Transcription Regulation And Gene Expression in Eukaryotes (Cycle G2 #13709-01)

NUCLEAR HORMONE RECEPTORS AND COREGULATORS
Brief history of steroid signaling (100 years on)

1902-1905 - Starling refers to bioactive chemicals extracted from glands as „hormones“
1915 - Kendall crystallizes thyroid hormone
1925 - Kendall and Reichstein complete structural analysis of cortisol from adrenal cortex
1946 – Selye coins the term glucocorticoid, needed for survival and response to stress
1949 – Hench administers cortisone to arthritic patients with dramatic effects
1950 – Kendall, Hench and Reichstein get the Nobel Prize
1950-1985 – Classical model of steroid hormone action
1986 – today - Receptor identification : „reverse endocrinology“ GR, ER, TR, RAR, RXR ...
Nuclear Receptors and Small Lipophilic Ligands

# The family of nuclear hormone receptors

<table>
<thead>
<tr>
<th>Members</th>
<th>Type I</th>
<th>Type II</th>
<th>Type III/orphan</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucocorticoid receptor</td>
<td>Retinoic acid receptor (RAR)</td>
<td>NGFI-B</td>
</tr>
<tr>
<td></td>
<td>Estrogen receptor</td>
<td>Retinoid X receptor (RXR)</td>
<td>ELP</td>
</tr>
<tr>
<td></td>
<td>Androgen receptor</td>
<td>Vitamin D3 receptor (VD3R)</td>
<td>Nurr77</td>
</tr>
<tr>
<td></td>
<td>Mineralocorticoid receptor.</td>
<td>Thyroid hormone receptor (T3R)</td>
<td>SHP</td>
</tr>
<tr>
<td></td>
<td>Progesteron receptor</td>
<td>Peroxisome proliferator activated receptor (PPAR)</td>
<td>DAX</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Liver X receptor (LXR)</td>
<td>LRH1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Farnesol X receptor (FXR)</td>
<td>ERR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pregnane activated X receptor (PXR)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Steroid+xenobiotic X receptor (SXR)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Benzoate X receptor (BXR)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Androstane receptor(CAR)</td>
<td></td>
</tr>
<tr>
<td>Localisation</td>
<td>Cytoplasm – nuclear (HSP90 complexation)</td>
<td>nuclear</td>
<td>nuclear</td>
</tr>
<tr>
<td></td>
<td>GR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dimerisation</td>
<td>homo</td>
<td>hetero (RXR)</td>
<td>hetero (RXR)</td>
</tr>
<tr>
<td>Binding</td>
<td>IR 3</td>
<td>DR</td>
<td>Single Repeat + extension</td>
</tr>
<tr>
<td>Mode of action</td>
<td>Transactivation “systemic”</td>
<td>Transactivation AP-1 antagonism</td>
<td>?</td>
</tr>
</tbody>
</table>

(48 in human; 230 in C. elegans; 21 in D. melanogaster)
Nuclear Hormone Receptors are Modular in Nature (operationally defined from A-F)

- **Ligand independent trx**
  - “AD”
  - AF-1 function

- **DNA binding**
- **dimerization**

- **Hinge**
- **NLS**
- **Hsp90**

- **Ligand dependent trx**
  - “AD”
  - dimerization
  - AF-2 function
  - co-regulator recruitment
  - NLS
  - Hsp90
C domain (DBD): 2 cys-cys Zinc Fingers eg. ERα
Cooperative Binding (homodimer/heterodimer)
Homodimer/heterodimer (NR/RXR) formation involves both the C and E domains.
Nuclear Hormone Receptors are Transcriptional Regulators

P. Chambon and co-workers, IGBMC, Illkirch France
The family of nuclear hormone receptor: unified nomenclature 
(based on the two well conserved domains C and E)
Induction of steroid responsive genes involves hormone dependent dissociation of the receptor from hsp90 (type I nuclear receptors).
Regulation of Specific Gene Expression

### Table 1: Some examples of selective or specific hormonal induction of gene expression

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Species, tissue induced de novo</th>
<th>Gene regulated</th>
<th>Expression</th>
<th>Rate of transcription</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oestrogen</td>
<td>Chick oviduct</td>
<td>Ovalbumin, egg white proteins, Vitellogenin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Chick liver</td>
<td>Vitellogenin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Frog liver</td>
<td>Vitellogenin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glucocorticoid</td>
<td>Rat liver</td>
<td>Tyrosine aminotransferase</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Progesterone</td>
<td>Rabbit uterus</td>
<td>Uteroglobin</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Testosterone</td>
<td>Rat, mouse prostate</td>
<td>Prostate specific antigen</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Thyroid hormone</td>
<td>Rat heart, frog tadpole liver, frog tadpole intestine, frog tadpole skin</td>
<td>Myosin light chain, Carbomyl phosphate synthetase, Stromelysin 3, Adult keratin</td>
<td>–, –, +</td>
<td>+, +, +</td>
</tr>
<tr>
<td>Ecdysone</td>
<td>Insect larval salivary gland</td>
<td>Glue protein</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Juvenile hormone</td>
<td>Larval fat body</td>
<td>Vitellogenin</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Steroid/thyroid dependent gene regulation: gene expression induced *denovo* and gene expression regulated (rate of transcription) by the hormone
LBD of GR Mediates Translocation to the Nucleus in presence of Hormone

Proteins expressed:

- (a) - Dex
  - β-Galactosidase
- (b) + Dex
  - Glucocorticoid receptor
- (c) + Dex
  - GR ligand-binding domain
The adrenal cortex is responsible for production of 3 major classes of steroid hormones: 
glucocorticoids, which regulate carbohydrate metabolism; mineralocorticoids, which regulate the 
body levels of sodium and potassium; and androgens, whose actions are similar to that of 
steroids produced by the male gonads.
PPAR/RXR-dependent nuclear signaling (type II nuclear receptors)

- PPAR - Peroxisome Proliferator Activated Receptor
- RXR - Rexinoid Receptor

NHR are the final effectors of a complex cytoplasmic/nuclear transduction cascade
E domain: canonical structure of the LBD

- Characteristical sandwich architecture with 3 layers built in by 12 alpha antiparallel helices and 1 antiparallel beta sheet
- Structure-AA sequence relationship for NHR LBD’s
- Ligand binding dependent pocket remodeling
- Receptor dimerization surface
- Co-regulator proteins interface
- Control of agonist vs. antagonist modes of action
### Nuclear Hormone Receptor Superfamily: Well Conserved DBD, Poorly Conserved LBD

<table>
<thead>
<tr>
<th>Receptor</th>
<th>DNA-Binding Domain</th>
<th>Ligand Binding Domain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progesterone Receptor</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>NGF1B</td>
<td>40%</td>
<td>18%</td>
</tr>
<tr>
<td>Retinoid X Receptor</td>
<td>35%</td>
<td>18%</td>
</tr>
<tr>
<td>Thyroid Hormone Receptor</td>
<td>28%</td>
<td>16%</td>
</tr>
</tbody>
</table>

Well conserved at sequence level

Well conserved at structural level

Poorly conserved at sequence level

Well conserved at structural level
Corepressor vs. Coactivator Interfaces  Structure Remodeling

“mouse trap”
unliganded co-repressor binding
liganded co-activator binding
Protein:protein interaction in vivo screen: “two hybrid-screen”

(a) Hybrid proteins
DNA-binding domain  Bait domain
Bait hybrid

(b) Fish domain  Activation domain
Fish hybrid

(c) Fishing for proteins that interact with bait domain
Bait gene  TRP
Bait vector
Fish cDNA from library  LEU
Fish vector

1. Transfect into trp, leu, his mutant yeast cells
2. Select for cells that grow in absence of tryptophan and leucine
3. Plate selected cells on medium lacking histidine

Colony formation  No colony formation

Coactivator CBP/p300, Corepresssor Ncor, etc

Combinatorial roles of multiple cofactor complexes are required to switch between transcriptional repression and activation functions
Structure and function of the PGC-1 family coregulators: binding to the HAT and TRAP/DRIP/Mediator complexes at the amino and carboxyl termini, respectively. SirT1 and p160 bind to the repression domain, which also contains three p38 MAP kinase phosphorylation sites.

PGC-1 Coactivator Functions in Maintenance of Glucose, Lipid and Energy Homeostasis

Cold exposure
Fasting
Physical exercise

Hormones

PGC-1

other NRs
GR
/ HNF4
PPARα
TR/PPARγ
ERR/

PGC-1 Coactivator Functions in Maintenance of Glucose, Lipid and Energy Homeostasis

GLUONEOGENESIS
FATTY ACID OXIDATION
MITOCHONDRIAL BIOGENESIS
RESPIRATION

?
Coactivator and Corepressor Complexes with the Basal Machinery are Involved in the Regulation of NHR Transcriptional Activity.
Nuclear Receptor Coregulator Interaction Motifs
Nuclear receptors and coregulators manifest in reproduction, development, central and basal metabolism and energy homeostasis
Molecular Bases for Agonism vs partial Agonist vs Antagonism: Selective Modulator Concept eg. ER$\alpha$

Co-regulator box LXXLL
Estrogen: Hormone with Ambivalent Functions
Molecular Action of Estradiol and of SERM Tamoxifen

Selective Estrogen Receptor Modulators

Estrogens

SERMs

SERMs- designed to act in specific ways at each of the estrogen receptor sites in different tissues

Phytoestrogens

Anti Estrogens

ERDR
The Liver-X-Receptor Genes

\( \alpha \)

\( \beta \)
Functions of Key LXR/RXR-regulated genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCA1</td>
<td></td>
</tr>
<tr>
<td>ABCG1</td>
<td></td>
</tr>
<tr>
<td>Cxx7A1</td>
<td></td>
</tr>
<tr>
<td>CETP</td>
<td></td>
</tr>
<tr>
<td>SREBP1</td>
<td></td>
</tr>
</tbody>
</table>
Molecular Function of the Liver-X-Receptors

\( \alpha \)  \( \beta \)

CYP7A1-LXRE  CCTTCTGGTCACCTCAAGTGG
             GGAAACCAGTGGTTCAAGGTTAC
               DR4

ABCA1 LXRE  TTTGACCAGATAGTGATTGCTGCG
             ACACTGCCATTGGTTCAAGGCG
               DR4

SREBP-1c LXRE GTCACTGGCAGGTGCTATTGGGT
            CAGTGACCGTGTTACCCAGCA
               DR4
Role of Cyp7A1 in Bile Acid Synthesis
Reverse Cholesterol Transport and Bile Acid Metabolism

- **Liver**: ABC1,8, BA, LXR, CYP7A1, CYP8B1, BSEP, FXR, NTCP, OATP-1, LRH-1, SHP
- **Macrophages**: ABC1,8, LXR
- **Gut**: BA, LXR

**Key Terms**:
- **LDL R**: LDL Receptor
- **VLDL**: Very Low Density Lipoprotein
- **HDL**: High Density Lipoprotein
- **FXR**: Farnesoid X Receptor
- **OxC**: Oxidized Cholesterol
- **C**: Cholesterol
- **BA**: Bile Acid
- **OATP-1**: Organic Anion Transporter Protein-1
- **SHP**: Sterol Regulatory Element-Binding Protein-1
- **NTCP**: Na+Ta+ Copporter 1
- **LRH-1**: Liver X Receptor
- **Enterohepatic circulation**: The exchange of bile acids between the gut and liver.

**Diagram Notes**:
- The diagram illustrates the interaction between liver, macrophages, and the gut in the context of reverse cholesterol transport and bile acid metabolism.
Reverse Cholesterol Transport and Bile Acid Metabolism
Cholesterol and Triglyceride Synthesis Pathways

LXR
LXRα Null Mice Shows Defects in Cholesterol Disposal

A

Chow
Chow + 2% Chol.

<table>
<thead>
<tr>
<th>Cyp7a</th>
<th>+/+</th>
<th>−/−</th>
<th>+/+</th>
<th>−/−</th>
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<tr>
<td>1.0</td>
<td>5.9</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cyp7b</th>
<th>+/+</th>
<th>−/−</th>
<th>+/+</th>
<th>−/−</th>
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</thead>
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</table>

<table>
<thead>
<tr>
<th>β−Actin</th>
<th>+/+</th>
<th>−/−</th>
<th>+/+</th>
<th>−/−</th>
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<tr>
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</tr>
</tbody>
</table>

B

Hepatic Cholesterol

C

Facial Bile Acid Excretion

D

Bile Acid Pool Composition

E

Bile Acid Pool Composition

F

Bile Acid Pool Composition

G

Bile Acid Pool Composition

H

Bile Acid Pool Composition

I

Bile Acid Pool Composition

J

Bile Acid Pool Composition

K

Bile Acid Pool Composition

L

Bile Acid Pool Composition

M

Bile Acid Pool Composition

N

Bile Acid Pool Composition

O

Bile Acid Pool Composition

P

Bile Acid Pool Composition

Q

Bile Acid Pool Composition

R

Bile Acid Pool Composition

S

Bile Acid Pool Composition

T

Bile Acid Pool Composition

U

Bile Acid Pool Composition

V

Bile Acid Pool Composition

W

Bile Acid Pool Composition

X

Bile Acid Pool Composition

Y

Bile Acid Pool Composition

Z

Bile Acid Pool Composition

α
Integrated Physiology by PPAR Isoforms

- Liver
  - Fatty acid oxidation
  - Fasting response
  - Lipogenesis and lipid storage

- Fat
  - Adipogenesis
  - Lipogenesis and lipid storage
  - Adipokine production
  - Major target of TZDs
  - Fatty acid oxidation
  - Energy uncoupling

- Muscle
  - Regulation of whole-body insulin sensitivity
  - Fatty acid oxidation
  - Energy uncoupling

- PPAR-γ
- PPAR-α
- PPAR-β
Peroxisome proliferator-activated receptors PPAR Isoforms

- Inducing the proliferation of peroxisomes in rodents
- Intimately connected to the cellular metabolism and cell differentiation

<table>
<thead>
<tr>
<th>Simplified overview of current understanding of the metabolic roles of the 3 PPAR isoforms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sites of highest expression</strong></td>
</tr>
<tr>
<td><strong>Cellular processes activated</strong></td>
</tr>
<tr>
<td><strong>Physiological function</strong></td>
</tr>
<tr>
<td><strong>Examples of target genes</strong></td>
</tr>
<tr>
<td><strong>Metabolic phenotype of knockout mice</strong></td>
</tr>
</tbody>
</table>
Ligands and Functions of the PPARα and -γ Isoforms

**PPARα**
- FIBRATES
  - Gemfibrozil, Benzafibrate, Fenofibrate
- POLYUNSATURATED FATTY ACIDS
  - Docoehexaenoic acid, eicosapentaenoic acid, linoleic acid, linolenic acid and arachidonic acid
- HDL RAISE, LIPID CATABOLISM
- PEROXISOME PROLIFERATION
- CONTROL OF INFLAMMATION

**PPARγ**
- THIAZOLIDINEDIONES
  - Rosiglitazone, Pioglitazone, Ciglitazone
- GLUCOSE HOMEOSTASIS
- LIPID STORAGE
- ADIPOCYTE DIFFERENTIATION
- CONTROL OF INFLAMMATION
**Ligands and Functions of PPAR**

- **PPAR**\(_{\beta}/\delta\)

**POLYUNSATURATED FATTY ACIDS**

**PROSTAGLANDINS**

- **GW501516**

- **DIFFERENTIATION** of oligodendrocytes, epithelial cells, keratinocytes and adipocytes

- **LIPID METABOLISM IN THE BRAIN**

- **EMBRYO IMPLANTATION AND DECIDUALIZATION**

- **TUMORIGENESIS IN THE COLON**

- **REVERSE CHOLESTEROL TRANSPORT**

- **WOUND HEALING**
**Synthetic \’s**

- thiazolidinediones (TZDs)
  - treatment of Type II Diabetes

- PPAR alpha specific
  - GW 7647

- PPAR beta specific
  - GW 50-1516

<table>
<thead>
<tr>
<th>Compound</th>
<th>EC$_{50}$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Troglitazone</td>
<td>0.55</td>
</tr>
<tr>
<td>Ploglitazone</td>
<td>0.58</td>
</tr>
<tr>
<td>Rosiglitazone</td>
<td>0.043</td>
</tr>
</tbody>
</table>

*with nM affinity*
Correlations between PPARγ-activity, insulin sensitivity and adipogenesis:

- The relationship between PPARγ-activation and adipogenesis is linear, while bell-shaped with insulin sensitivity.
- Thus, neither PPARγ antagonism (lipodystrophic state) nor full agonism (obese state) results in optimal insulin sensitization (mouse/rat/human genetic models).
Selective modulator concept: SPPARMs

**Agonists < partial > antagonists**

**Ligand-specific genomic & non-genomic effects**

**SRC1, PGC1**

- **PPARγ1**
- **RXR**

**Ligand-dependent differential coactivator/corepressor recruitment**

- **ligand-specific expression of “LEAN” genes**

**TIF2, PGC1, DRIP/TRAP**

- **PPARγ2**
- **RXR**

- **ligand-specific expression of “FAT” genes**
Selective Modulator Concept

- Gave boost to the continued research for SERMs.
- Concept of profiling selective modulators has been established since then eg. (SFXRM‘s, SPPARM‘s, SLXRM‘s)
- Ligand dependent selective co-regulator recruitment likely to elicit specific target gene repertoire linked to desirable pharmacological effects, (eg: LXR ligands which promote reverse cholesterol transport but do not induce hypertriglyceridaemia (SREBP1c gene pathway activation))
A Pro12Ala substitution in PPARγ2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity

Samir S. Deeb¹, Lluis Fajas², Masami Nemoto¹, Jussi Pihlajamäki³, Leena Mykkänen³, Johanna Kuusisto³, Markku Laakso³, Wilfred Fujimoto¹ & Johan Auwerx²

Table 1 • Clinical characteristics and genotypes of Finnish populations

<table>
<thead>
<tr>
<th></th>
<th>Pro/Pro</th>
<th>Pro/Ala</th>
<th>Ala/Ala</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Middle-aged subjects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>257</td>
<td>71</td>
<td>5</td>
<td>NS</td>
</tr>
<tr>
<td>Men/women</td>
<td>126/131</td>
<td>36/35</td>
<td>3/2</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>45±1</td>
<td>44±1</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.2±0.2</td>
<td>25.0±0.4</td>
<td></td>
<td>0.027</td>
</tr>
<tr>
<td>Insulin sensitivity</td>
<td>3.92±0.15</td>
<td>4.56±0.30</td>
<td></td>
<td>0.047</td>
</tr>
<tr>
<td>Fasting insulin (pM)</td>
<td>56±2</td>
<td>48±2</td>
<td></td>
<td>0.011</td>
</tr>
<tr>
<td>Fasting glucose (mM)</td>
<td>4.5±0.0</td>
<td>4.4±0.0</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td><strong>Elderly subjects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>695</td>
<td>258</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Men/women</td>
<td>249/446</td>
<td>102/156</td>
<td>6/14</td>
<td>NS</td>
</tr>
<tr>
<td>Age (years)</td>
<td>70±0</td>
<td>70±0</td>
<td>70±1</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.3±0.2</td>
<td>27.9±0.3</td>
<td>25.7±0.9</td>
<td>0.015</td>
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<tr>
<td>Fasting insulin (pM)</td>
<td>94±2</td>
<td>98±4</td>
<td>73±7</td>
<td>0.063</td>
</tr>
<tr>
<td>Fasting glucose (mM)</td>
<td>6.3±0.1</td>
<td>6.3±0.1</td>
<td>6.5±0.5</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol (mM)</td>
<td>1.27±0.01</td>
<td>1.24±0.02</td>
<td>1.52±0.09</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Initially, middle-aged subjects were studied; subsequently, results were confirmed in elderly subjects. Middle-aged Pro/Ala and Ala/Ala subjects were analysed together. Insulin sensitivity as determined by the frequently sampled intravenous glucose tolerance test and Bagmen’s minimal model³⁸; mean±10⁻⁴ μU⁻¹ ml⁻¹±s.e.m.; BMI, mean kg/m²±s.e.m.; fasting insulin levels (picomolar), mean±s.e.m. Fasting glucose and high-density lipoprotein (HDL) cholesterol levels (mM), mean±s.e.m.; NS, not significant.
Association of PPARγ polymorphisms with the metabolic syndrome?

The Pro12Ala substitution in PPARγ2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. 

Proline to Alanine substitution at codon 12 of PPARγ2

The codon Ala confers reduced activity compared to the Pro isoform


<table>
<thead>
<tr>
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<th>Genotype</th>
<th>P-value</th>
</tr>
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<tr>
<td></td>
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<td>25.0±0.4</td>
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<tr>
<td></td>
<td>4.56±0.30</td>
<td>0.047</td>
</tr>
<tr>
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<td>48±2</td>
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<td>4.4±0.0</td>
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<tr>
<td>Ala/Ala</td>
<td>5</td>
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</tr>
<tr>
<td></td>
<td>2</td>
<td>NS</td>
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<tr>
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<td>3/2</td>
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</table>

<table>
<thead>
<tr>
<th>Elderly subjects</th>
<th>Genotype</th>
<th>P-value</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Pro/Pro</td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>695</td>
<td></td>
</tr>
<tr>
<td>Men/women</td>
<td>249/446</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>70±0</td>
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</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.3±0.2</td>
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<tr>
<td>Fasting insulin (pM)</td>
<td>94±2</td>
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<tr>
<td></td>
<td>6.3±0.1</td>
<td>0.001</td>
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<tr>
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<td>1.27±0.01</td>
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<td>102/156</td>
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<tr>
<td></td>
<td>70±0</td>
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<tr>
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<td>27.9±0.3</td>
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<tr>
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<td>63±4</td>
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<tr>
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<td>6.5±0.5</td>
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</tr>
<tr>
<td></td>
<td>6</td>
<td>NS</td>
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<tr>
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<td>25.7±0.9</td>
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<tr>
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<td>73±7</td>
<td>0.063</td>
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<td>1.52±0.09</td>
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<tr>
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<td>6/14</td>
<td>NS</td>
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<td>25.7±0.9</td>
<td>0.015</td>
</tr>
<tr>
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<td>73±7</td>
<td>0.063</td>
</tr>
<tr>
<td></td>
<td>1.52±0.09</td>
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</tbody>
</table>

Initially, middle-aged subjects were studied; subsequently, results were confirmed in elderly subjects. Middle-aged Pro/Ala and Ala/Ala subjects were analysed together. Insulin sensitivity as determined by the frequently sampled intravenous glucose tolerance test and Bagmen's minimal model: mean±10⁻⁴ µU⁻¹ ml⁻¹ s.e.m.; BMI, mean kg/m²±s.e.m.; fasting insulin levels (picomolar), mean±s.e.m. Fasting glucose and high-density lipoprotein (HDL) cholesterol levels (mM), mean±s.e.m.; NS, not significant.
Association of PPARγ polymorphisms with the metabolic syndrome?

Haplotype analysis of the PPARgamma Pro12Ala and C1431T variants reveals opposing associations with BMI


Figure 1
PPARG Pro12Ala and C1431T have an opposing association with BMI in a type 2 diabetic population. A. Shown are the mean BMIs of subjects with type 2 diabetes within derived haplotype, expressed as a difference in mean BMI from the common Pro-C haplotype. The standard error of the mean BMI is indicated B. The individuals with a BMI in the upper quartile of BMI (BMI > 75th centile, 33.65 kg/m²) were analysed as above.

Figure 3
PPARG haplotype associations are replicated in army volunteers in response to exercise. Shown is the mean change in BMI observed during basic training by haplotype.
NHR are the second Most Precedented Drug Target Family

Other: (single member families) 56

- monoamine oxidase 2
- short-chain dehydrogenases/reductases 2
- serine proteinase - S1 (trypsin-like) 3
- cyclooxygenase 3
- phosphodiesterase 3
- sodium-neurotransmitter symporter 3
- monoamine oxidase 2
- SLC12A family 2
- oxidoreductase 2
- cytokine receptor - type II 2
- voltage gated calcium channel 2
- cation transport ATPase 2
- rhodopsin-like GPCR 37
- nuclear hormone receptor 14
- cytokine receptor - type I 6
- penicillin-binding protein 1a; 2; 2B homolog 4
- ligand-gated ion channel 4
- cytochrome P450 4
- topoisomerase II 4
- serine proteinase - S11 3
- dihydrofolate reductase 3

35% Other: (single member families)
23% rhodopsin-like GPCR
9% nuclear hormone receptor
4% cytokine receptor - type I
Competition Radioligand Binding Screening by Scintillation Proximity Detection

NON competitor

GOOD competitor
Principle of Transactivation Assay

receptor plasmid

P → HR

mRNA

HR

reporter plasmid

HRE

reporter
Principle of Transactivation Assay II

receptor plasmid

P → HR

mRNA → HR

+ ligand

reporter plasmid

HRE

ligand

mRNA → Protein
Reporter Gene Transactivation Assay for NHR’s

Receptor

\( \text{ß-actin promoter} \quad \text{NHR} \quad + \quad \text{NHR RE Adh basal promoter} \quad \text{FF Luciferase} \)

\[ \text{Pool Transient transfection} \]

\[ \text{Seed 96 well format / DRC Ligands} \]

\[ \text{Assay for Luciferases activity (Lumistar)} \]
Nuclear Hormone Receptors, Natural and Synthetic PPAR ligands

A

Wy 14,643

Clofibrate

GW2331

GW2433

B

ETYA

Bezafibrate

Indomethacin

BRL49653
(Rosiglitazone)

Linolenic acid

9-HODE

Arachidonic acid

Leukotriene B4

Linoleic acid

13-HODE

8(S)-HETE

15-deoxy-Δ12,14-Prostaglandin J2

Eicosapentaenoic acid