childhood-onset AATs is not rare, as in adults. However, this study has several limitations. First, this study was retrospective, and not all patients with AAT had their TSBAb levels measured; therefore, selection bias might be present. Second, there is no standardized thyroid function test kit or timing of TRAb and TSBAb measurements available. Third, the incidence of AAT in children is rare, and therefore the study cohort we studied was significantly small. To confirm these results, further prospective studies that unify the methods and timing of TSBAb measurement and encompass large number of cases are needed.

## Conclusion

In summary, we determined that either TSBAb or TRAb were present in 38.8% (CI 95%: 18.3–59.5%) of patients with childhood-onset AAT, as reported in adults. There were no significant differences in clinical and laboratory characteristics between the TSBAb-positive and -negative groups. These results provide new insights into the etiology of the onset of AAT during childhood. The long-term course of TSBAb-positive patients should be investigated in the future studies.

**Conflict of interests:** The authors of this manuscript declare no conflicts of interest.

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