



## PAPER

# Gender, biology and human disease: report of a conference

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**Keyword:** gender

## Introduction

In mid-March 1999, epidemiologists, clinicians, geneticists, and biologists gathered in New York to discuss gender.† In the context of *Lupus*, they tried to answer the question: Why does lupus attack women? and, in a larger context, How does gender alter human disease? Most of the participants had not previously met; several had not investigated human biology, let alone illness, but the independent ideas of each enlightened all. Unlike other conferences, the focus was the biology of gender, not the influence of hormones on immune function. The papers in this issue of *Lupus* derive from this meeting. This introduction provides an overview.

Most physicians believe that gender affects auto-immune disease incidence and severity, but definitions of gender, incidence, and severity are inexact. From fertilization through puberty gender is plastic; prevalence figures are as much hearsay as fact; and social and environmental influences on illness occur.

## Diagnostic and social aspects of gender definition

Sherine Gabriel, emphasizing that community-based studies minimize bias, reviewed the effects of gender-specific health care use and gender-specific criteria on disease incidence and outcome.<sup>1</sup> Harvinder Luthra described striking discrepancies, due to variable

selection and diagnostic criteria, in female:male (f/m) ratio among different studies of spondyloarthropathy. However, in his transgenic mice with spondyloarthropathy, males have higher incidence and severity of disease.

Male death rates from heart disease have declined more rapidly than have female. Using autopsy studies of coronary arteries, Veronique Roger, finding no trend difference in atherosclerosis, concluded that a reason is that men receive more aggressive medical intervention.<sup>2</sup> Indeed, Cynthia Leibson pointed out that cardiovascular mortality of diabetic women but not men worsened between 1970 and 1980<sup>3</sup> and that gender-specific data are very complex.

Cathleen Raggio said that the concept that scoliosis is female predominant is an artifact of curve severity.<sup>4</sup> Small curves, seldom diagnosed, affect 2–3% of the population and are *male* predominant (f/m = 0.7), while moderate (0.3–0.5% prevalence, f/m = 5.4) and severe (0.1–0.3%, f/m = 10) curves are strongly female predominant. Although almost all identical twins with scoliosis are concordant, scoliosis is neither X-linked nor Mendelian dominant. Examining mouse models of scoliosis, Robert Blank found multiple mutations, including those of inter- and intra-cell signaling, structural proteins, and matrix metabolism, some but not all of which affected females more.<sup>4</sup>

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Women are more susceptible to anterior cruciate ligament injury than are men engaged in the same sport and given the same training. Although training and conditioning may account for more than half of the gender difference, Jo Hannafin demonstrated that, even in highly trained athletes, women have slower hamstring reaction times and more quadriceps activation in cutting and pivoting maneuvers. Fibroblast function in tissue culture, a proxy for ligament strength, does not explain differences between genders, but intrinsic differences in kinematic neuromuscular control may.

### Gender definition in the zygote and gamete

Richard Lockshin pointed out that the X and Y chromosomes evolved late, that chromosomes have non-gender functions in lower animals (autosomes determine gender).<sup>5</sup> Birds and even some mammals have no Y chromosomes; invertebrates have no sex hormones; lower vertebrates are either hermaphroditic or switch sexes according to external stimuli. Carmen Sapienza pointed out that imprinting (phenotype depends on maternal or paternal origin of identical genes) can cause as much as a 2:1 gender skew of a clinical phenotype and that X inactivation (silencing of one of the maternal chromosomes) may be dictated across three maternal generations. Imprinting and X inactivation take place at the second meiotic division. As a result, depending on twinning stage, even 'identical' twins may widely diverge. Peter Gregersen applied this concept to rheumatic illness, hypothesizing that gender-skewed imprinting of thymic or dendritic cells may lead to inefficient thymic deletion of X-linked autoantigens. Harry Ostrer supported this argument and pointed out that sex determination occurs in a series of closely spaced stages, allowing multiple variants.<sup>6</sup>

### Gender definition in the embryo; uterine influences

Random, physical intrauterine events, some taking place in very short time windows, profoundly influence lifetime phenotypes, according to Frederick vom Saal. A male mouse fetus located *in utero* between two females has different sexual behavior as an adult than does a male located between a male and a female or between two females. Transfer of (hormonal?) signals between embryos at critical time periods influences that embryo's adult prostate size, behavior, longevity, and other seemingly unconnected

phenomena. Parallels are found in human twins. Vom Saal warned that transient exposure to toxins, environmental estrogens, for instance, may cause such aberrations. Lee Nelson added imaginative data.<sup>7</sup> Pregnancy results in two-way engraftment of maternal and fetal cells, which last in the mother for decades, and which span more than one generation. *In utero* twin chimerism occurs. The biological effects of fetal-maternal engraftment are unknown, but non-self cells are both more frequent and quantitatively greater in scleroderma patients than in controls; they occur in sites of disease in both scleroderma and primary biliary cirrhosis. From studies of androgen- and estrogen-treated 5 $\alpha$ -reductase deficient pseudohermaphrodites, Julianne Imperato-McGinley emphasized that gender phenotype follows chromosomal *and* hormonal sex, that phenotype is determined in several very narrow embryonic, fetal, and pubertal windows, and that it is subject to social constraints.

### Physiology of gender

Sex hormone receptors are widely distributed among tissues and independent of the apparent sexual nature of the tissue in question; gender may not depend on hormones at all. Zahra Zakeri subjected primary brain, heart and liver cell cultures, taken from mouse fetuses at the earliest points of sex determination, to noxious stimuli, with no exogenous hormones.<sup>8</sup> Regardless of the organ, cells from females were more susceptible to injury. Judy Spitzer described gender differences of phagocytosis, chemotaxis, and TNF $\alpha$  secretion by Kupffer cells and alveolar macrophages.<sup>9</sup> Nitric oxide production and susceptibility to ethanol differed between the genders, possibly because of hormones. In myocarditis, an illness of young men and postpartum women, Sally Huber found a testosterone-dependent increase in viral receptors in myocardium,<sup>10</sup> resulting in a larger viral load in males. The result is activation of  $\gamma/\delta$  T cells which suppress a protective Th2 cell response. She thus challenged the notion that sex hormones regulate disease susceptibility by acting directly on immune effector cells. Keith Elkon found that evidence for hormonal control of apoptosis is contradictory but not well-studied.

Brain function varies according to hormonal gender, chromosomal gender, and external stimuli. Testosterone, Erich Jarvis says, cerebrally wires songbirds to sing and activates genes in relevant brain areas. However, songs vary with context. The songs a male sings to another male (and consequent

brain activation and development) differ from those he sings alone or to a female. Neil MacLusky described different recovery patterns of male and female brains after injury, in part due to greater plasticity of the female brain (male brains are more lateralized) and in part due to estrogen-associated cellular repair responses. Jon Levine's description of the divergent behavior of narcotic analgesics in men and women was striking. Nalbuphine, a  $\kappa$  opioid agonist, *increases* pain in men at low doses and decreases it at high doses; it has a linear dose-response curve in women. Hormone-responsive bradykinin-induced inflammation, worse in female rats, is mediated by denervation and by the adrenal medulla, possibly because estrogen receptors exist in adrenal medullary cytoplasm and androgen receptors in dorsal root ganglion neuronal cytoplasm.

Russell Turner emphasized that bone remodeling is focal.<sup>11</sup> In space flight, neurectomy, and limb-unloading models, estrogen and weight bearing have independent effects on bone remodeling, mechanical strain being more important. Theresa Hefferan warned that conclusions about estrogen effects vary with experimental conditions; many assumptions are directly contradicted by studies using different doses, times, solutes, and cell sources. Sundeep Khosla suggested that parathyroid hormone is far more controlling of type II (senile) osteoporosis than is sex hormone, and that, in men, estrogen more than testosterone controls bone metabolism.<sup>12</sup>

Lorraine Fitzpatrick noted that pharmaceutical preparations such as Premarin<sup>®</sup> contain an array of estrogenic compounds, as does the human body.<sup>13</sup> Most of the 'minor' estrogens may be of greater biologic importance than estriol or estradiol, so conclusions based on studies of single estrogens may be unphysiologic. Estrogen receptor  $\beta$  is quantitatively greater in coronary arteries of females than in those from males. Estradiol downregulates vascular smooth muscle cell proliferation in female coronary (but not pulmonary) arteries and upregulates it in males. Niki Dietz found blunted forearm blood flow in women compared to men; estradiol modulated both nitric oxide dependent and independent vascular responses.<sup>14</sup> Virginia Miller found that estrogen upregulates estrogen receptors, and that receptors influence both non-genomic (rapid) responses and genomic (chronic) responses to hormones.<sup>15</sup> Estrogens initiate vascular responses in males as well. Dr. Miller added provocative data: vascular responses of transsexuals change in the direction predicted by the administered hormone, but there is no apparent change in the morbidity or mortality predicted by the chromosomal gender. Males receiving female heart transplants have lower survival than do same-

sex transplants or females receiving male hearts, perhaps because of the need for an acute unidirectional hormone change in the transplanted heart's regulatory mechanisms.

## Conclusions: never assume

This conference brought to light numerous ways in which males differ from females, and many more ways in which simple conjectures about gender differences are confounded by epidemiological biases and by unwarranted conclusions from unphysiologic experiments. Richard Lockshin summarized the lessons of the conference by warning us never to assume that:

- gender definitions are correct, because gender is not biologically conserved, because genetic, social, and hormonal gender is plastic, and because small precisely timed interventions change phenotype;
- hormones are responsible for observed differences, because a multiplicity of unknown hormones and unknown other effectors, for instance weight-bearing in osteoporosis, or imprinting, or athletic training, may be operative;
- apparent target tissues are insensitive to hormones, because hormone receptors occur in surprising locations;
- conclusions from cell culture apply, because the tested hormone may require co-factors present *in vivo* but not *in vitro*, or may be the wrong hormone at the wrong dose or wrong time period;
- animal models replicate human, because in many instances they do not;
- diagnostic criteria and counting are accurate, because social biases or phenotypic variation may have influenced the data.

Finally, Virginia Miller offered a wonderful acronym to summarize the issues anyone who studies gender and disease must consider:

- G—Genome;
- I—Integrated physiology (consider the whole organism, not just the organ or tissue);
- R—Receptors;
- L—Log-shifts (consider dose-responses and experimental conditions); and
- S—Specificity (is the observed effect truly due to gender or is it epiphenomenal?).

The conference did not answer the question: Why is lupus a women's disease? It did identify many questions worthy of future research.



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