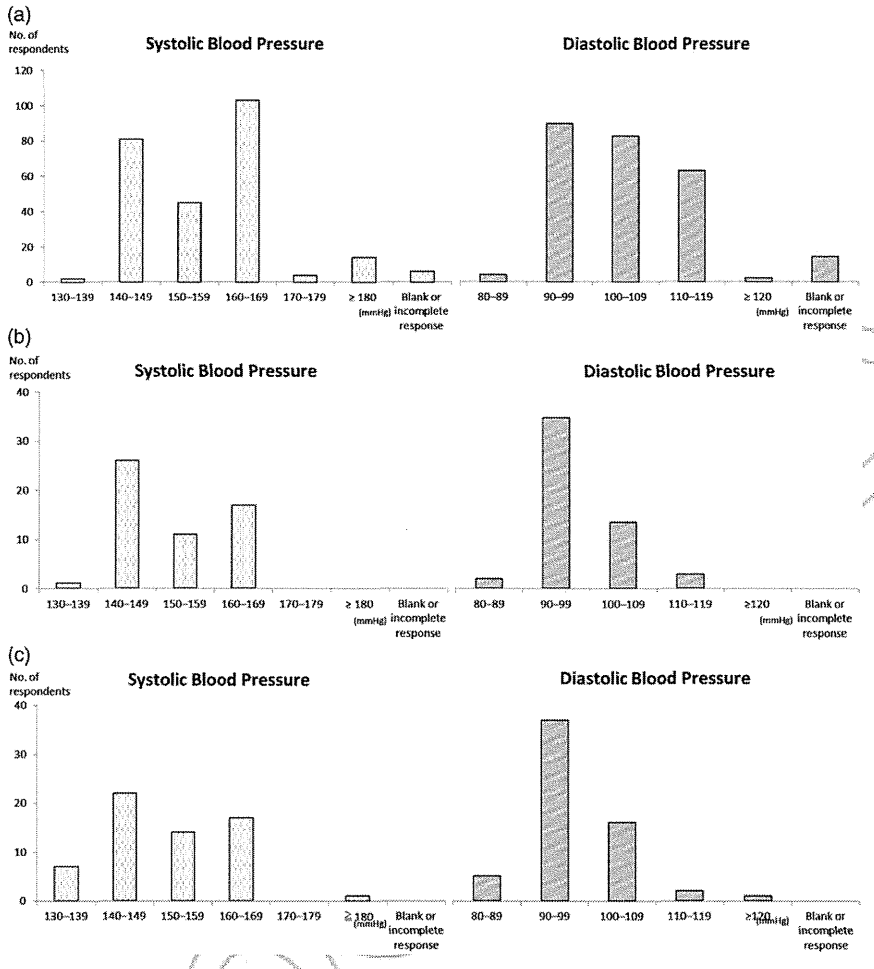

Figure 3. Distribution of threshold blood pressure levels to initiate antihypertensive therapy. (a) Chief obstetricians For systolic blood pressure, 40.2% (103/256), 31.6% (81/256) and 17.6% (45/256) of chief obstetricians indicated 160–169, 140–149 and 150–159 mmHg, respectively. For diastolic blood pressure, 35.2% (90/256), 32.4% (83/256) and 24.6% (63/256) indicated 90–99, 100–109 and 110–119 mmHg, respectively. (b) Chief internists For systolic blood pressure, 47.3% (26/55), 30.9% (17/55) and 20.0% (11/55) of chief internists indicated 140–149, 160–169 and 150–159 mmHg, respectively. For diastolic blood pressure, 65.4% (36/55) and 25.5% (14/55) responded with 90–99 and 100–109 mmHg, respectively. (c) Hypertension specialists For systolic blood pressure, 36.1% (22/61), 27.9% (17/61) and 23.0% (14/61) of hypertension specialists indicated 140–149, 160–169 and 150–159 mmHg, respectively. For diastolic blood pressure, 60.7% (37/61) and 26.2% (16/61) responded with 90–99 and 100–109 mmHg, respectively. Physicians who routinely prescribed antihypertensives to patients with postpartum hypertension responded to this question. *For an explanation of chief obstetricians (a), chief internists (b) and hypertension specialists (c), see the footnotes to Figure 1.

Table 3. Drug therapy for postpartum hypertension.

	Chief obstetricians[b]		Chief internists[b]		Hypertension specialists[b]	
	No.	Proportion (%)	No.	Proportion (%)	No.	Proportion (%)
Panel a: Class of drugs prescribed to treat postpartum hypertension[a]						
Calcium channel blocker	265	61.6	60	54.1	76	49.0
Methyldopa	90	20.9	22	19.8	34	21.9
Hydralazine	40	9.3	10	9.0	13	8.4
αβblocker	15	3.5	6	5.4	10	6.5
βblocker	3	0.7	3	2.7	5	3.2
αblocker	1	0.2	1	0.9	2	1.3
Diuretics	1	0.2	2	1.8	3	1.9
Angiotensin-converting enzyme inhibitor	5	1.2	2	1.8	4	2.6
Angiotensin receptor blocker	9	2.1	5	4.5	8	5.2
Combination drug	1	0.2	0	0	0	0
Total	430	100	111	100	155	100
Panel b: Calcium channel blockers indicated in the questionnaire response Nifedipine and amlodipine accounted for the majority of calcium channel blockers.[a]						
Agent						
Nifedipine	210	88.2	28	58.3	31	63.3
Amlodipine	16	6.7	18	37.5	16	32.7
Nicardipine	10	4.2	0	0	1	2.0
Cilnidipine	2	0.8	2	4.2	1	2.0
Total	238	100	48	100	49	100

[a]Calcium channel blockers were the most widely prescribed in departments of internal medicine and obstetrics, followed by central nervous system depressants and vasodilators.
This question was applicable only to the physicians who routinely prescribed antihypertensives to treat postpartum hypertension. Multiple choices were allowed.
[b]For an explanation of chief obstetricians, chief internists and hypertension specialists, see footnotes to Figure 1.

(6) Please choose the types of antihypertensives that you regularly prescribe (multiple responses allowed). (This question was applicable only to the physicians who responded positively to Question No. 4.)

Calcium channel blockers were most widely prescribed in departments of internal medicine and obstetrics, followed by central nervous system depressants and vasodilators (Table 3, panel a). Calcium channel blockers were used by 61.6% (265/430) of chief obstetricians, 54.1% (60/111) of chief internists and 49.0% (76/155) of hypertension specialists. Central nervous system depressants were prescribed by 20.9% (90/430) of chief obstetricians, 19.8% (22/111) of chief internists and 21.9% (34/155) of hypertension specialists. Vasodilators were administered by 9.3% (40/430) of chief obstetricians, 9.0% (10/111) of chief internists and 8.4% (13/155) of hypertension specialists. Alpha-beta-blockers were reported by 3.5% (15/430) of chief obstetricians, 5.4% (6/111) of chief internists and 6.5% (10/155) of hypertension specialists.

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Table 4. Use of antihypertensive agents in breastfeeding patients.

	Allow	Not Allow	Other	Total
Chief obstetricians[a]	224 (87.5%)	12 (4.7%)	20 (7.8%)	256 (100%)
Chief internists[a]	38 (69.1%)	14 (25.5%)	3 (5.5%)	55 (100%)
Hypertension specialists[a]	28 (45.9%)	20 (32.8%)	13 (21.3%)	61 (100%)

A total of 85.5% (224/256) of chief obstetricians, 69.1% (38/55) of chief internists and 45.9% (28/61) of hypertension specialists allowed breastfeeding in patients receiving antihypertensive medications, while 4.7% (12/256), 25.5% (14/55) and 32.8% (20/61), respectively, recommended discontinuation of lactation for patients who were on antihypertensive treatment.

This question was applicable only to the physicians who routinely prescribed antihypertensives to treat postpartum hypertension. Figures not enclosed in parentheses represent the numbers of respondents, and the percentages in parentheses their proportions.

[a]For an explanation of chief obstetricians, chief internists and hypertension specialists, see footnotes to Figure 1.

Angiotensin-converting enzyme inhibitors were prescribed by 1.2% (5/430) of chief obstetricians, 1.8% (2/111) of chief internists and 2.6% (4/155) of hypertension specialists, while angiotensin receptor blockers were used by 2.1% (9/430), 4.5% (5/111) and 5.2% (8/155), respectively (Table 3, panel a).

Nifedipine and amlodipine accounted for the majority of calcium channel blockers. Nifedipine was prescribed by 88.2% (210/238) of chief obstetricians, 58.3% (28/48) of chief internists and 63.3% (31/49) of hypertension specialists, while amlodipine was administered by 6.7% (16/238), 37.5% (18/48) and 32.7% (16/49), respectively (Table 3, panel b).

(7) Question: What guidance do you give concerning breastfeeding when you prescribe antihypertensive agents to patients with postpartum hypertension?

A total of 85.5% (224/256) of chief obstetricians, 69.1% (38/55) of chief internists and 45.9% (28/61) of hypertension specialists allowed breastfeeding in patients receiving antihypertensive medications, while 4.7% (12/256), 25.5% (14/55) and 32.8% (20/61), respectively, recommended discontinuation of lactation for patients who were on antihypertensive treatment (Table 4).

DISCUSSION

Awareness of Long-term Risks and Clinical Care for Women with HDP

In the Effectiveness-Based Guidelines for the Prevention of Cardiovascular Disease in Women-2011 Update (21) published by the American Heart Association, a history of abnormal pregnancy was identified as a risk factor for cardiovascular disease. The guidelines underline the importance of appropriate postpartum referral by the obstetrician to a primary care physician or hypertension specialist to ensure that risk factors can be carefully monitored and controlled after pregnancy. The current study showed that

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among the leading practicing obstetricians and internists in Japan, HDP are recognized as a risk factor for chronic hypertension and cardiovascular disease. At the same time, this study showed that over a third of chief obstetricians terminated their postpartum follow-up of HDP patients without referring them to primary care physicians or hypertension specialists. The finding that only 41% of chief internists and 36% of hypertension specialists were routinely seeing patients with postpartum hypertension suggested that such patients were not fully aware of the implications of HDP, and that there is a lack of appropriate referral by the obstetricians. This could be partly attributable to the fact that postpartum hypertension often resolves shortly after delivery. Pregnancy represents a metabolic "stress test" to predict cardiovascular events in women (22). In order to prevent future development of disease, it is important to keep track of women whose high cardiovascular risk has been unmasked by pregnancy-related events. For this purpose, coordination between the departments of obstetrics and internal medicine, such as a referral system that will enable smooth transition of high-risk patients from the obstetrician to the primary care physician or to the specialist, is necessary. At the same time, our results suggested the need to create effective education and long-term monitoring programs for women at high cardiovascular risk.

Drug Therapy for Postpartum Hypertension

Compared to internists, obstetricians applied higher blood pressure thresholds for prescribing drug therapy. This reflected the likelihood of obstetricians preferring to avoid medication during breastfeeding and that they hold the view that blood pressure can change significantly in the early postpartum period. The literature reports a variety of criteria for starting drug therapy in patients with postpartum hypertension. A systematic review found that antihypertensive therapy should be advised for severe hypertension (systolic blood pressure $\geq$ 170 mmHg or diastolic blood pressure $\geq$110 mmHg) to prevent maternal cerebral hemorrhage and other acute vascular injuries (6). However, there was no consensus regarding antihypertensive interventions for treating mild-to-moderate hypertension (systolic blood pressure 140 to 169 mmHg, or diastolic blood pressure 90 to 109 mmHg) (6). In the 1997 Canadian Hypertension Society Consensus report (23), initiation of antihypertensive drug treatment was recommended for severe hypertension and mild-to-moderate hypertension with a variety of comorbid conditions. The guidelines reported no particular criteria for the threshold to initiate therapy or treatment goal based on the study of Japan Society for the Hypertension in Pregnancy, the national high blood pressure education program work group set up by NIH (24) and the consensus statement by the ASSHP (25). This might possibly reflect the general idea that postpartum hypertension is only temporary, and that blood pressure will decrease within a certain time. However, studies revealed that approximately one-fifth of women with preeclampsia later developed chronic hypertension (11,26). In addition, a systematic literature review found that preeclampsia increased the risk of vascular disease and chronic hypertension (17). These findings emphasize the need to extend short-term postpartum care into a long-term follow-up and management program.

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Our survey showed that patients with postpartum hypertension were mostly frequently treated with calcium channel blockers by obstetricians and internists, followed by centrally acting sympatholytic drug methyldopa and vasodilator hydralazine. The profile of postpartum antihypertensive use was similar to that of the antepartum period. The wide acceptance of calcium channel blockers in pregnancy was probably attributable to their excellent blood pressure-lowering effect and that nifedipine have not been contraindicated in women after 20 weeks of gestation in Japan. Our data suggested that obstetricians preferred nifedipine to amlodipine, whereas internists favored amlodipine more than obstetricians. Appropriate antihypertensives should be chosen for the breastfeeding mother, taking into consideration the effects on maternal blood pressure levels, exposure of the lactating child to the drug, and the mother's daily life patterns. These factors suggested that calcium channel blockers were drugs of choice for breastfeeding mothers. Use of the centrally acting sympatholytic drug methyldopa in postpartum patients should be accompanied by careful consideration. The NICE advised that methyldopa should be replaced with an alternative drug for the management of postpartum hypertension because of associated postpartum depression (27).

Our data revealed a discrepancy of opinion between obstetricians and internists regarding the use of antihypertensives during breastfeeding. Obstetricians generally supported it, whereas internists were more cautious. Breastfeeding has benefits for infants with respect to nutrition, gastrointestinal function and host defense (28). Recent research showed that breast milk was superior to infant artificial formula in terms of reduction in systolic blood pressure (29,30), risk of type 2 diabetes mellitus (31) and obesity (32) of infants in later life. A generational study showed that children born to mothers with HDP had higher systolic and diastolic blood pressures at the age of nine years (33). These findings highlight the benefits of breast-fed children. Also, breastfeeding provides metabolic advantages to the mother. Recent studies of the relationship between breastfeeding and cardiovascular risk factors reported a lower prevalence of lipid metabolism abnormalities (34) and lower blood levels of glucose and insulin (35) in mothers with a history of breastfeeding than those without. In addition, lower incidences of metabolic syndrome were observed in mothers with longer lactation periods (36).

Breastfeeding offers a significant advantage in reducing cardiovascular risks in both mothers and children; it should therefore be recommended, in particular, for patients with HDP. If the mother requires antihypertensive therapy, appropriate drugs that enable breastfeeding, while achieving effective blood pressure control should be selected. The use of such medications will contribute to reducing future risks to the mother and her children.

CONCLUSION

Pregnancy and childbirth represent an excellent occasion to identify women who are at high risk of cardiovascular disease. An adequate referral system that enables smooth transfer to a local primary care physician or hypertension specialist will help reduce the likelihood of future cardiovascular disease.

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DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Disease specificity of anti-tryptophan hydroxylase-1 and anti-AIE-75 autoantibodies in APECED and IPEX syndrome

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KEYWORDS
Tryptophan hydroxylase;
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IPEX syndrome

Abstract Autoantibodies to autoimmune enteropathy-related 75 kDa antigen (AIE-75) and villin are disease markers of immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome which is characterized by a peripheral tolerance defect. On the other hand, anti-tryptophan hydroxylase-1 (TPH-1) antibodies are detected in autoimmune polyendocrinopathy, candidiasis, ectodermal dystrophy (APECED), a central tolerance defect, especially when complicated with gastrointestinal dysfunction. However, to date, anti-AIE-75 and anti-villin antibodies or anti-TPH-1 antibodies have not been tested in APECED or IPEX syndrome, respectively. In the present study, we confirmed the disease specificity of both anti-AIE-75 and anti-TPH-1, although anti-villin antibodies were detected in some patients with APECED. Our observation suggests that immunotolerance to AIE-75 depends on the peripheral mechanism, whereas the tolerance to TPH-1 depends on the central mechanisms.
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Abbreviations: AIE-75, autoimmune enteropathy-related 75 kDa antigen; AIRE, autoimmune regulator; APECED, autoimmune polyendocrinopathy, candidiasis, ectodermal dystrophy; CMC, chronic mucocutaneous candidiasis; FOXP3, forkhead box transcription factor 3; GST, glutathione-S-transferase; IPEX syndrome, immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome; mTEC, medullary thymic epithelial cells; TPH-1, tryptophan hydroxylase-1; Treg, regulatory T; TSA, tissue-specific antigen

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1. Introduction

Both T cells and B cells acquire their diversification by random recombination of T cell receptor (TCR) and B cell receptor (BCR) genes, respectively. This results in generation of a significant number of self-reactive T and B lymphocytes, but the majority of them are eliminated or suppressed by several mechanisms that contribute to immunological tolerance [1,2]. Autoimmune regulator, *AIRE*, is involved in the intrathymic expression of tissue-specific antigens (TSAs) and plays a critical role in the negative selection of self-reactive T cells, also known as central immunotolerance [1]. Although some self-reactive T cells escape negative selection and efflux to periphery, they are in anergic state or inactivated by regulatory T (Treg) cells [2,3]. Forkhead box transcription factor 3, *FOXP3*, is a master gene in the development of Treg cells and contributes to peripheral dominant immunotolerance [2,3]. Failure of the immunotolerance mechanisms causes multiple organ-specific autoimmune disorders. Mutations of *AIRE* gene result in autoimmune polyendocrinopathy, candidiasis, ectodermal dystrophy (APECED) which is characterized by autoimmunity to endocrine tissues such as parathyroid gland and adrenal gland, and to cytokines critical for antifungal immunity, interleukin-17 [1]. Mutations of *FOXP3* genes cause immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome, which is characterized by autoimmune enteropathy and endocrinopathies such as type-1 diabetes mellitus and thyroiditis [4,5].

We have identified autoimmune enteropathy-related 75 kDa antigen (AIE-75) and an actin binding protein, villin, as target autoantigens of enteropathy in IPEX syndrome [6–8]. Recent studies have confirmed the high specificity and sensitivity of these two antibodies regardless of ethnicity [9,10]. On the other hand, gastrointestinal dysfunction is observed in about 10% of APECED patients. Autoantibodies against tryptophan hydroxylase (TPH)-1 are detected in 89% of the patients with APECED complicated by gastrointestinal dysfunction and 34% of the patients without gastrointestinal complications [11–14]. Recently, Sayar et al. have suggested that some cases of APECED with gastrointestinal dysfunction could mimic IPEX syndrome [15]. Nevertheless there have been no studies that tested anti-AIE-75 or anti-villin antibodies in APECED and anti-TPH-1 antibodies in IPEX syndrome. In the present study, we examined autoantibodies to TPH-1, AIE-75 and villin in APECED and IPEX syndrome.

2. Materials and methods

2.1. Patients and sera

We investigated 7 patients with IPEX syndrome (6 Japanese and 1 American) and 23 patients with APECED (20 Italian, 2 Japanese and 1 American) (Tables 1 and 2). This work was approved by the Institutional Review Board of Hokkaido University Hospital with written informed consent from the patients or guardians. Clinical and laboratory features and genetic mutations of some patients have previously been reported [8,14,16,17]. Among the twenty Italian patients with APECED, 10 were positive for anti-TPH-1 antibodies as judged from immunoprecipitation (IP-positive), whereas the other 10 were negative for the antibodies (IP-negative) [13,14]. Sera from 2 Japanese and 1 American (Irish/Spanish) patients have not been tested for the antibodies by IP (IP-NT). Sera were obtained from the patients and stored at −20 °C until use.

2.2. Production of recombinant fusion proteins

Recombinant human TPH-1 was expressed as a fusion protein with glutathione-S-transferase (GST). Briefly, the primer pair was designed to amplify whole coding region with *BamHI* restriction site at the 5′ end and *XhoI* site at the 3′ end as the following; 5′-GGATCCATGATTGAAGACAATAAGGAG-3′, and 5′-CTCGAGTTAGATACTCGGCTTCCTGCT-3′. Complimentary DNA encoding TPH-1 (NM_004179) was amplified by polymerase chain reaction (PCR) using λgt11 human duodenal cDNA library (BD Biosciences Clontech, Palo Alto, CA) as a template. The PCR product was inserted into pCR2.1-TOPO TA cloning vector (Invitrogen, Carlsbad, CA), digested with both *BamHI* and *XhoI* and then subcloned into a GST fusion protein expression vector, pGEX4T-2. *Escherichia coli, BL-21,* was transformed with the plasmid containing correct nucleotide sequence of TPH-1. Fusion protein, GST-TPH-1, was expressed in the presence of 0.5 mM isopropylthiogalactoside (IPTG) and purified with glutathione-sepharose beads (Amersham Biosciences, Piscataway, NJ). Recombinant AIE-75 and GST-villin were expressed and used for immunoblotting as previously reported [7,8].

2.3. Immunoblotting

A 60 ng of the recombinant antigens was subjected to electrophoresis on sodium dodecyl sulfate-polyacrylamide gel, and electrically transferred to polyvinylidene difluoride membrane (Millipore, Bedford, MA). After blocking with 5% skim milk, the membranes were incubated with 1:200 diluted rabbit polyclonal anti-TPH-1 antibody (Sigma Aldrich), 1:1000 diluted goat anti-GST antibody (Amersham Biosciences), or 1:80–1:5120 diluted human sera. Human sera were diluted with Tris-buffered saline containing 0.1% Tween-20 (TBST) and crude lysate prepared from *E. coli* expressing GST to block potential cross-reactivity with GST or components of *E. coli* except for some experiments. After incubation with primary antibodies, membranes were washed with TBST three times and incubated with diluted horseradish peroxidase (HRP)-conjugated goat anti-human IgG at 1:5000 (Biosource, Camarillo, CA), HRP-conjugated goat anti-rabbit IgG at 1:2000 (Biosource) or HRP-conjugated rabbit anti-goat IgG (Biosource) at 1:2000 for 1 h at room temperature. All the secondary antibodies were diluted with TBST. After washing with 50 mM Tris–HCl pH 7.6, immunoreactive bands were detected by 3,3′-diaminobenzidine (Sigma, St. Louis, MO) and nickel ion (0.03% $NiCl_2$).

3. Results

3.1. Production of recombinant fusion protein

The fusion protein was immunoreactive on blots with either anti-GST or anti-TPH-1 antibody (Fig. 1). The apparent

Table 1 Clinical and laboratory features of patients with APECED.

Case no.	Clinical manifestation	Intestinal dysfunction	Autoantibodies	Anti-TPH-1 Abs by IP [binding index]	Ethnicity
1	AD, HP, CMC, M	Yes	Not tested	Not tested	Japanese
2	HP, CMC, AIH	No	Not tested	Not tested	Japanese
3	AD, HP, CMC, POF, K	Yes	IFNωAbs	84.13	Italian
4	AD, HP, CMC, EH, demyelinating polyneuropathy	No	IFNωAbs, AADC	167.6	Italian
5	AD, HP, AIT, POF, Ch, CTD, AG, PA, AIH, M, K, ND, A	Yes	IFNωAbs, AADC	90.1	Italian
6	AD, HP, CMC, AIT, POF, AG, PA, Co	No	IFNωAbs, AADC	200	Italian
7	AD, HP, CMC, POF, CTD, AG, PA, K, Va	No	IFNωAbs, AADC	56.7	Italian
8	AD, HP, CMC, AG, M, K, V, A	Yes	IFNωAbs, AADC	137	Italian
9	AD, HP, CMC, AIT, GHD, AG, M, Co, V	Yes	IFNωAbs, AADC	114.9	Italian
10	AD, HP, CMC, POF, AG, AIH, A	No	IFNωAbs, AADC	74.4	Italian
11	AD, HP, CMC, ND	No	IFNωAbs	7.7	Italian
12	AD, HP, CMC, ND, A	No	IFNωAbs, AADC	201.22	Italian
13	AD, HP, CMC, POF, Co, CTD, AG	No	AADC	Negative	Italian
14	HP, POF	No	IFNωAbs	Negative	Italian
15	AD, CMC, POF, ND, A, EH	No	IFNωAbs	Negative	Italian
16	AD, HP, CMC, POF	No	IFNωAbs, AADC	Negative	Romanian
17	AD, HP, CMC, POF, GHD, ND, EH	Yes	IFNωAbs	Negative	Italian
18	AD, HP, CMC,	No	IFNωAbs	Negative	Italian
19	AD, HP, AG	No	IFNωAbs	Negative	Italian
20	AD, HP, CMC, Ch, CTD, AG, A, TMC	No	IFNωAbs, AADC	Negative	Italian
21	AD, HP	No	IFNωAbs	Negative	Italian
22	AD, HP, CMC, AIH	No	IFNωAbs, AADC	Negative	Italian
23	AIH, AIT, GN, IDDM	Yes	Not tested	Not tested	Irish/ Spanish

Abbreviations; AADC, antibodies to aromatic L-amino acid decarboxylase; IFNωAbs, antibodies to interferon-ω; Abs, antibodies; A, alopecia; AD, Addison's disease; AG, atrophic gastritis; AIH, autoimmune hepatitis; AHA, autoimmune hemolytic anemia; AIT, autoimmune thyroiditis; As, asplenia; CD, Celiac Disease; Ch, cholelithiasis; CMC, chronic mucocutaneous candidiasis; Co, constipation; CTD, connective tissue disease; EH, enamel hypoplasia; GHD, growth hormone deficiency; GN: glomerulonephritis; HP, hypoparathyroidism; IDDM: insulin-dependent diabetes mellitus; IP, immunoprecipitation; K, keratoconjunctivitis; M, malabsorption; ND, nail dystrophy; PA, pernicious anemia; POF, premature ovarian failure; Ps, psoriasis; TMC, tympanic membrane calcification; TIN, tubulointerstitial nephritis; V, vitiligo; Va, vasculitis.

molecular size of the fusion protein was approximately 75 kDa, consistent with the sum of GST (26 kDa) and TPH-1 (51 kDa).

3.2. Autoantibodies to TPH-1

As shown in Fig. 2A, an IP-NT serum (APECED-2) reacted with GST-TPH-1 but not with GST alone. GST-TPH-1 reacted with 8 of 10 IP-positive APECED patients, but none of the sera from 10 IP-negative or 2 IP-NT patients (Fig. 2B and data not shown). However, there was no correlation between the titers of antibodies measured by IP and immunoblot (Fig. 3). On the other hand, none of the sera from 7 patients with IPEX reacted with GST-TPH-1 (Fig. 4).

3.3. Reactivity of the sera to AIE-75 and villin

Anti-AIE-75 antibodies were detected in 5 of 7 patients with IPEX syndrome but not in any patients with APECED (Fig. 5 and Table 2). Anti-villin antibodies were detected in 4 of 7 patients with IPEX syndrome and 3 of 23 patients with APECED (Fig. 6 and Table 2).

4. Discussion

In the present study, we demonstrate that anti-AIE-75 antibodies and anti-TPH-1 antibodies are specific to IPEX syndrome and APECED, respectively. These confirm the specificity and diagnostic value of anti-TPH-1 and anti-AIE-75 autoantibodies in APECED and IPEX, respectively [6–14]. On the other hand, anti-villin antibodies were detected in sera from 3 of 23 patients with APECED (1 with and 2 without GI dysfunction). Low levels (1:160 or lower) of anti-villin antibodies are also detected in some patients with other collagen vascular diseases such as SLE and mixed connective tissue disease [8]. Thus, villin could be highly immunogenic, and anti-villin autoantibodies are produced in several autoimmune diseases. Anti-TPH-1 antibodies were detected in 8 of 10 IP-positive patients with APECED. Interestingly, there were no correlations between the titers determined by immunoblotting and the binding index by the IP assay. IP assays using deletion mutants of TPH-1 have revealed three major epitopes of the antigen [13]. Although the conformation of antigens is preserved in IP, immunoblot detects antibodies against linear peptide sequences of denatured antigens. Our result suggests that

Table 2 Clinical and laboratory features of IPEX patients.

Case no.	Clinical manifestation	Anti-AIE-75 Abs (IB)	Anti-villin Abs (IB)	Ethnicity	Outcome
1	Diarrhea	1:80	1:2560	Japanese	Alive
2	Diarrhea, NS, T1DM	1:640	Negative	Japanese	Alive
3	AHA, Diarrhea, AIT, TIN	1:320	1:320	Japanese	Died
4	Diarrhea, AIT, TIN	1:160	1:320	Japanese	Died
5	Diarrhea	Negative	1:160	Japanese	Died
6	Alopecia, psoriasiform dermatitis, Diarrhea	Negative	Negative	Caucasian	Alive
7	Diarrhea, food allergy, eczema, NS, AIH	1:2560	Negative	Japanese	Alive

Abbreviations; AIE-75, autoimmune enteropathy-related 75 kDa antigen; Abs, antibodies; AHA, autoimmune hemolytic anemia; AIT, autoimmune thyroiditis; AIH, autoimmune hepatitis; NS, nephrotic syndrome; TIN, tubulointerstitial nephritis.

the autoantibodies to conformation of TPH-1 are dominant in some patients with APECED, whereas those to linear peptides are dominant in the others. However, our study could not clarify whether autoantibodies against the conformational or linear epitopes are associated with clinical GI tract dysfunction.

Most of APECED patients enrolled in our study were Italian, whereas most IPEX patients were Japanese. Both anti-AIE-75 and anti-villin antibodies are prevalent in patients with IPEX regardless of their ethnicities [7–10]. On the other hand, although the most frequent mutation in *AIRE* gene differs between races, genotype–phenotype correlations have not been clarified except for the association between large truncations of AIRE and candidiasis [18]. Thus, the influence of the bias in the ethnicities on our results remains unclear.

APECED and IPEX syndrome share several common features such as type-1 diabetes mellitus and thyroiditis, however, Addison's disease and hypoparathyroidism are prevalent in APECED but not in IPEX syndrome [1,3,19]. AIE-75 and villin are expressed in the duodenal epithelial cells, whereas expression of TPH-1 is limited to enterochromaffin cells in the intestine [7,8,14]. These are consistent with histopathological findings; severe inflammation is associated with villous atrophy of the duodenal tissue in IPEX syndrome, whereas only minimal inflammation and loss of enterochromaffin cells without apparent villous atrophy are observed in the intestine of anti-TPH-1 antibody-positive APECED [3,14,20]. Coincidence of the target cells and distribution of the autoantigens suggest a pathological role of antigen-specific autoimmunity in the development of enteropathy in both diseases. Because all of the three antigens are cytoplasmic proteins, circulating autoantibodies may not directly bind them. Indeed, interstitial nephritis is not necessarily observed in IPEX syndrome positive for autoantibodies to these antigens despite the expression of both AIE-75 and villin in the renal tubules [7,8]. Furthermore, anti-TPH-1 antibodies are positive in one-third of the cases of APECED without gastrointestinal dysfunction [11–14]. Given that autoreactive T cells share the same antigen-specificity with autoantibodies in autoimmune diseases [21–23], autoreactive T cells likely play a critical role in the tissue- or cell type-specific destruction in both diseases. Autoantibodies or autoreactive BCR may bind cytoplasmic antigens that had leaked from apoptotic or necrotic cells and facilitate the activation of T cells by antigen presenting cells [24]. Indeed, depletion of B cells in *Scurfy* mice, counterparts of human IPEX syndrome, reduces tissue damages [25]. Furthermore, follicular Treg cells which are derived from thymic naturally occurring Treg cells directly suppress autoreactive B cells in the peripheral lymphoid tissues [26–29]. As a result, Treg cell dysfunction allows the accumulation of autoreactive B cells in IPEX syndrome [30].

AIRE is expressed mainly in the medullary thymic epithelial cells (mTEC), acts as a transcription factor responsible for the

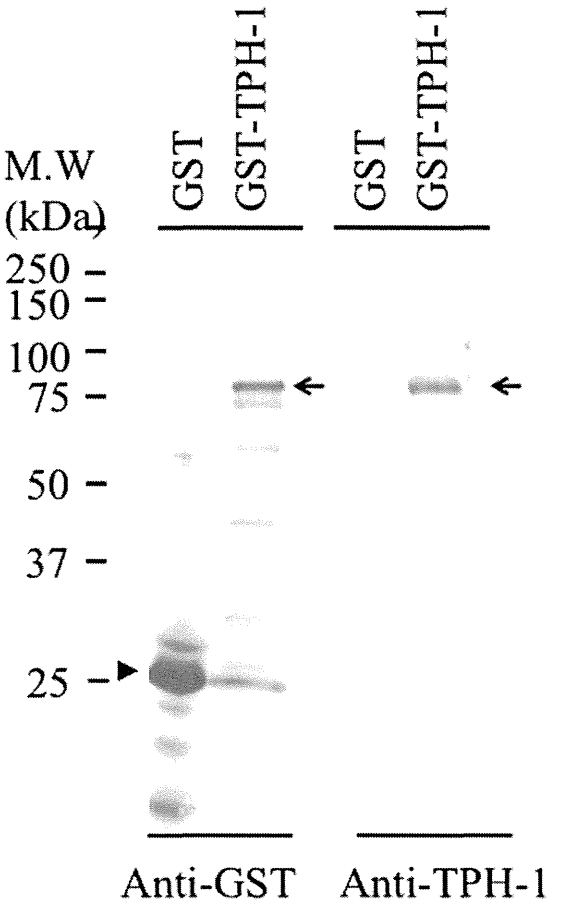

Figure 1 Expression of recombinant GST-TPH. GST or GST-TPH-1 is immunoblotted with anti-GST (A) or anti-TPH-1 (B) antibodies. In this experiment, the serum was diluted with TBST without *E. coli* extract. Both GST and GST-TPH-1 reacted with anti-GST antibodies, whereas only GST-TPH-1 reacted with anti-TPH-1 antibodies. Arrow and arrowhead indicate recombinant GST-TPH-1 and GST alone, respectively.

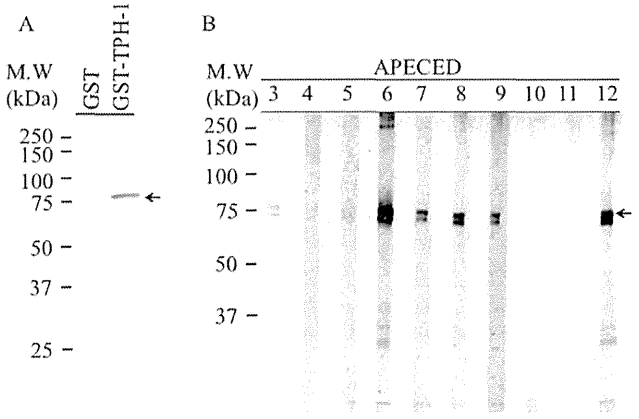

Figure 2 Anti-GST-TPH-1 antibodies in sera from APECED patients. (A) The serum of APECED-2 reacted with GST-villin but not GST alone. In this experiment, the serum was diluted by TBST without *E. coli* extract. (B) GST-TPH-1 reacted with 10 APECED sera (cases 3–12) in which the autoantibodies have been detected by immunoprecipitation. Anti-TPH-1 antibodies were not detected in cases 10 and 11 by immunoblotting. Arrows indicate GST-TPH-1.

thymic expression of TSAs, and contributes to the elimination of self-reactive T cells [1]. In addition, *AIRE* is expressed in the peripheral lymphoid tissues, and accordingly, may play a role in the peripheral tolerance. On the other hand, *FOXP3* is a master gene of Treg cells which play a central role in peripheral immunotolerance [2,3]. Thus, immunotolerance mechanisms may differ between autoantigens; tolerance to TPH-1 depends on intrathymic negative selection or AIRE-dependent peripheral tolerance, whereas tolerance to AIE-75 depends on Treg cells. Both TPH-1 and villin are expressed in mTEC of *AIRE*-deficient mice, although the expression of AIE-75 has not been studied [31]. Some target autoantigens associated with APECED are also expressed in mTEC in *AIRE*-independent manners [32–34]. Thus, AIRE must have roles in negative selection through a mechanism distinct from intrathymic transcription of TSA, e.g., antigen presentation by mTEC or interdigitating reticular cells in the peripheral lymphoid organs [35]. Otherwise, expression of TSAs in human mTEC may be different from those in mice. To date, whether AIRE contributes to the selection of Treg cells in the thymus is still controversial [34,36]. However, given the severe clinical features of IPEX syndrome compared with APECED, at least some of Treg cells may develop independent of AIRE.

5. Conclusions

Autoantibodies to AIE-75 and TPH-1 could be used for the differential diagnosis of IPEX syndrome and APECED. Coincidence of the distribution of autoantigens and target cell types

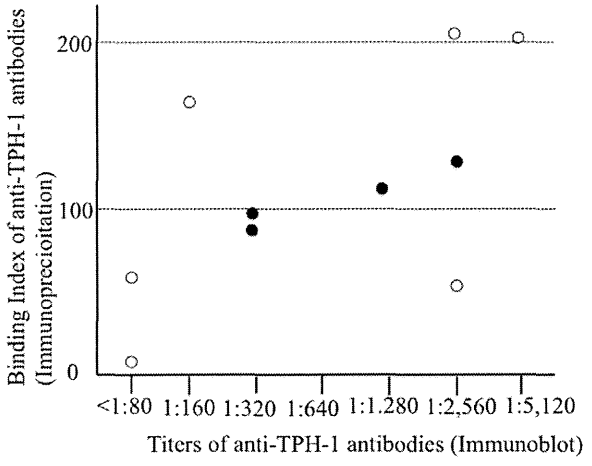

Figure 3 Anti-TPH-1 measured by immunoprecipitation and immunoblotting. The titers of anti-TPH-1 antibodies in APECED patients were compared with the binding unit of the antibodies measured by immunoprecipitation. There is no significant correlation between the binding unit by immunoprecipitation and titers by immunoblotting (Spearman's rank correlation coefficient; rs = −0.56, p = 0.088). Closed circles indicate patients with gastrointestinal manifestations.

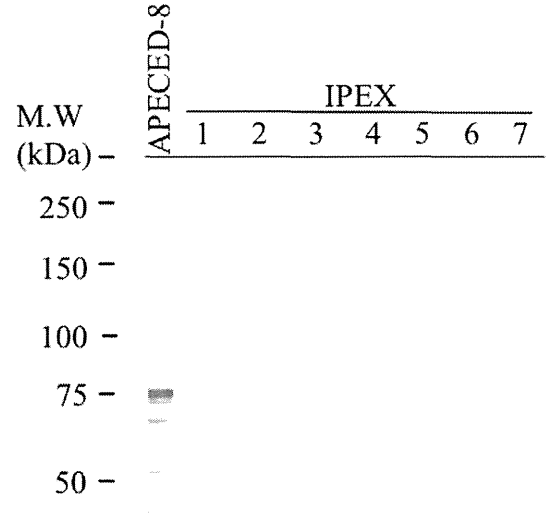

Figure 4 Autoantibodies to TPH-1 are specific to APECED. GST-TPH-1 reacted with the serum of APECED-8 but none of IPEX syndrome. All the sera were diluted with TBST containing extracts of *E. coli* expressing GST.

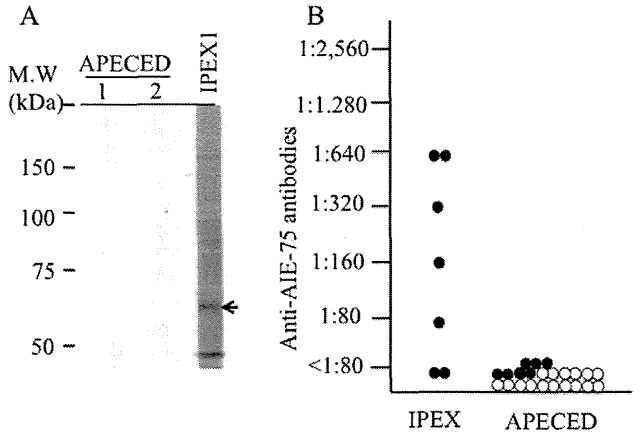

Figure 5 Autoantibodies to AIE-75 are specific to IPEX syndrome. (A) AIE-75 reacted with the serum from IPEX-1 but not with sera from APECED-1 and -2. (B) Anti-AIE-75 antibodies were detected in 5 of 7 sera from IPEX syndrome patients but none of 23 APECED sera. An arrow indicates recombinant AIE-75. Closed circles indicate patients with gastrointestinal manifestations.

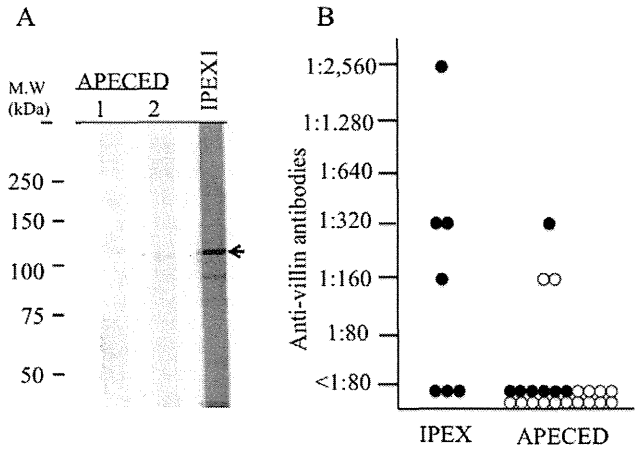

Figure 6 Autoantibodies to GST-villin. (A) GST-villin reacted with the serum from IPEX-1 but not with sera from APECED-1 and -2. The molecular weight of GST-villin is approximately 120 kDa which is consistent with that calculated from GST (26 kDa) and villin (91 kDa). (B) Anti-villin antibodies were detected in 4 of 7 sera from IPEX syndrome patients and 3 of 23 sera from APECED patients. All of the sera were diluted with TBST containing extracts of *E. coli* expressing GST. An arrow indicates GST-villin. Closed circles indicate patients with gastrointestinal manifestations.

suggests the involvement of antigen-specific mechanisms in the intestinal dysfunctions in both diseases. Immunotolerance to AIE-75 and TPH-1 may depend on the peripheral and central mechanisms, respectively.

Conflict of interest statement

The author(s) declare that there are no conflicts of interest.

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特集 糖尿病と妊娠における新たな展開

糖代謝異常合併妊娠と甲状腺疾患

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key words 糖尿病／妊娠／甲状腺機能異常

はじめに

自己免疫性甲状腺疾患は妊娠可能年齢の女性に好発し，甲状腺機能異常や自己免疫異常は妊娠成立，妊娠維持，妊娠中の母体や児に悪影響を及ぼす[1]。また，甲状腺ホルモン過不足は糖代謝に影響を及ぼすため，糖代謝異常合併妊娠においては，甲状腺機能も念頭においた血糖コントロールと母体の管理が必要である。特に，1型糖尿病は自己免疫甲状腺疾患合併率が高いので，妊娠前から甲状腺自己抗体や甲状腺機能のチェックを行い，甲状腺機能を正常化しておく必要がある。産後甲状腺機能異常は一般女性でも10～20人に1人の割合でみられるが，1型糖尿病ではその3～4倍のリスクとされている。糖尿病女性，特に1型糖尿病の場合には，産後甲状腺機能異常合併に注意を要する。

Ⅰ．一般女性および妊婦の甲状腺疾患の頻度

日本における妊娠可能年齢における甲状腺疾患の罹病率に関する確かなデータはないが，平成13年国民生活基礎調査によると，25～45歳までの甲状腺疾患で通院している女性は人口1,000人あたり約6～9人であった。潜在的な甲状腺疾患の存在を考慮すると，妊娠可能年齢女性における甲状腺疾患の頻度は高率と予想される。

また，Kasagiらは1,818名の検診データを基に，甲状腺自己抗体陽性頻度を報告した（表1）[2]。これによると，抗甲状腺ペルオキシダーゼ抗体（抗TPO抗体）陽性率は，男性7.2%，女性15.0%，抗サイログロブリン抗体（抗Tg抗体）陽性率は男性13.1%，女性29.4%であり，女性での甲状腺自己抗体陽性率は高率であった。北米からの報告も，抗TPO抗体の頻度は男性8.7%，女性17.0%と報告されており[3]，日本人の結果とほぼ一致していた。また，Kasagiらは[2]，40歳未満女性105名，いわゆる妊娠可能年齢女性においての抗TPO抗体陽性率は全年齢とほぼ同様に15.2%，抗サイログロブリン抗体（抗Tg抗体）の陽性率は35.2%と報告しており（表1），妊娠可能年齢での潜在的甲状腺自己免疫を有する可能性のある女性は数名に1名ともいえる。

妊娠女性においては，Oritoらの妊娠7～15週の連続的な568名の日本人妊婦の甲状腺自己抗体陽性頻度に関する報告がある[4]。それによると，平均妊娠週数10.5週にスクリーニング検査が行われ，抗TPO抗体は6.7%に陽性であった。また，抗TPO抗体陽性者のTSH値は非陽性者のTSHに比較して有意に高値を，抗TPO抗体陽性者のFT_4値は非陽性者のそれに比較して有意に低値を示すことも示された[4]。

妊娠成立後は，液性免疫の抑制による抗体価の低下のために自己抗体陽性率は過少評価になる可能性はあるが，それでも約15妊娠女性に1例の割合で，抗TPO抗体が陽性であった。

表1．年齢と性別ごとの抗サイログロブリン抗体(TgAb)，抗甲状腺ペルオキシダーゼ抗体(TPOAb)陽性率

女性

	対象者の数(頻度%)		
年齢(歳)	症例数	TgAb陽性	TPOAb陽性
＜40	105	37(35.2%)	16(15.2%)
40～50	308	86(27.9%)	40(13.0%)
50～60	487	140(28.7%)	79(16.2%)
＞60	114	35(30.7%)	17(14.9%)
全体	1,014	298(29.4%)	152(15.0%)

男性

	対象者の数(頻度%)		
年齢(歳)	症例数	TgAb陽性	TPOAb陽性
＜40	96	14(14.6%)	4(4.2%)
40～50	225	30(13.3%)	14(6.2%)
50～60	329	38(11.6%)	22(6.7%)
＞60	154	23(14.9%)	18(11.7%)
全体	804	105(13.1%)	58(7.2%)

(文献2より引用)

Ⅱ．1型糖尿病と自己免疫性甲状腺疾患

1型糖尿病に他の自己免疫疾患を合併することはよく知られており，特に自己免疫性甲状腺疾患の合併は有名である。わが国からは，内野らが東京女子医科大学糖尿病センターで1998年から3年間の間に治療，管理を行った糖代謝異常合併妊婦171例中12例(約7.0%)に自己免疫性甲状腺疾患が合併していることを報告した[5]。特に，1型糖尿病合併妊婦では，11.4%と高率に自己免疫性甲状腺疾患を合併していた。また，大崎らは88名の1型糖尿病女性のうち12.5%に自己免疫性甲状腺疾患を合併していることを報告しており[6]，この頻度は内野らの頻度に一致した。1型糖尿病女性の自己免疫疾患の内訳は，バセドウ病9.1%，橋本病3.4%であり，1,073名の2型女性でのバセドウ病合併0.9%，橋本病合併1.1%に比べ高頻度であった[6]。

また，1型糖尿病女性における抗TPO抗体陽性率は31.2%[7]，15～20歳の1型糖尿病女性では23.5%と海外で報告されている[8]。1型糖尿病女性の抗TPO抗体陽性率はいずれも，一般女性の同陽性率に比較して高率であり，1型糖尿病女性は潜在性自己免疫性甲状腺疾患を合併している可能性が高い。わが国での報告によると，抗TPO抗体あるいは抗Tg抗体いずれかが陽性である者は，インスリン依存型糖尿病患者102名中51例(50.0%)であり，健常者50名中2例(4%)に比較して非常に高率であった[9]。

Gallasらの，82名の1型糖尿病女性における妊娠前，妊娠中，産後の甲状腺機能異常の頻度を検討した報告によると[10]，妊娠前で11%，妊娠第1三半期に22.5%，第3三半期に18.4%の甲状腺機能異常を認め，多くは潜在性甲状腺機能低下であった(図1)。また，妊娠前に抗TPO抗体陽性のもののほうが陰性のものに比べてTSHの高値を示している。

以上のように，1型糖尿病合併妊娠の場合は，自己免疫性甲状腺疾患の合併率が高いことから，米国産婦人科学会[11]や国際内分泌ガイドライン[12]は，1型糖尿病妊婦における妊娠初期の甲状腺機能スクリーニングを推奨している。

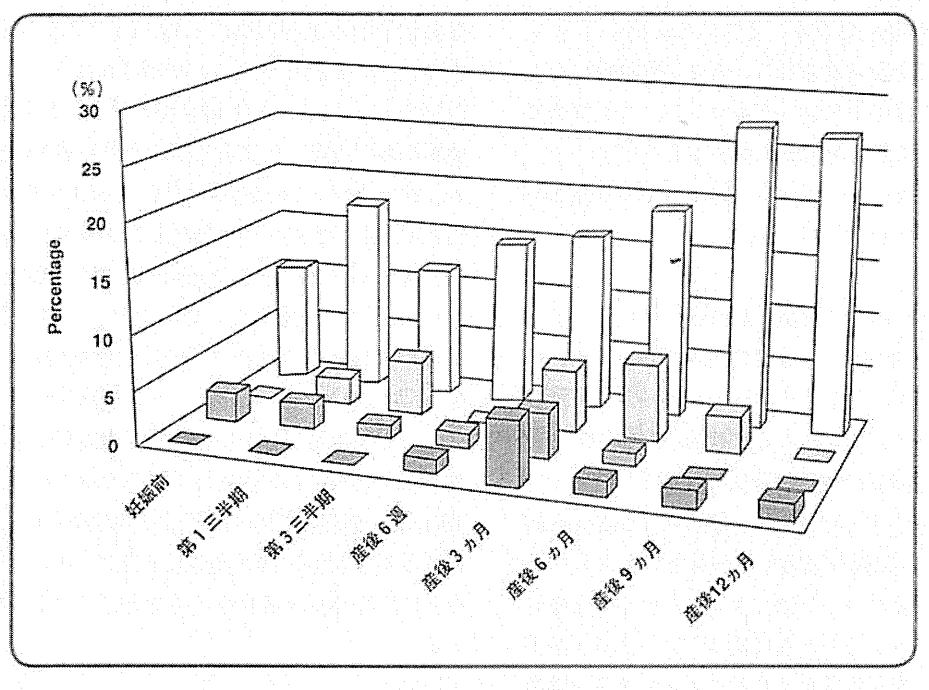

図1．1型糖尿病合併妊婦の妊娠前，妊娠中，産後の甲状腺機能異常の頻度
■：Overt Hyperthyroidism, ▨：Subclinical hyperthyroidism, □：Overt Hypothyroidism, ▨：Subclinical hypothyroidism

（文献10より引用）

Ⅲ．甲状腺機能異常が妊娠中の耐糖能に与える影響

1　甲状腺中毒症と耐糖能異常

甲状腺ホルモンは，腸管からの糖吸収亢進と肝臓での糖新生亢進，また肝臓，末梢組織におけるインスリン抵抗性など引き起こすことにより糖代謝に影響を与えると考えられており，甲状腺ホルモンの過剰は一般的に耐糖能異常の原因となる。妊娠中の甲状腺ホルモン過剰も妊娠糖尿病発症に関与している可能性があるが，妊娠中の甲状腺機能亢進症で妊娠糖尿病が増加するかどうかを検討された報告はほとんどない。日本のある施設からの報告で，妊娠糖尿病40例中3例(7.5%)に甲状腺機能亢進症を合併したことを示されたが[9]，これは前記を裏付ける所見と考えられる。また，妊娠10週をピークに胎盤での絨毛性ゴナドトロピン産生による妊娠性一過性甲状腺機能亢進症を呈する例が，妊婦全体で数パーセント存在する。その時期の糖尿病合併妊婦は，悪阻も関連して，血糖コントロールが不安定にある可能性がある。

1）糖代謝への影響

甲状腺中毒症による耐糖能異常の原因として，消化管でのブドウ糖吸収促進による血糖上昇作用が古くからいわれている[13]。しかし，経静脈ブドウ糖負荷試験でも，経口ブドウ糖負荷試験と同様に，インスリン分泌の亢進と耐糖能異常が認められることから[14]，消化管からのブドウ糖吸収の亢進のみでは説明できない。

次のメカニズムとして，甲状腺ホルモン過剰による内因性の糖産生亢進が考えられる。甲状腺ホルモンは肝細胞のGLUT2，すなわち肝臓での主な糖輸送因子の細胞膜内濃度を増加させる[15][16]。その結果，肝臓の血糖放出が増加し，糖代謝異常の原因になる。さらに，甲状腺中毒症時にみられる脂肪分解増加は，遊離脂肪酸(FFA)増加につながり，それは肝臓の糖新生を刺激する[17]。甲状腺ホルモン過剰によって引き起こされたカテコラミン刺激による脂肪分解が，FFA放出増加の理由の一部として考えられる。さらに，甲状腺ホルモン過剰時には，非酸化的糖処理能は増強

し，その結果乳酸過剰産生となる。それはCori cycleに入ってさらに肝臓の糖新生を亢進させる。また，甲状腺ホルモン過剰の際には，成長ホルモン，グルカゴン，カテコラミンは増加し，間接的に耐糖能異常に影響する[18)-20)]。

これらいくつもの複合のメカニズムによって甲状腺ホルモン過剰は耐糖能異常を引き起こす。

2)ケトン体産生亢進

甲状腺中毒症患者では甲状腺機能正常者に比較し，有意に血中ケトン体が増加する[21)]。糖尿病患者においては，甲状腺ホルモン過剰はさらに糖尿病ケトアシドーシスの誘因となる[22)23)]。甲状腺ホルモンによる直接的な脂肪分解の機序は，脂肪酸合成系酵素の遺伝子発現に重要な転写因子であるSREBP1-1c遺伝子とLPL活性を阻害するangiotensin-like protein 3遺伝子の抑制が原因していると考えられている[24)25)]。また，甲状腺ホルモンは，βアドレナリン系を介して交感神経を亢進させるとともに過剰なグルカゴン分泌を惹起させ，脂肪分解を促進することによってケトン体産生を亢進させる。インスリン作用不足の状況下では，過剰な甲状腺ホルモンによってさらにケトン体産生が促され，甲状腺機能亢進時には，著明な高血糖を呈さずともケトアシドーシスを起こしやすいと考えられている。

バセドウ病と糖尿病性ケトアシドーシスを同時に発症した症例報告がいくつか散見される。甲状腺クリーゼや重篤な合併症，劇症1型糖尿病を除いた症例についての検討では，平均血糖値は358mg/dLであり，甲状腺ホルモン過剰時には，著明な高血糖を呈さずとも糖尿病性ケトアシドーシスを容易に発症する可能性が高く，妊娠中においても注意が必要となる[26)]。

2　甲状腺機能低下症が糖代謝へ与える影響

甲状腺機能低下は肝臓の糖産生率低下の原因となり[27)]，糖尿病患者での甲状腺機能低下症合併はインスリン必要量の減少の原因となりうる。また，甲状腺機能低下症の際にみられる繰り返す低血糖エピソードは，甲状腺ホルモンの補充療法によってその血糖変動を改善させることができる[28)]。

3　顕性および潜在性甲状腺機能低下症とインスリン抵抗性

顕性および潜在性の甲状腺機能低下症時には，インスリン感受性が低下することが近年報告されている。このメカニズムとして，*in vivo* と *in vitro* の研究において，甲状腺機能低下症は末梢組織におけるインスリン刺激による糖利用低下の原因となることが示された[29)-31)]。このことは，最近のメタボリック症候群が健常者に比して潜在的，顕性甲状腺機能低下症患者で頻度が多い報告の説明となりうる。2012年に，Tudelaらは[32)]，24,883名の妊婦において，FT_4は正常値でTSHのみ増加している潜在性甲状腺機能低下症を妊娠前半に呈した妊婦の妊娠糖尿病合併率は6.3%であることを報告した。この値は，FT_4もTSHも正常である甲状腺機能正常妊婦の妊娠糖尿病合併率の4.2%に比較し，有意に高率であった。母体年齢，体重，人種，分娩歴などで調整後は，潜在性甲状腺機能低下の妊娠糖尿病発症リスクは減少し有意差は消失したが，妊娠前半の妊婦のTSH値と妊娠糖尿病発症率は正の相関を示した。これは，妊娠中の潜在性甲状腺機能低下症が，インスリン抵抗性を介して妊娠糖尿病発症リスクを上げている可能性を示している。

Ⅳ．産後の甲状腺機能異常症について

一般的に，潜在的に存在する自己免疫性甲状腺炎が出産後増悪し，産後甲状腺機能異常が発生するといわれている。そのほとんどは，抗甲状腺自己抗体のみが陽性で臨床症状，所見がない潜在性自己免疫性甲状腺炎が出産後増悪して発生するもので，図2に示す5つのタイプに分類される[33)]。最も典型的な臨床経過をとり，しばしば経験されるものは図2のⅢ型で，一過性破壊甲状腺中毒症から一過性甲状腺機能低下症へ移行するものである。出産後に発生した無痛性甲状腺炎であることから，出産後甲状腺炎といわれる。出産後発症するバセドウ病も含めて全体を出産後甲状腺機能異常症といわれる。本症は一般女性の出産後に5～10%の頻度で出現し[34)]，妊娠初期に甲状腺自己抗体陽性であった女性の約6割が産後甲状腺機能異常症を発症するといわれている[35)]。

1型糖尿病患者は，前述のように自己免疫性甲状腺疾患を合併しやすいことから，産後甲状腺機能異常症の合併頻度も一般女性に比較して高い。産後甲状腺炎の一般発生頻度が3.3～8.8%に対し，1型糖尿病では10.5～25%と報告され，一般女性に比較して約3～4倍の発症リスクと考えられる[36)]。82名の1型糖尿病女性における妊娠前，妊

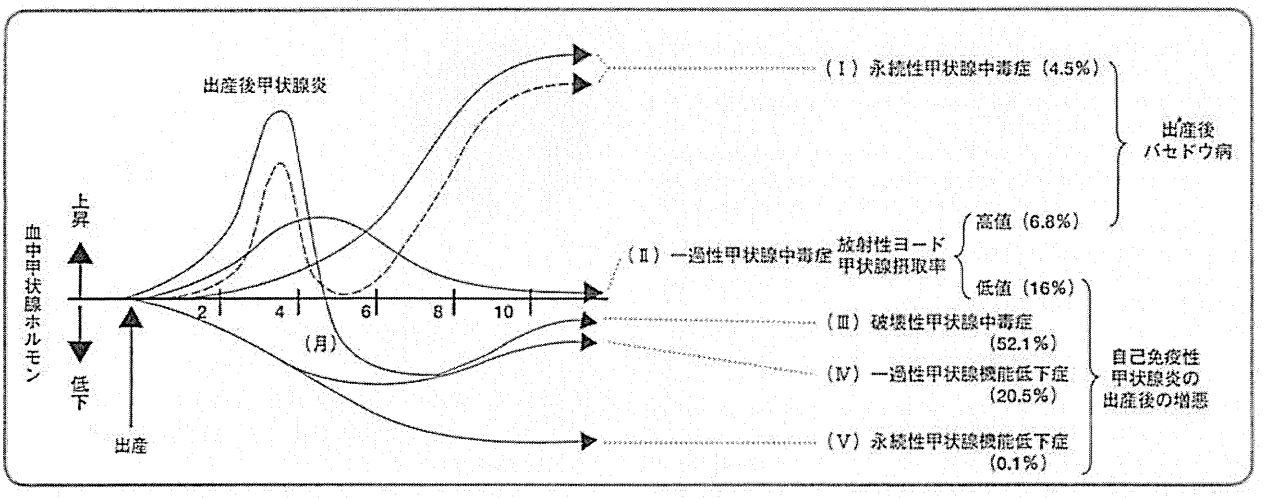

図2．出産後甲状腺機能異常症の5病型

（文献33より引用）

娠中，産後の甲状腺機能異常の頻度を検討した報告結果によると[10]，明らかな産後甲状腺機能異常症は15.9%にみられ，産後甲状腺機能異常症発症者の妊娠初期の抗TPO抗体陽性率は46.2%で，非発症者の15.9%より高値であった。また，妊娠初期のTSH値も中央値で産後甲状腺機能異常発症者の2.2 μU/mLと非発症者の1.1 μU/mLに比較して高値を示した。このように，1型糖尿病患者では，特に抗体陽性者および妊娠初期TSH増加のある例には産後も甲状腺機能異常に留意する必要がある。

おわりに

妊娠初期や産後の甲状腺機能異常は一般健常女性においてもしばしば経験される。とりわけ1型糖尿病患者の場合は自己免疫性甲状腺疾患の合併率が高いことから，妊娠前，妊娠中，産後を通じて甲状腺機能異常への留意が必要である。甲状腺機能異常，特に甲状腺中毒症は妊娠結果に影響するのみならず，妊娠中の耐糖能の悪化やケトアシドーシスの誘因となることから，糖代謝合併妊娠の管理の際には，甲状腺機能の正常化に努めることが重要であろう。

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