Metabolism & Reproduction

From the Ovary

To the Pancreas

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Feinberg School of Medicine, Northwestern University
Chicago, IL

De Sedibus et Causis Morborum per Anatomen Indagata

(*The seats and causes of diseases investigated by anatomy*)

74-year-old female patient who died from an ictal event with severe obesity and android aspect (*valde obesa et virili aspectu*). The abdomen is described as prominent and filled by a large amount of fat.

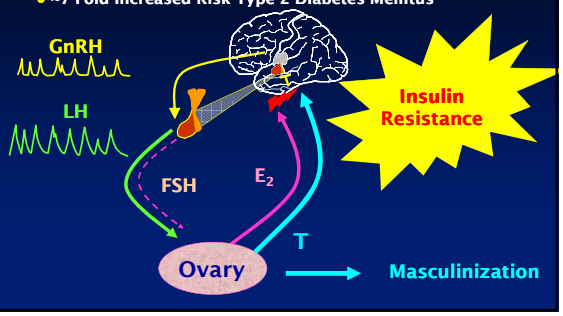
Morgani, JB. *Vol. I, 2nd edn., Remondini: Padova, 1765*

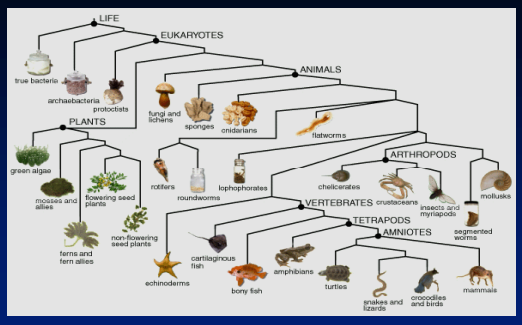
Mendelian Disorders
HAIR-AN

- Type A Adolescent girls, insulin receptor mutations and unknown
- Leprechaunism Infants, insulin receptor mutations
- Lipodystrophy Syndromes
  - Lamin A/C mutations
  - PPARγ mutations
  - unknown

Polycystic Ovary Syndrome

- ~7% Prevalence Anovulatory PCOS
- Leading Cause Hormonally-Related Infertility
- ~7-Fold Increased Risk Type 2 Diabetes Mellitus



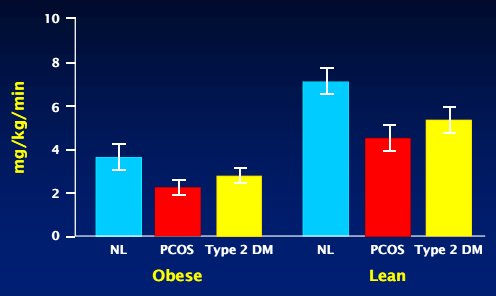


Metabolism & Reproduction

- Insights from PCOS
- Reproductive Actions of Insulin
- Metabolic Actions of Reproductive Hormones
- Genetic Analyses

Insulin-Mediated Glucose Uptake

Decreased by ~40% in PCOS

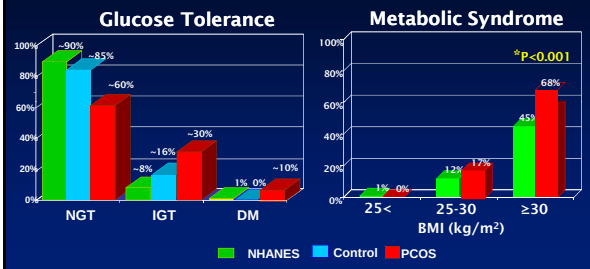


Dunaif A. *Endocr Rev* 18:774, 1997

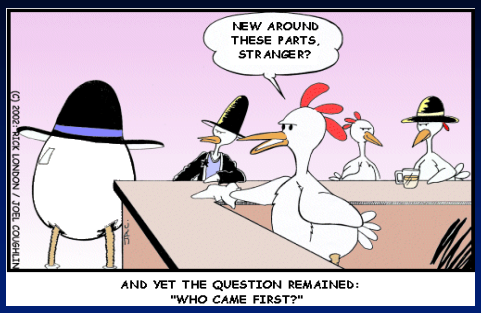
PCOS

Leading Risk Factor Diabetes & MetS

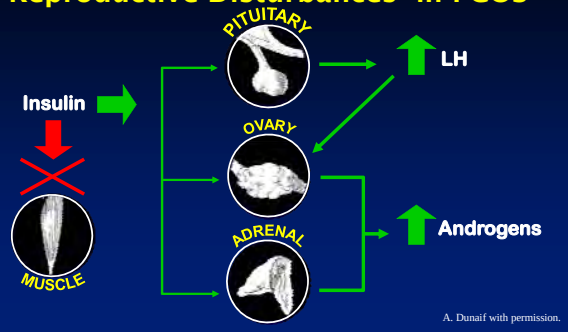
Ages 14 – 40 Years



Adapted from Legro et al. *J Clin Endocrinol Metab 84:165-169, 1999*
Palmert et al. *J Clin Endocrinol Metab 87:1017-1023, 2002*

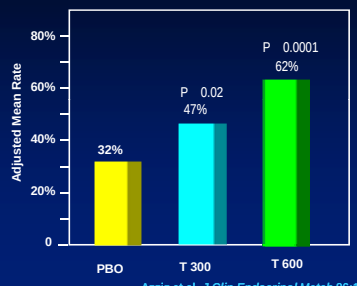


Hyperinsulinemia Amplifies Reproductive Disturbances in PCOS



A. Dunaif with permission.

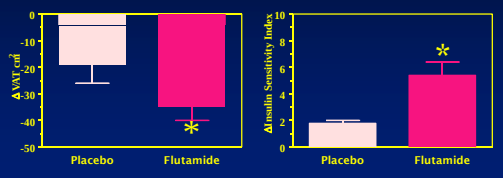
Insulin Sensitizing Agents Increase Ovulation in PCOS



Azziz et al. *J Clin Endocrinol Metab* 86:1626-1632, 2001

Androgen Receptor Blockade

Reduces Visceral Fat & Improves Insulin Sensitivity



Gambineri et al. *J Clin Endocrinol Metab* 91:3970, 2006

Metabolism & Reproduction

- Insights from PCOS
- Reproductive Actions of Insulin
- Metabolic Actions of Reproductive Hormones
- Genetic Analyses

Insulin Action & Reproduction

IRS-2 pathways integrate female reproduction and energy homeostasis

Deborah J. Burks*, Jaime Font de Mora†, Markus Schubert*, Dominic J. Withers‡, Martin G. Myers*, Heather H. Towery*, Shari L. Altamuro*, Carrie L. Flint* & Morris F. White*

*Howard Hughes Medical Institute, Joslin Diabetes Center, Harvard Medical School, One Joslin Place, Boston, Massachusetts 02215, USA
†Centro de Investigacion del Cancer, Facultad de Medicina, Universidad de Salamanca, Salamanca 37007, Spain

Nature 407:377, 2000

Insulin Action & Reproduction

Role of Brain Insulin Receptor in Control of Body Weight and Reproduction

Jens C. Brüning,[1*] Dinesh Gautam,[1] Deborah J. Burks,[2] Jennifer Gillette,[1] Markus Schubert,[1] Paul C. Orban,[3†] Rüdiger Klein,[3] Wilhelm Krone,[1] Dirk Müller-Wieland,[1] C. Ronald Kahn[2*]

Science 289:2122, 2000

Pituitary Insulin Sensitivity & Reproduction

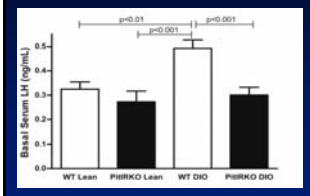
Cell Metabolism
Article

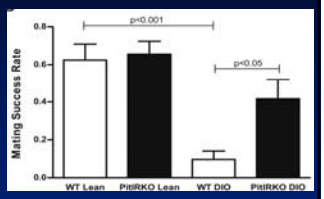
Rescue of Obesity-Induced Infertility in Female Mice due to a Pituitary-Specific Knockout of the Insulin Receptor

Kathryn J. Brothers,[3] Sheng Wu,[1] Sara A. DiVall,[1] Marcus R. Messmer,[2] C. Ronald Kahn,[4] Ryan S. Miller,[1] Sally Radovick,[1] Fredric E. Wondisford,[2,5,6,7] and Andrew Wolfe[1,5,6,*]
[1]Division of Pediatric Endocrinology
[2]Division of Metabolism
[3]Department of Physiology
Johns Hopkins University School of Medicine, 600 North Wolfe Street, Baltimore, MD 21287, USA
[4]Joslin Diabetes Center, Harvard Medical School, One Joslin Place, Boston, MA 02215, USA
[5]These authors contributed equally to this work
*Correspondence: awolfe38@jhmi.edu
DOI 10.1016/j.cmet.2010.06.010

Brothers et al. *Cell Metab* 12:295, 2010

Pituitary Insulin Resistance Protects Against DIO-Mediated Reproductive Dysfunction





Brothers et al. *Cell Metab* 12:295, 2010

Metabolism & Reproduction

- Insights from PCOS
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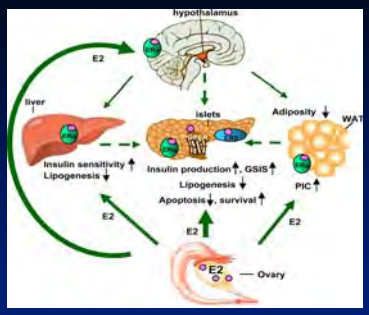
- Metabolic Actions of Reproductive Hormones
- Genetic Analyses



Liu S and Mauvais-Jarvis M. *Endocrinology* 151:859, 2010

Prenatally Androgenized Rhesus

Phenocopy of PCOS

David Abbott & Dan Dumesic

Developmental Exposure to Androgens Programs MetS

Am J Physiol Endocrinol Metab 295: E262–E268, 2008.
First published June 10, 2008; doi:10.1152/ajpendo.90508.2008.

TRANSLATIONAL PHYSIOLOGY

Transient prenatal androgen exposure produces metabolic syndrome in adult female rats

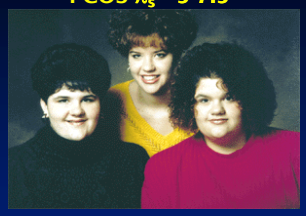
Marek Demissie,[1,3*] Miloš Lazic,[1*] Eileen M. Foecking,[2] Fraser Aird,[1] Andrea Dunaif,[1†] and Jon E. Levine[2†]

[1]Division of Endocrinology, Metabolism, and Molecular Medicine, Northwestern University, Chicago; [2]Department of Neurobiology and Physiology, Northwestern University, Evanston, Illinois; and [3]Department of Endocrinology, Diabetology, and Isotope Therapy, Wroclaw Medical School, Wroclaw, Poland

Submitted 2 February 2008; accepted in final form 5 June 2008

Complex Genetic Disease
Non-Mendelian Familial Aggregation

PCOS λ_s= 5-7.5



Correlation Twins

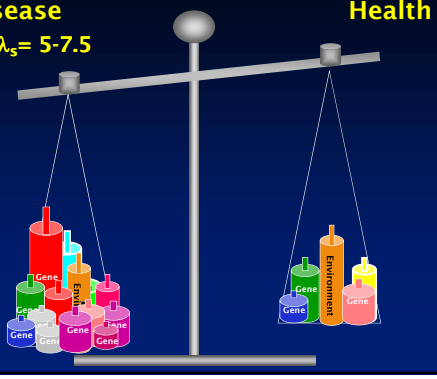
Monozygotic R^2=0.71

Complex Genetic Diseases

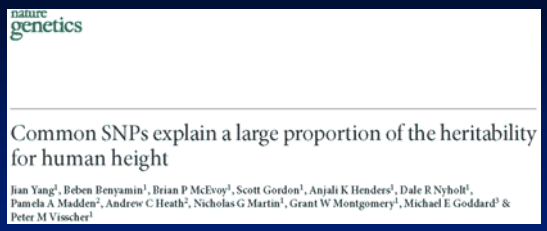
Disease

Health

PCOS λ_s= 5-7.5



Most of the heritability is not missing but has not previously been detected because the individual effects are too small to pass stringent significance tests.


nature genetics

Common SNPs explain a large proportion of the heritability for human height

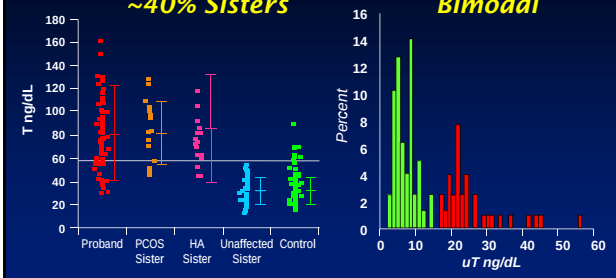
Jian Yang[1], Beben Benyamin[1], Brian P McEvoy[1], Scott Gordon[1], Anjali K Henders[1], Dale R Nyholt[1], Pamela A Madden[2], Andrew C Heath[2], Nicholas G Martin[1], Grant W Montgomery[1], Michael E Goddard[3] & Peter M Visscher[1]

Nat Genet 42:565, 2010

Elevated Testosterone Levels

Sisters n=224

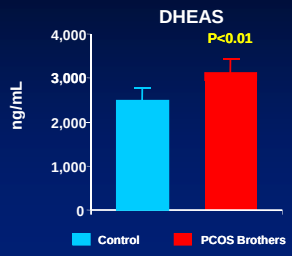
~40% Sisters *Bimodal*



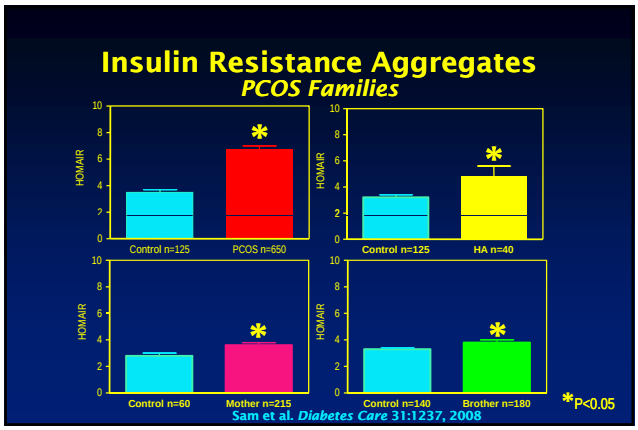
Legro et al. *Proc Natl Acad Sci* 95:14956, 1998

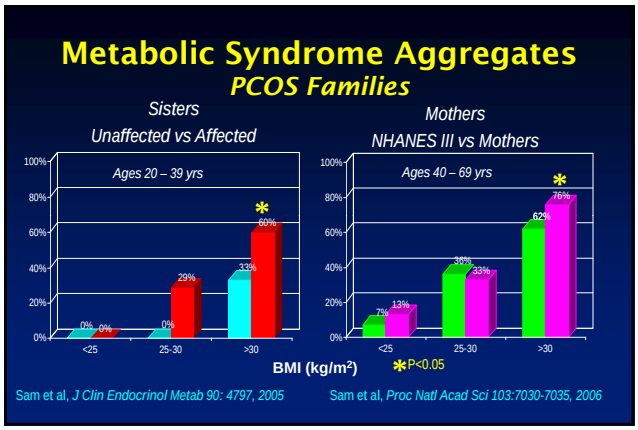
Male Reproductive Phenotype

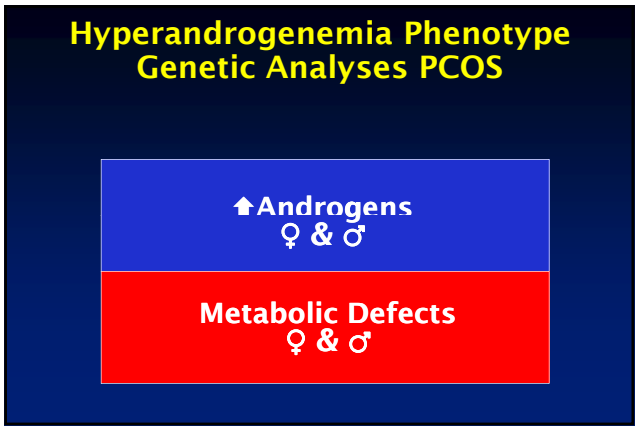
Brothers are Hyperandrogenemic

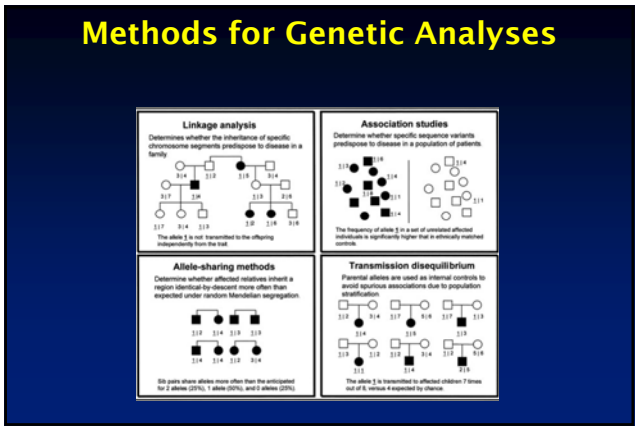


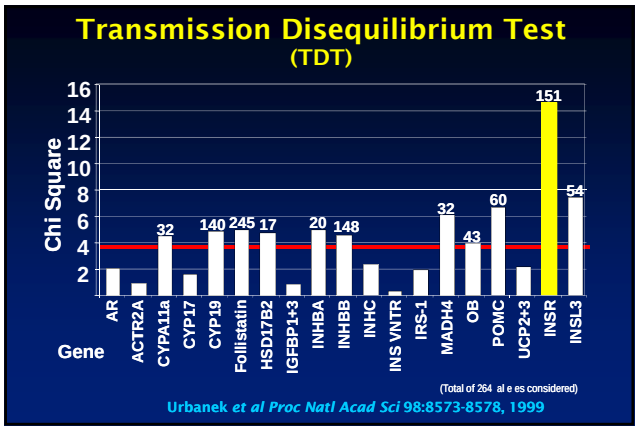
Legro et al *J Clin Endocrinol Metab 87:2134-38*, 2002

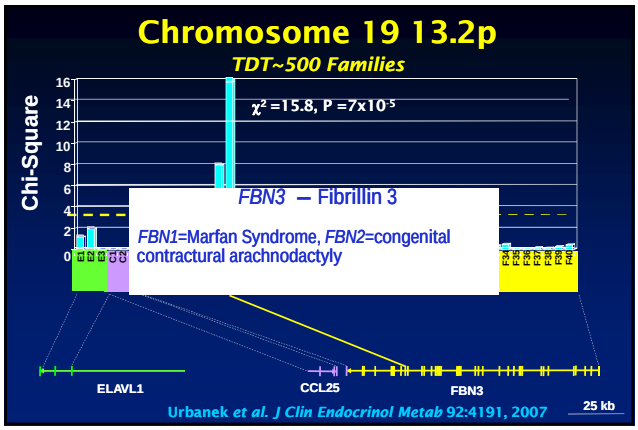

Insulin Resistance Aggregates
PCOS Families
HOMAIR
Control n=125
PCOS n=650
Control n=125
HA n=40
Control n=60
Mother n=215
Control n=140
Brother n=180
*P<0.05
Sam et al. Diabetes Care 31:1237, 2008


Metabolic Syndrome Aggregates
PCOS Families
Sisters
Unaffected vs Affected
Ages 20 – 39 yrs
Mothers
NHANES III vs Mothers
Ages 40 – 69 yrs
<25
25-30
>30
BMI (kg/m²)
*P<0.05
Sam et al, J Clin Endocrinol Metab 90: 4797, 2005
Sam et al, Proc Natl Acad Sci 103:7030-7035, 2006

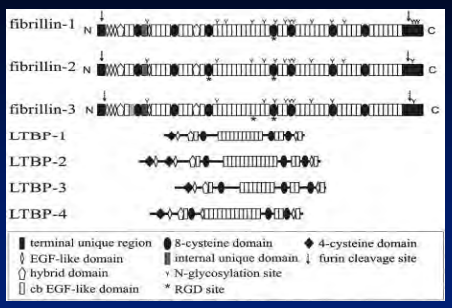

Hyperandrogenemia Phenotype
Genetic Analyses PCOS
Androgens
♀ & ♂
Metabolic Defects
♀ & ♂


Methods for Genetic Analyses
Linkage analysis
Association studies
Allele-sharing methods
Transmission disequilibrium


Transmission Disequilibrium Test
(TDT)
Chi Square
16
14
12
10
8
6
4
2
32
140
245
17
20
148
32
43
60
151
54
Gene
AR
ACTR2A
CYPA11a
CYP17
CYP19
Follistatin
HSD17B2
IGFBP1+3
INHBA
INHBB
INHC
INS VNTR
IRS-1
MADH4
OB
POMC
UCP2+3
INSR
INSL3
(Total of 264 al e es considered)
Urbanek et al Proc Natl Acad Sci 98:8573-8578, 1999

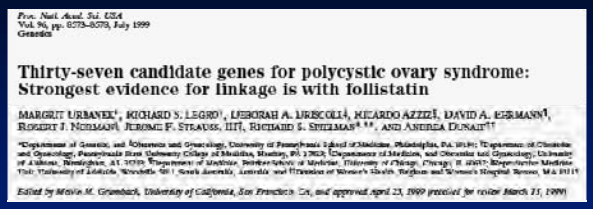

Chromosome 19 13.2p
TDT~500 Families
Chi-Square
16
14
12
10
8
6
4
2
0
χ² =15.8, P =7x10⁻⁵
FBN3 – Fibrillin 3
FBN1=Marfan Syndrome, FBN2=congenital contractural arachnodactyly
ELAVL1
CCL25
FBN3
25 kb
Urbanek et al. J Clin Endocrinol Metab 92:4191, 2007

Fibrillin & LTBP Superfamily



Charbonneau et al. *Birth Defects Research* 72:37-50, 2004

Follistatin Linked with PCOS


Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 8573–8578, July 1999
Genetics

Thirty-seven candidate genes for polycystic ovary syndrome: Strongest evidence for linkage is with follistatin

MARGRIT URBANEK[*], RICHARD S. LEGRO[†], DEBORAH A. DRISCOLL[‡], RICARDO AZZIZ[§], DAVID A. EHRMANN[¶], ROBERT J. NORMAN[‖], JEROME F. STRAUSS, III[†], RICHARD S. SPIELMAN[*,**], AND ANDREA DUNAIF[††]

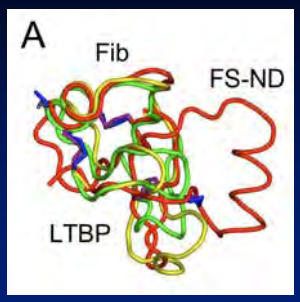
[*]Department of Genetics, and [‡]Obstetrics and Gynecology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104; [†]Department of Obstetrics and Gynecology, Pennsylvania State University College of Medicine, Hershey, PA 17033; [‡]Department of Medicine, and Obstetrics and Gynecology, University of Alabama, Birmingham, AL 35233; [§]Department of Medicine, Petrie-Schoen of Medicine, University of Chicago, Chicago, IL 60637; [‖]Reproductive Medicine Unit, University of Adelaide, Woodville 5011, South Australia, Australia; and [††]Division of Women's Health, Brigham and Women's Hospital, Boston, MA 02115

Edited by Melvin M. Grumbach, University of California, San Francisco, CA, and approved April 23, 1999 (received for review March 15, 1999)

Follistatin

- Activin-binding protein
- Neutralizes effects of activin
- Activin decreases:
  - Production of androgen by thecal cells
- Activin increases:
  - Development of ovarian follicles
  - Secretion of pituitary FSH
  - Insulin secretion

Structural Homology Follistatin, Fibrillin & LTBP TB Domains

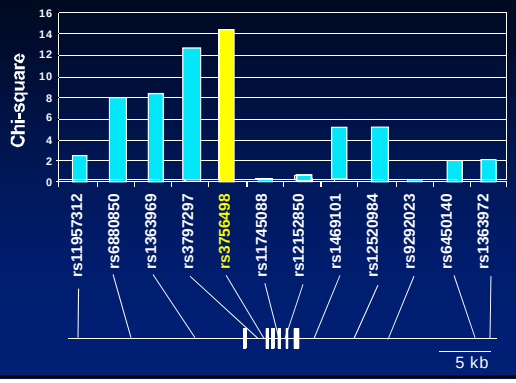


Thompson et al. *Dev Cell* 9:535, 2005

Follistatin
Case – Control Studies

- ~630 PCOS Cases
- ~540 Controls Most unselected for androgens
- Included SNPs 20 kb upstream & downstream of the gene & all intronic sequences

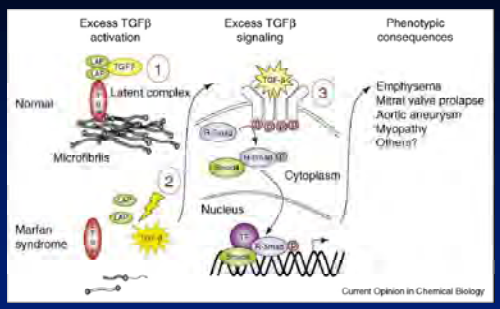
Follistatin χ^2=14.4, P=0.0001



TGFβ Signaling Pathway

- Ligands
  - Inhibins, Activins, Myostatin, BMPs, GDFs, AMH, TGFβ1-3, NODAL
- Extracellular Antagonists
  - Follistatin, Fibrillins, Noggin, Chordin, DAN/Cereberus
- Type I Receptors
  - ACVR1, ACVR1B, ACVR1C, BMPR1A, BMPR1B, TGFBR1
- Type II Receptors
  - ACVRL1, ACVR2A, ACVR2B, BMPR2, AMHR2, TGFBR2
- Type III Receptors
  - TGFBR3, ENG, IGSF
- Intracellular Modulators of Signaling
  - SMAD1-8, Smurf1-2, SARA, TGFBTRAP1, BAMBI, GSC

Marfan Syndrome



Ramirez & Dietz Current Opinion in Genetics & Development 17:252, 2007

Follistatin & Metabolism

Role of Follistatin in Promoting Adipogenesis in Women

John N. Flanagan, Kristina Linder, Niklas Mejhert, Elisabeth Dungner, Kerstin Wahlen, Pauline Decaunes, Mikael Ryden, Peyman Bjorklund, Stefan Arner, Shalender Bhasin, Anne Bouloumie, Peter Arner, and Ingrid Dahlman

Andrology Center (J.N.F., S.A.), and Department of Medicine (J.N.F., K.L., N.M., E.D., K.W., M.R., S.A., P.A., I.D.), Huddinge, Karolinska Institutet, S-141 86 Stockholm, Sweden; Institut National de la Santé et de la Recherche Médicale (P.D., A.B.), Unité 858 and Université Toulouse III-Paul Sabatier, Institut de Médecine Moléculaire de Rangueil, 31432 Toulouse, France; Department of Surgical Sciences (P.B.), Uppsala University Hospital, SE-751 05 Uppsala, Sweden, and Yale Endocrine Neoplasia Laboratory, Yale University School of Medicine, New Haven, Connecticut 06520; and Section of Endocrinology, Diabetes, and Nutrition (S.B.), Boston University School of Medicine, Boston, Massachusetts 02118

J Clin Endocrinol Metab 94:3003, 2009

Fibrillin-3 Not Expressed Adult Ovary

Molecular Human Reproduction, Vol.15, No.12 pp. 829–842, 2009
Advanced Access publication on August 19, 2009 doi:10.1093/molehr/gap072

MHR ORIGINAL RESEARCH

Genetic and gene expression analyses of the polycystic ovary syndrome candidate gene fibrillin-3 and other fibrillin family members in human ovaries

Mark J. Prodoehl[1], Nicholas Hatzirodos[1], Helen F. Irving-Rodgers[1], Zhen Z. Zhao[3], Jodie N. Painter[3], Theresa E. Hickey[1], Mark A. Gibson[2], William E. Rainey[4,5], Bruce R. Carr[5], Helen D. Mason[6], Robert J. Norman[1], Grant W. Montgomery[3], and Raymond J. Rodgers[1,7]

[1]Research Centre for Reproductive Health, Robinson Institute and School of Paediatrics and Reproductive Health, University of Adelaide, Adelaide, SA 5005, Australia [2]School of Medical Science, University of Adelaide, Adelaide, SA 5005, Australia [3]Molecular Epidemiology, Queensland Institute of Medical Research, Brisbane, QLD 4029, Australia [4]Department of Physiology, Medical College of Georgia, Augusta, GA 30912, USA [5]Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, University of Texas Southwestern Medical Center at Dallas, Dallas, TX 75390-9032, USA [6]Division of Basic Medical Sciences, St. George's University of London, Cranmer Terrace, London SW17 0RE, UK

[7]Correspondence address. E-mail: rjr.rodgers@adelaide.edu.au

Fibrillin-3 Decreased Polycystic Ovaries

Volume 58(10): 903–915, 2010
Journal of Histochemistry & Cytochemistry
http://www.jhc.org

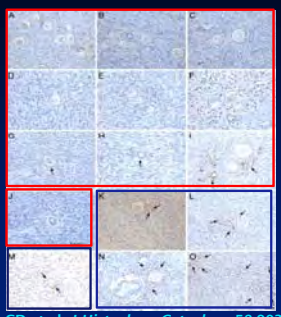
ARTICLE

Fibrillins in Adult Human Ovary and Polycystic Ovary Syndrome: Is Fibrillin-3 Affected in PCOS?

C. Diana Jordan, Sandra D. Bohling, Noe L. Charbonneau, and Lynn Y. Sakai

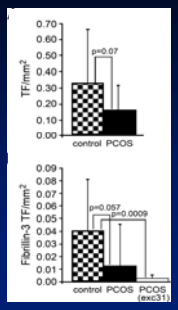
Department of Pathology, University of Washington Medical Center, Seattle, Washington (CDJ,SDB); Shriners Hospital for Children, Portland, Oregon (NLC,LYS); and Department of Biochemistry and Molecular Biology, Oregon Health & Science University, Portland, Oregon (LYS)

Fibrillin-3 Decreased Polycystic Ovaries



Jordan CD et al. *J Histochem Cytochem* 58:903, 2010

Fibrillin-3 Decreased Polycystic Ovaries



Jordan CD et al. *J Histochem Cytochem* 58:903, 2010

What about genes implicated in Type 2 Diabetes and Obesity?

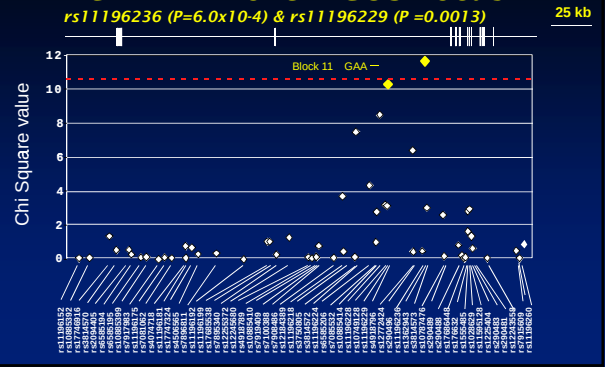
TCF7L2, FTO, etc?

TCF7L2
Case – Control Studies

- ~630 PCOS Cases
- ~540 Controls Most unselected for androgens
- ~260 kb *TCF7L2* genomic region

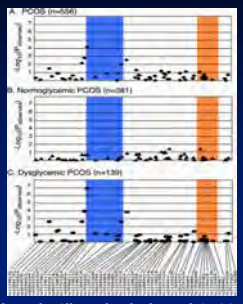
TCF7L2 – Novel PCOS Locus

rs11196236 (P=6.0x10-4) & rs11196229 (P =0.0013)



TCF7L2 T2D Locus

Significant Association Proinsulin:Insulin Molar Ratio



Urbanek *et al J Clin Endocrinol Metab* 94:2617, 2009

FTO Associated with BMI not PCOS

Diabetologia (2008) 51:1153–1158
DOI 10.1007/s00125-008-1028-6

ARTICLE

Association of variants in the fat mass and obesity associated (*FTO*) gene with polycystic ovary syndrome

T. M. Barber · A. J. Bennett · C. J. Groves · U. Sovio · A. Ruokonen · H. Martikainen · A. Pouta · A.-L. Hartikainen · P. Elliott · C. M. Lindgren · R. M. Freathy · K. Koch · W. H. Ouwehand · F. Karpe · G. S. Conway · J. A. H. Wass · M.-R. Järvelin · S. Franks · M. I. McCarthy

Proving Causation of a Variant

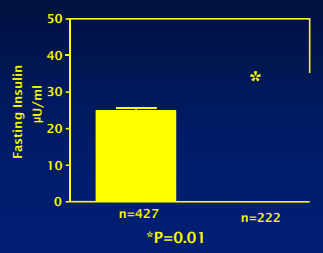
Especially difficult for complex diseases!

- Variation rather than mutation
- Implicated genomic regions are large
- Variation at any given gene is neither necessary nor sufficient
- Variants may have subtle effects on expression levels or function

Insulin Resistance *FBN3*+ PCOS

Independent of BMI

~650 Caucasians PCOS European Ancestry

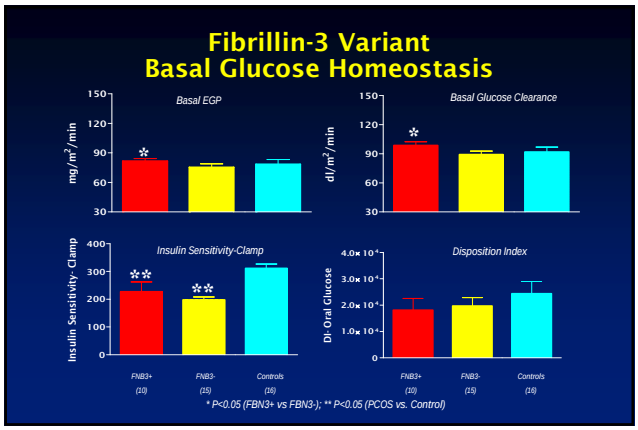


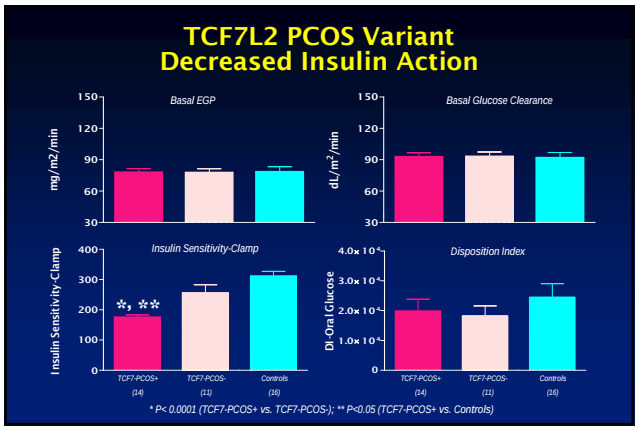
Urbanek *et al. J Clin Endocrinol Metab 92:4191, 2007*

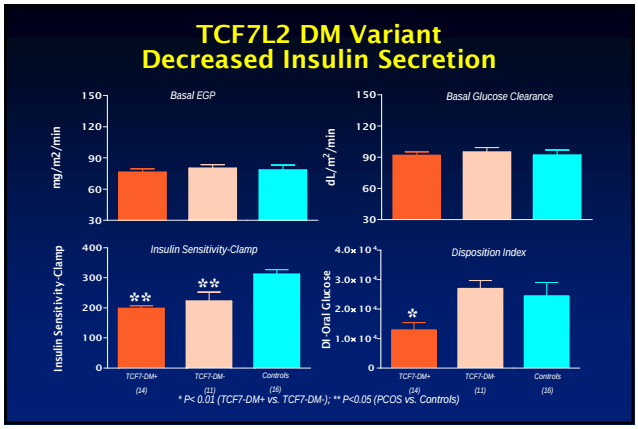
Baseline Features PCOS & Controls

Age, BMI & VAT Similar by Genotype

	FBN3-	*FBN3+*	*TCF7L2* PCOS-	*TCF7L2* PCOS+	*TCF7L2* DM	*TCF7L2* DM+	Control
n	19	12	12	18	13	14	18
Age	28±5	30±4	30±5	28±4	29±4	28±5	30±4
BMI	35±7	31±9	32±10	35±7	36±10	32±7	34±9
VAT	99±56	87±50	86±52	106±55	101±53	92±54	81±43
LBM	59±15	69±17	53±12	55±9	57±12	51±8	55±7
T	72±29	68±21	59±22	74±26	59±22	75±25	28±12
uT	26±12	21±9	19±6	27±11	23±9	24±12	9±4
SHBG	52±34	57±53	61±66	51±22	47±52	60±37	47±29
DS	239±114	207±112	178±80	251±128	206±84	231±136	145±53
Glu	91±6	94±9	92±8	94±7	95±7	91±7	94±6
Glu-2 h	131±31	125±25	97±28	127±32	121±29	134±31	108±21
IRI	21±12	15±7	20±13	20±9	23±11	16±10	15±9


Fibrillin-3 Variant
Basal Glucose Homeostasis
Basal EGP
mg/m²/min
Basal Glucose Clearance
dl/m²/min
Insulin Sensitivity-Clamp
Insulin Sensitivity- Clamp
Disposition Index
DI- Oral Glucose
FNB3+ (10)
FNB3- (15)
Controls (16)
* P<0.05 (FBN3+ vs FBN3-); ** P<0.05 (PCOS vs. Control)


TCF7L2 PCOS Variant
Decreased Insulin Action
Basal EGP
mg/m2/min
Basal Glucose Clearance
dL/m²/min
Insulin Sensitivity-Clamp
Insulin Sensitivity-Clamp
Disposition Index
DI-Oral Glucose
TCF7-PCOS+ (14)
TCF7-PCOS- (11)
Controls (16)
* P< 0.0001 (TCF7-PCOS+ vs. TCF7-PCOS-); ** P<0.05 (TCF7-PCOS+ vs. Controls)

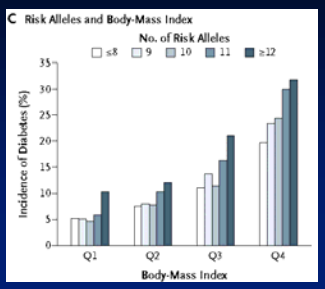

TCF7L2 DM Variant
Decreased Insulin Secretion
Basal EGP
mg/m2/min
Basal Glucose Clearance
dL/m²/min
Insulin Sensitivity-Clamp
Insulin Sensitivity-Clamp
Disposition Index
DI-Oral Glucose
TCF7-DM+ (14)
TCF7-DM- (11)
Controls (16)
* P< 0.01 (TCF7-DM+ vs. TCF7-DM-); ** P<0.05 (PCOS vs. Controls)

Stepwise Regression Analysis- All Genotypes With Clinical Risk Factors

- *TCF7L2*-PCOS Predicts Insulin Sensitivity (Partial R^2=0.34, P<0.01)
- *TCF7L2* DM Predicts Insulin Secretion (Partial R^2=0.60, P=0.001)
- Trend *FBN3* Predicting Glucose Clearance & Basal Insulin Secretion

Increased Risk DM with Increasing Number Risk Alleles

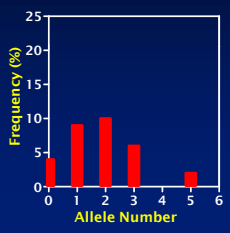
TCF7L2, PPARG, FTO,KCNJ11, NOTCH2, WFS1, CDKAL1, IGF2BP2, SLC30A8, JAZF1, and HHEX

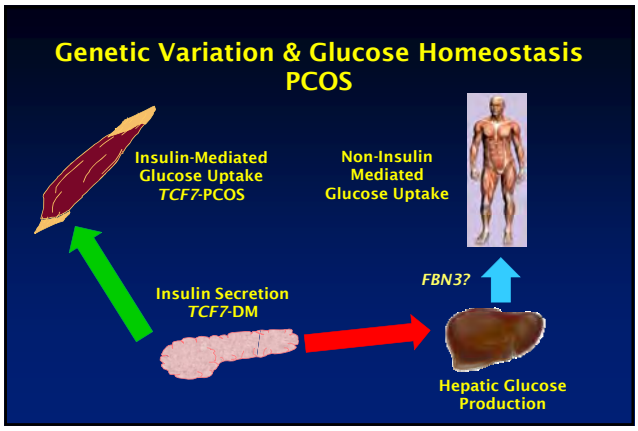


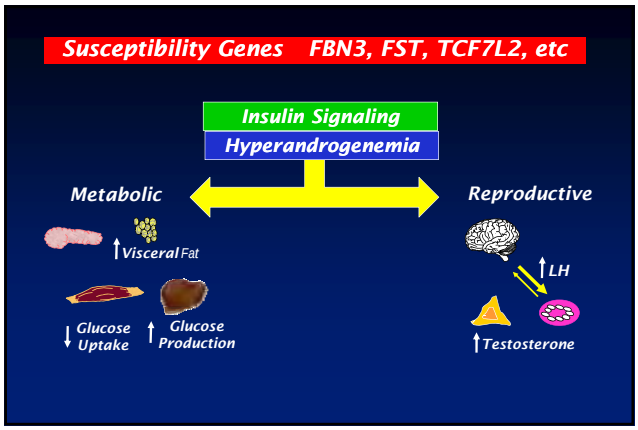
Lyssenko et al. *N Engl J Med* 359:2220, 2008

Pancreatic β-Cell Dysfunction Associated with Increasing Number Risk Alleles PCOS

r^2= 0.36, P< 0.05




Genetic Variation & Glucose Homeostasis
PCOS
Insulin-Mediated
Glucose Uptake
TCF7-PCOS
Non-Insulin
Mediated
Glucose Uptake
Insulin Secretion
TCF7-DM
FBN3?
Hepatic Glucose
Production


Susceptibility Genes FBN3, FST, TCF7L2, etc
Insulin Signaling
Hyperandrogenemia
Metabolic
Reproductive
Visceral Fat
Glucose
Uptake
Glucose
Production
LH
Testosterone

Northwestern University
Specialized Center Of Research
Genes, Androgens and Intrauterine Environment in PCOS
Northwestern University
Andrea Dunaif, MD
Jon Levine, PhD
Margrit Urbanek, PhD
Susan Sam, MD
Milos Lazic, Grad Student
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Fraser Aird, PhD
Sudha Yalamanchi, MD
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David A. Abbott, PhD
Current: P50 HD44405, R01 DK073411 & R01 HD057223
Past: U54 HD034449