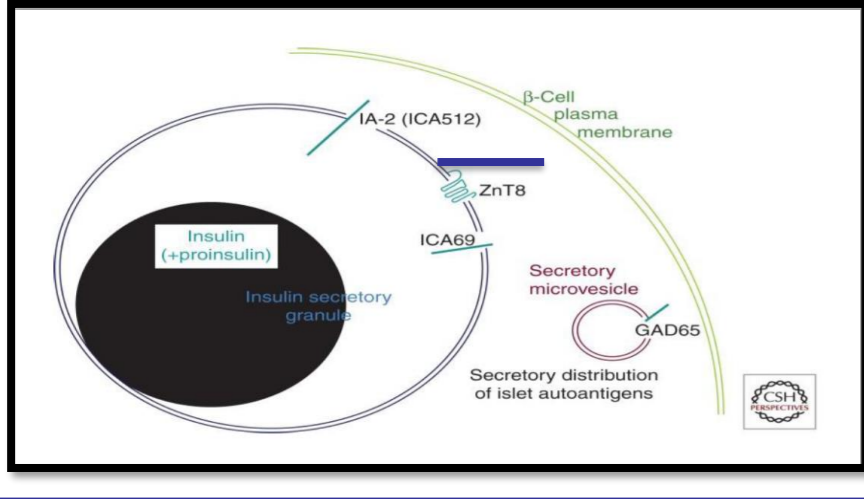


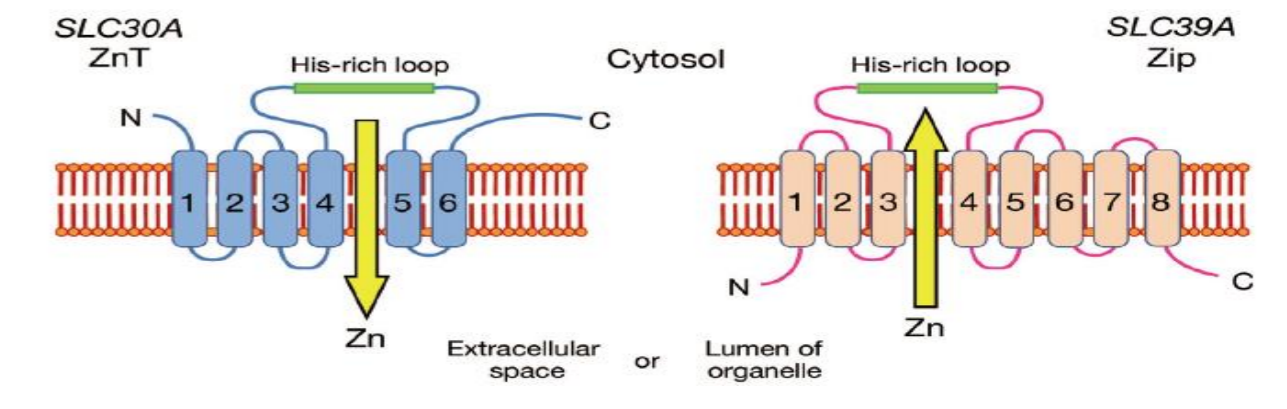


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INTRODUCTION



Zinc is essential for human health due to its unique role in multiple biological processes including promoting immunity, growth, development and reproduction. Zinc has a key role in all cell types for the maintenance of cellular metabolism and gene expression, as well as catalyzing and regulating numerous cellular processes. Cytoplasmic zinc homeostasis is regulated by zinc-transporter proteins (ZnTs), which are products of the *SLC30* gene. To date, 10 ZnT family members (ZnT1-ZnT10) have been identified. ZnTs are expressed in endocrine glands and this suggests that they play a role in regulating zinc concentration in endocrine cells. ZnT8 is a pancreatic islet cell secretory granule membrane protein which is essential for regulating insulin synthesis and secretion. In addition, ZnT8 is an islet cell autoantigen in type 1 diabetes mellitus (T1DM). Autoantibodies (Abs) to ZnT8 (ZnT8Abs) together with autoantibodies to insulin (IABs), glutamic acid decarboxylase (GADAb)s and protein tyrosine insulinoma antigen 2 (IA-2Abs) are markers of autoimmune diabetes mellitus.

Material and Methods

The study group consisted of 44 patients with Graves' disease (GD) (mean age, 14.8 ± 3.1 years; 27% male and 73% female), 65 patients with Hashimoto's thyroiditis (HT) (mean age, 13.3 ± 3.3 years; 12% male and 88% female) and 199 patients with T1DM (mean age, 12.5 ± 4.2 years; 47% male and 52% female) who were hospitalized between 2012 and 2015 in pediatric hospitals in Poland. The control group was recruited from children hospitalized with suspicion of mild heart diseases, but discharged from hospital with diagnoses of functional murmurs or non-cardiovascular syncopes. Serum samples from 58 children (mean age, 13.3 ± 3.5 years; 57% male and 43% female) who had no autoimmune conditions and no family history of autoimmunity were included in the control group.

On the day of sample collection, fasting venous blood was drawn, serum separated and stored at -80 °C.

***ZnT8Abs** were measured by enzyme-linked immunosorbent assay (ELISA) using kits from RSR Ltd. (Cardiff, UK, www.rsrltd.com) and values of **ZnT8Ab ≥15 units/mL** were considered positive.

****GADAbs** were measured by ELISA using kits from RSR Ltd and values of **GADAb ≥5.0** were positive.

*****IA-2Abs** were measured using an immunoprecipitation assay (IPA) based on 125I-labeled IA-2 using kits from RSR Ltd. **IA-2Ab levels >125 WHO units/mL** were considered positive.

******IABs** were measured using an IPA based on 125I-labeled insulin using kits from RSR Ltd. **IAB levels ≥0.4 units/mL** were considered positive.

^Serum 21-hydroxylase autoantibodies (21-OHABs) were measured by an IPA based on 125I-labeled recombinant 21-OH using kits from RSR Ltd. **21-OHAb levels >1.0 unit/mL** were considered positive in the assay according to the kit instructions.

^^Acetylcholine receptor autoantibodies (AChRABs) were measured by IPA using kits from RSR Ltd. and values **≥0.5 nmol/L** were considered positive.

TPOAbs, TgAbs and TRAbs were measured by ECLIA using a Modular Analytics E170 analyzer (Roche Diagnostics). The normal values were 0-34 IU/mL for TPOAb, 0-115 IU/mL for TgAb and 0-1.75 IU/L for TRAb.

RESULTS

Tab.1 Prevalence of Abs in patients with GD, HT and controls

	Graves' disease (GD)	Hashimoto's thyroiditis (HT)	Type 1 Diabetes mellitus (T1DM) (-) AITD	T1DM (+) AITD	Controls
Number	44	65	163	36	58
Female/Male	32/12	57/8	77/86	26/5	25/33
Age, years (mean ± SD)	14.8 ± 3.1	13.3 ± 3.3	12.4 ± 4.1	13.2 ± 4.3	13.3 ± 3.5
Disease duration, years (mean ± SD)	1.3 ± 2.3	2.2 ± 2.1	4.5 ± 3.6	6.4 ± 3.8 (T1DM) 2.1 ± 2.1 (AITD)	N/A
GADAb	4 (9.1%)	4 (6.2%)	108 (66%)	25 (69%)	2 (3.4%)
IA-2Ab	2 (4.5%)	4 (6.2%)	91 (56%)	18 (50%)	0 (0%)
ZnT8Ab	4 (9.1%)	6 (9.2%)	109 (67%)	19 (53%)	2 (3.4%)
IAb	1 (2.2%)	3 (4.6%)	125 (77%)	32 (89)	0 (0%)
21-OHAb	2 (4.5%)	5 (7.7%)	Not tested	1 (3%)	0 (0%)
AChRAB	0 (0%)	0 (0%)	Not tested	0 (0%)	Not tested

Tab.2 Autoantibody-positive AITD patients and controls

Gender	Age, years	Disease duration, years	GADAb, U/mL Pos ≥5.0	IA-2Ab, U/mL Pos >125	ZnT8Ab, u/mL Pos ≥15.0	IAb, u/mL Pos ≥0.4	21-OHAb, u/mL Pos >1.0	AChRAB, nmol/L Pos ≥0.5
Graves' disease patients								
F	17	Recent	Neg	Neg	21.9	Neg	Neg	Neg
F	17	1	31.9	Neg	Neg	Neg	Neg	Neg
F	11	Recent	55.5	213	1078	1.4	Neg	Neg
F	17	1	Neg	Neg	20.5	Neg	Neg	Neg
M	13	0.4	Neg	Neg	Neg	Neg	82.6	Neg
F	13	0.4	Neg	Neg	Neg	Neg	39.7	Neg
M	15	Recent	Neg	150	Neg	Neg	Neg	Neg
F	16	4	1034	Neg	30.3	Neg	Neg	Neg
F	15	Recent	34.1	Neg	Neg	Neg	Neg	Neg
Hashimoto's thyroiditis patients								
F	17	Recent	Neg	Neg	Neg	Neg	78.7	Neg
F	9	8	811	1562	74	50	Neg	Neg
F	16	9	Neg	Neg	49.3	Neg	Neg	Neg
F	16	3	Neg	Neg	23.8	Neg	Neg	Neg
F	17	0.2	Neg	Neg	33	Neg	Neg	Neg
F	12	4	Neg	188	Neg	Neg	Neg	Neg
F	18	4	7.4	6038	1699	Neg	Neg	Neg
F	14	1	198	Neg	Neg	Neg	Neg	Neg
F	14	0.3	Neg	Neg	Neg	Neg	12.9	Neg
F	13	3	6.6	Neg	Neg	36.4	Neg	Neg
F	17	7	Neg	Neg	Neg	Neg	11.9	Neg
F	12	1	Neg	Neg	Neg	Neg	910	Neg
F	11	1	Neg	Neg	Neg	Neg	Neg	Neg
F	8	1	Neg	225	104.1	15.5	Neg	Neg
M	14	2	Neg	Neg	Neg	Neg	15.1	Neg
Controls								
M	17	N/A	23	Neg	Neg	Neg	Neg	Not tested
F	7	N/A	Neg	Neg	18.2	Neg	Neg	Not tested
M	15	N/A	9.6	Neg	Neg	Neg	Neg	Not tested
F	16	N/A	Neg	Neg	27.1	Neg	Neg	Not tested

Tab.3 Characteristics of 21-OHAb-positive children

Patient	Gender	Age, years	Disease duration	Diagnosis	GADAb, U/mL	IA2Ab, U/mL	ZnT8Ab, u/mL	IAb, u/mL	21-OHAb, u/mL	TgAb, IU/mL	TPOAb, IU/mL	Adrenal function
1	M	13	6 months	GD	Neg	Neg	Neg	Neg	82.6	444.06	>1000	Normal
2	F	13	6 months	GD	Neg	Neg	Neg	Neg	39.7	149.83	>1000	Normal
3	F	17	Recent onset	HT	Neg	Neg	Neg	Neg	78.7	80	196.1	Normal
4	F	14	4 months	HT	Neg	Neg	Neg	Neg	12.9	617.9	>600	Adrenal insufficiency
5	F	17	7 years	HT	Neg	Neg	Neg	Neg	11.9	14.3	689.6	Normal
6	F	12	1 year	HT	Neg	Neg	Neg	Neg	910	240	115	Adrenal insufficiency
7	M	14	2 years	HT	Neg	Neg	Neg	Neg	15.1	350	>600	Adrenal insufficiency
8	M	16	2 months	T1DM, GD	2000	150	Neg	22.1	748	39.78	770.19	Normal
Positive cut-off					≥5.0	>125	≥15.0	≥0.4	>1.0	>115	>34	

Conclusions

Overall, our study showed that diabetes-associated autoantibodies including ZnT8Ab were found in children and adolescents with GD and HT, and future studies should be carried out to better understand the pathogenesis of the co-occurrence of thyroid and diabetes autoimmunity.

Ref: Rydzewska M, Michalak J, Bossowska A, Chen S, Black S, Powell M, Furmaniak J, Rees Smith B, Bossowski A. Analysis of diabetes-associated autoantibodies in children and adolescents with autoimmune thyroid diseases J Pediatr Endocrinol Metab. 2019 Apr 24;32(4):355-361