Low frequency of pendrin autoantibodies detected using a radioligand binding assay in patients with autoimmune thyroid disease

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Background and Aim

•Autoimmune thyroid disease (ATD), encompassing autoimmune hypothyroidism (AH) and Graves' disease (GD), is caused by autoreactivity against several autoantigens: thyroglobulin (TG), thyroid peroxidase (TPO), the thyroid-stimulating hormone receptor (TSHR) and the sodium-iodide symporter. The existence of other autoantigens is possible as autoimmune processes escalate during chronic tissue damage.

•Pendrin is a transmembrane protein located at the apical end of the thyrocyte and mediates iodide efflux through the thyroid cell into the colloidal space. Individuals with Pendred syndrome, which results in abrogation of thyroid iodide transport, feature mildly impaired thyroid function.

•Autoantibodies against pendrin have been reported in 81% of ATD patients and 9% of controls using an immunoblotting method. This technique, however, is largely qualitative in nature and can lack reproducibility of results. In contrast, pendrin autoantibodies could not be detected in ATD using an ELISA or immunofluorescence.

•Given the discrepancy in previous studies, the aim of the present study was to screen a panel of ATD patient sera for pendrin autoantibodies using a novel RBA.

Methods

1. Participants

•71 unrelated patients (63 female, 8 male; mean age: 37±14 yr) with GD defined by the presence of documented biochemical hyperthyroidism in combination with either: 1) a diffuse goiter on a scan, 2) positive for autoantibodies against TSHR, TG, or TPO, 3) Graves' ophthalmopathy, or 4) confirmation of a lymphocytic infiltrate in thyroid histology. 16 patients were newly diagnosed and untreated, and 55 had been treated with anti-thyroid drugs. Subsequent to anti-thyroid drug treatment, 11 patients had received radio-iodine, three had undergone a thyroidectomy and one had received both radio-iodine and surgery. 12 patients had another autoimmune disease. 66 unrelated patients with AH (62 female, 4 male; mean age: 42±14 yr) defined as documented biochemical hypothyroidism and either: 1) positive autoantibodies to TG or TPO, 2) histological confirmation of a lymphocytic infiltrate in a fine needle aspirate, or 3) presence of a goiter on clinical examination. All patients were euthyroid on thyroxine replacement. 11 patients had an additional autoimmune disease. 28 healthy individuals (10 male, 18 female; mean age, 34±11 yr) were used as controls.

•The study was approved by the Sheffield Research Ethics Committee, Sheffield, UK, and all subjects gave informed consent.

2. Radioligand binding assay for pendrin autoantibodies

•[^{35}S]-methionine-labelled pendrin was produced from pcDNA3-PDS (encoding pendrin) in a TnT® T7-Coupled Reticulocyte Lysate System (Promega) (Fig. 1a).

•For RBAs, *in vitro* translated pendrin, equivalent to 100,000 cpm of TCA-precipitable material, was suspended in 50 µl of immunoprecipitation buffer . In duplicate, patient or control sera were added to a final dilution of 1:100. Anti-pendrin antibody sc-16894 (Santa Cruz Biotechnology, Inc.) was a positive control.

•Following overnight incubation at 4°C, 50 µl of protein G Sepharose 4 Fast Flow (GE Healthcare UK, Ltd.) were added and incubated for 1 h at 4°C. Protein G Sepharose-antibody complexes were collected by centrifugation and washed extensively. Immunoprecipitated radioactivity was evaluated by scintillation counting.

•A pendrin antibody (Ab) index for each serum was: cpm immunoprecipitated by tested serum/mean cpm immunoprecipitated by 28 healthy control sera. Each serum was tested in duplicate in at least two experiments and the mean pendrin Ab index calculated.

•The upper limit of normal for the assay was: the mean pendrin Ab index + 3SD of the population of 28 healthy individuals. Serum samples with a pendrin Ab index above the upper limit of normal were designated as positive.

•Comparisons of the prevalence of pendrin autoantibodies in patient and control groups were carried out using Fisher's exact test for 2x2 contingency tables. Pendrin Ab indices were compared using paired Student's *t* tests. *P* values (two-tailed) <0.05 were regarded as significant.

•To determine pendrin autoantibody titres, pendrin autoantibody-positive patient sera were analysed in RBAs at final dilutions of 1:100, 1:200, 1:500, 1:1000 and 1:2000.

•To confirm the antigen specificity of pendrin autoantibodies, pendrin autoantibody-positive patient sera were pre-absorbed with non-radiolabelled pendrin, tyrosinase or melanin-concentrating hormone receptor 1 (MCHR1), which were produced from plasmids pcDNA3-PDS, pcDNA3TYR and pcMCHR1, respectively, in an Expressway *In Vitro* Protein Synthesis System (Invitrogen). Sera were pre-incubated in immunoprecipitation buffer at a 1:200 dilution at 37°C for 2 h with 1, 2, 5, 7.5 and 10 µg of non-radiolabelled ligand. Pre-absorbed samples were spun at 20,000 *g* for 30 min at 4°C, before analysis in the RBA. Pendrin autoantibody-positive patient sera without pre-absorption were included in each assay set.

Results

1. Radioligand binding assays for autoantibodies against pendrin

•Sera from GD (*n*=71) and AH (*n*=66) patients, and healthy controls (*n*=28) were evaluated for pendrin autoantibodies in RBAs. A pendrin Ab index was determined for each serum sample (Fig. 1b).

•Anti-pendrin antibody was included in each assay set as a positive control and displayed a pendrin Ab index of 10.8 ± 1.43.

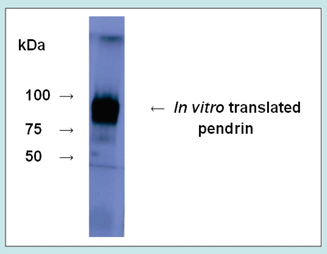
•The intra- and inter-assay coefficients of variation were 6.0% and 9.2%, respectively.

•The upper limit of normal for the RBA was estimated as a pendrin Ab index of 1.41.

•None of the healthy individuals was positive for autoantibodies against pendrin. For ATD patients, 7/71 (9.9%) of the GD and 5/66 (7.6%) AH patient sera, respectively, were positive for pendrin autoantibodies. Of 16 untreated and 55 treated GD patients, 1 (6.3%) and 6 (10.9%), respectively, had pendrin autoantibodies.

•The prevalence of pendrin autoantibodies did not differ significantly between the ATD patient cohorts and the healthy control group (*P*=0.186 and 0.317 for GD and AH patients, respectively) nor between the GD and AH patient groups (*P* 0.766).

(a)



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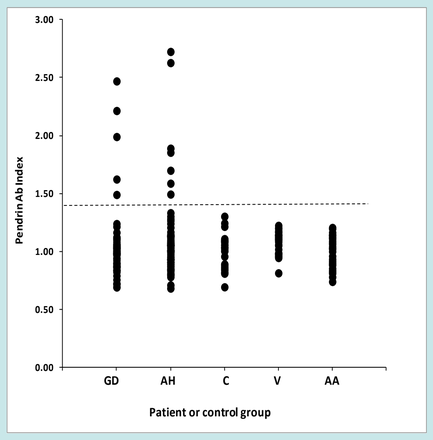


FIG. 1 (a) *In vitro* translated pendrin analysed by SDS-PAGE and autoradiography. (b) Radioligand binding assays for autoantibodies against pendrin in patients and controls. Pendrin Ab indices are shown for GD (*n*=71) and AH (*n*=66) patient sera and control sera (*n*=28). The upper limit of normal of 1.41 for the RBA is indicated by the *dotted line*.

2. Pendrin autoantibody titres

•Immunoreactivity reactivity against pendrin could be detected in 3/5 AH and 5/7 GD patient sera at a dilutions of up to 1:500 (as determined from a pendrin Ab index above the upper limit of normal for the RBA) (Fig. 2a).

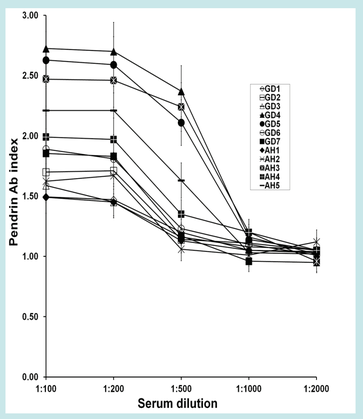
•For 2/5 AH and 2/7 GD patients, pendrin autoantibodies were detected in serum dilutions of up to 1:1000 (Fig. 2a).

3. Pendrin autoantibody specificity

•Pre-absorption with increasing amounts of non-radiolabelled pendrin reduced pendrin autoantibody binding of all 12 pendrin autoantibody-positive ATD patient sera in the RBA (Fig. 2b): pendrin Ab indices of serum samples pre-absorbed with pendrin were significantly lower than those of unabsorbed sera (*P* values were < 0.05).

•Pre-absorption of pendrin autoantibody-positive ATD patient sera with either tyrosinase or MCHR1 did not affect pendrin autoantibody binding in the RBA (data not shown).

(a)



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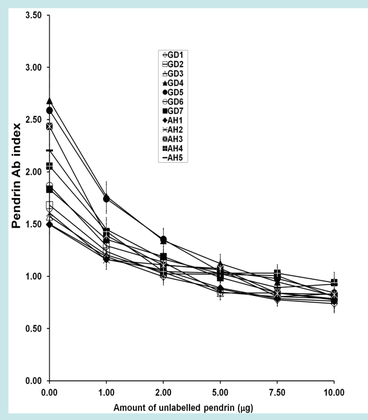

FIG. 2. (a) Pendrin autoantibody-positive GD (*n*=7) and AH (*n*=5) patient sera were evaluated for pendrin autoantibodies in RBAs at dilutions ranging from 1:100 up to 1:2000. The pendrin Ab index (± SD) at each dilution is shown for each patient serum sample and is the mean of two experiments. (b) Pendrin autoantibody-positive GD (*n*=7) and AH (*n*=5) patient sera were pre-absorbed with non-radiolabelled pendrin before analysis of pendrin autoantibody binding in the RBA. The pendrin Ab index (± SD) of each patient serum sample is shown for sera pre-absorbed with non-radiolabelled pendrin (1-10 µg) and is the mean of two experiments.

4. Associations of pendrin autoantibodies

•In both AH and GD patient groups, there were no associations between the presence of pendrin autoantibodies with patient sex, the presence of TPO or TG autoantibodies or the presence other autoimmune diseases (*P*>0.05).

•In the case of GD, there were no associations between the presence of pendrin autoantibodies and whether the patients were untreated or had received anti-thyroid drugs, radio-iodine or a thyroidectomy (*P*>0.05).

Summary of Findings

•A novel radioligand binding assay for pendrin autoantibodies was designed.

•Pendrin autoantibodies were found occur at a low prevalence in patients with ATD.

Acknowledgment

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