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Association between autoimmune thyroid disease and Familial Alzheimers disease

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Summary

OBJECTIVE To determine the prevalence of autoimmune thyroid disease in Familial Alzheimer's Disease kindreds and to ascertain whether there is any evidence for genetic linkage between the two conditions.

DESIGN Retrospective study of Familial Alzheimer's Disease kindreds.

PATIENTS Seventy affected and unaffected family members from 12 kindreds.

MEASUREMENTS Anti-thyroglobulin and anti-microsomal autoantibody status was determined using an enzyme-linked immunosorbent assay. Thyrotrophin levels were determined by an immunoradiometric assay.

RESULTS Of the family members, 41·4% had evidence of autoimmune thyroid disease, with significant co-segregation between the presence of thyroid autoantibodies and the development of Alzheimer's disease ($P < 0{\cdot}01$).

CONCLUSIONS This study demonstrates a very high prevalence of autoimmune thyroid disease in Familial Alzheimer's Disease kindreds and suggests that a genetic factor contributing towards the development of autoimmune thyroid disease may be located on chromosome 21 within close proximity to the Familial Alzheimer's Disease gene.

Genetic factors are important in the aetiology of Alzheimer's disease, (AD); there is an increased frequency of the disease amongst patients' relatives (Heston *et al.*, 1981; Heyman *et al.*, 1984) and several large kindreds of familial Alzheimer's disease (FAD) demonstrating autosomal dominant inheritance have been reported (Cooke *et al.*, 1979; Nee *et al.*, 1983). Observations that patients with Down's Syndrome (DS) surviving over 40 years invariably develop clinical and neuropathological manifestations of AD (Burger & Vogel, 1973) suggested that the genetic defect in AD might be on chromosome 21. Subsequent genetic linkage studies (St George-Hyslop *et al.*, 1987; Goate *et al.*, 1989) in FAD kindreds have localized the gene for FAD to the long arm of chromosome 21 (Fig. 1). Over-expression of this gene in DS (trisomy 21) is thought to be responsible for the early development of the histopathological features of AD (Editorial, 1987).

There is a well recognized association between DS and autoimmune thyroid disease (AITD) (Burgio *et al.*, 1966; Lobo *et al.*, 1980); anti-thyroid auto-antibodies (anti-thyroglobulin (Tg) and anti-microsomal (TMA)) have been reported in up to 30% of children with DS (Sare *et al.*, 1978) and biochemical evidence of hypothyroidism detected in up to 17% (Fialkow *et al.*, 1971). As in the case of AD, these associations with DS suggest that an abnormality on chromosome 21 may be important in the development of AITD.

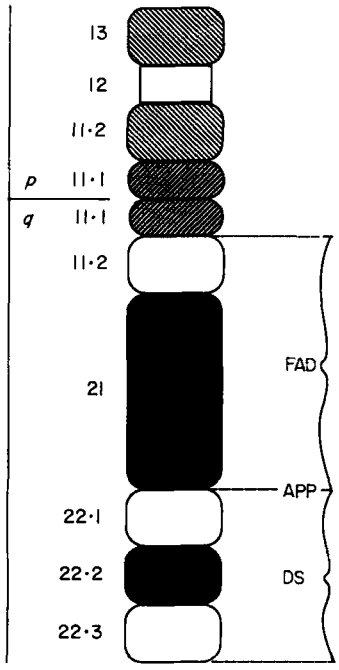

Fig. 1 Diagrammatic representation of chromosome 21 showing obligate DS segment (DS), location of the gene for the amyloid precursor protein (APP) and region believed to encompass the FAD gene (FAD).

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To investigate whether this putative genetic factor is linked to the gene for FAD we looked for evidence of AITD (thyroid auto-antibodies and abnormal thyroid function) in a large group of FAD kindreds.

Materials and methods

Families

Serum samples were obtained from FAD kindreds. These were families in which two or more living individuals were clinically diagnosed as having AD, according to National Institutes for Neurological and Communicative Disorders and Stroke (NINCDS) criteria (McKhann *et al.*, 1984). In addition, in the majority of families the diagnosis has been subsequently confirmed histologically. The dementia was all of early onset (<65 years) and in the majority of cases symptoms were manifest before the age of 50. A total of 70 members from 12 kindreds were studied, having a mean age of 48 years (range 29–84).

Thyroid auto-antibodies

Test sera were assayed for anti-thyroglobulin (Tg) and anti-microsomal (TMA) auto-antibodies by an enzyme-linked immunosorbent assay (ELISA). ELISA plates (Linbro, Flow Laboratories) were coated with 200 μl Tg or TMA, derived from Graves' thyroid glands (Weetman *et al.*, 1984), at 10 μg/ml in carbonate–bicarbonate buffer (pH 9·6), incubated overnight at 4°C and then washed with PBS–Tween (pH 7·4). Each well was filled with 200 μl of 1:100 dilution (in PBS–Tween) of control or test serum and incubated for 2 hours at room temperature prior to washing. Two hundred microlitres of alkaline phosphatase-conjugated anti-human IgG (Sigma, Poole), diluted 1:1000 in PBS–Tween, was added and the plates were incubated for a further 2 hours at room temperature and then washed. *p*-Nitrophenylphosphate (Sigma, Poole) at 1 g/l in diethanolamine buffer, was added (200 μl) to each well and the optical density (OD) was recorded at 490 nm after 30 minutes using a micro ELISA plate reader (Titertek Multiscan MCC/340, Flow Laboratories). Each sample was assayed in triplicate and test samples regarded as positive if they gave an OD value >2SDs above the mean value for the 50 control sera. The control serum samples were obtained from 50 hospital in-patients with no known personal or family history of thyroid or other autoimmune disease. The purpose of the control sera was to provide a negative control for the ELISA against which the presence of autoantibodies in the test sample could be assessed.

Table 1 Auto-antibody (Tg and TMA) status of patients and unaffected relatives in FAD kindreds

	Antibody+	Antibody−	Total
FAD	9 (82%)	2	11
Unaffected relatives	20 (34%)	39	59
	29	41	70

$\chi^2 = 8{\cdot}77$, $P < 0{\cdot}01$.
95% confidence interval: 8–44%.

Thyroid function tests

Thyroid stimulating hormone (TSH) was measured in all samples by an immunoradiometric assay (Sucrosep, Celltech Diagnostics Ltd, Cambridge). In samples with a suppressed TSH (TSH <0·4 mU/l) the free tri-iodothyronine (FT3) levels were assayed by an analogue radioimmunoassay method (Amerlex M, Amersham International).

Statistics

The distribution of thyroid autoantibodies or the presence of hypothyroidism in the patients with FAD compared to their unaffected relatives was assessed by the Chi-squared test. Assay (ELISA) precision was studied looking at the coefficient of variation of the OD results.

Results

The 70 members of FAD kindreds comprised 32 females and 38 males; 11 (seven female, four male) had Alzheimer's disease (mean age 59 years, range 39–84), and there were 59 unaffected relatives (mean age 46 years, range 29–79) at the time of the study. Serum samples from 29 (41·4%) of 70 family members were positive for anti-thyroid antibodies (Tg and/or TMA); 27 (38%) were anti-TMA positive, and 12 (17%) were anti-Tg positive. Two (3%) of the family members had (previously unrecognized) biochemical evidence of hypothyroidism (TSH >5·5 mU/l), and a further two (3%) were on thyroid replacement therapy for previously documented hypothyroidism. Thus four (6%) had treated or newly diagnosed hypothyroidism. Subjects with positive autoantibody results were detected in nine of the 12 kindreds studied (75%), and those with treated or newly diagnosed hypothyroidism were from two of the 12 kindreds (17%).

The 11 patients who had manifest FAD at the time of the study included nine (82%) who were auto-antibody positive (Table 1) and two (18%) who were biochemically hypothyroid or already on replacement therapy (Table 2). Twenty

Table 2 Thyroid status of patients and unaffected relatives

	Thyroid dysfunction	Normal thyroid function	Total
FAD	2 (18%)	9	11
Unaffected relatives	2 (3·4%)	57	59
	4	66	70

$\chi^2 = 2{\cdot}5$, $0{\cdot}1 < P < 0{\cdot}05$.
95% confidence interval: 0–28%.

(34%) of the, as yet, unaffected relatives were auto-antibody positive (Table 1), and two (3·4%) had treated or newly documented hypothyroidism (Table 2). Only two patients had suppressed TSH levels; both were on thyroxine replacement therapy and had normal FT3 levels. In the ELISA the within-assay and between-assay coefficients of variation were 6 and 8% respectively.

Discussion

The prevalence of AITD (defined as the presence of antibodies to TMA or Tg, with or without an elevated TSH) has been determined in several population surveys, with the highest values of 14% (Tunbridge *et al.*, 1977) and 15% (Hawkins *et al.*, 1980) being recorded in middle-aged females. A recent study (Lazarus *et al.*, 1984) looking specifically at an elderly population (> 70 years) detected the presence of thyroid autoantibodies in 18·6%. Thus several large studies in the United Kingdom have demonstrated evidence of AITD in less than 20% of the population. The prevalence of thyroid autoantibodies in the patient sample was compared with the expected population prevalence rather than that of non-affected relatives because some of these may go on to manifest AD.

Our results demonstrate a very high prevalence of AITD (41%) in FAD kindreds. This is substantially higher than would be predicted for a similar age and sex-matched population using the known population data. It could be argued that the higher incidence of AITD detected in our FAD kindreds could be explained by a high prevalence of thyroid disease in some of the pedigrees leading to a higher than expected total incidence. However, as patients with positive thyroid autoantibodies were detected in the majority of the families (75%), this is not the case. The known strong association of AITD with DS, and the localization of the 'FAD gene' to the long arm of chromosome 21 proximal to the obligate DS region (St George-Hyslop *et al.*, 1987; Goate *et al.*, 1989) (Fig. 1) suggests that this chromosome is of importance in the link between FAD and AITD. Our results show there is an increased prevalence of AITD in patients with FAD (82%) compared to their, as yet, unaffected relatives (34%). This demonstrates statistically significant co-segregation between FAD and AITD suggesting that any contributory genetic loci are closely linked. It could be argued that, as the mean age of auto-antibody positive unaffected family members (46 years) is lower than that of their affected relatives (59 years), a proportion of them may yet go on to manifest FAD, thus increasing the statistical evidence for genetic linkage. The observed increased prevalence of hypothyroidism (treated and newly diagnosed) in patients with FAD (18%) compared to their unaffected relatives (3·4%) could be, at least partially, explained by the increased age of these patients compared with their unaffected relatives. These findings support and extend previous work (Heyman *et al.*, 1984) which, in a small family study of AD, demonstrated biochemical evidence of thyroid dysfunction in 19% ($n = 9$) of female probands and 12% ($n = 9$) of their sisters as compared to only 6% ($n = 5$) of their spouses' sisters. A recent post-mortem study of elderly patients with AD (Lopez *et al.*, 1989), looking only at pathological and retrospective clinical features, failed to demonstrate any increased prevalence of thyroid disease compared to age-matched controls.

In addition to AITD, patients with DS have an increased risk of developing other organ-specific autoimmune disorders (alopecia areata, vitiligo (Duvivier & Munro, 1975) and chronic active hepatitis (McCulloch *et al.*, 1982)), as well as having an increased susceptibility to infections (Levin *et al.*, 1979) and risk of developing leukaemia (Miller, 1970). These factors suggest a deficiency in cell mediated immunity (CMI), as evidenced by documented histological changes in the thymus (Miller, 1970), thymic hormone deficiency (Fabris *et al.*, 1984) and diminished number and function of peripheral T cells (Wisnewski *et al.*, 1979). The implication is that trisomy 21 is associated with a deficiency in CMI. Our study would suggest that there may be a genetic factor on chromosome 21 in close proximity to the FAD gene responsible for the deficiency in CMI predisposing to AITD. Theoretical candidates would include the factor, encoded by chromosome 21, believed (Rabinowe *et al.*, 1989; Jung *et al.*, 1987) to be involved in the signal transduction of the response to interferon—a lymphokine known to induce aberrant class II antigen expression by thyrocytes which may have a critical role in the development and/or maintenance of AITD (Bottazzo *et al.*, 1983).

It will be important to ascertain in the future whether this very high prevalence of AITD is a feature of sporadic as well as familial AD, and whether cases of DS secondary to balanced translocations involving various segments of chromosome 21 are also associated with autoimmunity.

Such information will further help to localize genetic factors involved in AITD.

In summary, our results demonstrate a very high prevalence of AITD in FAD kindreds which is more than double that which would be expected from population studies. We have also shown significant co-segregation between the two conditions suggesting that genetic factors on chromosome 21 are of importance in the development of AITD and may be located close to the FAD gene.

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