Epitopes, Specificity, IgG Subclasses and Functional Effects of Anti-Calcium-Sensing Receptor Autoantibodies in Patients with Autoimmune Polyendocrine Syndrome Type 1

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Background

- **Autoimmune polyendocrine syndrome type 1** (APS1) is a rare disorder caused by mutations in the autoimmune regulator (*AIRE*) gene [1].
- **Major diseases** are chronic mucocutaneous candidiasis (100% of APS1 patients), hypoparathyroidism (80%), and Addison's disease (70%).
- **Pathology** includes chronic inflammation of internal organs and organ-specific and anti-cytokine (e.g., IFN-α and IFN-ω) antibodies.
- **Autoantibodies** against the **calcium-sensing receptor** (CaSR) **(Figure 1)**, which is highly expressed on the parathyroid**,** are found in 36% of patients with APS1 [2].

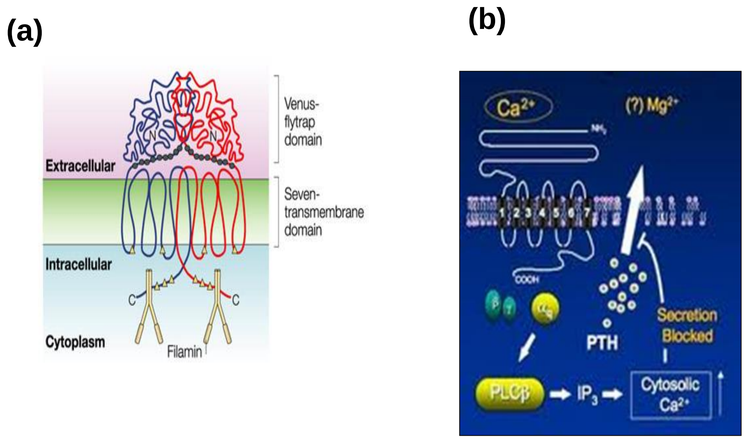

Figure 1: (a) The CaSR is composed of a dimer pair, which is shown in red and blue. The bi-lobed, venus-flytrap domain of the CaSR is modelled on the known crystal structure of the metabotropic glutamate receptor type 1. **(b)** Increases in serum [Ca^{2+}] suppress PTH secretion from the parathyroid 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 caused by activating mutations or stimulating autoantibodies in the presence of low serum [Ca^{2+}] results in lowering of PTH secretion and resultant hypoparathyroidism and hypocalcaemia.**

Aims

To characterise anti-CaSR autoantibodies in APS1 patients in relation to:-

- Epitopes (binding sites)
- Specificity
- IgG subclass
- Effects on CaSR function

Patient and study details

- **Participants:** 16 unrelated APS1 patients (8 female, 8 male; mean age 28 years with range 9-51 years). 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 written informed consent.

Details of APS1 patients

***AIRE* mutations:** 15 R257X homozygotes; 1 R257X/967-979del13 compound heterozygote.
Antibodies against: IFN-ω, 10/16 APS1 patients; IFN-α, 15/16; IFN-λ, 2/16; IL-22, 16/16; IL-17F, 14/16; IL-17A, 13/16; CaSR, 16/16.
Disease components: chronic mucocutaneous candidiasis, 16/16 APS1 patients; hypoparathyroidism, 15/16; Addison's disease, 16/16; alopecia 6/16; vitiligo, 2/16; keratitis, 5/16; hypogonadism, 6/16; type 1 diabetes mellitus, 4/16; autoimmune thyroid disease, 2/16.

Anti-CaSR autoantibody epitopes

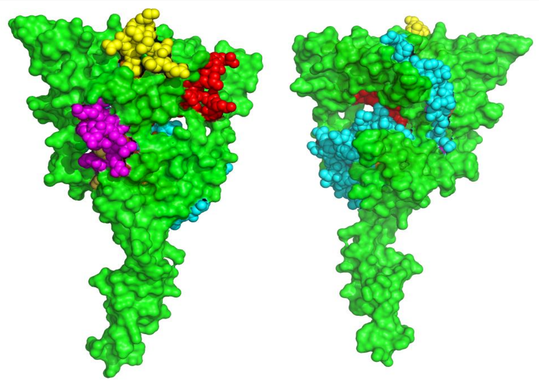

Figure 2: All epitopes were identified by phage-display and CaSR peptide ELISAs, and were in the CaSR extracellular domain (ECD). Autoantibodies against **epitope 1** (amino acids 41-69) found in 16/16 (100%) patients; **epitope 2** (amino acids 114-126) - 5/16 (31%); **epitope 3** (amino acids 171-195) - 6/16 (38%); **epitope 4** (amino acids 260-340) - 7/16 (44%).

Anti-CaSR autoantibody IgG subclass

- Investigated in CaSR peptide ELISAs with IgG subclass-specific secondary antibodies.
- Anti-CaSR autoantibodies recognising epitope 1 (41-69), epitope 3 (171-195), and epitope 4 (260-340) were of the **IgG1 subclass**.
- Anti-CaSR autoantibodies recognising epitope 2 (114-126) were of the **IgG1 and IgG3 subclasses**.

Anti-CaSR autoantibody specificity

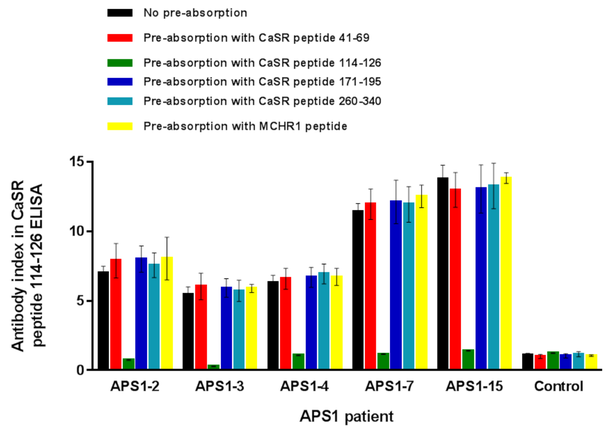

Figure 3: APS1 patient sera were pre-absorbed with a panel of CaSR peptides prior to measuring CaSR binding reactivity in a specific CaSR peptide ELISA. The results are shown for antibody binding in a CaSR peptide 114-126 ELISA which indicated that antibodies against epitope 2 were **specific** for that binding site. Similar results were obtained for all four identified epitopes.

Anti-CaSR autoantibody functional effects

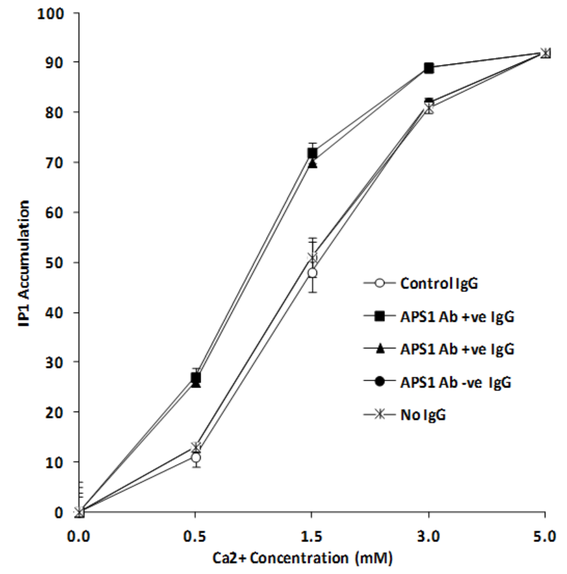

Figure 4: Effect of APS1 patient IgG on the response of the CaSR to Ca^{2+} stimulation by measuring inositol phosphate (IP1) accumulation in HEK293-CaSR cells. The results showed that IgG from two patients **stimulated significantly IP1 accumulation** when compared with control IgG at [Ca^{2+}] of 0.5, 1.5 and 3 mM (P values < 0.05).

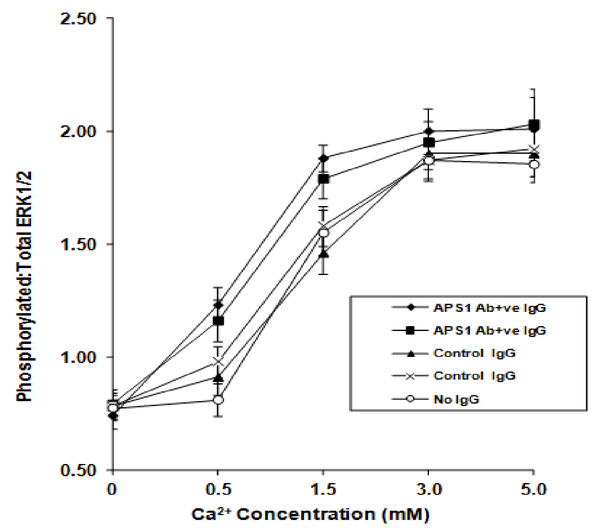

Figure 5: Effect of APS1 patient IgG on the response of the CaSR to Ca^{2+} stimulation by measuring ERK1/2 phosphorylation in HEK293-CaSR cells. The results showed that IgG from two patients **stimulated significantly ERK1/2 phosphorylation** when compared with control IgG at [Ca^{2+}] of 0.5, 1.5 and 3 mM (P values < 0.05).

Conclusions

- Anti-CaSR autoantibody **binding sites** are located in the surface accessible **ECD** of the receptor.
- Anti-CaSR autoantibodies are mainly of the **IgG1 subclass**. This subclass of antibody can activate complement and bind to Fcγ receptors and therefore cause cellular damage. This aspect requires further investigation in relation to the parathyroid.
- A minority of APS1 patients have anti-CaSR autoantibodies that can **activate** the CaSR. Further studies are required to determine if these CaSR-stimulating antibodies can prevent PTH secretion from parathyroid cells.

References

[1]. Ahonen et al. N Eng J Med 1990;322:1829-1836.
[2]. Kemp et al. J Endocrinol Metab 2014; 99:1064-71.

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