Premature Ovarian Failure in Patients with Autoimmune Addison’s Disease: Clinical, Genetic, and Immunological Evaluation


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**Design:** The design of the study was to investigate the prevalence of the following: 1) premature ovarian failure (POF) in patients with autoimmune Addison’s disease (AD); 2) steroid-producing cell antibodies (StCA) and steroidogenic enzymes (17α-hydroxylase autoantibodies and P450 side-chain cleavage enzyme autoantibodies) in patients with or without POF; and 3) the value of these autoantibodies to predict POF.

**Patients:** The study included 258 women: 163 with autoimmune polyendocrine syndrome type 2 (APS-2), 49 with APS-1, 18 with APS-4, and 28 with isolated AD.

**Methods:** StCA were measured by an immunofluorescence technique and 17α-hydroxylase antibodies and P450 side-chain cleavage enzyme autoantibodies by immunoprecipitation assays.

**Results:** Fifty-two of 258 women with AD (20.2%) had POF. POF was diagnosed in 20 of 49 (40.8%) with APS-1, six of 18 (33.3%) with APS-4, 26 of 163 (16%) with APS-2, and none of 28 with isolated AD. In patients with APS-1 and APS-4, POF developed after AD, whereas it preceded AD in patients with APS-2. StCA were detected in 31 of 43 with POF (72%) and 51 of 198 without POF (25.7%). StCA were present in 22 of 38 with APS-1 (57.9%) (11 of 13 with POF); in five of 13 with APS-4 (38.5%) (three of four with POF); in 53 of 162 with APS-2 (32.7%) (17 of 26 with POF), and in one of 28 isolated AD patients (3.6%). Twelve of 13 patients with POF with a duration less than 5 yr (92.3%) and 18 of 25 with duration longer than 5 yr (72%) were StCA positive. Twenty-eight of 31 with POF (90.3%) were positive for at least one steroidogenic antibody. Forty-one women with AD less than 40 yr were followed up for a mean period of 9 yr. Eight of 21 women (38%) positive or seroconverted for steroidogenic autoantibodies developed POF at a mean age of 23 yr (six with APS-1, one with APS-2, and one with APS-4), and none of the 20 patients negative for steroidogenic autoantibodies developed POF.

**Conclusions:** This study indicates that AD is frequently associated with POF and that steroidogenic antibodies are markers of patients with POF. Steroidogenic autoantibodies are predictive markers of POF in patients with AD. (*J Clin Endocrinol Metab* 96: E1255–E1261, 2011)
gery, irradiation, or medication that could have had an effect on ovarian function (4). Autoimmune POF may be associated with AD or other autoimmune diseases, or it may be isolated (3).

Antibodies to steroid-producing cells (StCA) are found by immunofluorescence (IF) tests (5) in the majority of patients with AD and POF (6–9). In contrast, patients with isolated POF or POF associated with other autoimmune diseases (excluding AD) are rarely StCA positive or if positive are generally diagnosed with subclinical or potential AD (10, 11). Furthermore, there is a close correlation between the presence of StCA and a lymphocytic infiltration of the ovarian tissue (12).

In patients with autoimmune AD who do not have POF, StCA positivity was found to be helpful in the prediction of development of POF, however, only in patients with autoimmune polyendocrine syndrome (APS) type 1 (13, 14).

The main gonadal autoantigens recognized by StCA in patients with POF associated with AD are steroid 17α-hydroxylase (17α-OH) and cytochrome P450 side-chain cleavage enzyme (P450scc) (15). Measurement of autoantibodies to 17α-hydroxylase (17α-OHAb) and to P450scc (P450sccAb) by immunoprecipitation assays (IPA) (16) in different patient groups confirmed that 17α-OH and P450scc are the main targets of StCA in patients with POF and AD (9, 10, 11).

We have studied the prevalence of POF in a cohort of Italian females with AD referred to our clinic from 1971 to 2009. Furthermore, we have measured StCA (using an IF test) and 17α-OHAb and/or P450sccAb (using IPA) in patients with and without POF. Autoantibody-positive patients without POF were followed up to assess whether the presence of StCA, 17α-OHAb, and/or p450sccAb was predictive for POF.

Materials and Methods

Patients

In all, 258 women with autoimmune AD were included in the study. Neufeld's criteria (1, 17) for APS were used to classify the patients to APS-1 group when AD was associated with chronic candidiasis and/or chronic hypoparathyroidism, APS-2 when AD was associated with thyroid autoimmune diseases and/or type 1 diabetes mellitus, APS-4 when AD was associated with any other autoimmune diseases excluding the previous associations, and isolated AD when other autoimmune diseases were absent. One hundred sixty-three women had APS-2, 49 had APS-1, 18 had APS-4, and 28 had isolated AD. POF was diagnosed using the criteria proposed by Nelson (4).

Specifically, POF was diagnosed when the patients had an hypergonadotropic amenorrhea with serum LH and FSH concentrations greater than 15 U/liter and estradiol less than 150 pmol/liter on two occasions below the age of 40 yr.

Forty-one patients with AD (StCA positive or negative) without POF were followed up to assess the risk of developing POF (10 patients under 12 yr and 31 aged between 12 and 39 yr of age at the start of follow-up).

The study was approved by the local ethics committee, and all the patients gave signed written informed consent to the study; the study was performed according to the Helsinki Declaration.

Measurements

StCA were measured in 241 women with AD (44 with POF and 197 without POF) using the IF technique on unfixed cryostatic sections of normal human tissue from adrenal gland and testis, as described elsewhere (9).

17α-OHAb and P450sccAb were measured in 159 women with AD (31 with and 128 without POF) by IPA using recombinant antigens labeled with 35S-methionine as reported previously (16).

Genetic evaluation

HLA-DRB1 typing was carried out in 29 AD patients diagnosed with POF by the PCR sequence-specific primers method at low resolution using commercial kits (Olerup SSP, Sweden) (18). Phenotypic frequencies of the patients were compared with the frequencies of 206 sex-matched controls using Fisher exact test.

Results

Prevalence of POF in patients with AD

POF was found in 52 of 258 women with AD (20.2%) (Fig. 1). The duration of POF in the patients included in the study ranged from the onset up to 30 yr after diagnosis, and the mean values of serum FSH measurements were 60 ± 15 U/liter, serum LH measurements 35 ± 10 U/liter, and serum 17β-estradiol levels 35 ± 10 pmol/liter.

In particular, POF was found in 20 of 49 patients who had AD (40.8%) in the context of APS-1 (in two, POF had manifested as primary amenorrhea); in six of 18 women with AD (33.3%) in the context of APS-4; in 26 of 163 patients with AD (16%) in the context of APS-2 (in one, POF developed as primary amenorrhea). None of the 28
women with isolated AD had POF (Fig. 1). The mean age at the onset of POF and AD in various groups of AD patients is summarized in Table 1. In general, AD preceded POF in patients with APS-1 and APS-4 and followed POF in those with APS-2.

**StCA in patients with AD with or without POF**

Positive StCA were found in 81 of 241 patients with autoimmune AD (33.6%); in particular, StCA were detected in 31 of 43 women with POF (72%), with various duration of disease, and in 50 of 198 without POF (25.3%) (Fig. 2A).

StCA tested positive in 22 of 38 women with APS-1 (57.9%) of whom 11 of 13 with POF (84.6%) and 11 of 25 without POF (44%) (Fig. 2B). Two of the 13 patients with POF and APS-1 had primary amenorrhea and both were StCA negative. Genetic analyses carried out in these two StCA-negative patients showed a karyotype typical of the Turner’s syndrome in one patient. The other patient had a normal female karyotype but however tested positive for 17α-OHAb on a subsequent serum sample collected many years after initial assessment.

Of 13 women with APS-4, five (38.5%) were StCA positive; three of four with POF (75%) and two of nine without POF (22.2%) (Fig. 2C), whereas 53 of 162 APS-2 patients (32.7%) were StCA positive, of whom 17 of 26 with POF (65.4%) and 36 of 136 without POF (26.5%) (Fig. 2D). One of the patients with APS-2 had primary amenorrhea and was StCA positive. Only one of 28 patients with isolated AD (3.6%) was positive for StCA.

**StCA in relation to duration of POF**

StCA measurements were available for 38 women with POF with different disease duration and 12 of 13 patients with POF of less than 5 yr from the diagnosis (92.3%) were positive compared with 18 of 25 patients with more than 5 yr POF duration (72%) (Fig. 3).

**StCA, 17α-OHAb, and P450sccAb in patients with AD, with and without POF**

All three autoantibodies (StCA, 17α-OHAb, and P450sccAb) were tested in 159 women with AD and 31 with and 128 without POF. Twenty-eight of the 31 patients with POF (90.3%) and 50 of 128 patients without POF (39%) were found to be positive for at least one of the autoantibodies (Fig. 4).

The overall results and the correlation between StCA, 17α-OHAb, and/or P450sccAb in women with autoimmune AD with and without POF are summarized in Fig. 5.

**Genetic testing**

Genetic testing carried out in 29 patients with both AD and POF showed a significantly increased frequency of HLA-DRB1*04 (P < 0.000005) and a significant reduc-
tion of HLA-DRB1*01 (P < 0.003) in the patients compared with the controls.

Follow-up of patients with AD with and without StCA

Forty-one women with AD younger than 40 yr of age were followed up for a mean 8.9 yr. The 10 young females aged younger than 12 yr (nine with APS-1 and 1 with APS-2) were followed up for a mean 12.2 yr (range 1–30 yr) (Fig. 6). Four of these 10 patients were initially positive for StCA, and another four seroconverted for StCA during the follow-up. Five of the eight StCA-positive patients (62.5%) developed POF at a mean age of 21.7 yr, whereas the other three are still being followed up (to date one is 22 yr old and has normal menses, and the other two are 6 and 11 yr old). Of the six initially StCA-negative patients, two remained persistently negative for StCA; to date one is 9 yr old; the other is 21 yr old and has normal menses. All the patients who developed POF in this group had APS-1.

Thirty-one patients aged between 12 and 40 yr (five with APS-1, 24 with APS-2, and two with APS-4) were followed up for a mean 7.9 yr. Thirteen of these 31 women were positive for StCA at the beginning or seroconverted during the follow-up and three of 13 StCA-positive patients (23%) developed POF (one with APS-2, one with APS-1, and one with APS-4 after 3, 18, and 9 yr, respectively). Overall, during the follow-up, eight of 21 StCA-positive or seroconverted patients (38%) developed POF (six with APS-1, one with APS-2, and one with APS-4). In contrast, none of the 20 persistently StCA-negative patients developed POF during the period of observation.

Discussion

We assessed the prevalence of POF, StCA, and antibodies to steroidogenic enzymes (17α-OHAb and P450sccAb) in the largest cohort of women with AD published so far. Of 258 females studied, 20.2% had autoimmune POF, which suggests that autoimmune AD is strongly associated with autoimmune POF.

Autoimmune AD may present as an isolated disease, or it can occur in association with other autoimmune diseases as a component of APS, for example, in association with chronic candidiasis and/or chronic hypoparathyroidism (APS-1), in association with autoimmune thyroid disease and/or type 1 diabetes mellitus (APS-2) and in association with other autoimmune diseases (APS-4) (1).

In our study we have found that the prevalence of POF in patients with different types of APS varied; the highest prevalence was detected in patients with APS-1 (>40%), lower in patients with APS-4 (30%), and the lowest in APS-2 (16%).

In patients with both AD and POF, AD tended to develop before POF (mean age at onset 27 yr of age and 28.5 yr, respectively). The age at onset of POF in patients with different forms of APS differed and POF presented earlier in life in patients with APS-1 than in patients with the other forms of APS. Women with APS-1 developed POF at approximately 24 yr of age; however, some patients presented with primary amenorrhea. It is likely that in patients who presented with primary amenorrhea an autoimmune attack of the ovary had started and caused significant damage before the patient’s puberty. In women with APS-1, POF usually developed after AD had been diagnosed. Similar sequence of disease onset was observed also in patients with APS-4 who developed POF at a mean age of 25.8 yr and AD at a mean age of 23.2 yr. This would
suggest that women with autoimmune AD in the context of APS-1 and APS-4 may be at risk of developing POF and consequently should be tested for adrenal cortex autoantibodies (ACA) and/or 21-hydroxylase autoantibodies (21-OHAb) to confirm adrenal autoimmunity and for StCA and/or steroidogenic enzyme autoantibodies to aid the prediction of the risk of POF.

Women with APS-2 tended to develop POF later in life compared with patients with APS-1 or APS-4 and usually before the onset of AD. In particular, POF was diagnosed at a mean age of 32.3 yr and AD at 35.5 yr. Consequently, patients with POF and autoimmune thyroid diseases or type 1 diabetes mellitus should be tested for StCA or steroidogenic enzyme autoantibodies to confirm autoimmune POF and also for ACA and/or for 21-OHAb to assess a risk of autoimmune AD (potential AD) (1).

In our study the majority of patients with APS-1 and POF were positive for StCA (11 of 13; 84.6%), suggesting an autoimmune background in development of POF. One of the two StCA-negative patients with APS-1 and POF who presented with primary amenorrhea had Turner’s syndrome. This observation suggests that genetic and karyotyping assessments should be carried out in StCA-negative APS-1 patients with primary amenorrhea to clarify the background cause of ovarian insufficiency. This is particularly relevant because most of the component diseases manifest early in life in patients with APS-1 due to the autoimmune regulator gene mutations that are responsible for loss of tolerance to self-antigens (19).

We have found StCA positivity in approximately 70% of women with AD and POF, and this confirms the strong relationship between StCA and POF as reported previously (11). Furthermore, StCA were positive in more than 92.3% of patients when tested within 5 yr from the onset of POF. Overall, these observations indicate that StCA are very good markers of POF in patients with AD. The prevalence of StCA in AD patients at the onset of POF is similar to that reported for thyroid autoantibodies in patients with chronic thyroiditis (20), ACA or 21-OHAb in patients with AD (9) or autoantibodies to islet cell antigens in patients with type 1 diabetes mellitus (21). In our study StCA positivity showed tendency to decrease slowly over time and approximately 75% of women with POF duration longer than 5 yr tested positive for StCA. A similar trend in decreasing autoantibody positivity has been described in the majority of the above-mentioned autoimmune diseases.

Two steroidogenic enzymes (17α-OH and P450scc) expressed in the adrenals and the gonads have been identified to be the major target autoantigens recognized by StCA (1, 15) and serum 17α-OHAb and P450sccAb can be measured using sensitive IPAs (16). StCA, 17α-OHAb, and P450sccAb were tested in many but not all the patients included in this study and the relationship between the autoantibodies and POF analyzed. Unfortunately, we were unable to measure all three types of autoantibodies (StCA, 17α-OHAb, and P450sccAb) in all patients because serum samples were no longer available for some of
the patients, particularly those referred to our department before 17α-OHAb and P450sccAb tests had become available.

Although there was an overall good correlation between StCA, 17α-OHAb, and P450sccAb, there were some discrepancies. These discrepancies may be related to the differences in the methods used to measure these antibodies. For example, IPA to measure 17α-OHAb and P450sccAb are likely to be more sensitive than an IF test for StCA, and this could account for some samples tested positive for 17α-OHAb and/or P450sccAb and negative for StCA. In the case of samples positive for StCA and negative for 17α-OHAb and/or P450sccAb, it is possible that serum reactivity to yet-unidentified autoantigen (different from 17α-OH or P450scc) was responsible for a positive signal in the IF test (11).

Our study showed that autoimmune AD in the context of APS is strongly associated with autoimmune POF. Furthermore, the prevalence of autoantibodies to the steroidogenic enzymes is higher in patients with autoimmune AD and APS associated with POF compared with AD patients without POF. However, the autoantibody positivity varies in different types of APS.

The outcome of the long-term follow-up of StCA-positive patients indicated that, overall, eight of 21 patients with autoantibodies to steroidogenic enzymes (38%) developed POF after a long period of observation. This suggests that StCA, 17α-OHAb, and/or P450sccAb may serve as predictive markers of POF not only in patients with APS-1 as reported before (13, 14) but also in patients with APS-2 or APS-4. Furthermore, StCA, 17α-OHAb, and/or P450sccAb detected before puberty may herald primary amenorrhea of autoimmune etiology, whereas primary amenorrhea in the absence of the autoantibodies would indicate to nonautoimmune background of the gonadal failure.

Genetic assessments in our patients with both AD and POF showed that the presence of class II human leukocyte antigen (HLA) D-related antigens was associated with both susceptibility (HLA-DRB1*04) and protection (HLA-DRB1*01) from the disease.

Overall our study showed that autoimmune AD is strongly associated with POF. The prevalence and the onset of POF vary in different types of APS. In patients with APS-1 and APS-4, POF tends to develop after the onset of AD, whereas POF tends to precede AD in patients with APS-2. StCA, 17α-OHAb, and/or P450sccAb are detectable in the majority of patients with POF associated with AD at the onset of POF. StCA, 17α-OHAb, and/or P450sccAb positivity in AD patients without clinical POF may indicate potential POF in APS-1, APS-2, and APS-4. Monitoring serum StCA, 17α-OHAb, and P450sccAb positivity in female patients with AD should be helpful in the clinical management, particularly in ovarian function assessment.

Acknowledgments

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