

PROCEEDINGS FROM
THE SECOND ANNUAL
CONFERENCE ON
**SEX AND
GENE EXPRESSION**

MARCH 8-11, 2001

GRAYLYN INTERNATIONAL CONFERENCE CENTER
WINSTON-SALEM, NC



SOCIETY FOR
WOMEN'S HEALTH RESEARCH

Inside front cover

BLANK



PROCEEDINGS FROM
THE SECOND ANNUAL
CONFERENCE ON
**SEX AND
GENE EXPRESSION**

MARCH 8-11, 2001
GRAYLYN INTERNATIONAL CONFERENCE CENTER
WINSTON-SALEM, NC



SOCIETY FOR
WOMEN'S HEALTH RESEARCH

Conference and proceedings
made possible by grants from
Aventis Pharmaceuticals, Inc.
and
The David and Lucille Packard Foundation

©2001

Society for Women's Health Research
1828 L Street, NW, Suite 625
Washington, DC 20036
Phone: (202) 223-8224
Fax: (202) 833-3472
Web site: www.womens-health.org

This report written by Beth Schachter for the Society for Women's Health Research.

TABLE OF CONTENTS

| | |
|--|----|
| Introduction..... | 5 |
| Sex Differences in Development | 6 |
| Metals and Protamines: Effects of Toxic Metals on Sperm Chromatin Stability and Integrity, <i>Ellen K. Silbergeld, Ph.D.</i> | |
| Differential Timing of Methylation of Imprinted Genes in the Male and Female Germ Lines, <i>Jacquetta Trasler, M.D., Ph.D.</i> | |
| A Pure Maternal Effect Mutation in the Mouse that Affects Genomic Imprinting, <i>Tim Bestor, Ph.D.</i> | |
| Sex Differences in Cognitive Development | 9 |
| Nuclear Receptor Co-Activators Modulate Hormone-Dependent Development And Gene Expression in Brain, <i>Marc Tetel, Ph.D.</i> | |
| Genetic and Hormonal Influences on Cognitive Function in Turner Syndrome, <i>Judith L. Ross, M.D.</i> | |
| Prenatal Androgen Effects on Cognitive and Social Development, <i>Sheri Berenbaum, Ph.D.</i> | |
| Neuroendocrine Mechanisms Underlying Hormonal Effects on Arousal: Molecular and Genetic Approaches, <i>Donald W. Pfaff, Ph.D.</i> | |
| The Use of Mitochondria and Y-Chromosomes in Population Genetics | 14 |
| The Mitochondrial Genome in Population Biology, <i>Douglas Wallace, Ph.D.</i> | |
| Mitochondrial and Y-Chromosomal Genetics—Windows into Male and Female Population Histories, <i>Mark Seielstad, Ph.D.</i> | |
| Contrasting Male and Female Demographic Histories with Y-Chromosome and mtDNA Variation, <i>David B. Goldstein, M.Sc., Ph.D.</i> | |
| MtDNA and Y-Chromosome Perspectives on the Peopling of the Pacific, <i>Mark Stoneking, Ph.D.</i> | |
| Mitochondrial Diseases | 19 |
| An Overview of Mitochondrial Diseases, <i>Doug Wallace, Ph.D.</i> | |
| Oxidative Phosphorylation Diseases: Maternally Inherited Diseases, <i>John Shoffner, M.D.</i> | |
| Mitochondrial Abnormalities in Alzheimer’s Disease, <i>Gary Gibson, Ph.D.</i> | |

| | |
|--|----|
| Interaction of Genotype and Environment in Complex Diseases | 22 |
| Nature vs. Nurture: Coronary Artery Disease as Example, <i>Craig Hooper, Ph.D.</i> | |
| Gene-Environment Interaction in Common Complex Diseases, <i>Molly Bray, Ph.D.</i> | |
| Female Hormones and Thrombosis: Interactions of Genes and Environment, <i>Frits Rosendaal, M.D.</i> | |
| The Importance of X-Inactivation | 27 |
| The Biology of the X-Chromosome: Compensating for Sex Differences, <i>Huntington Willard, Ph.D.</i> | |
| About the Society for Women’s Health Research | 29 |
| Second Annual Conference on Sex and Gene Expression Planning Committee | 30 |
| Society for Women’s Health Research | |
| Staff | 30 |
| Board of Directors | 31 |

SECOND ANNUAL CONFERENCE ON SEX AND GENE EXPRESSION

The Society for Women's Health Research held its Second Annual Conference on Sex and Gene Expression at Wake Forest University's Graylyn Conference Center on March 8-11, 2001. For the second year in a row, the event created a forum for basic and clinical scientists to share research findings that contribute to the emerging field of sex-based biology.

The 2001 conference covered a range of topics:

- *Sex differences that shape the earliest events in mammalian embryologic development*
- *The impact of gonadal hormones, both during fetal development and later in life, on cognitive functions*
- *Maternally inherited mitochondrial DNA and paternally inherited Y chromosome DNA as tools for studying human evolution and migration*
- *Mitochondrial diseases—contributions from nuclear and mitochondrial genes*
- *Strategies for understanding gene-environment interactions underlying common diseases*
- *X chromosome contributions to sex differences in mammals*

These subjects received extensive coverage at the morning and evening presentations and at the afternoon poster sessions. In addition, they served as the basis for lively discussions among investigators who do not often have the opportunity to speak with one another. As cardiologist Frits Rosendaal noted in the introduction to his presentation, "At conferences, I'm accustomed to talking with people who do thrombosis research. I seldom have breakfast conversations like the one we had this morning where we discussed whether the fruit fly dreams and what those dreams might mean." Such flights of fancy, along with new insights into the molecular basis of sex differences, characterized the Second Annual Conference on Sex and Gene Expression.

SEX DIFFERENCES IN DEVELOPMENT

Developmental biologists who study mammals have long suspected that the events taking place in the preimplantation embryo critically affect subsequent developmental events. Until recently, technical limitations in working with tiny embryos composed of just a few cells prevented researchers from testing many interesting hypotheses. Technological developments, including extensive use of DNA amplification methods and genetic engineering of mice to selectively knock out genes, now make such studies possible. The presentations on sex differences during development provide a sampling of the fascinating results emerging from experiments utilizing such methods.

Metals and Protamines: Effects of Toxic Metals on Sperm Chromatin Stability and Integrity *Ellen K. Silbergeld, Ph.D.*

When we think about the effects of environmental toxins on embryonic development, we usually consider maternal effects, either before or during the pregnancy. In some cases, however, the father may be the source of the risk that affects the embryo, explained environmental toxicologist Ellen Silbergeld, Ph.D., University of Maryland. Regrettably, as Silbergeld noted with references to the Environmental Protection Agency and the Occupational Safety & Health Administration, “protection of male reproductive function is quite minimal in terms of occupational standards as well as the information needed to set environmental standards.” That is why Silbergeld has devoted part of her research to studying the effects of toxic agents that affect the father.

Silbergeld spoke about how the father’s exposure to moderate levels of lead affects the

embryo. It is well known that men exposed to high lead levels become sterile due to hormonal dysregulation or testicular toxicity, but little attention has been paid to the developmental effects in children of men exposed to moderate levels, particularly in the workplace. It has been difficult to determine whether the risk of toxicants such as lead might be transmitted from the father to the fetus because, as Silbergeld noted, lead is “a classic take-home toxin”—carried by the worker and transferred to people in the home. Therefore, animal studies had to be conducted to look separately at the effects of paternal and maternal lead exposure on embryonic development.

Silbergeld’s former student, Robin Gandley, studied early embryos derived from male rats that had either been exposed to moderate lead levels or not exposed, then mated with unexposed female rats. The lead levels used in this work had no effect on fertility. The study looked at newly synthesized proteins made at the two-cell stage when the embryo starts using its own unique genome to transcribe mRNA for protein synthesis rather than translating protein from maternal mRNA transcripts.

The researchers found a pronounced difference in expression of one particular protein between embryos from lead-exposed and unexposed males. By contrast, exposing the female to lead before mating had no such effects on this protein in the two-cell embryos. Preliminary characterization suggests that the protein may be a laminin, a type of extracellular matrix protein. This result proposes that the paternal exposure to lead influences a biochemical process occurring well after fertilization.

Silbergeld and her colleagues next sought to determine which mechanisms were disturbed in males that might contribute to the toxic effects on the offspring. When the investigators began this work, they knew that many cellular processes depend on zinc-binding proteins, and under certain conditions, lead may compete for

zinc-binding sites in these proteins. Because the lead ion is four to five times larger than zinc, substituting lead for zinc in a binding pocket may alter a protein's shape, and therefore, its activity.

As Silbergeld noted, one of the most important nuclear proteins in mature spermatazoa is a zinc-binding protein known as protamine. Protamines replace histones in sperm chromatin, permitting highly condensed packaging of DNA in sperm heads. Protamines are also thought to protect DNA during the sperm's long journey in search of an egg. Therefore, the researchers decided to test the hypothesis that lead distorts the shape of the zinc-binding protamine and consequently alters its interaction with DNA in mature sperm.

Using various spectroscopic methods, the researchers learned that the shape of protamine differs when it binds to lead rather than zinc. In addition, once protamine has bound lead, zinc cannot displace it. Finally, whereas zinc has just one binding site in the protamine molecule, lead appears to have two.

Silbergeld and colleagues next determined that lead reduces the binding affinity between protamine and DNA, resulting in a decrease in sperm decondensation after fertilization. The researchers now plan to determine whether lead also reduces the ability of protamine to protect DNA against harmful effects of mutagenic agents.

While increasing attention gets paid to the effects of maternal exposure to toxins on adverse affects on the offspring, the research discussed by Ellen Silbergeld makes clear why we must not overlook the health and safety of the father.

Differential Timing of Methylation of Imprinted Genes in the Male and Female Germ Lines

Jacquetta Trasler, M.D., Ph.D.

The vast majority of autosomal gene pairs behave the same in mammals, whether they are inherited from the mother or the father. However, a few autosomal genes—approximately 40 or so in humans—show selective expression from just the maternal or the paternal allele. Uniparental expression, resulting from a process called genomic imprinting, often involves DNA methylation of one or the other allele.

Many imprinted genes regulate key aspects of growth and early development. Consequently, inappropriate methylation of any imprinted gene may have severely detrimental, and often lethal, consequences. These observations have prompted investigations into the mechanisms that establish this parent-of-origin epigenetic marking of genes, which is faithfully transmitted during development. Jacquetta Trasler, M.D., Ph.D., McGill University, and Tim Bestor, Ph.D., Columbia University, gave tandem presentations on studies to address these issues, including their collaborative efforts.

Trasler has been tackling the question by inspecting mature and immature mouse germ cells of both sexes to look at the methylation status of known imprinted genes. In males, she started with H19, a paternally methylated gene in somatic cells. Focusing on germ cells at different postnatal stages of differentiation, Trasler and her colleagues used a strategy that separately inspected maternal and paternal alleles in the male germ cells. The researchers found that spermatogonia, which are premeiotic, have full methylation of the paternal allele. This heavy methylation of the paternal H19 persists throughout the meiotic stages of spermatogenesis. By contrast, the maternally inherited allele is undermethylated in the spermatogonia and acquires methylation during the meiosis prophase.

Trasler noted that University of Pennsylvania researchers M.S. Bartolomei and T.L. Davis, who looked at fetal male mice, found that the primordial germ cells in the midgestation fetus lack detectable methylation on H19. As the germ cells develop in the male gonad, methylation appears first on the paternal allele then later on the maternal one. These results suggest that the paternal and maternal alleles carry some mark that permits the methylating system to distinguish between them.

Turning to studies on the female germ cells, Trasler reminded the audience that oocyte maturation proceeds to a more advanced stage prenatally than does sperm maturation, arresting at meiotic prophase 1. Despite the fact that female germ cells at birth have already entered meiosis, methylation of the imprinted genes has only just begun, and it continues to increase until puberty. According to Trasler, full methylation of maternally imprinted loci occurs at least two weeks after birth in these mice.

To start learning about the enzymatic control of imprinting, Trasler and colleagues looked at the temporal pattern of expression of the predominant DNA methyltransferase, DNMT1, which is essential for normal development. Using immunostaining, they detected the enzyme in male germ cells at midgestation, with a decrease in expression just before birth. On the first postnatal day, the enzyme disappeared from male germ cells but was present once again several days later. In the adult, DNMT1 expression remained in early meiotic cells but not in mature sperm. In females, DNMT1 is present in primordial germ cells. At the onset of meiotic prophase 1, which is prenatal in the female germ cell, the enzyme level is high. DNMT1 levels then decline during the middle of prophase 1, as in the male.

Trasler and colleagues then found that postnatal oocytes have a smaller form of DNMT1 (now designated DNMT1o) than somatic cells. In collaboration with Tim Bestor, Trasler discovered that the two forms differ at their

amino termini because of differential use of two transcription start sites, which give different first exons. In a developmental study they found DNMT1o, but not DNMT1, in oocytes from new born and five-day old mice.

To their surprise, the researchers also found DNMT1o—not DNMT1—in preimplantation embryos. At the two-, four-, and 16-cell stages, DNMT1o resides exclusively in the cytoplasm whereas in eight-cell embryos it is largely nuclear. Given this intracellular distribution, DNMT1o appears to be positioned to methylate DNA only at the eight-cell stage. As described below, Tim Bestor discussed work that extends this finding and suggested a physiological role for DNMT1o in early embryogenesis.

Trasler concluded by noting that the findings about the timing of imprinting should be informative for people interested in reproductive technologies such as cryopreservation of germ cells or ovaries for later use. Knowing that proper germ cell maturation and embryogenesis involves specific imprinting events, clinical researchers will have new tools for evaluating the quality of the biological specimens.

A Pure Maternal Effect Mutation in the Mouse that Affects Genomic Imprinting *Tim Bestor, Ph.D.*

Tim Bestor, Ph.D., Columbia University, continued the thread spun by Jacquetta Trasler about the process of setting down genomic imprints during development. As Bestor noted, when the researchers found DNMT1o expression to be concurrent with genomic imprinting in immature oocytes, they tested the hypothesis that this DNMT1 isoform is necessary for establishing genomic imprints. For this test, they created mice incapable of expressing DNMT1o but fully able to make DNMT1, the somatic isoform that they knew was needed for viability.

These studies, which were a collaboration with Trasler and with J.R. Chaillet, University of

Pittsburgh, and K.E. Latham, Temple University, showed that imprints were indeed established in the absence of DNMT1o, suggesting that another enzyme must catalyze de novo methylation in oocytes. Moreover, mice that were homozygous for the DNMT1o deletion had no obvious phenotypic abnormalities.

Only when the researchers bred the mutant females did they start to see the consequences of the genetic knockout: almost no live births. Subsequent studies confirmed that fetal death was not due to a poor uterine environment in the knockout animals, since the mutant mice could carry wild-type fetuses to term. Bestor and his colleagues began a systematic inspection of the fetal progeny of the homozygous knockout females and saw that death occurred over a wide window of time, mainly covering the last one-third of gestation. The phenotypic abnormalities in these animals also showed considerable variation. Taken together, the results suggest the problems are specific to the process of genomic imprinting rather than a more general defect in DNA methylation.

Next, the researchers inspected the abnormal progeny of the homozygous knockout females for the methylation status of known imprinted genes. They found that the animals had only one-half the amount of methylation on imprinted DNA found in control mice. Because DNMT1o appears in the nucleus only at the eight-cell preimplantation embryo, Bestor suggests that DNMT1o plays a maintenance role, adding methyl groups to the unmethylated strand of hemimethylated DNA only following the third S-phase in embryogenesis.

Since the somatic form of DNMT1 is absent throughout the preimplantation stage, other methylating enzymes probably fulfill the maintenance functions except during the one cell cycle when DNMT1o acts.

What causes so much phenotypic variability among the failed fetuses of the homozygous knockout females? Bestor suspects that gene

shuffling, through a process known as sister chromatid exchange, underlies this variability because it occurs at a particularly high rate during the preimplantation mitoses in mice. Since such shufflings happen randomly, different hemimethylated genes might escape full methylation in different animals. This would lead, in subsequent cell cycles, to an absence of methylation of some previously imprinted genes.

Bestor concluded by observing that the process of establishing and maintaining genomic imprints in a sex-specific pattern is far more dynamic and complex than biologists once thought it was. As he noted, future studies will focus on finding the genes involved in setting up sex-specific methylation patterns at imprinted loci and determining the functions of these genes.

SEX DIFFERENCES IN COGNITIVE DEVELOPMENT

Our maleness and femaleness depend greatly on three key gonadal steroid hormones—testosterone, estrogen and progesterone. Early in development the hormones cause permanent changes, and then later in life they control reversible ones. Biologists call the developmental actions of sex steroids organizational and the reversible actions activational. As organizers, these hormones determine features such as the developmental course of the reproductive organs; as activators, they control such processes as spermatogenesis and ovulation.

The brain is a key target for both the organizational and the activational effects of these hormones. While researchers have known for several decades that sex hormones act directly on the brain, the underlying mechanisms by which these hormones act are only partially understood. The speakers in this session gave the audience a picture of how animal models and human subjects are contributing to this understanding of sex hormone action on brain functions.

Nuclear Receptor Co-Activators Modulate Hormone-Dependent Development and Gene Expression in Brain

Marc Tetel, Ph.D.

The research of Marc Tetel, Ph.D., Skidmore College, explores the molecular signaling that takes place when sex steroids act in the brain. The principle mechanism of steroid hormone action has been elucidated for non-neuronal cells in culture, and Tetel is trying to determine whether brain cells in the living animal use this same mechanism during both the organizational and the activational actions of these hormones. Given the anatomical and molecular complexity of the brain, researchers try to avoid extrapolating from information gathered from other systems; rather, they use such data to generate hypotheses about mechanisms that might be at work in the brain.

Tetel reminded the audience that steroid hormones act by binding to specific intracellular receptors that function as ligand-dependent transcription factors. Hormone binding activates receptors, moving them to specific sites on DNA, which alters the transcription rates of the hormone-responsive genes. Until recently, researchers were perplexed by this mode of biochemical communication because the receptors often bind to DNA sequences that are fairly far away on the linear molecule from the promoters of the genes they activate. According to Tetel, the missing pieces of the puzzle that explain this apparent “action at a distance” are proteins called co-activators, which form bridges between the DNA-bound receptors and the transcription initiation complexes sitting atop promoters.

Tetel wanted to determine if known co-activators participate in either the organizational or the activational effects that the sex steroids mediate in the rat brain. He and his colleagues first studied a well-characterized organizational action: the hormonal effect on the size of the sexually dimorphic nucleus (SDN) of the hypothalamus.

The SDN is much larger in adult male rats than in adult females; the volume difference results from exposure of the male brain to steroids produced by the testis shortly after birth. The SDN size can be experimentally manipulated during a narrow window of time in both male and female rats—in the male by neonatal castration, and in the female by injection of gonadal steroids.

Tetel, in collaboration with Margaret McCarthy, Ph.D., University of Maryland, used this paradigm to ask whether one of the co-activators, steroid receptor coactivator-1 (SRC-1), participates in the steroid-induced increase in SDN size. The researchers selectively decreased expression of SRC-1 in the hypothalamus of neonatal female rats by infusing antisense oligonucleotides that block translation of SRC-1 mRNA. Control rats received a scrambled oligonucleotide that does not decrease SRC-1 expression. All animals were then treated with steroid hormone and the brains were examined two weeks later. The treatment that diminished SRC-1 expression resulted in less hormone dependent growth in the SDN, supporting the hypothesis that this coactivator participates in the gonadal steroid hormone mediated organizational action in the brain.

The researchers then explored the involvement of SRC-1 and another co-activator, CBP (CREB binding protein), in mating behavior in the adult female rat. This stereotypic and quantifiable behavior, termed lordosis, is the arched-back posture taken by many female quadrupeds during mating. In rodents, lordosis is a transient hormone-dependent activation function, requiring the sequential action of estrogen and progesterone in the hypothalamus. A necessary component of estrogen's action is the induction of hypothalamic progesterone receptors. Experimentally, the behavior is induced in rats that have been ovariectomized as adults, then given estrogen followed by progesterone.

Using antisense oligonucleotides to decrease expression of both SRC-1 and CBP in the

hypothalamus, the researchers found that oligonucleotides directed against either coactivator mRNA reduced the induction of hypothalamic progesterone receptors by estrogen compared to animals treated with control oligonucleotides. Then, the scientists took the work a step further by showing that SRC-1 and CBP antisense oligonucleotides attenuated hormone-mediated lordosis.

Collectively these studies help to inform us about the mechanism used to mediate both fixed and transient actions of sex steroids in the brain. In addition, they validate the feasibility of experimental strategies such as antisense oligonucleotides to decrease protein levels for studies of gene expression in the brain. As Tetel noted, the molecular tools are now in hand to ask questions about how sex steroid hormones affect cognitive functions.

Genetic and Hormonal Influences on Cognitive Function in Turner Syndrome *Judith L. Ross, M.D.*

In female mammals, X-inactivation silences most genes on one or the other X-chromosome. However, a few genes escape this silencing, permitting bi-allelic expression within individual cells. Women and girls who have just a single X chromosome—the condition known as Turner syndrome—serve as natural experiments demonstrating the need for a double dose of certain X-linked genes. One of the most consistent features of Turner syndrome is the failure of ovarian development, including a lack of estrogen production.

As Judith Ross, M.D., Thomas Jefferson University, explained, people with Turner syndrome frequently experience certain neurological difficulties. They have no problems with verbal tasks but have trouble in a range of non-verbal learning areas. “They have particular difficulty with spatial function, visual-spatial ability, motor functions, attention, behavior and memory,” according to Ross. Given the growing awareness that estrogen acts throughout the brain and can influence a variety of cognitive

and motor skills, Ross wondered whether young girls with Turner syndrome might benefit from taking estrogen.

In the study by Ross and colleagues, girls between the ages of 5 and 11 with Turner syndrome received estrogen for periods of time ranging from one to seven years, then they were tested at ages eight or 12. This treatment with ethinyl estradiol gave the subjects’ hormone levels approximating what the ovary produces early in puberty. The study compared hormone-treated girls to untreated Turner syndrome girls and to controls, matching between groups for age, verbal IQ and socioeconomic status. The researchers assessed the girls’ memory of verbal information by tests such as having subjects listen to a list of several words then reporting back what they heard. The tests for nonverbal memory included tasks such as reciting back a list of digits in forward and then in reverse order.

Estrogen treatment helped the eight-year old Turner syndrome girls with their verbal recall skills, since they performed as well as the control group and much better than the untreated Turner subjects. Estrogen also improved their non-verbal memories, but less impressively than with their verbal recall.

Tests of the 12-year olds showed no real difference in verbal memory among the three groups, suggesting that estrogen had no beneficial effect on verbal memory in girls of this age. Results of the tests for memory of non-verbal information showed that estrogen had no effect on the ability to recall and reorder numbers, but the hormone had a slightly beneficial effect on the ability to recall a non-representational figure, another non-verbal memory test.

In the same study, Ross and her colleagues asked whether estrogen therapy helped Turner syndrome girls of either age with motor skills. First, as Ross noted, they determined that untreated Turner syndrome girls were much slower than controls “on very simple motor, repetitive tasks, like finger and foot tapping.”

In addition, on more spatially mediated motor tasks such as lining up pegs on a pegboard, the untreated Turner syndrome girls took much longer than did control subjects. On other motor tasks the Turner syndrome girls moved much faster and more impulsively.

Using this information, the researchers then asked whether estrogen influenced nonverbal processing speed or motor function among the Turner syndrome girls. In the eight-year olds, hormone treatment failed to improve these skills, whereas it helped the 12-year olds.

These results establish roles for estrogen in both cognitive and motor functions in prepubertal human females. Ross argues that, despite the complexity of the results, the study shows some benefits from estrogen on brain function at each age studied. Therefore, she suspects that, with further optimization of dose and duration, young girls with Turner syndrome may be helped with treatments such as estrogen therapy.

Prenatal Androgen Effects on Cognitive and Social Development *Sheri Berenbaum, Ph.D.*

Recent studies on girls with a genetic defect that overexposes them to testosterone in utero gives us insight into how this hormone helps organize the male brain, reported Sheri Berenbaum, Ph.D., Pennsylvania State University. Normally, early in development, the adrenal gland starts making the hormone cortisol from a steroid precursor, with the help of the enzyme 21-hydroxylase. In fetuses with a condition known as congenital adrenal hyperplasia (CAH), caused by defective 21-hydroxylase genes, the steroid precursor accumulates and instead gets converted to testosterone, which the adrenal gland secretes. Cortisol replacement right after birth can successfully treat this condition, which is so common that over one third of U.S. states mandate testing for it in all newborns.

Prenatal exposure to testosterone gives the CAH girls masculinized genitals at birth. For psychol-

ogist Berenbaum, this finding raised questions about other ways in which in utero exposure to testosterone contributes to masculine traits, in particular whether exposure to the hormone affects learning and behavior. To answer these questions, Berenbaum established a long-term program to assess CAH girls, comparing each subject with an unaffected sibling, most often a sister, several times between ages three and 18 years.

One such study looked at toy preferences. Berenbaum and colleagues first compared unaffected girls and boys. The researchers monitored the proportion of time each child played with girls' toys, such as dolls and kitchen items; boys' toys, including transportation toys and building blocks; or gender-neutral items such as board games and books. Girls and boys showed decidedly different toy preferences. CAH girls had preference ratings that fell between the unaffected girls and the boys. Moreover, the girls with the most severe CAH—those with null mutations in both 21-hydroxylase alleles—preferred boys' toys more than did the less severely affected girls with residual enzymatic function.

In another study, Berenbaum and colleagues used a questionnaire to assess gender identity. The investigators asked the girls questions such as, "If there were a magical way to turn you into a boy, would you do it?" Most CAH girls answered "no" to this question. Overall, the CAH girls and their unaffected sisters overlapped substantially on questionnaire replies, and the data showed no association between response outliers and severity of the CAH syndrome.

Berenbaum and her colleagues then compared CAH girls and their sisters for spatial abilities in which boys tend to outscore girls. For example, the children were asked to perform two- or three-dimensional spatial rotations of displayed objects. The CAH group significantly outperformed their sisters in three of the five tests of spatial ability. The study group is still small, and therefore the results must be interpreted

cautiously. However, Berenbaum cited a similar study of younger girls showing that the CAH group had stronger spatial abilities than the control girls.

Might these differences in spatial ability be secondary to the toy preference, a consequence of CAH girls practicing spatial tasks more with their chosen toys? As Berenbaum commented, "If so, we could eliminate the sex difference in spatial abilities if we just gave girls boys' toys." Some small studies suggest exactly the opposite causal relationship. According to Berenbaum, "Children with high spatial ability select activities that facilitate that behavior."

Together with results of related studies, Berenbaum's findings argue that prenatal exposure to testosterone has certain organizational, lasting effects on many distinct aspects of cognition in humans. The effects seem to be somewhat independent of each other, since the CAH girls differ from their unaffected sisters in some but not all of the male/female differences measured in these studies.

Berenbaum now wants to determine which parts of the brain are involved in which testosterone-dependent behavioral changes. Given the increasing popularity of "real time neural imaging" methods, applying these becomes an obvious next step. While Berenbaum is planning such studies, she cautioned that anatomical differences don't necessarily represent the cause of behavioral differences. She related the following anecdote to support her caution:

Verbal ability is strongly associated with temporal lobe function. Since verbal ability differs between the sexes, it has been assumed that this difference must relate to a sexual dimorphism in this region of the brain. Berenbaum and her collaborators had the opportunity to test this hypothesis, in studying people who were having temporal lobectomies to treat otherwise refractory epilepsy. Assessments of patients before surgery confirmed the sex difference in verbal abilities. Testing of each patient following sur-

gery showed that the sex difference remained after "crucial" regions were lost.

Berenbaum concluded by stressing that prenatal androgen exposure in CAH females affects their behavior in complex ways. "The interesting question now is not whether androgens affect behavior, but how they do so," she said.

Neuroendocrine Mechanisms Underlying Hormonal Effects on Arousal: Molecular and Genetic Approaches *Donald W. Pfaff, Ph.D.*

Donald Pfaff, Ph.D., Rockefeller University, a pioneer behavioral and molecular neuroendocrinologist, has made key contributions to understanding the neural circuitry of female rodent sexual behavior and the roles played by estrogen and progesterone in regulating these brain functions. Now Pfaff has started to decipher the effects of estrogen on brain functions that are even more basic than mating behavior, namely the manifestations of arousal, the elemental neural processes needed for cognitive, motor and emotional functions.

Studies by Pfaff and his colleagues have shown that estrogen stimulates lordosis, the female mating behavior, through a biochemical pathway in the brain that requires estrogen receptor (ER)-dependent induction of new RNAs followed by synthesis of new proteins. Until recently, researchers assumed that all estrogen-regulated biology depended on activating the estrogen receptor (ER), an intracellular ligand-dependent transcription factor. However, a few years ago, scientists discovered a second ER, the product of a separate gene, which raised questions about the actions and interactions of the two ER isoforms, now designated ERalpha and ERbeta.

Pfaff and colleagues have started to isolate the contributions that each ER isoform makes to the control of lordosis, as well as other estrogen-regulated behaviors, in studies using knockout mice lacking one or the other ER gene, there-

fore called AERKO and BERKO mice. For example, the investigators found that AERKO females show no hormone-mediated lordosis behavior. By contrast, the BERKO females have increased estrogen-stimulated lordosis responses compared to their wild-type counterparts. This example of ERalpha and ERbeta working in apparent opposition reflects only a few of the results comparing ERalpha and ERbeta functions. As Pfaff noted, while the hypothesis of strictly equal and opposite roles for the two isoforms is appealing, the data reveal a more complex picture. Many functions subserved by the two isoforms seem independent and unrelated to each other, perhaps because the cell and tissue distribution of the two isoforms differs greatly.

Studies of these knockout animals have turned up certain unexpected sex differences in behavior regulated by ER. For example, in female mice, ERalpha not only facilitates mating behavior but also attenuates aggression. By contrast, ERalpha potentiates both mating and aggressive actions in males. Speaking generally about the complex contributions of specific genes to behavior in mammals, Pfaff suggested that the function of certain genes in mammalian behavior may depend upon context, such as ambient stress levels or social experience in addition to sex-based differences.

Turning to how estrogen might contribute to arousal, Pfaff explained that his student, John Frohlich, devised a set of assays for quantifying sensory, motor and fear-conditioning parameters of arousal. This scheme can give information about the substructures of arousal by looking, for example, at which parameters vary together within individual animals.

In new studies that look at estrogen's role in arousal, Pfaff and his colleagues have found several parameters that the hormone affects. In tests comparing ovariectomized mice with and without hormone replacement, the researchers found that estrogen stimulates certain measures of motor activity and fearfulness. The researchers

also discovered subtle modulatory effects of estrogen on exploratory behavior. Finally, rat studies revealed that estrogen was antidepressive. These studies collectively argue that estrogen helps activate arousal in female rodents. The Pfaff group is now beginning to separately investigate the roles of ERalpha and ERbeta in these tests to discover their genetic contributions to arousal.

THE USE OF MITOCHONDRIA AND Y-CHROMOSOMES IN POPULATION GENETICS

The Mitochondrial Genome in Population Biology *Doug Wallace, Ph.D.*

Emory University geneticist Doug Wallace, Ph.D. began his plenary lecture with the reminder that transmission of mitochondrial DNA to progeny of both sexes seems to be exclusively maternal. Mature oocytes each have well over 100,000 mitochondrial DNA molecules while mature sperm carry fewer than 100 per cell. After fertilization, those few paternal mitochondria in the zygote are selectively destroyed.

Neither the mitochondrial genome nor the paternally transmitted Y-chromosome undergoes much, if any, recombination, in contrast to the autosomal chromosomes whose DNA sequences are subject to extensive shuffling between the maternal and paternal chromosomes during gametogenesis. Consequently, the inter-individual variations among mitochondrial genomes trace female lineages, while Y-chromosome variations identify male lineages.

Having identified mitochondrial DNA haplogroups, linked sets of polymorphic mtDNA markers, research teams are using these marker sets to test hypotheses about human origins

and migration patterns. For example, investigators have found that Africans show the greatest heterogeneity in mtDNA polymorphisms, consistent with the notion that *Homo sapiens* had their origins on that continent. The migration proceeded first to Eurasia and then to Europe, according to data collected from mtDNA haplogroup studies.

The African lineage studies Wallace described included analyses of two groups of pygmies, the Biaka (Western), who live in the Central African Republic, and the Mbuti (Eastern) from Zaire. While the two groups share physical similarities, the mtDNA haplotype data shows only a distant relationship between the groups. In addition, a third pygmy group, the Negritos of Southeast Asia, had been thought to have African pygmy origins. However, the mtDNA data from the Negritos argues against that notion. Noting that all three pygmy groups live in tropical rainforest areas, Wallace hypothesizes that their short stature represents separate adaptations to similar physical environments. “It shows that it is easy to change our physiognomy based on minor environmental selection pressures over relatively short periods of time. The Mbuti Pygmy are about 40–50,000 years old as a population whereas the Biaka Pygmies are about 125,000 years old.”

Wallace then shifted his focus from Africa and Asia to the Americas, describing studies that ask whether all Native Americans descended from a single ancestral migration across the Bering land bridge, or whether there were multiple major migrations to this hemisphere. As he noted, anthropologic studies of language differences between groups suggest that three major migrations may have taken place. The mtDNA haplogroup data suggests that most present-day Native Americans descended from a small number of females who crossed the Bering landmass about 20–30,000 YBP, or years before present. The Pacific Northwest and Southwest Navajo and Apache ancestors (the Na-Déné) came from a trans-Bering migration 7–9,000

YBP, and the Eskimo-Aleut ancestors arrived 3–4,000 YBP.

One somewhat surprising result emerged during a study of the Ojibwa in the Great Lakes area. In that study, researchers found the presence of a European heritage in about 25 percent of the population. The European marker held in common here was very rare in Europe. As Wallace commented, “it would be very bizarre for a few women with this marker to have gotten into the Native American tribe and then suddenly constituted 25 percent of all the lineages. Rather, it implies that the marker was in the population a lot longer ago.” Wallace estimates that the founders of this group arrived about 15,000 YBP.

This evidence for an ancient European migration has stimulated discussion about the origin of people capable of killing large mammals, including the woolly mammoth, the giant sloth and the giant deer. These animals appear to have become extinct about 10,000 years ago, presumably at the hands of Amerindians who had Siberian ancestors. Archeological evidence suggests that the weapon used to kill these beasts was a bifacial stone spear. The discovery of such tools in Iberia has prompted some researchers to link the demise of the giant mammals to the people of European rather than Siberian origin. The issue remains unresolved, but as Wallace noted “it’s interesting how molecular genetics and archeological data are coming together.”

Mitochondrial and Y-Chromosomal Genetics—Windows Into Male and Female Population Histories *Mark Seielstad, Ph.D.*

Mitochondrial DNA and Y-chromosomal DNA respectively hold the histories of female and male lineages. Consequently, studies of their diversity in humans can tell us about female and male population structures and migration patterns. Mark Seielstad, Ph.D.,

Harvard School of Public Health, introduced the panel discussion on that topic by noting that, until recently, it has been difficult to find variation on the Y-chromosome. “We have only recently been able to compare the paternal with the maternal genetic lineages. But, when we began to do so, we found some striking differences.”

One such difference, according to Seielstad, is the apportionment of genetic diversity throughout the world’s populations. Polymorphism studies of mtDNA (and of autosomal DNA) show that greater than 80 percent of the genetic diversity of the entire human species resides within any particular population. By contrast, similar analyses of Y-chromosomal DNA show that as little as 35 percent of the total genetic variation on the Y is found within any one population. This distinction, Seielstad suspects, reflects differences between female and male migration patterns. In most traditional cultures, when a woman and a man marry, the couple remains in the man’s family group. Consequently, women migrate more than men do, resulting in a greater global distribution of women’s than men’s genes. To formally test this hypothesis, Seielstad hopes to conduct mtDNA and Y-chromosome diversity studies in so-called matrilineal cultures in which a woman lives with her children and sometimes her daughter’s children, without husbands or other adult men.

Demographers have begun using mtDNA and Y-chromosomes to look at the impact that social factors such as kinship, class, language and religion have on genetic diversity in human populations.

Contrasting Male and Female Demographic Histories with Y-Chromosome and mtDNA Variation *David B. Goldstein, Ph.D.*

Common diseases, such as asthma, obesity and certain cancers appear to have hereditary com-

ponents. However, unlike certain rare genetic diseases caused by mutations in one or another single gene, the hereditary contribution to these diseases probably involves subtle effects at multiple loci. This complexity makes it difficult to find the disease genes using population genetics. David Goldstein, Ph.D., University College, London, UK, added some new wrinkles to the picture in his description of how the demographic histories of the populations being studied can affect the hunt for disease genes.

Scientists often start honing in on disease-related genes by doing linkage disequilibrium (LD) studies. Researchers have found that if particular alleles at two or more loci are closely linked on the same chromosome, they are likely to segregate together during recombination. The closer the physical linkage, the greater the likelihood of co-segregation, and thus the higher the LD value. Therefore, case-control studies that test a panel of known polymorphic markers to look for strong associations between the disease trait and certain alleles with high LD values are a starting point for finding the disease-causing gene or genes. However, as Goldstein reported, demographic factors such as genetic admixture, the shuffling of alleles following the mixing of two genetically distinct populations, or genetic drift, the random loss of certain alleles, can have important effects on patterns of LD.

To demonstrate the impact of admixture on LD, graduate student Jim Wilson focused on a tribe in South Africa known as the Lemba. The Lemba descended from the Bantu, but both cultural and genetic evidence shows that intermarriage with a Semitic group occurred. Goldstein and Wilson reasoned that by using a panel of more than 60 polymorphic markers to walk down the X-chromosome of Lemba, and their presumed parent populations of Bantu and Ashkenazi Jewish males, they could show how much genetic admixture contributes to LD data. Since males carry only a single

X-chromosome, data comparing two markers at different X-linked loci in men directly identifies haplotypes, the collective genotypes of a number of closely linked loci on a chromosome.

The researchers compared pairs of markers separated from each other at increasing distances to learn how far apart the markers must be before they seemed to be unlinked. Results from the putative parental populations showed that markers separated by about two centimorgans or less maintained linkage disequilibrium, whereas those further apart behaved as though they were unlinked. However, in the Lemba, markers separated by over 20 centimorgans showed significant LD, suggesting that some genetic event occurred in the Lemba recently, relative to the parent populations, to cause LD over such a large physical distance. The researchers confirmed that this difference resulted from admixture by comparing pairs of markers that show little difference against those which have larger allele frequency differences between the parent populations. Because in the Lemba only the latter category had elevated LD, admixture is directly implicated as a source of the expanded LD. Goldstein noted that the admixture in the Lemba resulted exclusively from movement of males, observing that “Males and females have different demographic roles, and those roles can have dramatic consequences for the properties of a population in terms of an epidemiology study.”

Goldstein then described work from his group that sought to identify Eurasian populations that may have undergone bottlenecks, or major population reductions, that could influence LD. In this case, the researchers studied seven different Jewish populations worldwide, comparing each to the resident population. Because Jewish identity is maternally transmitted, the scientists wondered whether they would see genetic evidence of this aspect of the Jewish culture, and therefore studied both male and female populations by looking at Y-chromosomal and mtDNA markers.

For each of the groups—Ashkenazi, Moroccan, Iraqi, Iranian, Georgian, Uzbeki, Yemeni, Ethiopian and Indian—the study asked whether the Jewish population had less genetic diversity than the host population. Studies on males showed negligible differences between paired populations, but a much different picture emerged in the study of females. In all cases, the Jewish females showed less diversity than did the resident population of women. As Goldstein said, these findings are telltale signs of bottlenecks or founding events in each of the Jewish female populations.

Closer scrutiny of the mtDNA data shows that, in each of the non-Jewish reference populations, no single genotype occurs in more than 10 percent of the population. By contrast, in several of the Jewish groups, one particular genotype predominates. The Georgian females present the most extreme case, in which 70 percent of the population carries the particular mtDNA marker in question. A different genotype predominates in each of the six other groups considered, pointing to separate historical bottleneck events in each group.

Taken together, these studies on admixture and genetic drift demonstrate types of heterogeneity that occur between human populations. They reveal the importance of understanding the diversity of various populations and emphasize the need to study the demographic histories of both males and females.

MtDNA and Y-Chromosome Perspectives on the Peopling of the Pacific

Mark Stoneking, Ph.D.

Anthropologists have long debated the pattern of human migration to the Polynesian Islands. Mark Stoneking, Ph.D., Max Planck Institute for Evolutionary Anthropology, described how he and his team are using genetic polymorphism studies to help resolve the dispute. Speaking generally about the relative powers of

anthropology and genetics for learning about patterns of human migration, Stoneking observed that “anthropological evidence is very good for generating hypotheses, but often has a hard time distinguishing between the different hypotheses or trying to test them. By contrast, genetic evidence is not always well suited for coming up with new hypotheses, but we can use it to test our various ideas.”

Fossil records show that humans traveling from Southeast Asia reached Australia more than 40,000 YBP. At that time, Australia and New Guinea were a single landmass. Even so, that ancient journey involved at least two open-water crossings. Only much later, around 5,000 YBP, did humans start colonizing Polynesian islands, beginning with Fiji. This raises the question: Was there a single prominent migration from Asia to Polynesia? Or was the early habitation pattern a more complex process?

As Stoneking explained, the South Pacific region—Australia; Melanesia, including New Guinea and the Solomon Islands; Polynesia, including Fiji, Hawaii and Easter Island; and Micronesia—readily attracts anthropologists not only for the physical beauty of the area but also because the inhabitants display extensive cultural diversity. For example, Melanesia alone has over 750 different languages, meaning that this region of merely two million people accounts for about one-fifth of all the languages in the world. The so-called Austronesian languages comprise all other languages in the regions and have recently been classified into 10 groups, all tracing back to Taiwan.

Most recently, researchers have been trying to distinguish between two competing hypotheses about the colonization of Polynesia. The first, based on studies of language and pottery, is dubbed the “express-train” model. It argues for a rapid, direct migration from Taiwan or Southeast Asia to Polynesia, with little mixing between the migrants and the Melanesians. The alternative hypothesis, which is called the “entangled-bank”

model opts for a much slower rate of colonization of Polynesia, involving substantial interaction between Southeast Asians, Melanesians and Polynesians during waves of forward and back migrations. “So, given these two competing hypotheses, we can try to use the genetic evidence to ask: Which story do the data seem to fit better?” said Stoneking.

To get a picture of the genetic trail into Polynesia, Stoneking and colleagues studied the frequency distributions of polymorphic markers in longtime native inhabitants from throughout the South Pacific and Southeast Asia. First, they studied marker sets on mtDNA and then did similar studies on Y-chromosomal markers. For both studies, the researchers selected multiple markers in which one genotype or haplotype predominated in Polynesia and was more rare among the other populations. In each case, one marker was slowly evolving and the other more rapidly changing. By determining the genotype frequencies in each of the various populations, the investigators gathered evidence about the origins of the Polynesian genotype.

The results of these genetic studies led Stoneking to argue that both the “express-train” and the “entangled-bank” model are too extreme. Rather, he put forth a hybrid model, which he calls the “slow boat” pattern of migration. In this scenario, the populating of Polynesia started as a migration from Taiwan or Southeast Asia about 5,000 YBP. The migrants passed through Indonesia then through New Guinea and elsewhere in Melanesia. In New Guinea, these new arrivals mixed with Melanesian populations, both Asian males with Melanesian females, and the converse. Descendants who subsequently went to Polynesia at about 1,500 YBP carried Melanesian genes on their Asian genetic background. In addition, they left behind in Melanesia distinct genetic evidence of their presence.

MITOCHONDRIAL DISEASES

An Overview of Mitochondrial Diseases *Doug Wallace, Ph.D.*

Doug Wallace opened the session on mitochondrial diseases by summarizing what is known about mitochondrial structure and function. Mitochondria, as Wallace said, are chimeras assembled from locally synthesized proteins as well as many encoded by nuclear genes. The human mitochondrial genome encodes just 13 proteins. It also carries genes for its own two ribosomal subunits and for 22 tRNAs needed for local protein synthesis.

Mitochondria perform three notable functions: production of the high-energy molecule adenosine triphosphate (ATP); generation of reactive oxygen species (ROS); and organellar self-destruction leading to cell death (apoptosis). Speaking about the mitochondria as a functional unit, Wallace said, “What we have is a three-legged stool on which energy metabolism is balanced. As long as energy output is good and oxidative stress is low, the mitochondrial membrane pores stay closed. However, when energy output declines or oxidative stress increases, this impinges on the pore proteins, which act as a self-destruct switch and destroys not just the mitochondria but the entire cell within which it resides.”

Turning to mitochondrial diseases, Wallace noted that heritable mitochondrial DNA (mtDNA) diseases are typically diagnosed by their maternal inheritance pattern. One of the best-studied mtDNA genetic diseases is Leber’s hereditary optic neuropathy (LHON), an adult-onset form of blindness. Most of the documented cases of LHON result from one of several point mutations in a gene for a protein in Complex 1 of the electron transport chain.

Mitochondrial diseases vary substantially in their degree of severity, depending on the ratio of mutant and normal mitochondria an individual carries. For example, some LHON patients also develop severe muscle rigidity, called dystonia. This more severe affliction often correlates with having far more mutant than normal mitochondria. Similarly, a particular mutation in a mitochondrial tRNA gene presents as type-II diabetes and sensory neural hearing loss in individuals with low percentages of mutant mitochondria. However, when mitochondria with that mutation predominate, the individuals show growth retardation, lactic acidosis, progressive dementia and cardiac defects.

Phenotypic expression of mitochondrial diseases also varies among mtDNA genetic backgrounds. “Not all lineages are equal,” Wallace stated, noting that blindness associated with a particular LHON mutation was much more likely to occur in a relatively rare European mtDNA lineage than in other European or in African mtDNA lineages.

Diseases like LHON are rare because they afflict individuals before or during their reproductive prime. By contrast, mtDNA mutations leading to late-onset diseases might be maintained in our species. Drawing on this idea, Wallace and his colleagues have begun looking for such mutations in individuals suffering late onset conditions such as Alzheimer’s. The group has found at least two candidate mutations: one that changes a codon in a tRNA gene and another within a ribosomal gene. The first mutation associates with an increased risk of getting Alzheimer’s, and the second one correlates with disease severity rather than increased risk.

Wallace observed that, due to our aging population, late onset mtDNA-based diseases, including those due to somatic mutations, are likely to become more prevalent in this coun-

try. Treatment for such diseases requires a better understanding of the biochemical defects that are associated with the observed genetic and phenotypic defects.

With that goal in mind, Wallace and his colleagues developed a technique for making cellular hybrids, called cybrids: cells have their own mitochondria removed, then get mitochondria from another cellular source. This strategy has revealed causal links between particular mtDNA mutations and disease phenotypes through biochemical tests comparing cybrids with their donor cells. To study the relationship between mtDNA mutations and disease phenotypes, researchers have recently started constructing cybrid embryonic stem cells to create mouse models for mtDNA genetic diseases. Results from the initial studies show that embryonic chimeras made up of a mix of unmanipulated and cybrid ES cells that can develop normally, and females generated in this way can transmit the introduced mitochondrial genome to their progeny. Wallace noted, "We've been able to recapitulate many of the phenotypes of the mitochondrial diseases through introduction of mtDNA mutations into the mouse germ line."

Oxidative Phosphorylation Diseases: Maternally Inherited Diseases

John Shoffner, M.D.

Many genetic diseases lend themselves to easy diagnosis, but mitochondrial diseases are often exceptions. "There are probably 80 to 100 different clinical presentations of mitochondrial diseases," said John Shoffner, M.D., Children's Health Care of Atlanta. The result is that the clinician may be overwhelmed when trying to decide if the patient meets the criteria for having a mitochondrial disease. Many mitochondrial functions use protein complexes assembled from several to dozens of different gene products. Thus, certain of these biochemical functions can be perturbed by defects in any of a number of different genes. Some of

these are encoded in the nucleus and others by the mitochondrial genome. Consequently, a given mitochondrial disease, such as Leigh syndrome, may result from either mitochondrial or nuclear DNA mutations.

Heritable mtDNA defects should be easy to recognize from their maternal mode of transmission. In fact, the phenotypes caused by any particular mtDNA mutation may differ widely among affected family members. As Shoffner noted, individuals with mtDNA mutations are frequently heteroplasmic, that is, they have both normal and mutant mitochondria. Variability in the degree of heteroplasmy accounts for differences in both severity of disease and in which organ is impaired within members of the same family. Shoffner estimates that at least 60 to 70 percent of the mitochondria must be the mutant form to damage cellular energetics.

When the physician has sufficient evidence that a patient has a mitochondrial defect that might stem from a mtDNA mutation, direct sequencing of the mitochondrial DNA can be carried out. By contrast, it is harder to establish whether the disease stems from a recessive mutation of a nuclear gene. Shoffner described a clever approach for finding such nuclear DNA mutations, which was used by Eric Shoubridge, McGill University, and coworkers for studies of certain Leigh syndrome patients. Having determined the biochemical defect—diminished activity of cytochrome oxidase (COX), which is the final complex in the respiratory chain—Shoubridge and colleagues used a technique called microcell-mediated complementation to track down the gene that restored COX activity to fibroblasts from Leigh syndrome (LS) patients. The technique, which involved separately transferring each human chromosome from wild-type cells into LS cells, showed that wild-type chromosome 9 restored COX activity. The study led to the identification of the protein Surf-1, encoded by a gene on chromosome 9q34, which contained mutations in both alleles in the LS patients.

Shoffner concluded that physicians must determine whether a patient has a maternally transmitted disease, a new mtDNA mutation, or an autosomal recessive defect, as is the case for most nuclear DNA-encoded mitochondrial diseases. These distinctions are important, especially to provide better genetic counseling.

Mitochondrial Abnormalities in Alzheimer's Disease *Gary Gibson, Ph.D.*

In the final talk on mitochondria and disease, Gary Gibson, Ph.D., Weill Medical College, Cornell University, described research that tests an intriguing notion: oxidative stress and abnormalities in oxygen metabolism lead to Alzheimer's disease. The brain, as Gibson pointed out, has only two percent of the body's mass but uses about 20 percent of the body's glucose for mitochondrial ATP synthesis. Since ATP synthesis is linked to the production of reactive oxygen species (ROS), perturbations in the pathway leading to ATP production can cause a build-up of potentially cytotoxic ROS. "While it has been known for decades that Alzheimer's involves a decline in brain glucose," Gibson said, "no one understood how this fit into the pathophysiology of the disease." To make the case for the proposed connection, Gibson first laid out the pieces of the Alzheimer's puzzle, including the pathology, the genetics and the clinical symptoms. Then he showed how mitochondrial defects, in particular a decline in alpha ketoglutarate dehydrogenase (AKGD), the least active enzyme in the Krebs' cycle, contribute to each of these aspects of the disease.

The histopathologic hallmarks of Alzheimer's brains include amyloid plaques and neurofibrillary tangles. Plaques form mainly from a peptide called amyloid-beta (A-beta), and recent studies have shown that plaques also contain markers of oxidative stress. Tangles develop from hyperphosphorylated forms of a protein called Tau.

These structures have now been found to harbor oxidized proteins as well as lipid peroxidation products.

Gibson said that genetic links to the disease fall into two classes, the 100 percent penetrant group and the genes with alleles that predispose individuals to the disease. The fully penetrant mutations, which are rare, lead to early onset of the disease. About 500 families exist worldwide with these mutations. In one such family, bearing the so-called Swedish mutation, Gibson's group found a selective reduction in AKGD.

The best-studied genetic predisposition for Alzheimer's links apolipoprotein E, specifically the Apo E4 allele of the gene, with this disease. A recently established connection between AKGD activity and Apo E4 incorporates a clinical symptom of Alzheimer's disease as well. Specifically, among patients carrying one E4 allele, there is a strong correlation between AKGD and the extent of clinical dementia. As Gibson stated, "The genetic background alters the importance of the deficits in mitochondrial metabolism."

These and other results suggest that AKGD plays a key role in the progression of Alzheimer's disease and lead investigators to ask: Which comes first, the neurodegeneration or the metabolic deficit? To answer this question, scientists needed to find peripheral markers for Alzheimer's disease in order to study people prior to the onset of clinical symptoms. Since mitochondrial defects alter calcium distribution, the investigators tested fibroblasts from healthy subjects and Alzheimer's patients with a drug that stimulates calcium release. The recovery pattern after calcium release differed between the patient and control cells, demonstrating that defective calcium redistribution is a feature of the disease in peripheral tissues. Evidence that this abnormality occurs in the brain as well came from studies on a transgenic mouse model of Alzheimer's disease. Both

fibroblasts and brain tissue from the transgenic animals showed the same defect in calcium behavior found in Alzheimer's patients. In addition, the transgenic animals also showed evidence of considerable oxidative stress. Investigators also found that, in humans, sporadic Alzheimer's is associated with a reduction in fibroblast levels of AKGD.

Another question that the investigators are asking deals with the interaction between oxidative changes and the plaque peptide A-beta. Recently, investigators have provided a link by showing that tiny concentrations of A-beta peptide added to cells in culture inhibit AKGD activity. Interestingly, other types of studies show that oxidative stress impacts specifically on A-beta metabolism, and more generally on plaque and tangle formation.

Gibson then provided a link between oxidative stress and the memory deficits seen in Alzheimer's. As he stated, a connection between lack of oxygen and decreased short-term memory has been known for many decades. Studies in animals have associated transient hypoxia-induced memory loss with reduced levels of the neurotransmitter acetylcholine. Given that Alzheimer's brains classically show a deficit of the neurotransmitter acetylcholine, Gibson and coworkers verified in an animal model of long-term hypoxia that an increase in acetylcholine improved the animals' memory.

Having presented a convincing argument linking oxidative stress to Alzheimer's disease, Gibson turned briefly to one possible mode of intervention, namely dietary restriction. The link between dietary restriction and increased longevity of laboratory rodents has been well established. Many studies show that this dietary restriction helps the animal handle oxidative stress. Consequently, dietary restriction may offer a means of protecting against the onset of Alzheimer's disease.

INTERACTION OF GENOTYPE AND ENVIRONMENT IN COMPLEX DISEASES

Nature vs. Nurture:

Coronary Artery Disease as Example *Craig Hooper, Ph.D.*

While some common diseases may result from the interaction of environmental and genetic risk factors, identifying and quantifying such factors poses enormous challenges. Craig Hooper, Ph.D., Centers for Disease Control and Prevention (CDC), opened the panel on this topic, using coronary artery disease as an example of current research dealing with gene-environment interactions. He emphasized findings that differ by sex or by race, both of which may have genetic as well as environmental, or social, components.

Beginning with sex differences, Hooper noted that, for coronary artery disease (CAD) in the US, death rates are declining for men but rising for women. Since the risk of CAD in women increases substantially after menopause, many researchers suspect the involvement of hormones.

Another sex difference in risk for CAD appears among diabetics. In the study Hooper described, diabetes was a strong risk factor for both sexes, but in contrast to men, women with diabetes showed an almost 100 percent incidence of CAD.

Hooper also noted a new report from the CDC on the interaction between heart disease and obesity in young people. This study of males and females between 15 and 34 years old found that, while obesity was linked to an increased risk for heart attack in both sexes, the risk was much higher in young women.

What factors contribute to these sex differences? According to Hooper, some investigators have

suggested that women have a greater tendency to develop blood clots than do men. “This has led some people to suggest that women may benefit from earlier pharmacotherapy than men,” Hooper explained.

Certain sex differences associated with CAD appear in some populations. Hooper cited the example of endothelial cell nitric oxide synthetase (ECNOS). He and his colleagues studied an allelic variant of the ECNOS gene that was associated with myocardial infarctions (MI). The Australian investigators who initially described this genetic variant found MI occurred at higher rates in both male and female smokers harboring this allele, whereas a Japanese group found this association only in men. A study of African-Americans with this allele from Hooper’s group reported an increased risk for MI but no difference between the sexes.

Hooper then described other racial differences in heart disease, citing data from a West Virginia University-CDC study. Using data from death certificates, the investigators noted that African-American women are most likely to die from heart disease; Caucasian women fall in the middle; and Asian and Pacific women have the lowest death rate from heart disease among women in the U.S. Further inspection showed that, among the Caucasian women, 66 percent had ischemic heart disease and just three percent died from hypertensive heart disease. By contrast, among African-American women, 54 percent died from ischemia while nine percent died from hypertensive heart disease.

Another important racial disparity appeared in the area of premature death due to heart disease. Premature death is defined as death before age 65, and therefore possibly preventable. The CDC found that seven percent of Caucasian, 24 percent of Native American

and 19 percent of African-American women die from premature heart disease. A similar trend was observed in men.

Because CAD develops slowly over several decades, researchers have found it useful to consider separately the initiation, progression and expression of the disease when they attempt to learn about the factors causing the disease. As Hooper stressed, learning about the contributions that sex and race may make to each component of the disease will help improve prevention and intervention programs.

Gene-Environment Interaction in Common Complex Diseases *Molly Bray, Ph.D.*

Sir Winston Churchill’s parents gave their son a strong complement of genes. Hence, despite enduring a stress-filled public life, favoring a rich diet, and enjoying a pint of whiskey and a big Cuban cigar most days of his adult life, Churchill survived to the age of 91.

Jim Fixx, the 1970s joggers’ guru, fared less well, having inherited genes that marked him for a lethal heart attack, to which he succumbed at age 54. But, as speaker Molly Bray, Ph.D., University of Texas-Houston, pointed out, Fixx’s reshaping of his environment, by changing his diet and exercise plan, may have added a decade or more to his life, given that his father’s fatal heart attack happened when the elder Fixx was only 40. Bray cited these contrasting situations to introduce her own research on gene-gene and gene-environment interactions associated with obesity.

Currently, more than half of the U.S. population weighs more than they should, and obesity plagues a significant portion of that group. Moreover, women and minorities are over-represented in the ranks of the obese. Since obesity often accompanies other illnesses, such

as hypertension, diabetes, cardiovascular disease, orthopedic and pulmonary conditions and an increased risk for cancer, the problem raises great concern about the health of our citizenry.

Maintaining body weight, by balancing the feelings of satiety and hunger, as well as energy metabolism, involves biochemical communication between brain, fat and muscle cells. To achieve this balance, intercellular signaling molecules activate specific cell surface receptors and consequently alter the activities of the recipient cells. The first such signaling molecule shown to function in regulating body weight was leptin. Beginning with its identification in 1994, researchers discovered that adipocytes, or fat cells, synthesize leptin and that brain cells make the leptin receptors.

The scientists found that circulating levels of leptin rise and fall with body weight. Subsequent studies revealed that obese rodents given leptin lost weight. Unfortunately, the peptide was far less effective as an anti-obesity drug in human clinical trials. The failure of leptin to become the miracle anti-obesity drug instigated further research into the genetic contributions to obesity.

In one such project, Bray and co-workers used linkage analysis to find genetic similarities among obese families, and also hunted for genetic differences between obese and slim siblings. The investigators focused on eight genes that play some part in the utilization and storage of body fat. The results of the study pointed to the involvement of the neuropeptide Y (NPY) gene. Then, DNA sequencing of the NPY gene revealed a two base deletion that lies in a regulatory region near the promoter, and may therefore influence the amount of NPY made in response to incoming regulatory signals.

In a follow-up study, Bray and colleagues found the NPY allelic association with obesity in a separate sample of individuals. This replication of the original finding showed small but significant associations between the two-base-deleted-allele and several quantitative measures of obesity,

including weight, waist/hip ratio and hip circumference. Despite the small magnitude of difference, the NPY allele's appearance in an independent population encourages Bray to pursue further studies of the gene.

To look for gene-environment interactions leading to obesity, Bray and her colleagues are tapping the resources of the cohort study called Atherosclerosis Risk in Communities (ARIC), which has monitored 12,000 Caucasian men and women and 4,000 African-Americans from four communities in Minnesota, Maryland, North Carolina and Mississippi since 1986. The study gathered demographic and medical data, as well as DNA for later genotyping.

To date, the researchers have completed genotyping of the 4,000 African-Americans from Jackson, MS, focusing on a set of polymorphic markers in a pre-selected group of candidate genes for obesity and hypertension. Because the average man in the population is considered overweight, the average woman is obese and almost half of the Mississippi group also suffers from hypertension, this population is a prime target for studies of genetic factors related to the two diseases.

The researchers found an intriguing association between obesity, hypertension and a particular allele for the gene for GNB3 (G protein beta3 subunit). The variant allele (so-called 825T) was associated with increased risk for hypertension only among the obese subjects in the study. By contrast, among the non-obese group, this same variant appeared to protect against hypertension. Furthermore, the association between the 825T allele and hypertension, which showed a weak but significant positive association in most of the obese group, became a negative association in the subset of physically active people. The allele in question appears not to cause a function change, and therefore Bray suspects that it may be a marker for an authentic mutation.

Bray's presentation highlights the challenges researchers face in trying to learn about the

gene–environment interactions that may explain many common diseases in humans. The strategies she discussed, including the use of large, well–defined populations and collection of extensive medical, behavioral and genetic data, should help tease out the contributions of genetics and environmental factors to development of these diseases in the coming decades.

Female Hormones and Thrombosis: Interactions of Genes and Environment *Frits Rosendaal, M.D.*

Hundred of millions of women regularly take oral contraceptives (OCs) or hormone replacement therapy (HRT). The major health risks associated with these medications involve cardiovascular problems. As Frits Rosendaal, M.D. of Leiden University Medical Center in The Netherlands noted, both genetic factors and the dose and chemical composition of OC or HRT taken may influence these risks. To understand such medication–induced risks for cardiovascular diseases, it is necessary first to distinguish between factors affecting the arterial system and those influencing the venous system. As Rosendaal stressed, this distinction is often overlooked “not just by the lay press but by physicians as well.”

Rosendaal explained that thrombosis, vessel blockage due to clot formation, typically develops in veins in the leg, causes local pain and can lead to pulmonary embolisms which may sometimes be fatal. By contrast, arterial thrombosis leads to myocardial infarctions—heart attacks—or strokes.

Thrombosis can have any of three underlying causes: changes in blood flow, or stasis; changes in blood composition, or coagulability; or changes in vessel walls. Empirical evidence shows that changes in blood flow result in venous thrombosis whereas factors that change vessel walls cause arteriosclerosis, or arterial thrombosis. Changes in blood composition, once assumed to underlie only venous

thrombosis, seem to contribute to myocardial infarctions as well.

Blood composition, Rosendaal noted, is the composite of pro- and anti-coagulation factors and pro- and anti-fibrinolytic or –clot resorbing factors. The numerous coagulation factors, designated by Roman numerals, include factors II, V, VII, VIII, IX, X and XI while the balancing anticoagulant proteins include protein C, protein S and antithrombin. Genetic alterations in these proteins may lead to a tendency toward bleeding or thrombosis. Most of these mutations are rare. Two common mutations are alterations in factor V, factor V Leiden, and in factor II, prothrombin 20210A. Both increase the risk of thrombosis. Rosendaal discussed the results of recent studies from his group that looked at the contribution of these genetic variants on venous and arterial thrombosis.

In one large case–control study, researchers found that the Leiden variant of factor V, present in three to seven percent of healthy people, is a strong risk factor for venous thrombosis in both men and women because about 20 percent of the case group had the Leiden variant. A separate study found that factor V Leiden was also a weak risk factor for myocardial infarction. That study included only middle-aged men because, as Rosendaal said, “We thought at the time that there were so few women with myocardial infarction that we included just men.”

Subsequently, Rosendaal, together with colleagues in Seattle studied women, specifically those who had suffered myocardial infarctions before age 44. In this case–control study, the researchers determined the genotype of participants for both the factor V and the prothrombin variants. The results showed that the variant forms of both genes were significant risk factors for myocardial infarction in this group of younger women.

In the same study, the researchers wanted to determine which women were most vulnerable.

Therefore, they looked for interactions of the genetic variants with environmental risk factors. “The one that really stood out was smoking,” said Rosendaal. “Smokers without such clotting abnormalities had a 9-fold increased risk. If they also had one of these mutations, their risk was increased over 35-fold. However, the risk was not increased in women with the mutation who did not smoke.” According to Rosendaal, “This is a clear example of a gene-environment interaction, where the effect of the gene is amplified by the environmental risk factor.”

Turning to the topic of the impact of postmenopausal estrogens on thrombosis, Rosendaal summarized the information about HRT. Data on venous thrombosis indicate a four-fold increased risk with HRT. Studies on arterial thrombosis suggest that HRTs increase the risk during the first years of treatment, but then may have a protective effect over longer times of treatment. As Rosendaal noted, these findings on arterial thrombosis suggest that different subgroups may respond differently to HRT. Hence, studies are underway to test whether lipid or clotting abnormalities underlie the increased risk of arterial thrombosis seen during the first years of therapy.

Overall, OC use increases the risk for venous thrombosis approximately four-fold. Because the estrogen dosage in OCs has dropped over the past few decades, people have assumed that the associated risks also declined. However, as Rosendaal explained, the actual data show that dropping the dosage from 100 to 50 micrograms resulted in a small decline in risk for venous thrombosis, and the further dosage reduction to 30 micrograms or even lower has had no noticeable effect.

The progestin component of OCs has been changed in chemical composition rather than dosage. Most women currently take OCs with either second or third generation progestins. Concern has been raised that third generation progestins put women at higher risk for venous

thrombosis than do the second-generation compounds. The results from several different studies show an increased risk of 2.5 fold for women using the newer rather than the older drugs, i.e. an almost 10-fold increased risk compared to non-users.

As Rosendaal explained, controversy about study designs and interpretation of data has surrounded these findings, heightening the effort to learn more about how progestins and estrogen affect thrombosis. A recent study by J. Rosing and colleagues of Maastricht University, The Netherlands, found that blood from women taking the third-generation OCs had greater clotting potential than did blood from women on second-generation compounds. Because each subject received both drugs in sequence, this study permits a direct comparison of the drugs. Therefore the results support the finding that newer OCs put women at higher risk for venous thrombosis than do the older drugs. Hence, as Rosendaal concluded, for the OC consumer and her physician, the problem is solved: “You use the safest pill. They all have equal efficacy, so the decision is easy.”

Finally, Rosendaal discussed certain genetic contributions to the risk for venous thrombosis among OC users. Whereas the factor V Leiden variant raises this risk about seven-fold among women who don't use OCs, the combination of the variant and the medication yield a 35-fold increase in risk. Does this combined risk create a strong case for a factor V screening program? Rosendaal argues that the case is not sufficiently strong since the absolute risk is still low, and may not be outweighed by the benefits from using OCs and the cost of such a screening program. Nonetheless, he sees this as a sign of movement into the post-genomic era in which learning about gene-environment interactions will at least permit us to modify our environment, based on our genes.

THE IMPORTANCE OF X-INACTIVATION

The Biology of the X Chromosome: Compensating for Sex Differences *Huntington Willard, Ph.D.*

Female mammals have a double dose of the X chromosome compared to males. But that does not mean that females make twice the amount of protein that males do from the X-encoded genes. Rather, as Huntington Willard, Ph.D., University Hospital of Cleveland and Case Western Reserve Medical School, noted in the final presentation of the conference, female mammals have a mechanism to silence most genes on one or the other X chromosome in each cell.

This process, called X-inactivation, begins in the first few days after conception of the fetus. Most often, the choice of which chromosome becomes inactivated occurs randomly. Placing X-inactivation in the context of biological difference between the sexes, Willard reminded the audience of two points: the randomness of X-inactivation makes females cellular mosaics and the mechanism for X-inactivation is a female-specific biochemical process.

Willard explained that X-inactivation is one of several mechanisms that have evolved to compensate for the difference in sex chromosome dosage. In earthworms, the female expresses all genes from both X chromosomes, but at an attenuated level. In the fruit fly, the male shows hyperactive transcription from its single X chromosome compared to that of the XX female.

The first clue about X-inactivation in mammals came in the late 1940s with the discovery of the Barr body, a dark-looking structure in the nucleus of interphase cells from females but not males. In the 1960s Mary Lyon correctly reasoned that Barr bodies were highly condensed, transcriptionally inactive X chromosomes.

As Willard explained, it is now known that the inactive X chromosome becomes covered with a non-coding RNA transcript called Xist, produced locally from that chromosome. Initially after conception, both X chromosomes transcribe the Xist gene, but then a signal silences the transcription of the gene from what becomes the active X.

Along with Xist, the Barr bodies also harbor some novel histones, known as macro-H2As. While these proteins appear elsewhere in the nucleus, they show marked enrichment on the inactive X. The researchers don't yet know what drives these distant relatives of the core histone H2A to the Barr bodies, but Brian Chadwick in Willard's laboratory found that two distinct domains of a macro-H2A contribute to its interaction with Barr bodies.

Chadwick recently found another novel histone that can distinguish the active from the inactive X chromosome. This protein, called H2ABB1 for Barr body deficient, binds to all chromosomes except the inactive X. The investigators are now trying to determine the sequence of events that attract macro H2A to the inactive X and H2ABB1 to the active X chromosome.

At the DNA level, selective silencing of genes on one X chromosome appears to be due to a number of chromatin changes, including DNA methylation. In typical cases, the methylated allele is the silenced one.

While most genes on the X chromosome fall subject to X-inactivation, there are many exceptions. If the X chromosome has, as is now believed from the Human Genome Project, about 2000 expressed genes, perhaps 200 to 300 of these "escape" inactivation and are thus expressed at different levels in males and females. As Willard noted, the data collected so far suggest that most X-linked genes that escape inactivation reside on the short arm, and indeed, on the distal part of that arm of the chromosome. Generally, those genes that

escape inactivation tend to be expressed at about the same level from both alleles. However, for a handful of genes escaping X-inactivation, expression from the inactive X varies either during development, as a function of cell type or among individuals.

“Can we say anything about the DNA sequences involved in either propagating or assisting in X-inactivation, or those sequences that prevent X-inactivation and block the spreading?” Willard asked. To address this issue, Laura Carrel, a member of Willard’s group, compared DNA sequences in the vicinity of genes on the X to see if any fit such predictions. Looking at DNA sequences 16 nucleotides long, Carrel found 74 that are well represented around X-inactivated genes but are highly underrepresented around the genes that escape this mono-allelic silencing. All 74 sequences are known repeated sequences and most are part of the Long, Interspersed Repetitive Element (LINE) class called L1. Given that only primates, but not other mammals, have these evolutionarily young L1 repeated sequences, their presence could not cause X-inactivation. Therefore Carrel will test the hypothesis that the L1 elements increase the likelihood of nearby genes being inactivated.

Finally, Willard mentioned new research suggesting that the proportion of cells with one versus the other inactive X can change over the life span, at least in the case of blood cells. In this research, most young females had a 50/50 distribution of gene expression from the two X-linked alleles. By contrast, more of the older women showed a skewed expression of genes from one of the two X chromosomes. “There may be some environmental factors over the life span that eventually give one lineage a competitive advantage...presumably because of differences in the particular alleles on one or the other X chromosome,” commented Willard, noting that the observation now opens up new questions for further investigation.

In fact, the general topic of skewed proportions of active versus inactive X chromosomes may be a very fruitful line of research. As Willard pointed out, individuals who are born with an extreme skewing in X-inactivation may carry important mutations on the preferentially silenced chromosome. Therefore, new studies will focus on these individuals and their male relatives to look for X-linked diseases. Hence, genetic studies initiated with females may help to promote the understanding of diseases in males.

ABOUT THE SOCIETY FOR WOMEN'S HEALTH RESEARCH

The Society for Women's Health Research is the nation's only non-profit advocacy group whose sole mission is to improve the health of women through research. The Society was founded in 1990 when it brought to national attention the need for the appropriate inclusion of women in major medical research studies and the resulting need for greater funding for research on conditions experienced by women. The Society works to increase public and private funding for research on women's health, promote the inclusion of women in medical research studies, and encourage the scientific examination of the basic biological and physiological differences between men and women. The emerging field of sex-based biology explores these differences and their effect on both health and the diagnosis and treatment of disease.

History

The Society was the force behind many major advances in women's health including increased federal funding for women's health research, passage of the federal law requiring women to be included in federally-funded medical research and establishment of the Office of Research on Women's Health at the National Institutes of Health. It was also responsible for the strengthened guidelines from the U.S. Food and Drug Administration to include women in all phases of drug testing. The current public awareness of gaps in women's health research is due in large measure to the ongoing efforts of the Society.

Outreach

One of the Society's priorities is to promote and support the efforts of basic and clinical researchers in the emerging field of sex-based biology. The Scientific Advisory Meetings (SAMs) bring together representatives of scientific,

medical, and health specialty organizations for annual updates on research in sex-based biology. Basic research into the molecular and cellular biology of sex differences is the focus of the Society's Annual Conferences on Sex and Gene Expression (SAGE). The Society co-sponsors a Scholars Grant Program to support the scientific and academic advancement of young physician researchers.

The Society works with policy makers, researchers, and the public to increase public dialogue and change public policies on women's health research issues. The Society's Women's Health Research Coalition of leaders from health, medical, and scientific organizations supports increased research funding for, and expansion of, sex-based research at academic research institutions.

The Society recently launched a public education campaign, *Some Things Only A Woman Can Do*, to educate women about the importance of participating in medical research. Additionally, the Isis Fund for Women's Health Research was established by the Society to focus on the scientific exploration of diseases and conditions that affect women solely, predominantly, or differently. The Society played a key role in urging and supporting the development of an April 2001 Institute of Medicine report, "Exploring the Biological Contributions to Human Health: Does Sex Matter?," which confirmed that differences between the sexes exist in the prevalence and severity of a broad range of diseases, disorders, and conditions.

The Society publishes reports, produces educational videotapes, maintains an award-winning Web site (www.womens-health.org) and sponsors the peer-reviewed *Journal of Women's Health and Gender-Based Medicine*.

SAGE II PLANNING COMMITTEE

Julia Amadio, MBA

Aventis Pharmaceuticals, Inc.
Parsippany, NJ

Geoffrey Cooper, PhD

Boston University
Boston, MA

Cheri Deal, PhD, MD

Hopital Ste-Justine
University de Montreal Teaching Hospital
Montreal, Quebec

Denise Faustman, MD, PhD

Harvard School of Medicine
Massachusetts General Hospital
Charlestown, MA

Myron Genel, MD

Yale School of Medicine
New Haven, Connecticut

Linda Giudice, MD, PhD

Stanford University
Stanford, California

Judith Hall, MD

University of British Columbia
Vancouver, British Columbia

Florence Haseltine, PhD, MD

National Institute of Child Health
& Human Development
National Institutes of Health
Bethesda, MD

Craig Hooper, PhD

Centers for Disease Control & Prevention
Atlanta, GA

Pamela Madden, PhD

Washington University School of Medicine
St. Louis, MO

Salli Tazuke, PhD

Stanford University
Stanford, CA

Rosanna Weksberg, MD, PhD

Toronto Hospital for Sick Children
Toronto, Ontario

Vince McLaughlin, PhD

Aventis Pharmaceuticals, Inc.
Washington, DC

SOCIETY FOR WOMEN'S HEALTH RESEARCH STAFF LIST

Phyllis Greenberger, MSW

President & CEO

Anita Bollt, MEd

Deputy Director & COO

Roberta Biegel, MA

Government Relations Director

Sherry Marts, PhD

Scientific Director

L. Jo Parrish, MA, MBA

Development Director

Michele Robinson

Communications Director

Kim Allman

Development Assistant

Kirsten Blanton

Assistant to the Deputy Director

Karyn Crichton

Assistant to the President

Stacey Fannon, MS

Programs Coordinator

Deonna Farr

Receptionist

Joi Foss, MS

Senior Development Officer

Sarah Gevers

Communications Manager

Jennifer Houtman

Development Officer

Melissa Kaplan, MA

Government Relations Assistant

Sarah Knab, MPH

Program Manager

Renee Lajeunesse

Communications Coordinator

Erin Ravelette, MA

Government Relations Associate

Regina Vidaver, PhD

Program Manager

Katie Wagner

Program Assistant

SOCIETY FOR WOMEN'S HEALTH RESEARCH BOARD OF DIRECTORS

CHAIR

Denise L. Faustman, MD, PhD
Associate Professor of Medicine
Harvard Medical School
Director, Immunology Laboratory
Massachusetts General Hospital

IMMEDIATE PAST CHAIR

Gloria E. Sarto, MD, PhD
Professor, OB/GYN
Co-Director, National Center of
Excellence in Women's Health
University of Wisconsin

VICE CHAIR

Lynne Wilcox, MD, MPH
Atlanta, Georgia

SECRETARY/TREASURER

Irma Goertzen, MA, RN
President and CEO
Magee Women's Hospital and
Research Institute

Janet Belle, RN
Basking Ridge, New Jersey

Mary J. Berg, PharmD
Professor, College of Pharmacy
University of Iowa

Colleen Conway-Welch, RN, PhD
Professor and Dean
Vanderbilt University School of Nursing

Rosemary Berkel Crisp, RN
President
Women for Health and Wellness

Gail Evans

Executive Vice President
Domestic Network, CNN

Florence P. Haseltine, MD, PhD
Bethesda, Maryland

Janet B. Henrich, MD
Associate Professor of Medicine and OB/GYN
Yale University School of Medicine

Mitzi Krockover, MD
Vice President, Women's Health
Humana

Ellen Leibenluft, MD
Clinical Associate Professor of Psychiatry
Georgetown University Medical Center

Celia J. Maxwell, MD, FACP
Assistant Vice President for Health Affairs
Director, Women's Health Institute
Howard University

Carmen Sapienza, PhD
Professor
Temple University Medical School

Nanette Kass Wenger, MD
Professor of Medicine
Emory University School of Medicine
Chief of Cardiology, Grady Memorial Hospital

Raymond L. Woosley, MD, PhD
Vice President
Arizona Health Sciences Center
Dean, College of Medicine
University of Arizona

BIOGRAPHICAL SKETCHES OF SPEAKERS

SHERI BERENBAUM, PH.D.

Sheri Berenbaum, Ph.D. is currently professor of physiology at Southern Illinois University School of Medicine in Carbondale. She earned her bachelor's degree in psychology and mathematics from City College of the City University of New York and her doctoral degree in psychology from the University of California, Berkeley. Dr. Berenbaum completed a postdoctoral fellowship in behavioral genetics at the University of Minnesota. Before joining the faculty at Southern Illinois University, she was assistant and then associate professor of psychology and of psychiatry and behavioral sciences at Finch University of Health Sciences/ The Chicago Medical School. Prior to her current position, she was professor of behavioral and social sciences and of psychology at Southern Illinois University.

Berenbaum's research focuses on the development of individual differences in cognition and social behavior from a neuroscience perspective. She is particularly interested in the effects of prenatal sex hormones on the development of sex-typed behaviors, and how these effects are mediated directly by the brain and indirectly through the social environment. Her behavioral studies of children and adults exposed to high prenatal levels of androgens have been supported by the National Institutes of Health since 1985.

TIM BESTOR, PH.D.

Dr. Bestor studied the role of microtubules in pronuclear fusion in sea urchin fertilization in Dr. Gerald Schatten's laboratory at Florida State University. He did postdoctoral studies with Dr. Vernon Ingram at the Massachusetts Institute of Technology, where he purified, characterized and cloned DNA methyltransferase-1 from mouse, the first eukaryotic DNA methyltransferase to be identified. He was assistant and associate professor of cell biology at Harvard

Medical School from 1988 to 1995, and was also a member of the staff of the Laboratory of Human Reproduction and Reproductive Biology. He is currently professor of genetics and development at the College of Physicians and Surgeons of Columbia University in New York City.

MOLLY BRAY, PH.D.

Dr. Bray is an assistant professor in the Human Genetics Center at the University of Texas-Houston School of Public Health. Dr. Bray began her graduate work with an interest in metabolic rate and obesity and has investigated aerobic fitness and resting and exercise energy expenditure in several samples of children and adolescents. She completed a master's degree in exercise science from the University of Houston in 1991 and obtained her Ph.D. in human and molecular genetics in 1998 from the University of Texas Graduate School of Biomedical Sciences. Dr. Bray specializes in population-based genetic analyses of complex disease data and has considerable interest and experience in research pertaining to the genetics of obesity and obesity-related interventions. She is currently funded to investigate the effects of gene-environment interaction on cardiovascular disease outcomes by genotyping and analyzing the entire 15,792 subjects in the Atherosclerosis Risk in Communities (ARIC) cohort for selected candidate genes for obesity and CVD-related co-morbidities. This will represent one of the largest sets of epidemiological genetic data yet created.

GARY GIBSON, PH.D.

Dr. Gary Gibson received his Ph.D. from Cornell University. After a position as junior faculty at the University of California at Los Angeles, he moved to Cornell University Medical College at Burke Medical Research Institute. He is currently professor of neuro-

science in the department of neurology and neuroscience. His research has always focused on the interaction of oxidative metabolism with brain function and dysfunction in disease and aging. He was the first recipient of the American Society For Neurochemistry's Jordi-Folch Pi award. Within the last year, he presented the Cornell Dean's talk, the NIH Directors' talk and the Visek Lectureship at the University of Illinois. He has served on numerous NIH review panels. He currently serves on editorial boards of four journals including the *Journal of Neurochemistry* and the new journal *Mitochondrion*. He has co-authored 180 research papers and chapters.

DAVID B. GOLDSTEIN, M.Sc., Ph.D.

Dr. Goldstein attended The University of California in Los Angeles where he received his Bachelor of Science degree. He then went on to the University of Connecticut to earn a masters of science. Finally, Dr. Goldstein received a Ph.D. from Stanford University in 1994. Since 1999 Dr. Goldstein has been a Wolfson Professor of Genetics at University College of London. He currently heads up research on gene expression in drosophila, the genetic structure of human intestinal nematodes, and the genetic structure of the British Isles.

Before coming to the University College of London, Dr. Goldstein held positions as a research associate at Interval Research Corporation in Palo Alto, CA, a NIH postdoctoral fellow at the Pennsylvania State University and a University lecturer in evolutionary biology at the University of Oxford.

Dr. Goldstein's awards include the Stanford Teaching Award in 1992, the Samuel Karlin Award for Mathematical Evolutionary Theory from Stanford University in 1994 and the NIH National Service Award for postdoctoral research. Dr. Goldstein has spoken at conferences nationally and internationally, and has published significantly within his field.

CRAIG HOOPER, Ph.D.

W. Craig Hooper, Ph.D. is section chief of the Molecular and Hemostasis Laboratory, Hematologic Diseases Branch, National Center for Infectious Diseases, Centers for Disease Control. He is involved in many thrombogenic population based and case-control studies which are focused on the identification of genetic polymorphisms that may be associated with cardiovascular disease. These studies range from thromboembolic complications of pregnancy to outcomes following interventional procedures in coronary artery disease. A special interest has been the influence of sex and ethnicity on disease progression and how these determinants may influence disease association with known genetic polymorphisms. He is also involved in basic research looking at the relationships between coagulation proteins and cytokine production.

DONALD W. PFAFF, Ph.D.

Dr. Donald Pfaff attended Harvard College and received his Ph.D. in 1965 from the Massachusetts Institute of Technology. In 1966 Dr. Pfaff returned to Rochester, NY, where he grew up, to do a postdoctoral fellowship at Rockefeller University. In 1969 he became an assistant professor and since 1978 has been a professor of neurobiology and behavior at Rockefeller.

Dr. Pfaff is on the editorial board for several journals including *Hormones and Behavior*, *Neuroendocrinology*, *Synapse*, *Endocrinology*, *Developmental Neuroscience* and *Neuroscience-Net*. He has done extensive research on sex hormone treatment and hormone receptors and has published extensively in this field.

FRITS R. ROSENDAAL, M.D.

Frits R. Rosendaal is professor of clinical epidemiology at the Leiden University Medical Center, The Netherlands. He has a medical degree from the Erasmus University in

Rotterdam, and obtained his Pd.D. in Leiden, Department of Hematology, with a thesis on hemophilia treatment. He currently holds joint positions in the departments of clinical epidemiology and hematology.

His main research interest is hemostasis and thrombosis. The Leiden center is active in studies on the etiology of thrombosis, management of thrombosis and quality control of anticoagulant therapy, and treatment and complications of bleeding disorders. These studies are performed in a close collaboration of clinical and laboratory scientists. Ongoing studies focus on genetic abnormalities predisposing to thrombosis, studies of gene-environment interaction, in particular with oral contraceptives and hormone replacement therapy, studies on safety of anti-coagulant treatment and national surveys of morbidity and mortality in hemophilia. Over the last few years, the Leiden center has been first to describe several new risk factors for thrombosis, such as factor V Leiden, prothrombin 20210A, high levels of factor VIII, of factor IX and of factor XI, and has also provided prevalence and risk estimates for these risk factors. It has also first described interactions of some of these genetic factors with oral contraceptives on the risk of venous thrombosis, and with postmenopausal estrogens on the risk of arterial disease.

JUDITH L. ROSS, M.D.

Dr. Ross is currently a pediatric endocrinologist at Thomas Jefferson University in Philadelphia, PA. Dr. Ross obtained her bachelors of arts degree from Wellesley College, graduating with high honors. She continued on to the University of Chicago Pritzker School of Medicine, doing graduate research in molecular virology and completing her M.D. in 1977. Dr. Ross fulfilled her residency in pediatrics at the Children's Hospital of Philadelphia after which she served at the National Institute of Child Health and Human Development in their Developmental Endocrinology branch. In

1982 Dr. Ross became board certified by the American Academy of Pediatrics and in 1983 she followed this with a certification in pediatric endocrinology.

MARK SEIELSTAD, PH.D.

Mark Seielstad is with the Program for Population Genetics at the Harvard School of Public Health in Boston. His research seeks to apply knowledge of a population's demographic and evolutionary history to the search for disease predisposing allelic variation. A particular interest is in female versus male differences in population structure and the effects of these differences on mitochondrial and Y-chromosomal variation.

JOHN M. SHOFFNER, M.D.

John M. Shoffner, M.D. is director of the Molecular Medicine Laboratory at Scottish Rite Children's Hospital in Atlanta, GA, and an attending physician at Egleston Children's Hospital. His research over the past 10 years has focused on clinical, biomedical and molecular genetic investigations of oxidative phosphorylation diseases as well as neurogenetic diseases.

Dr. Shoffner obtained his bachelor of arts in chemistry at Emory University in 1981. In 1985 he obtained his M.D. at Emory University School of Medicine where he went on to do his residency in neurology and genetics. Along with being the director of the Molecular Medicine Laboratory, Dr. Shoffner's appointments include attending physician at Egleston Children's Hospital.

Dr. Shoffner is on the editorial board of the journal *Neurogenetics* and has been a member of the Alpha Omega Alpha Honorary Medical Society since 1985. Dr. Shoffner lectures at many conferences throughout the year and has published extensively in his field.

ELLEN K. SILBERGELD, PH.D.

Dr. Silbergeld is trained in environmental toxicology, and her work has focused on understanding mechanisms of toxic agents that affect the development of the reproductive, nervous and immune systems. Her research combines laboratory-based studies using cellular and non-human models, with population-based studies of exposed persons. Dr. Silbergeld's laboratories also serve as a resource for studies using molecular epidemiologic methods, including genotyping, exposure assessment and biomarkers of early response.

Her research on lead poisoning has identified sources of exposure to lead for children, fetuses and adults; recent studies have demonstrated that lead, stored in bone, can be remobilized during pregnancy and lactation and also over the menopause. Other studies focus on understanding mechanisms of lead effects on the brain, reproductive system and regulation of blood pressure.

Current research interests also include understanding the potential adverse effects of chemicals that act to disrupt hormone function. Basic research studies are focused on effects of early developmental exposure on brain and reproductive system. A major interdisciplinary focus of both laboratory and epidemiological research in Dr. Silbergeld's group has concerned the interactions between mercury exposures and host resistance to major infectious diseases, including malaria. Using cell systems, they have found that mercury perturbs cytokine-dependent responses to pathogens, including malaria. Field epidemiological studies in Brazil and the Philippines are studying mercury exposures and human immune response among remote populations in the Amazon and in Mindanao.

MARK STONEKING, PH.D.

Mark Stoneking is a professor of biological anthropology at the Max Planck Institute for Evolutionary Anthropology and the University of Leipzig in Leipzig, Germany. His research

interests involve using analyses of molecular genetic variation in contemporary and ancient human populations to address questions about population origins, history and relationships.

MARC J. TETEL, PH.D.

Marc J. Tetel received his bachelor's degree in biology from Northwestern University and his Ph.D. in neuroscience from the University of Massachusetts. After completing a post-doctoral fellowship in molecular endocrinology at the University of Colorado Health Sciences Center, he joined the faculty at the University of Massachusetts in Amherst in 1998. Currently, he is an assistant professor in the Center for Neuroendocrine Studies and a member of the Neuroscience and Behavior Program and the Molecular & Cellular Biology Program. His research interests include how the gonadal steroid hormones, estradiol, progesterone and testosterone, act in the brain to regulate gene expression, development and behavior. His work has revealed much about the molecular mechanisms of action of gonadal steroid receptors in the mammalian brain. Most recently his work on nuclear receptor coactivators, proteins that dramatically enhance steroid receptor transcriptional activity, has pioneered the investigation of these important cofactors in gene expression in brain.

JACQUETTA M. TRASLER, M.D., PH.D.

Jacquetta M. Trasler, M.D., Ph.D., is associate professor in the departments of pediatrics, human genetics and pharmacology & therapeutics at McGill University and director of the Developmental Genetics Laboratory at the Montreal Children's Hospital Research Institute in the McGill University Health Centre in Montreal. She obtained her M.D. from McGill University in 1980, followed by two years of clinical training, including one year of obstetrics and gynecology. In 1987, she received a Ph.D. from McGill University under the supervision of Dr. Bernard Robaire, study-

ing the male-mediated developmental effects of anti-cancer drugs. After postdoctoral training in molecular biology with Dr. Norman Hecht at Tufts University in Boston, Dr. Trasler joined the faculty at McGill University in 1990. She is co-leader of the Medical Genetics and Genomics Axis of the McGill University Health Centre Research Institute and teaches pharmacology and genetics to undergraduate, graduate and medical students. At McGill, Dr. Trasler has mentored a number of pre-medical and graduate students and is director of the McGill University M.D./Ph.D. Program. Dr. Trasler's research interests focus on the molecular and developmental regulation of gene expression in the germ line and the implications for the resulting embryos, with specific interests in DNA methylation and genomic imprinting and the molecular and cellular targets for drug effects on germ cells.

DOUGLAS C. WALLACE, PH.D.

After growing up in Maryland and New York, Douglas Wallace received his bachelor's of science degree from Cornell University in Ithaca, New York. Following two years in the service, he moved to Yale University where he completed both masters and doctorate of philosophy degrees by 1975. After one year of postdoctoral study at Yale, he joined the faculty at Stanford University as assistant professor of genetics and remained in that position until 1983. He then moved to Emory University in Atlanta, Georgia as professor of biochemistry and associate professor of neurology, pediatrics and anthropology. Dr. Wallace was named the Robert W. Woodruff Professor of Molecular Genetics, director of the Center for Molecular Medicine in 1990 and chairman of the department of genetics and molecular medicine in 1992. In 1994 he was awarded the William Allan Award by the American Society of Human Genetics for his fundamental work on the genetics of mammalian mitochondria and for elucidating the role of mitochondrial DNA variation in human evolution, genetic disease and aging; was elected

to the National Academy of Sciences in 1995; was the first Emory Distinguished Faculty Lecturer in 1996 and presented the Lehninger Lecture at Johns Hopkins Medical School.

HUNTINGTON F. WILLARD, PH.D.

Trained in genetics at Harvard, Yale and Johns Hopkins Universities, Dr. Willard is a respected leader nationally and internationally in the field of human genetics. Prior to coming to University Hospitals of Cleveland and Case Western Reserve University in 1992, he held faculty positions at the University of Toronto and at Stanford University. Currently president of the American Society of Human Genetics, he has also served in elected leadership positions in the Association of Professors of Human/Medical Genetics and the Human Genome Organization. He is a former member and chair of the Mental Retardation and Developmental Disabilities Research Committee at the National Institutes of Health and is currently chair of the Mammalian Genetics study section at the National Institutes of Health. He also serves on the Genetics Review Board for the Howard Hughes Medical Institute, as well as on scientific advisory boards for several biotechnology companies. He has served on the editorial boards of numerous scientific journals and is co-founder and executive editor of *Human Molecular Genetics*. Dr. Willard has been the author or co-author of over 250 scientific publications. He is also co-author of *Genetics in Medicine*, a widely used textbook, now entering its sixth edition.

In 1999, Dr. Willard was appointed director and president of The Research Institute of University Hospitals of Cleveland. The Research Institute is among the nation's largest, with over \$75 million in funded research activities across the full spectrum of biomedical research, from fundamental investigation of biological processes to the development of new approaches to therapeutics and health care in the coming era of Genetic and Genomic Medicine.

Inside back cover

BLANK



SOCIETY FOR
WOMEN'S HEALTH RESEARCH

1828 L Street, NW
Suite 625
Washington, DC 20036
202-223-8224
www.womens-health.org