Sexual Dimorphism in the Liver

Impact on drug metabolism, disease and cancer

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Gender differences in hepatic function

- Lipid metabolism
  - Microsomal (smooth endoplasmic reticulum)
  - Mitochondrial
  - Peroxisomal
- Steroid metabolism
  - Sex steroids and other cholesterol derivatives
- Energy production
  - Females have higher mitochondrial function and metabolic activity

Sex and the single rat (liver)

- **Rats exhibit highest liver sexual dimorphism among tested species**
- Females sleep longer when given hexabarbital (1937)
- Gonadectomy or exogenous steroids affect drug metabolism (1950's)
- Classical species used in toxicology studies
  - Skewed results??

Liver dimorphism in non-rats

- Mice
  - Exhibit dimorphism in Cyp genes, but some reversed in gender specificity vs. rat
  - Less pronouced differences than in rat
- Other small animals
  - Dimorphism shown, but not pronounced
- Primates (non-human and human)
  - Differences in drug metabolism between sexes known
  - But no gender-dimorphic CYP 450 genes shown yet
  - Individual variation masks small gender differences

Mechanisms of liver dimorphism

- **Growth hormone periodicity**
  - GH is the greatest determinant of liver dimorphism
- Sex steroids
  - Determine GH periodicity by indirect means
  - Direct action on liver? Liver expresses androgen, estrogen and progesterone receptors
- Imprinting
  - Neonatal
  - Peripubertal
- Hepatocyte nuclear factors

Growth hormone regulation

- GH secreted by pituitary
- Regulated by neuropeptides from neurons in the hypothalamus
- Positive feedforward peptides
  - growth hormone-releasing hormone (GHRH)
  - ghrelin
- Negative feedback peptide
  - somatostatin
  - inhibits GH release by pituitary somatotrophes until a certain threshold is exceeded

Sex steroids and GH regulation

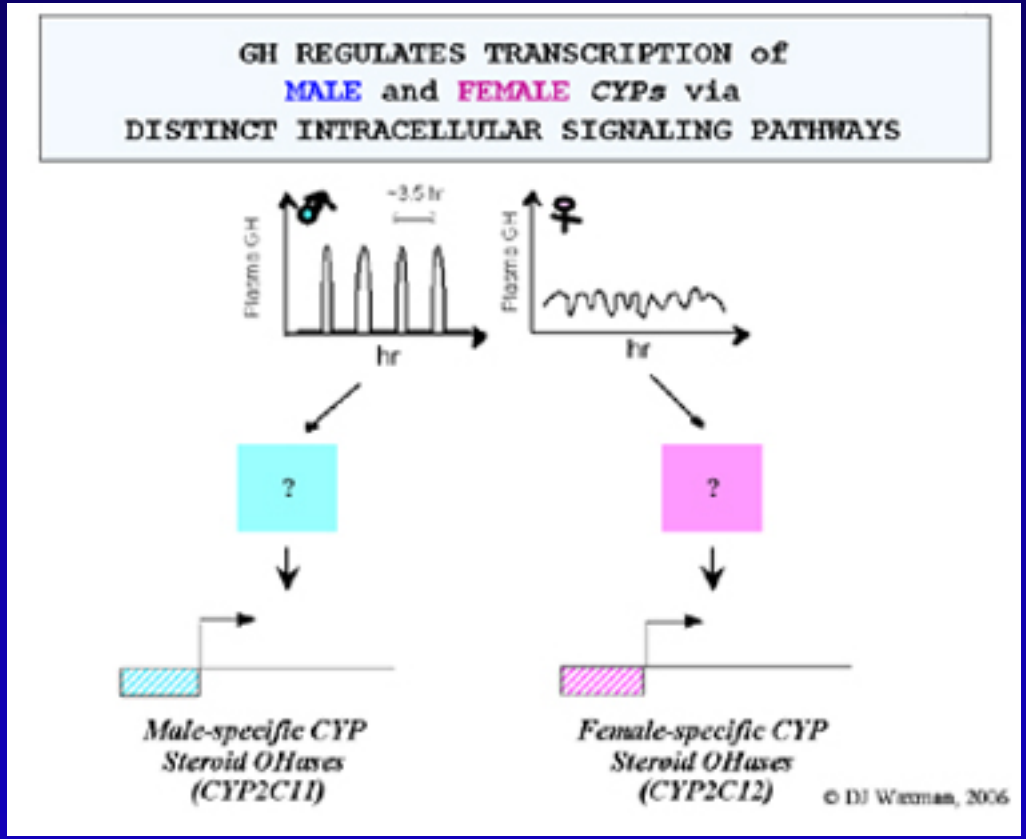
- Both androgens and estrogens stimulate GHRH and ghrelin secretion
  - Hypothalamic arcuate nucleus neurons bear both androgen receptors (AR) and estrogen receptors (ER)
- Only androgens stimulate somatostatin secretion
  - Hypothalamic periventricular nucleus neurons only AR
- Net effect of sex steroids on GH secretion:
  - Females: frequent unpredictable release of submaximal GH from pituitary
  - Males: diurnally regular large GH boluses followed by long nadirs with undetectable serum GH

Control of GH secretion (males)

Figure removed for copyright reasons.

GH periodicity and gender-specific CYP gene expression

- Rat
  - Cyp2c11 is male-predominant
  - Cyp2c12 is female
  - Regular GH cycle in males stimulates Cyp2c11 via Jak2/Stat5b signaling
- Interpeak period determines dimorphic gene expression, not wave amplitude



Waxman DJ

GH, sex steroids: affects on liver

Effects of Various Treatments on the Expression of Sex-Specific Isoforms of Cytochrome P450 in Rat Liver

Treatment	Males	Females
Steroid administration to intact animals	Estradiol reduces expression of male isoforms.	Testosterone reduces expression of female isoforms, but increases expression of some male-specific isoforms.
Castration[#]	Reduces male-specific isoforms.	Reduces female-specific isoforms.
Castration followed by steroid administration	Testosterone increases expression of male isoforms.	Estradiol restores levels of female-specific isoforms.
Hypophysectomy	Significantly reduces the level of male-specific isoforms.	Causes expression of male-specific isoforms.
Hypophysectomy followed by steroid administration	No effect of estradiol.	No effect of testosterone.
Hypophysectomy followed by growth hormone administration	Isoform expression reflects pattern of growth hormone secretion.	Isoform expression reflects pattern of growth hormone secretion.

[#]The age of the animal at the time of castration determines the effect on the composition of hepatic cytochrome P450 isoforms. For example, castration does not have an effect if animals are older than five weeks of age.

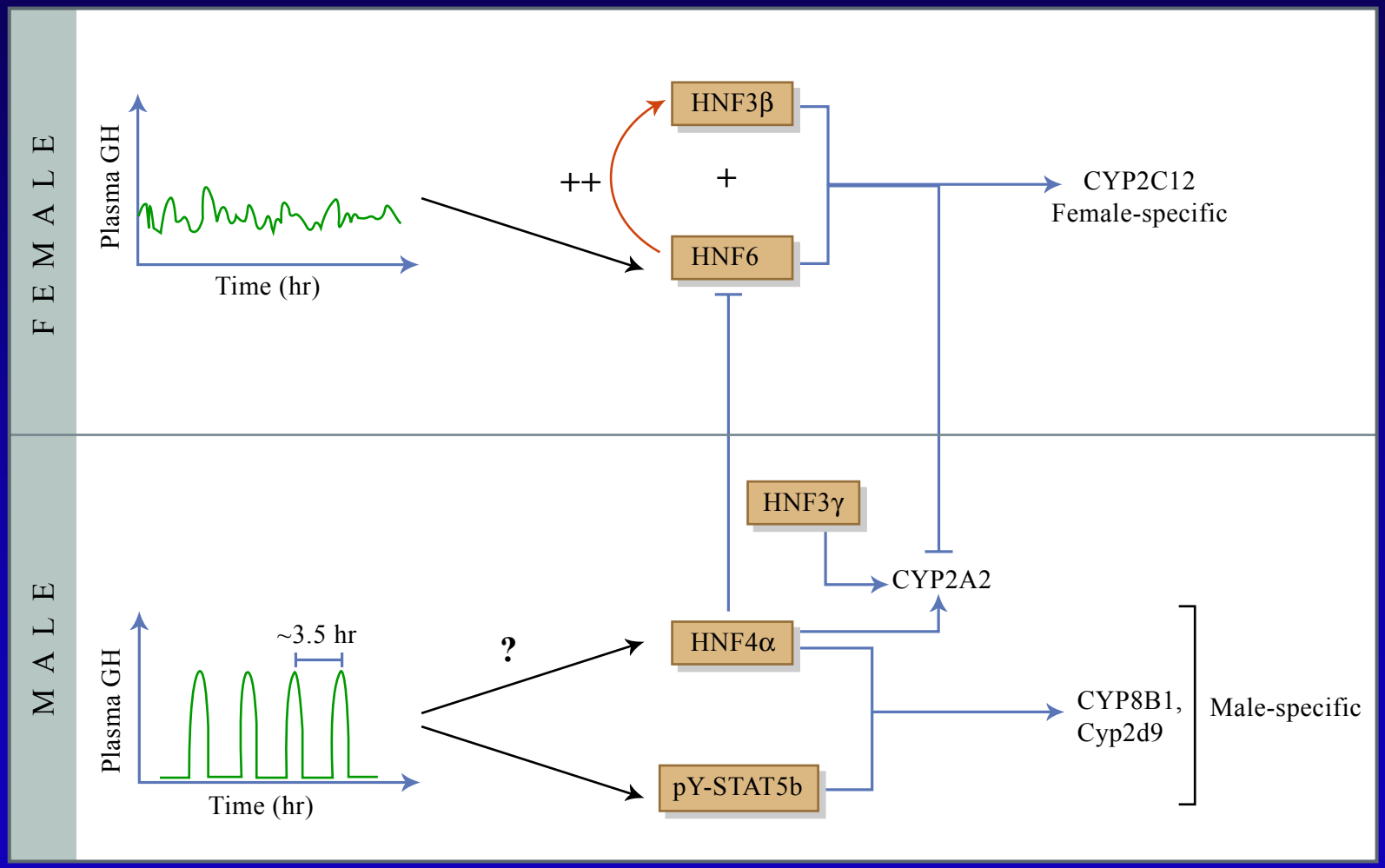
Summary: Most sex steroid effects mediated through GH

Imprinting

- Female rats given testosterone approach but do not achieve male phenotype
  - "Females are not just males without androgens."
- Certain male-specific genes retain male phenotype following post-pubertal castration
- Imprinting also occurs in the neonatal period
- Mechanisms poorly understood

Hepatocyte nuclear factor-mediated dimorphism: Rat Cyp2a2

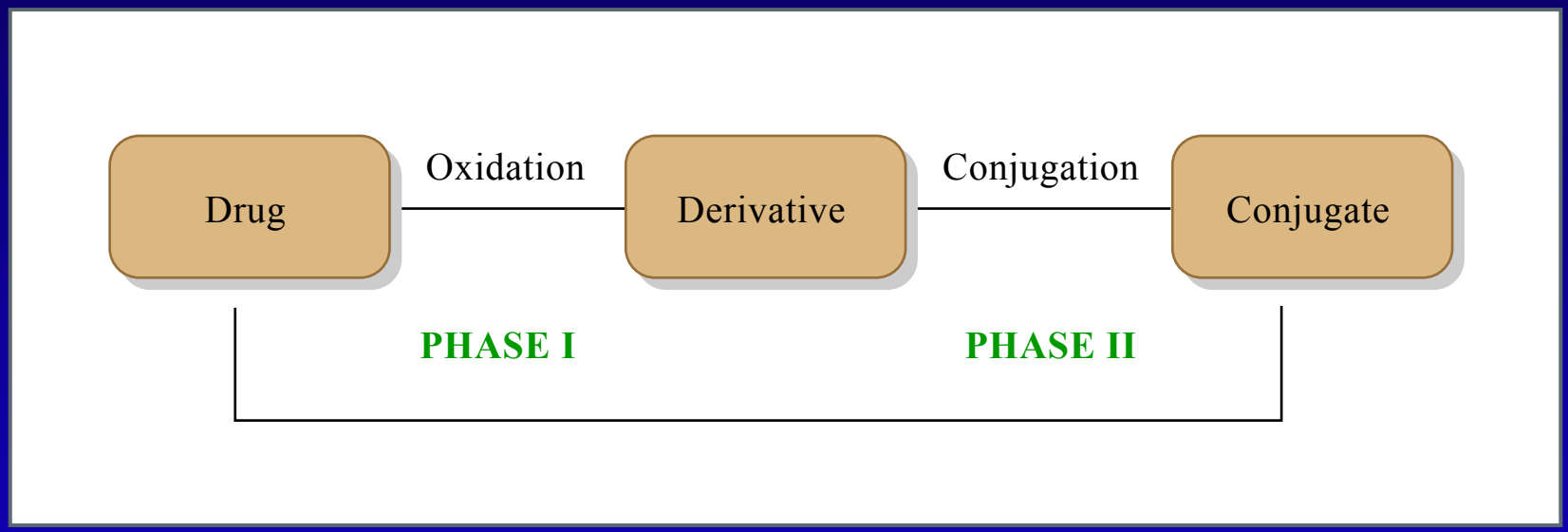
Other HNFs also important



Wiwi and Waxman, JBC 2005

Figure by MIT OCW.

Xenobiotic metabolism



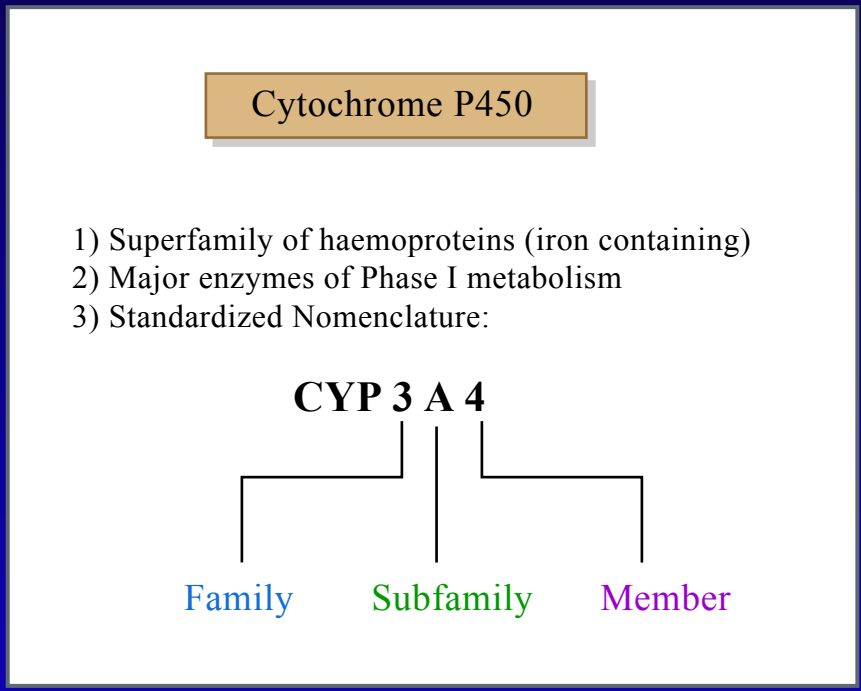
Cytochrome P450
Flavin monooxygenases
etc.

Glutathione S-transferases
Glucuronidyl transferases
etc.

Figure by MIT OCW.

Cytochrome P450

- Heme-thiolate enzymes absorb light at 450 nm when bound with CO
  = **P**igment **450** nm
- Phase I drug metabolism
- Monooxygenases or mixed function oxidases
  - Generally add hydroxyl (-OH) group to hydrophobic compounds to increase water solubility and prepare for phase II conjugation



Nomenclature
Human = **CYP** (all caps)
Nonhuman = **Cyp**

Figure by MIT OCW.

CYP 450 metabolism (human)

Graph removed for copyright reasons.
Source: Accelrys Software, Inc.

None of these enzymes have known sexual dimorphism

P450 species comparison

Figure removed for copyright reasons.
Source: Table 2 in Kedderis, G.L., and C. A. Mugford. "Sex-dependent metabolism of xenobiotics." *Drug Metab Rev* 30 (1998):441-498.

Implications for toxicology studies in animals

Many rodent P450 are dimorphic, but not human

Figure removed for copyright reasons.
Source: Table 3 in Kedderis, G.L., and C. A. Mugford. "Sex-dependent metabolism of xenobiotics." *Drug Metab Rev* 30 (1998):441-498.

Entries for 2A1, 2A2, 2A4, CYP2C, 3A2, and CYP4A are highlighted.

Dimorphic metabolism in rats

Drugs and Chemicals Showing Sex-Dependent Differences in Metabolism in Rats

Agent	Differences
Cocaine	Males metabolize the agent two times faster than females
Diazepam	Metabolism is greater in males than females
Hexobarbital	Metabolism in females is slower, resulting in higher blood levels & a prolonged sleep time
Indinavir	Males metabolize the agent three times faster than females
Morphine	Metabolism is greater in males than females
Pentobarbital	Metabolism in females is slower, resulting in higher blood levels & a prolonged sleep time
Tolbutamide	Metabolism is greater in males than females

Not surprising given known high dimorphism of Cyp genes but…

Kedderis and Mugford (1998)

Figure by MIT OCW.

Humans also show dimorphism!

Xenobiotics Showing Sex-Dependent Differences in Pharmacokinetics in Humans

Agent	Reported Difference
Acetaminophen	Higher parent plasma concentration in females due to lower glucuronidation.
Aspirin	Higher esterase activity in males; lower plasma levels in males.
Chloramphenicol	Higher plasma levels in females.
Chlordiazepoxide	Lower clearance in females as compared with males.
Diazepam	Lower clearance in females as compared with males.
Erythromycin	Higher clearance in females.
Lidocaine	Greater half-life & volume of distribution in females.
Mephobarbital	Greater total body clearance & shorter half-life in young males.
Nortriptyline	Higher metabolism in males; females have higher plasma levels of parent compound.
Oxazepam	Lower clearance levels in females.
Phenytoin	Higher plasma levels in males.
Propranolol	Lower clearance in females due to lower glucuronidation.
Rifampicin	Higher plasma levels in females; higher urinary excretion of parent compound.
Tetracycline	Higher plasma levels in females.

Non-CYP genes
Unidentified sexually dimorphic CYPs?

Kedderis and Mugford (1998)

Figure by MIT OCW.

Gender-dimorphic human liver diseases (besides drug metabolism)

Gender-specific liver diseases

- Male predominant
  - Chronic hepatitis
  - Primary sclerosing cholangitis
  - Hepatocellular carcinoma (HCC)
- Female predominant
  - Primary biliary cirrhosis
  - Autoimmune hepatitis
  - Alcohol intolerance
  - Gallstones and gallbladder cancer

Chronic hepatitis

- Male predominant
- HBV and HCV
- Alcoholic steatohepatitis
- *H. hepaticus* in mice

Photo of liver removed for copyright reasons.
Source: [MacSween].

Primary sclerosing cholangitis

- Male predominant
- Strong association with inflammatory bowel disease
- Affects large bile ducts
  - Inflammation
  - Fibrosis
  - Cholestasis
- Predisposes to HCC
- Walter Payton

Photo removed for copyright reasons.
Source: [MacSween].

Primary biliary cirrhosis

- Female predominant (older)
- Autoimmune disease of small bile ducts
- 9:1 female:male ratio
- Inflammation, obliteration, ductopenia
- Unresponsive to corticosteroids
- Leading noninfectious cause of liver transplants

Photo removed for copyright reasons.
Source: [MacSween].

Autoimmune hepatitis

- Female predominant
- Autoimmune disease affecting hepatocytes
- Steroid responsive

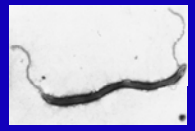
Gender and alcohol

- Alcoholism predominant in men
  - Major cause of liver failure and cirrhosis
  - Contributes to male predominance of HCC
- Less alcohol required to cause liver disease in women
  - ↓ metabolizing enzymes
- Also ethnic differences
  - ↓ Alcohol dehydrogenase in some Asian populations

Gallstones, cholecystitis and gallbladder cancer

- Female predominant
- High prevalence in certain isolated populations
  - Indigenous Americans of Alaska, US southwest, Chile
- Largest single US healthcare expense
  - >$6 billion annually
- Association with *Helicobacter* infection?

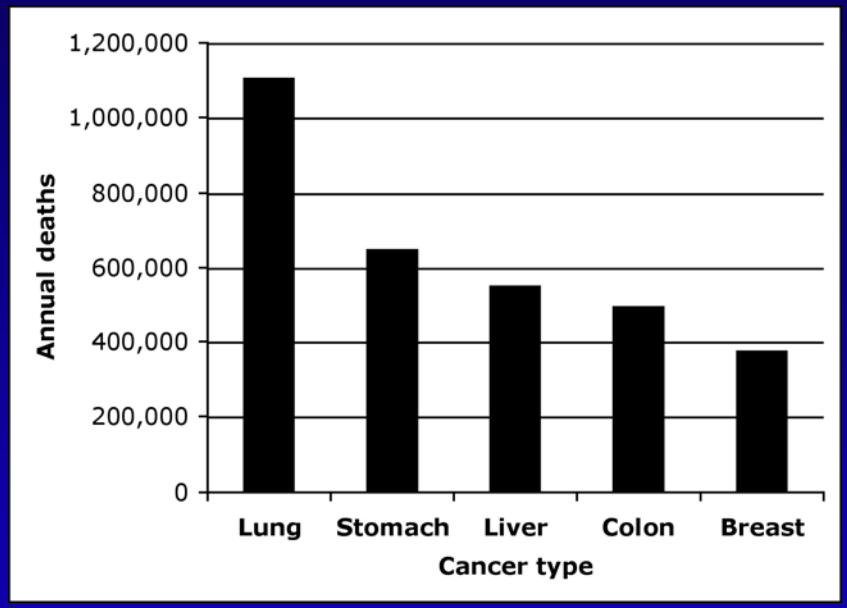
Diagram removed for copyright reasons.
Source: WebMD.



MIT DCM image

Hepatocellular carcinoma

- 5th most common cancer worldwide
- 3rd leading cause of cancer mortality
- Dismal prognosis
  - Only pancreatic cancer worse
- Male:female ratio 2:1 or higher
- Associated with HBV, HCV, alcoholism, chronic metabolic diseases, aflatoxin B1, many others



Data from Parkin, D. M. et al. "Estimating the world cancer burden: Globocan 2000." *Int J Cancer* 94 (2001):153-156.

Why do men get more HCC?

- CYP450 differences?
- Reactive oxygen species?
- Direct action of sex steroids?
  - Testosterone levels increased in men with HCC in Asia, but decreased in Europe
  - Hepatic feminization: Liver failure in men associated with ER upregulation and gynecomastia
  - Female oral contraceptives leading cause of hepatic adenomas, but almost never malignant cancer
- Indirect/direct GH tumor promotion?
- Lipid metabolism? (Arlin's hypothesis)

Summary

- Liver dimorphism is mediated by sex steroids and growth hormone
- Species differences in metabolic dimorphism (Rats high, mice intermediate, humans low)
- Gender dimorphism of drug metabolism a major factor in pharmaceutical industry
- Most major liver diseases are sexually dimorphic
- Men are at higher risk for chronic hepatitis and cancer, but women get more autoimmune disease

Who ya callin' dimorphic?

Image removed for copyright reasons.
Movie poster for "The Rats"
(http://www.imdb.com/title/tt0282418/)