SERUM AUTOANTIBODIES DIRECTED TO THE GONADOTROPIN RELEASING HORMONE RECEPTOR ECL2 ARE DIAGNOSTIC OF PCOS

PRESENTED BY
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PCOS BACKGROUND

8-10% of women of reproductive age

Two out of three are required to make the diagnosis:
• Oligo and/or anovulation
• Clinical and/or biochemical signs of hyperandrogenism
• Polycystic ovaries (by ultrasound)

Diagnosis of exclusion: No viable laboratory-based assay has been available

PCOS BACKGROUND

• In ovulatory women, GnRH is released in a pulsatile manner resulting in differential secretion of LH and FSH

• In PCOS there is a variable elevation of LH, presumed to be caused by accelerated GnRH-LH pulsatile activity

From The Physiological Society of New Zealand http://www.physoc.org.nz
HYPOTHESIS

• Our lab has previously demonstrated the presence of autoantibodies against the second extracellular loop of different G proteins including alpha 1AR, Beta 1/2AR, M2/3R, AT1R etc. in selected diseases of previously unknown etiology

• We hypothesized that autoimmune antibodies to the second extracellular loop of the GnRH receptor would be present in PCOS and be of pathogenic and diagnostic value
METHODS

The target peptide is the 28 amino acid 2nd extracellular loop of GnRHR

(synthesized by GenScript Inc., Piscataway, NJ)
METHODS: SAMPLES

- Obtained from the Reproductive Endocrinology and Infertility clinic and were blinded to the lab:

- 32 PCOS patients (based on the Rotterdam criteria)
- 38 age and BMI matched, ovulatory infertile women
- 7 patients with Tubal Factor (TF)
- 12 patient with Male Factor (MFI)
- 19 Unexplained infertile women
METHODS: ELISA

• Day 1:
  Coated the plates with the antigen at a concentration of 10mcg/ml
• Day 2:
  Sera diluted at 1:50 and added to the plates
• Day 3:
  Secondary antibody added (goat antihuman IgG 1:2000)
  Substrate added (para-nitrophenyl-phosphate 104)
• The optical density (OD) values are read at 405 nm at 10 min intervals
FIG. 1  ELISA DATA

\[ \text{(OD value)} \]

- PCOS (n=32)
- Other infertility (n=39)

\[ P < 0.01 \]

\[ \text{GnRHR ELISA (OD value)} \]

- PCOS (n=32)
- Other infertility (n=39)

\[ P < 0.01 \]
FIG. 2  PCOS vs Infertile subgroups

![Bar chart showing comparisons between PCOS (n=32), Tubal Factor (n=7), MFI (n=12), and Unexplained (n=19) groups. Significance levels are indicated by P<0.01 for all comparisons.](image)
• AUC = 0.94 ± 0.03  \( p < 0.0001 \)

• GnRHR Ab > 0.2062 indicates presence of PCOS

  Sensitivity = 91%
  Specificity = 87%
FIG. 4  Effect of PCOS IgG on GnRHR mediated Ca\(^{2+}\)I in CHO cells

PCOS dose response (747, 755, 580)

FIG. 5  Cetrorelix (antagonist) blocks IgG induced GnRHR activity in PCOS

Relative Fluorescence Units (% of Maximum)
CONCLUSIONS

- PCOS subjects harbor activating autoantibodies directed toward the GnRHR ECL2
  - ELISA positive
  - IgG specific
  - Dosage responsive activation of specific GnRHR transfected cells
  - Activity is blocked by a GnRH-specific antagonist

- It is likely these activating autoantibodies disrupt hypothalamic and pituitary LH/FSH signaling and lead to abnormal gonadal signaling in afflicted individuals

- The ELISA assay appears to fulfill the need for an inexpensive, sensitive and specific test to identify subjects with PCOS-AAb
FUTURE PLANS

• Animal studies
  • Passive transfer of IgG to mice

• Decoy peptide
  • Block the antibodies

• The variable presence and activity of these autoantibodies in females and in males raises the possibility that they are active in other conditions:
  • Hyperandrogenism including acne
  • Metabolic Syndrome
  • Hypogonadotrophic hypogonadism
  • Premature pubarche and puberty
  • Cancers including ovarian, endometrial, breast, prostate
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REI DEPARTMENT:
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- Anna C. Reynolds MS4

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- Christopher Aston PHD
Thank you!
FIG. 6  BMI

Bar chart showing BMI comparison between PCOS (n=32) and Other infertility (n=39) with a significance level of P>0.05.

Scatter plot showing BMI distribution across different infertility categories: PCOS (n=32), Tubal Factor (n=7), MFI (n=12), Unexplained (n=19), with significance levels: P=0.1731, P=0.4541, P=0.2659.
WITEBSKY’S POSTULATES FOR AUTOIMMUNE DISEASE

• Direct evidence from transfer of pathogenic Ab or pathogenic T cells

• Indirect evidence based on reproduction of the autoimmune disease in experimental animals

• Circumstantial evidence from clinical clues