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Autoantibody Response Against NALP5/MATER in Primary Ovarian Insufficiency and in Autoimmune Addison's Disease

Annalisa Brozzetti, Mohammad Alimohammadi, Silvia Morelli, Viviana Minarelli, Åsa Hallgren, Roberta Giordano, Annamaria De Bellis, Roberto Perniola, Olle Kämpe, and Alberto Falorni, on behalf of the Italian Addison Network

Department of Internal Medicine (A.B., S.M., V.M., A.F.), University of Perugia, 06126 Perugia, Italy; Department of Medical Sciences (M.A., O.K.), Science for Life Laboratory, Uppsala University, 750 03 Uppsala, Sweden; Centre of Molecular Medicine (M.A., A.H., O.K.), Department of Medicine (Solna), Karolinska Institutet, 171 76 Stockholm, Sweden; Division of Endocrinology, Diabetology, and Metabolism (R.G.), Department of Medical Sciences, University of Turin, 10126 Turin, Italy; Department of Cardio-Thoracic and Respiratory Science (A.D.B.), Endocrinology Unit, Second University of Naples, 80132 Naples, Italy; and Department of Pediatrics-Neonatal Intensive Care (R.P.), V. Fazzi Regional Hospital, 73100 Lecce, Italy

Address all correspondence and requests for reprints to: Al- berto Falorni, MD, PhD, Department of Medicine, University of Perugia, Via E. Dal Pozzo, 06126 Perugia, Italy. E-mail: alberto.falorni@unipg.it.

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Abstract

Context: NACHT leucine-rich-repeat protein 5 (NALP5)/maternal antigen that embryo requires (MATER) is an autoantigen in hypoparathyroidism associated with autoimmune polyendocrine syndrome type 1 (APS1) but is also expressed in the ovary. Mater is an autoantigen in experimental autoimmune oophoritis.

Objectives: The objectives of the study were to determine the frequency of NALP5/MATER auto- antibodies (NALP5/MATER-Ab) in women with premature ovarian insufficiency (POI) and in pa- tients with autoimmune Addison's disease (AAD) and to evaluate whether inhibin chains are a target for autoantibodies in POI.

Methods: Autoantibodies against NALP5/MATER and inhibin chains-a and -[3A were determined by radiobinding assays in 172 patients with AAD without clinical signs of gonadal insufficiency, 41 women with both AAD and autoimmune POI [steroidogenic cell autoimmune POI (SCA-POI)], 119 women with idiopathic POI, 19 patients with APS1, and 211 healthy control subjects.

Results: NALP5/MATER-Ab were detected in 11 of 19 (58%) sera from APS1 patients, 12 of 172 (7%) AAD sera, 5 of 41 (12%) SCA-POI sera, 0 of 119 idiopathic POI sera and 1 of 211 healthy control sera ($P < .001$). None of 160 POI sera, including 41 sera from women with SCA-POI and 119 women with idiopathic POI, and none of 211 healthy control sera were positive for inhibin chain-a/[3A autoantibodies.

Conclusions: NALP5/MATER-Ab are associated with hypoparathyroidism in APS1 but are present also in patients with AAD and in women with SCA-POI without hypoparathyroidism. Inhibin chains do not appear to be likely candidate targets of autoantibodies in human POI

Introduction

Autoimmune Addison's disease (AAD) is the consequence of the autoimmune destruction of adreno- cortical cells, with subsequent deficit of glucocorticoids and mineralcorticoids and a life-long need for a substitu- tive therapy (1, 2). The adrenal autoimmune reaction is accompanied by the production of 21-hydroxylase auto- antibodies (21OHAb), the major immune marker of AAD (3–7).

AAD is frequently associated with other endocrine and nonendocrine autoimmune (or immunomediated) dis- eases, in the so-called autoimmune polyendocrine syn-dromes (APS) (5, 8). Although APS type 2 is polygenic, with a major contribution of the HLA genes, APS type 1 (APS1) is a monogenic disorder caused by mutations in the autoimmune regulator (aire) gene on chromosome 21 (5, 9 –13). The serum from patients with APS1 expresses au- toantibodies directed against several targets (14 –19).

Immunoscreening of a human parathyroid cDNA li brary identified NACHT leucine-rich-repeat protein 5 (NALP5) as a novel autoantigen, target of autoantibodies, associated with hypoparathyroidism in APS1 patients (20). NALP5 is a 1200-amino acid-long protein also de- nominated maternal antigen that embryo requires (MA- TER) or NLRP5 (21). Interestingly, NALP5/MATER is predominantly expressed in parathyroid tissue but also in the ovaries, although to a lesser extent (20). Because bio- chemical and clinical signs of ovarian autoimmunity are frequently present in women with APS1 (5, 11), NALP5/ MATER may have a role also as an ovarian autoantigen. However, the association of NALP5/MATER autoanti- bodies (NALP5/MATER-Ab) with hypogonadism cannot be confirmed in APS1 sera because 85%–90% of patients with APS1 have hypoparathyroidism, which makes it problematic to discriminate a NALP5/MATER-specific ovarian autoimmunity, independently from parathyroid autoimmunity.

Approximately 1% of women from the general popu- lation develop primary ovarian insufficiency (POI), as de- fined by hypergonadotrophic hypogonadism with hypo- estrogenism and infertility before the age of 40 years (22, 23). The large majority of POI cases remains idiopathic in nature, but biochemical signs of ovarian autoimmunity can be detected in approximately 4%–5% of patients (24 – 26). Autoimmune POI is almost invariably associated with clinical or preclinical AAD and affected women are typi- cally positive for autoantibodies directed against steroid- ogenic cytochrome P450 enzymes, such as 21-hydroxylase (21OHAb), 17-hydroxylase (17OHAb), and side-chain cleavage enzyme (P450sccAb) (steroidogenic enzymes autoantibodies, StE-Abs) (24 –26). Presence of these auto- antibodies identifies POI due to autoimmunity to steroid- producing cells (SCA-POI) (26). Other potential ovarian

autoantigens have been proposed as target of human autoantibodies associated with POI, including LH receptor (27), FSH receptor (28), and zona pellucida proteins (29), but to date no reliable and reproducible immunoassay has been developed for any of these markers.

Little is known of the molecular mechanisms responsible for autoimmune-mediated ovarian autoimmunity. Nevertheless, some studies have shed light on the patho- physiology of human SCA-POI. Human ovarian autoim- munity is responsible for a selective mononuclear cell in- filtration of large, antral follicles (24) and for the selective autoimmune destruction of theca cells with concomitant preservation of granulosa cells. For several years after clin- ical diagnosis of SCA-POI, granulosa cells remain vital, as demonstrated by increased serum levels of inhibins, and the pool of functioning follicles is preserved, as demon- strated by normal serum concentrations of antimüllerian hormone (25).

The animal model of neonatal thymectomy has proven instrumental to unravel the critical role of CD4+CD25+ regulatory T cells in suppressing autoimmune processes (among which is oophoritis) in regional lymph nodes un- der a continuous stimulation by autologous antigens (30). The autoantigen identified in the animal model of neonatal thymectomy is the ooplasm-specific Mater protein (31, 32). Given that human NALP5/MATER is ortholog to the murine autoantigen Mater, studies are warranted to eval- uate the possible role of NALP5/MATER in human ovarian autoimmunity.

In the present study, we have analyzed the frequency of NALP5/MATER-Abs in a large group of patients with APS1, AAD with or without gonadic dysfunction, or idiopathic POI. In addition, we evaluated the frequency of autoantibodies against inhibin chains in women with SCA-POI and in women with idiopathic POI.

Subjects and methods

Subjects

The study design included serum samples from the following patients: 1) 172 patients with AAD, 2) 41 women with AAD and SCA-POI, 3) 119 women with idiopathic POI, 4) 19 patients with APS1, and 5) 211 healthy control subjects (Table 1).

The 172 AAD patients (median age 45 y, range 18 –70 y, 60 males and 112 females without POI, time from diagnosis: me- dian 6 y, range 0 – 45 y) were consecutively recruited by the Italian Addison Network between April 1998 and January 2014. The etiological classification of primary adrenal insufficiency was made according to a diagnostic flow chart that took in con- sideration the biochemical and imaging data (33), and AAD was defined by the presence of 21OHAb. None of the AAD patients had hypogonadism, hypocalcemia, or hypoparathyroidism, and none was suffering from chronic candidiasis. APS type 2 was diagnosed in 103 of 172 AAD patients (60%). Among the 112 females with AAD but with no clinical signs of POI, 11 (10%) were positive for 17OHAb and 16 (14%) were positive for P450sccAb. Among the 60 males with AAD, 17OHAb and P450sccAb were, respectively, found in four (7%) and in five (8%) subjects.

Among the women with POI (all with 46,XX karyotype and negative for fragile X mental retardation 1, mutations, or per- mutations), identified by the onset of clinical and biochemical signs of hypergonadotrophic hypogonadism before the age of 40 years, 41 were positive for StE-Abs and were diagnosed with SCA-POI. One hundred nineteen women with POI, but negative for StE-Abs, formed group 3 (idiopathic POI). Menarche oc- curred between 10 and 13 years of age in both SCA-POI and idiopathic POI patients and blood sampling for the present study was taken 0 – 19 years (median 6 y) after the diagnosis of SCA- POI and 0 –9 years (median 3 y) after the diagnosis of idiopathic POI. All women with SCA-POI had clinical AAD and were treated with substitutive doses of cortisone acetate and fludro-cortisone. Twenty-six of the 41 patients with SCA-POI (63%) were affected by other autoimmune diseases, including thyroid autoimmune diseases, type 1 diabetes mellitus, vitiligo, chronic atrophic gastritis, and rheumatoid arthritis. None of the women with SCA-POI suffered from hypocalcemia or hypoparathyroid- ism. All 41 women with SCA-POI were positive for 21OHAb, whereas 24 women were positive for 17OHAb and 32 were positive for P450sccAb. Of the 119

women with idiopathic POI, 12 had documented autoimmune diseases, including thyroid autoimmune diseases (n = 9), type 1 diabetes mellitus (n = 1), celiac disease (n = 1), or systemic lupus erythematosus (n = 1).

The 19 APS1 patients (median age 23 y, range 5– 40 y, nine males, and 10 females, five of whom suffering from SCA-POI) enrolled in the study represented the entire population of APS1 subjects recruited by the Italian Addison Network in the period April 1998 through January 2014. Sixteen of the 19 APS1 pa- tients (84%) suffered from hypoparathyroidism.

Serum samples from 211 Italian healthy control subjects (me- dian age 42 y, range 18 –56 y, 102 males, and 109 females) served as the control group to determine the upper level of normal in the NALP5/MATER-Ab assay.

The study was approved by the local Ethics Committee in the Umbria Region. All patients gave their written informed consent to be enrolled in the study.

NALP5/MATER and inhibin chain cDNA

A cDNA clone encoding for a truncated version of 1181 amino acids of human NALP5/MATER (catalog number SC306608; Origene Technologies) was initially used to produce the radiolabeled autoantigen. The deduced sequence of the pro- tein produced by this clone is missing a 19-amino acid sequence, corresponding to region 207–226 of the full-length protein. In a separate set of experiments, full-length NALP5/MATER (cata- log number SC318548; Origene Technologies) was also used. The 35S-radiolabeled NALP5/MATER was produced by cou- pled in vitro transcription and translation by using rabbit reticu- locyte lysate (Promega Co) and 35S-methionine (NEN Life Science Products, Inc) according to the manufacturer's instructions. The full-length cDNA clone for human inhibin-a (catalog number 4126990; Open Biosystems) was subcloned into the pGEM vector (Easy vector system, catalog numberA1380; Pro- mega Co) and the above.

The full length cDNA clone for human inhibin-[3A (catalog number 42699832; Open Biosystems) was used to produce the 35S-methionine radiolabeled antigen.

Development of radiobinding assays for NALP5/ MATER autoantibodies and inhibin chain autoantibodies

In the autoantibody assays, 20 000 counts per minute (cpm) of 35S-NALP5/MATER or 3H-inhibin-a or 35S-inhibin-[3A was immunoprecipitated in duplicate with human serum at a dilution of 1:25. The immunocomplexes were separated in 96-multiwell plates (Multiscreen; Millipore) by using Protein A-Sepharose (Pharmacia Biotech) according to the procedures described for the 65-kDa isoform of glutamic acid decarboxylase autoanti- bodies (34) and 21OHAbs (6). Finally, immunoprecipitated ra- dioactivity was evaluated in a microplate scintillation counter (Top Count-NXT; Packard Bioscience Co). To calculate auto- antibody levels, a positive standard serum and two negative stan- dard sera were used. Levels of autoantibodies were expressed as a relative index according to th*e following formula: (cpm sam*ple - mean cpm negative controls)/(cpm positive controls -mean cpm negative controls). The upper level of normal was estimated as the mean + 3 SD of the indices observed in healthy controls. In the NALP5/MATER-Ab assay, the positive standard serum was a serum from an APS1 patient, and the two negative standard sera were obtained from two healthy persons with no biochemical or clinical signs of autoimmune or other chronic diseases.

Antisera for inhibin-a and inhibin-[3A (H00003623 and H00003625-A01; Abnova) were used as positive controls for the inhibin autoantibody assays.

Statistical analysis

Differences in autoantibody frequency were analyzed by us- ing the x2 test, with Yates' correction when necessary, or by using the Fisher exact test, in 2 X 5 and 2 X 2 contingency tables. Differences in antibody levels were analyzed by a Kruskal-Wallis test followed by post hoc pairwise comparisons with Bonferroni correction. Statistical analyses, including complex sample

anal- ysis, were performed using IBM-SPSS version 22.0 (IBM Corp, 2013). $P < .05$ was considered significant in all tests.

Results

The immunoreactivity of the 35S-NALP5/MATER pro- duced by in vitro transcription and translation was tested by using the serum from an APS1 patient with hypopara- thyroidism. When 20 000 cpm of 35S-NALP5/MATER was used, 33.4% ± 7.3% of the tracer was immunopre- cipitated by the positive control serum as compared with 1.0% ± 0.2% immunoprecipitation obtained with the two negative control sera.

In 211 healthy controls, the mean ± SD of the NALP5/MATER index was 0.008 ± 0.009, and the upper level of normal was set at 0.035. Only 1 of 102 healthy control male sera, and 0 of 109 healthy control female sera, was associated with a NALP5/MATER index higher than the upper level of normal (NALP5/MATER index 0.125).

When a NALP5/MATER index cutoff value of 0.035 was applied, 11 of 19 sera from APS1 patients (58%) were positive for NALP5/MATER-Ab (Figure 1) (median NALP5/MATER index 0.769, range 0.042–1.17). On the other hand, 12 of 172 AAD sera (7%) (median NALP5/ MATER index 0.042, range 0.036 –1.102), 5 of 41 SCA-POI sera (12%) (median NALP5/MATER index 0.049, range 0.037– 0.511), and 0 of 119 idiopathic POI sera were positive for NALP5/MATER-Ab (Figure 1). To confirm these results, all weakly positive samples (NALP5/ MATER index < 0.05) and an equal number of negative samples were blindly reanalyzed by another laboratory participating in the study: concordance among the two laboratories was 100% for both positive and negative samples.

The overall contingency table (five groups, subdivided according to sex) documented that the differences in au- toantibody frequencies among the five groups of patients were statistically different ($P < .001$). When 2 X 2 con- tingency tables were applied (Table 2), APS1 patients

differed statistically from every other group both among males (P = .024 vs AAD and P < .001 vs healthy controls) and among females (P < .001), AAD females and SCA- POI patients differed statistically from idiopathic POI women (P = .017 and P < .001, respectively), AAD males differed statistically from healthy control subjects (P =.049), and SCA-POI patients differed statistically from healthy control females (P < .001), whereas no statistical difference was observed between AAD females and SCA- POI or between idiopathic POI and healthy control subjects.

To exclude that the results might have been influenced by the use of a truncated version of the autoantigen, all sera were reanalyzed using full-length NALP5. Similarly to what observed when using the truncated version of NALP5, 11 of 19 sera from APS1 patients (58%), 12 of 172 AAD sera (12%), 5 of 41 SCA-POI sera (12%), 0 of 119 idiopathic POI sera, and 1 of 211 healthy control sera resulted positive for NALP5/MATER-Ab with a 100% agreement between the two constructs.

In APS1 sera, the presence of NALP5/MATER-Ab was strongly associated with hypoparathyroidism because all positive samples were from patients affected by this dis- ease. More specifically, 11 of 16 patients with hypopara- thyroidism (69%) were positive for NALP5/MATER-Ab. Among women with APS1 and POI, four of five (80%) were positive for NAL*P5/MATER-Ab, but all those sub*jects were also suffering from hypoparathyroidism.

Of the 12 AAD patients positive for NALP5/MATER- Ab, five were males and seven were females. Four of these 12 patients had isolated AAD with no other sign of an- other autoimmune or immunomediated disease. Of the eight AAD patients positive for NALP5/MATER-Ab with other autoimmune diseases, two had type 1 diabetes mel- litus, four had Hashimoto's thyroiditis, four had vitiligo, and two had atrophic gastritis. The frequencies of con- comitant autoimmune diseases were not statistically dif- ferent from those observed among AAD patients negative for NALP5/MATER-Ab.

None of the 12 AAD patients positive for NALP5/MA- TER-Abs was positive for either 17OHAb or P450sccAb. Similarly, no statistical significant association was seen between the presence of NALP5/MATER-Ab and 17OHAb or P450sccAb in APS1 patients or in SCA-POI patients.

In NALP5/MATER-Ab-positive female patients, the index values were significantly higher for APS1 (median 0.123, range 0.06 –1.17) than AAD (median 0.045, range 0.036 –1.102) ($P < .05$) and SCA-POI (median 0.048, range 0.036 – 0.511) ($P = .03$) subjects. The difference in index values between the four NALP5/MATER-Ab-pos- itive APS1 males (median 0.459, range 0.042–1.02) and the five Ab-positive AAD males (median 0.037, range 0.036 – 0.529) did not reach a statistical significance.

None of 211 healthy control sera and none of 160 sera from women with POI, including 41 sera from women with SCA-POI and 119 sera with idiopathic POI, had an inhibin-a autoantibody index or an inhibin-alpha autoanti- body index higher than the upper level of normal of each assay.

Discussion

POI shows variable clinical manifestations with intermit- tent ovarian function and even occasional conception after diagnosis, suggesting a progressive decline of ovarian function (22, 23). Although clinical and biological fea- tures of ovarian insufficiency have been well characterized, the etiology of the disease remains unclear in most cases. An autoimmune origin has been proposed to ex- plain up to 30% of cases of POI (35), but a more accurate estimate indicates that an ovarian autoimmune reaction could be unequivocally documented in no more than 4%–5% of women with ovarian insufficiency, more spe- cifically in association with adrenal autoimmunity (24 –26).

A limited number of translational models for autoim- mune POI has been made available so far. Ovarian failure due to autoimmune destruction of the ovary can be in- duced by immunization with crude or defined ovarian an- tigens (36) or by thymectomy at day 3 after birth in various strains of inbred mice (30). In this latter 3-day thymectomy model, a 125-kDa oocyte-specific protein,

localized in the cytoplasm of growing oocytes, acts as an autoantigen (32), and autoantibodies reacting to this protein have been identified. This protein was further characterized (21) as a maternal protein required for early embryonic development, the so-called maternal antigen that embryo requires (MATER). Transgenic expression of MATER in antigen- presenting cells induces antigen-specific tolerance that mitigates the autoimmune phenomenon at the ovarian level (31) both in terms of incidence of autoantibodies production against oocyte proteins and in terms of lym- phocytic infiltration of the ovary. On the other hand, be- cause the oophoritis is reduced but not completely sup- pressed, the contribution of MATER to murine ovarian autoimmunity is not exclusive, and alternative or concomitant pathogenetic targets for T cells may exist.

NALP5, a parathyroid autoantigen in APS1 patients (20), is the human homolog of murine MATER (21). Be- cause NALP5 expression is restricted to parathyroid glands in men but is extended also to the ovaries in women (20), we hypothesized that this protein could act as an ovarian autoantigen in women affected by POI, and we performed radiobinding assays in large groups of women affected by SCA-POI or idiopathic POI to detect NALP5/ MATER-Ab. NALP5/MATER-Ab were present in 58% patients with APS1, which is in line with the results of previous investigations (20, 37). However, the results ob- served in APS1 patients could not be interpreted as a dem- onstration of an ovary-specific autoreactivity because all positive sera were from patients affected with hypopara- thyroidism. Nevertheless, the high frequency of NALP5/ MATER-Ab in APS1 patients is in line with the concept that autoimmune mechanisms in subjects with mutation of autoimmune regulator are similar to those observed in the thymectomy model (38).

Our study demonstrates that three groups of patients with different autoreactivity to NALP5-MATER exist: pa- tients with APS1 who show the strongest reactivity, pa- tients with AAD and SCA-POI who show a less frequent but still statistically significant reactivity, and, finally, pa- tients with idiopathic POI and healthy control subjects who do not exhibit any (or almost any) autoreactivity at all.

The finding that NALP5/MATER-Ab were detected in 12% of women with SCA-POI could be interpreted as an indirect sign of an oocyte-directed autoimmune process occurring in a small subgroup of these patients in con- comitance with the autoimmune attack against theca cells. However, it must be noted that NALP5 ovarian expres- sion has so far been demonstrated in humans only at the mRNA level (20) and that AAD women found positive for NALP5/MATER-Ab did not show clinical or biochemical signs of POI. In addition, in AAD patients, occurrence of NALP5/MATER-Ab was similar in males and in females. Furthermore, this novel marker of ovarian autoimmunity was present exclusively in women with an ongoing adrenal autoimmune process that confirms that SCA-POI is al- most invariably associated with clinical or preclinical AAD. For this reason, presence of 21OHAb remains the marker at highest diagnostic accuracy for the diagnosis of autoimmune POI (24 -26). On the other hand, the absence of NALP5/MATER-Ab in our group of so-called idio- pathic POI confirms that we cannot yet understand the true nature of the disease in this group of women.

Interestingly, NALP5/MATER-Ab were detected in 7% patients with AAD without hypogonadism. More spe- cifically, in 4 of 12 positive AAD sera, NALP5/MA- TER-Ab levels were as high as those typically detected in patients with APS1, ruling out the possibility that auto-antibody positivity was the result of an aspecific immu- noreaction. In addition, the presence of these autoanti- bodies in only 1 of 211 healthy control sera and their

absence in 119 women with idiopathic POI strengthen the conclusion that presence of NALP5/MATER-Ab in 7% patients with AAD is the expression of a consistent auto- immune phenomenon. This result is somehow surprising because the tissue expression pattern of this autoantigen could not predict its role as a potential autoantigen in adrenal autoimmunity. The frequent occurrence of other autoimmune diseases in patients with AAD in the context of the so-called APS suggests that NALP5/MATER-Ab may be more likely related to other autoimmune

diseases present in patients with AAD rather than to the adrenal autoimmune process per se and not necessarily a sign of an oocyte-directed autoimmune process. Although none of the tested 172 AAD patients had clinical or biochemical signs of either hypoparathyroidism or hypogonadism and frequency of concomitant autoimmune diseases was sim- ilar in NALP5/MATER-Ab positive and -Ab negative AAD patients, we cannot exclude that an initial autoim- mune process against parathyroid or ovary might have started, thus leading to the production of NALP5/ MATER-Ab. Further studies are needed to address the question whether NALP5/MATER-Ab may be an early marker of parathyroid or ovarian autoimmunity in pa- tients with AAD or a marker of atypical forms of APS1. NALP5 belongs to a family of cytoplasmic proteins comprising 14 members that share similar structure and are involved in apoptosis and inflammation as well as in reproduction. The NALP5 gene clusters with NALP 2, 4, 7, 8, 9, 11, 12, and 13 on human chromosome 19q13.4, reflecting a common phylogenetic origin that well fits with the expression patterns in human oocytes and embryos (39). Notably, NALP13 is closest to NALP5 on the phy- logenetic tree and has been reported to be expressed in the ovary. Similarly, also NALP9 has been reported to show an expression pattern similar to that of NALP5. Although NALP5 appears to be a strong parathyroid autoantigen in APS1 patients, the low levels of NALP5/MATER-Ab in women with SCA-POI raises the question whether ovarian NALP autoimmunity might be triggered by another mem- ber of the NALP family, with partial cross-reactivity to NALP5. At the moment we cannot resolve this issue, and further studies are warranted to address this specific question.

In our search for ovarian autoantibodies, we also in- cluded inhibins as potential autoantigens. The rationale for this approach was provided by an experimental model of autoimmune oophoritis induced in SWXJ female mice by immunization with the p215-234 sequence of in- hibin-a (36). To evaluate whether inhibins may be target of an autoreactive process also in human POI, we devel- oped RIAs for the detection of autoantibodies against in- hibin chains in human serum. Although immunoreactivity of inhibin-alpha could not be tested because we were not able to efficiently produce radiolabeled inhibin-[3B using the in vitro translation technique, no sample

resulted positive for inhibin a- or inhibin [3A-Ab, neither in SCA-POI nor in idiopathic-POI, which suggests that inhibins are not likely candidate targets of autoantibodies in human autoimmune oophoritis.

Here we report that the human MATER homolog, NALP5, is targeted by an autoimmune process in a small subgroup of women with SCA-POI. The involvement of several different target antigens in ovarian autoimmunity may reflect a variety of pathological mechanisms whose clinical and diagnostic relevance still needs to be investigated.

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Table 1. Characteristics of the Subjects Included in the Study

Subject Groups	n	Age at Blood Sampling, y	Males/Females	Associated Autoimmune Diseases, %[a]	21OHAb	17OHAb	P450sccAb
AAD	172	45 (18 –70)	60/112	103/172 (60%)	172/172 (100%)	15/172 (9%)	21/172 (12%)
SCA-POI	41	37 (18 – 44)	0/41	26/41 (63%)	39/41 (95%)	20/41 (49%)	28/41 (68%)
Idiopathic POI	119	38 (20 – 45)	0/119	12/119 (10%)	0/119	0/119	0/119
APS1	19	23 (5– 40)	9/10	19/19 (100%)	17/19 (89%)	13/19 (68%)	15/19 (79%)
Healthy controls	211	42 (18 –56)	102/109	0/211	0/211	0/211	0/211

[a] Autoimmune diseases listed in the text.

Table 2. Statistical Analysis (*P* Values) of the Comparisons of Frequency of NALP5/MATER-Ab in the Studied Groups

	APS1	AAD Without POI	SCA-POI	iPOI	HC
Females					
APS1		<.001	<.001	<.001	<.001
AAD without POI			NS	.017	.023
SCA-POI				<.001	<.001
iPOI					NS
Males					
AAD without POI	.024				
SCA-POI	—	—			
iPOI	—	—	—		
HC	<.001	.049	—	—	

Abbreviations: HC, healthy controls; iPOI, idiopathic POI; NS, Not significant.

Figure 1. NALP5/MATER-Ab levels in patients with APS1, AAD, SCA- POI, and idiopathic POI (iPOI) and in healthy control subjects (HC). Dotted line shows the upper level of normal of the assay. Filled circles, female subjects; open circles, male subjects.