Prevalence and clinical associations of calcium-sensing receptor and NALP5 autoantibodies in Finnish patients with autoimmune polyendocrine syndrome type 1

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Background

- **Autoimmune polyendocrine syndrome type 1 (APS1):** Rare autosomal recessive disorder caused by mutations in the autoimmune regulator (*AIRE*) gene.
- **Major diseases:** Chronic mucocutaneous candidiasis (100%), hypoparathyroidism (80%), and Addison's disease (70%).
- **Pathology:** Chronic inflammation of internal organs; organ-specific and anti-cytokine autoantibodies [1].
- **The calcium-sensing receptor (CaSR):** Highly expressed on the parathyroid **(Figure 1)**; CaSR autoantibodies detected in patients with APS1 [2], but association with hypoparathyroidism is unknown.
- **NALP5:** Parathyroid-expressed autoantibody target may be associated with APS1 hypoparathyroidism, but as yet unclear [3].

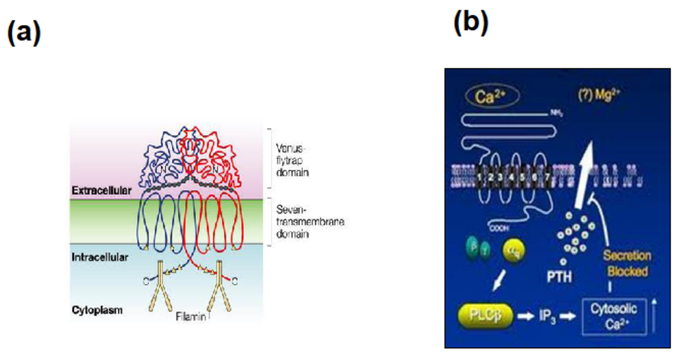

Figure 1: (a) The CaSR is composed of a dimer pair, which is shown in red and blue. The bi-lobed, venus-flytrap domain of the CaSR is modelled on the known crystal structure of the metabotropic glutamate receptor type 1. **(b)** Increases in serum [Ca^{2+}] suppress PTH secretion from the parathyroid as the CaSR signals to increase intracellular [Ca^{2+}] which inhibits PTH exocytosis. Reductions in serum [Ca^{2+}] lead to PTH release which causes uptake of Ca^{2+} by the intestine, release of Ca^{2+} from bone tissue and re-absorption of Ca^{2+} by the kidneys. Consequently, serum Ca^{2+} levels are returned to a normal baseline value. Abnormally elevated activity of the receptor (due to activating mutations or autoantibodies) in the presence of low serum [Ca^{2+}] results in lowering of PTH secretion and resultant hypoparathyroidism and hypocalcaemia.

Aims

- To determine the prevalence of CaSR, NALP5 and cytokine autoantibodies in Finnish APS1 patients.
- To determine *AIRE* genotypes.
- To identify associations between both CaSR and NALP5 autoantibodies and disease components and demographic characteristics.

Patient and study details

- **Participants:** 44 unrelated Finnish APS1 patients (26 female, 18 male; mean age 33 years with range 8-67 years). Clinical disease manifestations are given in **Figure 1**. Controls were 38 healthy individuals (22 females, 16 males; mean age 36 years with range 19-64 years).
- **Study approval:** Approved by the Medical Ethics Committee of Helsinki University Central Hospital. Patients participated after informed consent.

Clinical disease manifestations of APS1 patient group

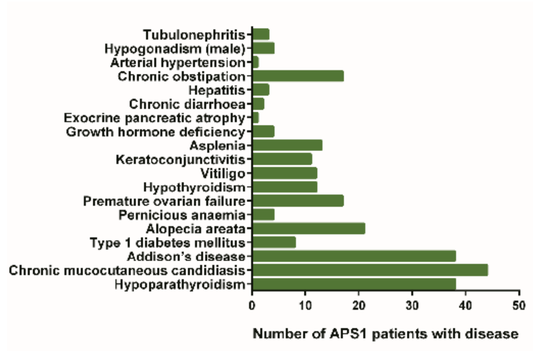

Figure 2: Clinical disease manifestations in APS1 patients. 43/44 (98%) patients had either two or three of the major APS1 disease components. 38/44 patients (86%) had at least one other disease component outside of the classic APS1 triad.

AIRE genotypes

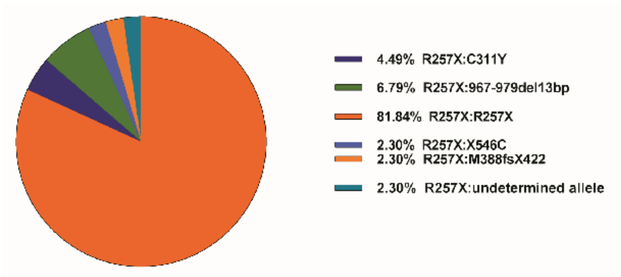

Figure 3: Percentage of APS1 patients with particular *AIRE* genotype. *AIRE* genotypes were determined by PCR and DNA sequencing [5]. At least one *AIRE* gene R257X mutation was present in every APS1 patient. 82% (36/44) of the patients exhibited the common Finnish *AIRE* mutant genotype R257X:R257X.

Autoantibody prevalence

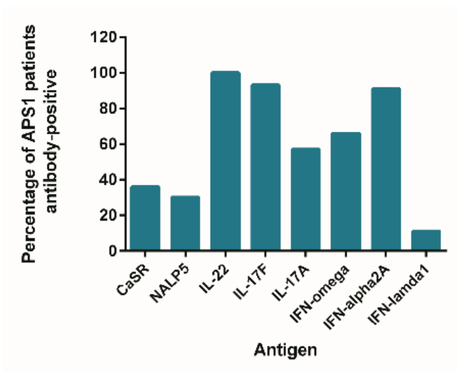

Figure 4: Prevalence of autoantibodies in APS1 patients. Cytokine, CaSR and NALP5 autoantibodies were detected using ELISAs [2], immunoprecipitation assays [3], and radioligand binding assays [4], respectively. All controls were negative for all tested antibodies. Except for IFN-lambda1, all tested autoantibodies were at a significantly increased prevalence in APS1 patients compared with controls (Fisher's exact test - $P < 0.05$).

Associations of CaSR and NALP5 autoantibodies

- Neither CaSR nor NALP5 autoantibodies were associated with hypoparathyroidism (**Table 1**).
- For hypoparathyroidism diagnosis, CaSR autoantibodies - sensitivity of 39% and specificity of 83%; NALP5 autoantibodies – sensitivity of 26% and specificity of 50% (**Table 1**).
- Neither CaSR nor NALP5 autoantibodies were associated with age, sex, or age at disease presentation: all *P* values from comparisons were > 0.05 (Fisher's exact test).
- CaSR autoantibodies were associated with a shorter APS1 disease duration of < 10 years (**Table 1; Figure 5**).

Table 1: Associations of CaSR and NALP5 autoantibodies

Patient detail	CaSR antibody-positive APS1 patients	CaSR antibody-negative APS1 patients	[1]*P* value	NALP5 antibody-positive APS1 patients	NALP5 antibody-negative APS1 patients	[1]*P* value
Hypoparathyroidism	15/38 (39%)	23/38 (61%)	**0.392**	10/38 (26%)	28/38 (74%)	**0.339**
No hypoparathyroidism	1/6 (17%)	5/6 (83%)		3/6 (50%)	3/6 (50%)	
Disease duration						
< 10 years	6/8 (75%)	2/8 (25%)	**0.019**	4/8 (50%)	4/8 (50%)	**0.209**
> 10 years	10/36 (28%)	26/36 (72%)		9/36 (25%)	27/36 (75%)	

[1]*P* values from Fisher's exact test

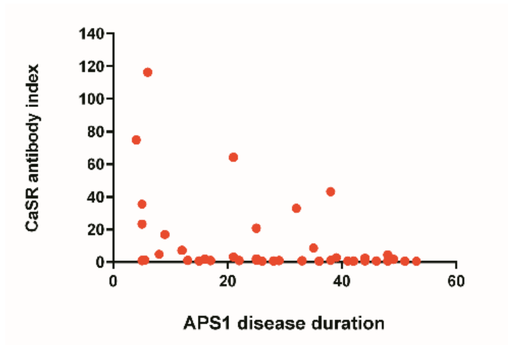

Figure 5: Correlation of CaSR antibody index with APS1 disease duration. Higher CaSR autoantibody indices correlated with a shorter duration of APS1 (Spearman's *r* = -0.452 with 95% confidence intervals: -0.666 to -0.170; *P* = 0.0021).

Conclusions

- Neither CaSR nor NALP5 autoantibodies were specific or sensitive markers for hypoparathyroidism in Finnish APS1 patients.
- Further investigations are required to:
  1. Identify a hypoparathyroidism-associated autoantigen which would allow serologic diagnosis of the disease.
  2. Determine the exact role of CaSR and NALP5 autoantibodies in APS1.
  3. Identify autoreactive T cells against both the CaSR and NALP5 to allow insights into the pathogenic processes leading to impaired parathyroid function in APS1.

References

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