A.D. MDLXII

Genes, Drugs and Gender

Sassari (Italy), September 23rd - 24th , 2011

Organized by

DEPARTMENT OF DRUG SCIENCES
UNIVERSITY OF SASSARI

DEPARTMENT OF THERAPEUTIC RESEARCH
AND MEDICINE EVALUATION
ISTITUTO SUPERIORE DI SANITÀ, ROME

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ABSTRACT BOOK

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CONTENTS

Systems biology asks new questions about sex differences

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A modern general theory of sexual differentiation starts with the sex chromosomes, which are the only factors that differ in female and male zygotes.[4] All sex differences originate from the inherent imbalance in expression of genes on the X and Y chromosomes. The male-specific expression of the testis-determining gene *Sry* from the Y chromosome is critically important, because *Sry* is the sex-specific factor that begins the divergent development of gonads in the two sexes leading to life-long differences in secretion of gonadal hormones. Gonadal hormones have permanent (differentiating) organizational effects, and reversible activational effects, that cause profound sex differences in the brain and numerous other organs.[1] *Sry* acts directly on non-gonadal tissues to have male-specific effects. Importantly, X and Y genes other than *Sry* directly cause sex differences in phenotypes such as gamete formation, autoimmune disease, obesity and metabolic disease, neural tube closure defects, and brain processes related to addiction and alcoholism.[2] Thus, numerous X and Y genes act along parallel direct pathways to create sex differences in development and function of diverse tissues.

Analyzing the origins of sex differences in phenotype and disease requires measuring the interaction of at least three major classes of sex-specific factors causing sex differences: (a) organizational and (b) activational effects of gonadal hormones, and (c) direct sex chromosome effects on various tissues.[1] Recent advances in systems biology offer novel methods for analyzing the aggregate behavior of most genes in the genome.[3] The new analytical tools empower the biologist to determine which gene networks are responsible for specific traits, which regions of the genome regulate specific networks, and how the genome is organized and has evolved in a sex-biased manner. The relatively small number of systems biology studies in this field so far suggest several conclusions. In some tissues such as adipose and liver, the majority of active genes are expressed at different levels in males and females.[6] The largest number of sex differences in gene expression are caused by sex differences in the acute (activational) effects of gonadal hormones, followed by permanent effects of gonadal hormones and sex chromosome effects. Analyzing the

coordinated group behavior of genes leads to discovery of new functional gene groups that respond to different sex-specific factors and are linked as a group to specific phenotypes.[5] Specific genes within these groups have characteristics that indicate their relative importance to physiology. We can expect that the new systems biology tools will be applied with increasing frequency to the understanding of sex differences in physiology and disease.

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NOTES

Epigenetic mechanisms in autoimmune diseases

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Autoimmune diseases include over 80 different disorders that cumulatively affect up to 5% of the population and represent the third leading cause of morbidity in Western countries. Genetic and environmental factors have been incriminated. Recent advances in epigenetics have contributed to our comprehension of how environmental factors (U.V. lights, drugs, sex, etc.) participate in the development of autoimmune diseases in genetically predisposed individuals. Epigenetics is referred as stable and heritable changes in gene expression without alteration in DNA sequence. Epigenetics modifications involve either methylation of cytosine in CpG dinucleotides, histone-tail modifications, and micro-RNAs alterations [1].

The first evidence of epigenetic involvement in the development of autoimmunity comes from the animal studies showing that prolonged treatment with DNA methylation inhibitors induces a lupus-like disease in rodents [2]. Interestingly, such an effect has been reproduced after adoptive transfer of DNA hypomethylated $CD4^+$ T cells and hypomethylated B cells [3-4]. The second evidence comes from the analysis of twins showing that changes in DNA methylation reflects twin discordances in lupus erythematosus systemic (SLE) when analyzing peripheral blood mononuclear cells (PBMC) [5]. The third evidence comes from the cellular specificity of the process [4-5]: $CD4^+$ T cells and B cells in SLE, synovial fibroblasts in rheumatoid arthritis and cerebral cells in multiple sclerosis.

In SLE, we and others have established that T and B cells are characterized by a profound DNA methylation defect and the degree of DNA hypomethylation correlates with disease activity [7]. As a consequence numerous methylation-sensitive genes are over-expressed, and these includes cytokine genes (IL-4, IL-6, IL-10 and IL-13), co-stimulatory molecules (CD6, CD11a, CD70, and CD43L/CD154), and human endogenous retrovirus [8, 9]. Demethylation of the female inactivated X-chromosome in SLE provide an explanation for female predominance in autoimmune diseases [10]. In SLE DNA hypomethylation is related in part to the reduction of the DNA methyl transferase activity by DNA-methyl-transferases (DNMTs) [11]. Such

effect has been related to a blocage in the PKC delta/Erk pathway and/or a growth arrest at the G0/G1 interface.

One of the important aspect of epigenetic regulation is the possibility of reversion, for exemple blocking the IL-6 autocrine loop in SLE B cells restores DNA methylation thus opening perspectives for the development of new therapeutics and diagnosis biomarkers [7].

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N O T E S

The glucocorticoid receptor: sex, inflammation and life and death

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Males and females show differences in the prevalence of many major diseases that have important inflammatory components to their etiology. These gender-specific diseases, which include autoimmune diseases, hepatocellular carcinoma, diabetes, and osteoporosis, are largely considered to reflect the actions of sex hormones on the susceptibility to inflammatory stimuli. However, inflammation reflects a balance between pro- and anti-inflammatory signals, and investigation of gender-specific responses to the latter has been neglected. Glucocorticoids are the primary physiological anti-inflammatory hormones in mammals, and synthetic derivatives of these hormones are prescribed as anti-inflammatory agents, irrespective of patient gender. We explored the possibility that sexually dimorphic actions of glucocorticoid regulation of gene expression may contribute to the dimorphic basis of inflammatory disease by evaluating the rat liver, a classic glucocorticoid-responsive organ. Surprisingly, glucocorticoid administration expanded the set of hepatic sexually dimorphic genes. Eight distinct patterns of glucocorticoid-regulated gene expression were identified, which included sex-specific genes. Our experiments also defined specific genes with altered expression in response to glucocorticoid treatment in both sexes, but in opposite directions. Pathway analysis identified sex-specific glucocorticoid-regulated gene expression in several canonical pathways involved in susceptibility to and progression of diseases with gender differences in prevalence. Moreover, a comparison of the number of genes involved in inflammatory disorders between sexes revealed 84 additional glucocorticoid-responsive genes in the male, suggesting that the anti-inflammatory actions of glucocorticoids are more effective in males. These gender-specific actions of glucocorticoids in liver were substantiated in vivo with a sepsis model of systemic inflammation.

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NOTES

Role of GILZ in inflammation

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Glucocorticoids (GC) are of extraordinary therapeutic value in a wide range of inflammatory, autoimmune and inflammatory diseases. Most of GC effects relates to regulation of gene transcription. Their therapeutic activity is due to regulatory effects on activation, cell growth and differentiation in a number of cells and tissues, including cells of the immune/inflammatory system such as T lymphocytes.
With the aim to deeply analyze the molecular mechanisms of GC action, we have identified a number of GC-induced genes including GILZ (Glucocorticoid-Induced Leucine Zipper), a protein rapidly induced by GC treatment. GILZ is an important mediator of the anti-inflammatory and immunosuppressive effects of GC. Moreover, we identified a new GILZ isoform, L-GILZ, involved in mediating the effects of GC on inflammation and on cell differentiation.
Using GILZ-TG and GILZ-KO mice we demonstrate GILZ and L-GILZ are anti-inflammatory molecules and mediate the GC-induced effects. In particular, GILZ regulates T cell activation and differentiation, cytokine production, including pro-inflammatory cytokines, and inflammatory process development. Notably, the Fluorescence-Activated Cell Sorting (FACS) analysis of frequency of CD4+/CD8+ cells on thymus showed no difference in the numbers of double negative, double positive or single positive cells when comparing GILZ-TG or GILZ-KO, vs Wild Type (WT) mice. On the contrary, analysis of polarized T lymphocytes subpopulation evidenced significant changes in the Th1/Th2 ratio with a significant decrease of Th1 in GIILZ-TG and an increase in GILZ-KO mice. Consistent with these changes we found an increased severity of DNBS-induced colitis in GILZ-KO and a reduction in GILZ-TG.
Results could provide new means to predict sensitivity to treatment with GC and to outline new therapeutic approaches.

NOTES

Sex differences in the brain

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The root causes of sexual differentiation of brain and behavior are well understood. Differential exposure to gonadal hormones during development (organizational effects) (Bakker and Baum, 2007; Schwarz and McCarthy, 2008) and in adulthood (activational effects) (Cooke et al., 1998), as well as direct effects of sex chromosomes (Arnold, 2009) influence developmental processes such as cell birth, cell migration, cell death, and differentiation. Ensuing differences in morphology and behavior trigger different interactions with the environment, which may lead to further differentiation (McCarthy and Arnold, 2011). An important new research trend on the molecular mechanisms underlying sexual differentiation involves epigenetic modifications (McCarthy et al., 2010). Considering such modifications is especially relevant if sexual differentiation is based on cell differentiation, as cells seem to 'remember' whether they should express male or female characteristics. Indeed epigenetic modifications have been found for several differentially expressed genes, e.g., sex differences in DNA methylation for genes coding for estrogen and progesterone receptors (Nugent and McCarthy, 2011). Epigenetic modifications may also play a role in differential cell death during development. Cells typically die several days up to a week before they die, again seemingly 'remembering' whether they were exposed to high or low levels of testosterone. Histone deacetylase inhibitors can indeed eliminate sex differences in cell number in areas where cell death happens at a different rate (Murray et al., 2009). No genetic targets, however, have been identified in this case.

Although currently hundreds of sex differences have been found in the brain in almost any parameter imaginable, in most cases we do not understand how these sex differences contribute to sex differences in brain function. At least two factors obscure this question. The first is that sex differences in behavior are often overstated (Södersten, 1984). The second is that the function of sex differences are too narrowly interpreted. Intuitively, such differences are thought to underlie sex differences in behavior or other overt functions controlled by the brain. Other options are typically not considered. Studying the sexually dimorphic

vasopressin/vasotocin (AVP/AVT) projections from the bed nucleus of the stria terminalis and the medial amygdala offers some answers. In most vertebrates studied, these projections are much denser in males than in females (De Vries and Panzica, 2006). The widespread presence of this sex difference among vertebrates suggest that it was important enough to conserve it through vertebrate evolution. These projections have been especially well studied in rats, where they have been implicated in social and reproductive behaviors as well as in autonomic functions (De Vries and Panzica, 2006). Comparative studies, however, suggest that AVP/AVT may have different roles in males and females. For example, in virgin voles, AVP stimulate parental behavior in males but may inhibit it in females as treatment AVP receptor antagonists inhibits parental behavior in males but stimulates it in females (De Vries, 2004). These and similar behavioral data obtained in different species suggest that sex differences in neuropeptide pathways do not always induce sex differences in function, but may also prevent them by compensating for differences in hormonal and physiological conditions that may otherwise cause undesirable sex differences in certain functions (De Vries, 2004). Such dual function may be a general phenomenon for sex differences in the brain. It implies that the neural substrate of functions that show no obvious sex differences may nonetheless differ between males and females. Functional imaging studies suggest that this is true for humans as well.

Recent data from our laboratory suggest that immune challenge during development reduces AVP expression in males but not in females. As we have also shown that AVP stimulates play behavior in males, this differential reduction may explain why we find that the same immune challenge reduces play behavior in male but not in female rats. Given the importance of the AVP system for social behavior in general (Caldwell et al., 2008), this research may provide insight in sex differences in vulnerability to behavioral disorders with a strong social component, such as autism, which affects males much more than females (Fombonne, 2009).

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N O T E S

Clinical biomarkers gender specific

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It is well established that males and females differ in their basic physiology, and in the susceptibility to and progression of diseases. The study of the clinical biomarkers in gender medicine has recently received a large attention in order to develop new procedures and adequate theragnostic strategies. The impact of the advancing knowledge in molecular biology and clinical genetics has rapidly improved our understanding of the sex differences variation during the different decades of life. In particular, recent studies emphasize that the adult female phenotypes suffer from several different multifactioral diseases such as autoimmune disease or cardiovascular problems or osteoarthritis. The loss of female hormones was considered a major cause of the disease starting and progression not only in the bone metabolism but also in renal dysfunction or, in cooperation with the inflammatory system, in the cardiovascular accident.
The oxidative stress which produces cytotoxic reactive species reacts and leads to dysfunction or tissues district damage or unbalance of the molecules antioxidant activity that induces the injury. By the new technologies, in particular by the “omics”, it is now possible to get a light on the different molecular mechanisms that have a major impact on the preventive medicine such us the drug metabolism intervention observed during the development of cholesterol lowering drugs as preventive treatment of CHD. In the new era of the gender specific medicine there is a growing evidence that it is the time to develop non-invasive diagnostic tests for critical care medicine and to expand the knowledge of new biomarkers role not only for the biomedical research advances but for the theragnostic application by the translational medicine.

NOTES

The role of regulatory agency

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A core principle of the pharmaceutical development is that patients enrolled in clinical trials "should be reasonably representative of the population that will be treated by the drug". Nevertheless, the traditional research model raised barriers to the recruitment and participation of women in clinical trials.
The European Medicines Agency (EMA) has not yet implemented guidelines for the analysis and evaluation of gender differences in the clinical evaluation of medicines, a policy developed by the FDA since late '90s.
In 2005 EMA published some considerations about gender in the conduct of clinical trials and argued against the need for a separate guideline on women as a special population group in clinical trials, since the guidelines issued by the International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use have addressed gender as part of an adequate demographic characterization, analysis and assessment of the patient population [1, 2, 3].
The evidences of a still not adequate women recruitment in clinical trials as well as the increasing findings on gender variation in medicines efficacy and safety profiles are some of the key factors leading to the revaluation of a gender-related policy for designing, conducting, reporting and analysing clinical trial. Furthermore, the concerns about the difficulties of testing medicines in pregnant women underscore the need for a more formal mechanism of women inclusion in the development of pharmaceutical that are likely to be used in pregnancy [4, 5, 6].
To improve preventive, diagnostic and therapeutic strategies in women and men, in the era of personalized medicine, gender specific research and use of gender disaggregated data are extremely needed in clinical research as well as in clinical practice.

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Estrogens and Cancer

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17β-estradiol (E2), the most effective female estrogen, is critical for the control of a plethora of biological responses that strongly influence several aspects of male and female physiology. Thus, it is not surprising that E2 and its receptors (*i.e.,* ERα and ERβ) are also considered to be risk factors for the initiation and progression of several endocrine-related cancers (*e.g.,* breast, prostate, ovarian and endometrial cancer) (Ascenzi et al., 2006). Estrogen is implicated in the development of breast cancer, based on data from both clinical and animal studies; risk factors associated with breast cancer reflect cumulative exposure of the breast epithelium to E2 (Henderson and Feigelson, 2000). Both steroid hormones and gonadotropins contribute to the etiology of ovarian cancer in humans (Deroo and Korach, 2006). Approximately 70-80% of sporadic endometrial carcinomas are distinguished as type I carcinomas and are associated with endometrial hyperplasia, hyperestrogenism, and expression of ERs. The remaining 20% constitute type II carcinomas, are generally unrelated to estrogen, and exhibit negative or low ER expression (Lax, 2004). E2 is required for prostate carcinogenesis. Estrogenic stimulation through ER in a milieu of decreasing androgens contributes significantly to the genesis of benign prostatic hyperplasia, prostate dysplasia, and prostate cancer (Steiner and Raghow, 2003). A completely opposing effect of E2 has been described in colon cancer. In fact, clinical studies indicate that the incidence of colon cancer is lower in women than in men, and data from the Women's Health Initiative indicate a significantly reduced incidence of colon cancer in postmenopausal women receiving combined 'Hormone Replacement Therapy' (HRT; estrogen plus progestin) (Rossouw et al., 2002)

Two current hypotheses exist to explain the relationship between estrogen and cancer. In the first, binding of estrogens to the ER stimulates proliferation of responsive cells, increasing the target cell number within the tissue. The increase in cell division and DNA synthesis elevates the risk for replication errors, which may result in the acquisition of detrimental mutations that disrupt normal cellular processes such as apoptosis, cellular proliferation, or DNA repair. In the second hypothesis, estrogen metabolism leads to the production of genotoxic by-products

that could directly damage DNA, again resulting in point mutations. This latter hypothesis has been recently revised by the analysis of ERs knockout mice in which E2 missed any proliferative effect (Deroo and Korach, 2006).

However, the E2 effects on cancer are often divergent and somewhat contrasting depending on the relative levels of the ER subtypes in a given cancer cell. These contrasting effects relate to the spectacular complexity of the E2 intracellular signaling triggered by the ERs (Ascenzi et al., 2006). The ERs are principally localized in the nucleus where they act by globally modifying the expression of the E2-target genes. The premise that E2 effects are exclusively mediated through the nuclear localized ERs has been rendered obsolete by research over the last 15 years demonstrating that ERα and ERβ proteins are also localized at the plasma membranes and in other extra-nuclear organelles. These effects, mediated by membrane-localized ERα and ERβ, also occur in tumor environments. Indeed, in cultured cancer cells, membrane-initiated ERα signaling mediates the proliferative effects of E2, whereas membrane-initiated ERβ signaling directs the anti-proliferative effects of E2 (Ascenzi et al., 2006; Marino and Ascenzi, 2008). Moreover, the progressive reduction in ERβ expression correlates with an increased ERα-mediated cell proliferation in breast cancer cells and uncontrolled colon cell proliferation (Marino and Ascenzi, 2008). Nonetheless, the role of the membrane-initiated ER signaling in tumors has been underestimated and, at the present, nuclear ERs localization is considered as both a negative and positive prognostic factor. Here our recent studies on the complex system of E2-induced signal transduction pathways, their impact on E2-induced cancer cell proliferation, and the participation of E2-induced membrane-initiated signals in tumor environment will be presented and discussed.

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N O T E S

Analgesic Therapy

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Chronic pain is a very great problem in our society, as it affects about 20% of the adult population and is severe in 13% of cases; in cancer pain patients percentages are also higher (Niv and Devor, 2007). Sex differences have been reported in experimental pain responses and in the prevalence and severity of clinical pain conditions (Kraft et al., 2004). Several experimental findings indicate that women demonstrate increased sensitivity to pressure, electrical, temporal summation, and muscle pain measures and a much higher incidence in many chronic painful syndromes. Moreover sex differences were reported in pain therapy and, in particular, in opioid use (Sibille et al., 2011).
Sex differences in preclinical studies indicate that male rodents demonstrate an increased μ-opioid analgesic response in comparison to their female counterparts, while evidence regarding sex differences in opioid analgesia among humans is less consistent. Clinical studies have demonstrated greater analgesic responses to mixed-action opioid agonist–antagonists among women compared with men, while studies examining sex differences in μ-opioid analgesia have yielded mixed results. Conclusions from a recent meta-analysis of clinical and experimental pain studies indicated that overall, women demonstrated a greater analgesic response to morphine compared with men with insufficient evidence to support sex differences among other exogenous opioids (Furlan et al., 2006). However, pharmacodyamic differences have been revealed, indicating that, despite similar analgesic responses across sexes, women experience a slower onset of analgesia and increased side effects to μ-opioids (Trescot et al., 2008, Sibille et al 2011).
A number of different genetic polymorphisms have been identified that may contribute to individual differences in opioid analgesia. Allelic variations of CYP2D6 resulting in either poor or excessive metabolizers have been associated with altered opioid metabolism. Another potential candidate gene is the μ-opioid receptor gene (*OPRM1*). In particular, the rare 118G allele has been associated with attenuated μ-opioid analgesic responses, as well as reduced basal pain sensitivity.

The need to treat pain compels physicians to use opioids for long periods, an important consequence is the effect on the hypothalamus-pituitary axis (Meczekalski et al., 2008). Indeed both endogenous (β-endorphin) and exogenous opioids are known to modulate the secretion of pituitary hormones, including gonadotropins. These effects were present in both sexes, although with some differences. Indeed the presence of hypogonadism was clearly demonstrated in men, while in women data are less clear (Aloisi et al., 2009; Daniell 2008).
Hypogonadism is a clinical syndrome associated with many physical and cognitive complaints (Cherrier 2009) since there is substantial evidence that gonadal hormones can influence the structure and functions of neurones not only during puberty but also in adulthood, including alterations of their dendrites and synaptic connections. For instance, it was shown that synaptic connectivity in the hippocampus and prefrontal cortex of male rats normally depends on androgens. When adult males are castrated, the number of synapses in each region decreases. It has also been suggested that androgens might be an effective therapy for certain neurological dysfunctions such as Alzheimer's disease and schizophrenia and that they can modulate pain.

The opioid-induced hypogonadism symptoms are fatigue, anaemia, changes in skin features, absence of libido, bad mood and depression (Aloisi et al., 2005). Therefore, it is necessary to treat pain but also to avoid other important dysfunctions that can increase the negative effects of pain, particularly when treatment is long-lasting.

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Gender specific tumour pharmacology

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As we are more and more approaching the era of individualised medicine, it may sound surprising that gender aspects of tumour pharmacology have been neglected over decades. Fortunately, novel treatment paradigms and therapeutic strategies all fuelled by a pipeline of promising compounds necessary to realize, at least in theory, the advocated approach of tailored therapies. On the other hand, these novel options are in sharp contrast with the clinical evidence about pharmacokinetic and pharmacodynamic differences among genders [1]. To refine the term "difference" further, we should clearly address the distinction between gender disparities observed in experimental systems (hypothesis generating) and clinical evidence requiring a completely different study design. Although there are convincing examples of pharmacokinetic differences for over 25 years (e.g. 5-fluorouracil) [2, 3] or pharmacodynamics (e.g. differential response in the therapy of lung cancer) [4], gender disparities are subject to a variety of other confounding variables. These variables include age, life style factors such as nutrition and individual fitness, the pathophysiology of impaired organ function, concurrent diseases, a differential genetic/epigenetic background and several more. To overcome this impasse, the limited evidence on gender specific tumour pharmacology requires a much broader knowledge base collected in prospectively randomized clinical trials. Basic problems simply arise from the fact that women are notoriously underrepresented in clinical cancer trials [5], particularly in early stages, thus soliciting not only gender oriented approaches from a scientific point of view, but also from organisational aspects including the awareness of health policy makers. Once initiated, these trials mandatorily rely on gender oriented primary PK/PD endpoints, integrated in PK/PD modelling to confirm gender as a determinant in cancer pharmacology with the ultimate goal to implement this knowledge into daily clinical practice.

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N O T E S

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To die or survive: a sexual challenge of the cell

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Different cell death processes have widely been investigated in the recent years in order to elucidate the different pathways involved in the complex machinery implicated in determining cell fate. Different forms of cell death have been described: apart from the "classical" form of death known as necrosis, a well characterized traumatic injury of the cell, several additional forms of cell death have been identified. Among these, apoptosis has been characterized in great detail. More recently, autophagic processes, previously considered as death-related, have also been analyzed and defined as a survival option of all cell types. Defects in the mechanisms of cell demise, i.e. an increase or a decrease of apoptosis or autophagy, have been associated with the pathogenesis of a number of human diseases. We recently started to work in this field taking into account "cell gender" and cell fate. We found that gender differences are detectable in terms of both apoptotic-susceptibility (Maselli et al 2009) and, more recently, autophagic process (Lista et al. 2011).
In consideration of the great importance of apoptosis and autophagy in the onset and progression of a number of human diseases we hypothesize that these differences could represent a new task in future studies in the field of pathogenesis and pharmacological management of human diseases. Several reports claim in fact for the straightforward importance of the control of cell fate either in cardiology or in vascular research as well as in the fields of cancer, neurodegeneration and immunopathology. The control of cell fate can be lost in all these diseases so that the induction, e.g. in cancer, or the inhibition, e.g. in neurodegeneration, of apoptosis represents one of the main goals of the therapeutic research. On the other hand, more recently, autophagy also became an innovative pharmacological target. Several autophagy-modulating drugs are already in the clinical practice and the so called molecularly targeted therapy became a novel strategy in the attempt to control all the human diseases cited above. Unfortunately, gender is still out of the match but we are convinced that it should become a key player in these new tasks of pharmacology. Our results, together with those obtained by other groups (e.g. by the group of R. Clark, JBC 2007) clearly suggest that a differential cell fate between cells from males and from females can

occur, providing new information as concerns the pathogenesis of the diseases but, also, their treatment.

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NOTES

Metabolic control of systemic autoimmunity: convergence of genetic and environmental factors on mitochondrial dysfunction and oxidative stress reveal regulatory checkpoints for selection of treatment targets in lupus

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Systemic lupus erythematosus (SLE) is characterized by the dysfunction of T cells, B cells, and dendritic cells (DC), the release of pro-inflammatory nuclear materials from necrotic cells and the formation of antinuclear antibodies (ANA) and immune complexes of ANA with DNA, RNA, and nuclear proteins (1). Oxidative stress and inflammation lead to parenchymal and vascular tissue damage, the latter resulting in accelerated atherosclerosis which is a major cause of mortality in SLE. Activation of the mammalian target of rapamycin (mTOR) has recently emerged as a key factor in abnormal activation of T and B cells in SLE (2). In T cells, increased production of nitric oxide and mitochondrial hyperpolarization (MHP) were identified as metabolic checkpoints upstream of mTOR activation. mTOR controls the expression T-cell receptor-associated signaling proteins CD4 and CD3ζ through increased expression of the endosome recycling regulator Rab5 and HRES-1/Rab4 genes (3), enhances Ca^{2+} fluxing and skews the expression of tyrosine kinases both in T and B cells, and blocks the expression of Foxp3 and the generation of regulatory T cells (4). MHP, increased activity of mTOR, Rab GTPases, and Syk kinases, and enhanced Ca^{2+} flux have emerged as common T and B cell biomarkers and targets for treatment in SLE (5). While inactivation and depletion of B cells have shown success in both animal models and patients, blockade of oxidative stress, mTOR, tyrosine kinases and T-B cell interaction are also being evaluated as targets for treatment in SLE.

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NOTES

Biological drugs and gender

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Numerous autoimmune diseases, including SLE, RA, and systemic sclerosis, have been shown to be more prevalent in women. In many of these diseases, sex hormones have been implicated to play an important role. In SLE, both estrogen and prolactin have been shown to be immunomodulators that can allow autoreactive B cells to evade tolerance mechanisms and proliferate, while treatment with testosterone, estrogen receptor antagonists, or inhibitors of prolactin secretion have been shown to alleviate disease in murine models of SLE as well as in certain groups of SLE patients.
Moreover, some studies have also point out that gender determinants that directly or indirectly influence. In particular, immune reactivity is more enhanced in females than in males, lymphocytes and monocytes from females show higher antigen presenting activity and mitogenic responses, females have higher immunoglobulin levels than males, an enhanced antibody production to both primary and secondary antigen stimulation, and a higher homograft rejection rate and males are more prone to infections.
Therefore, it has been demonstrated that gender may also influence the response to biological drugs. An example is rheumatoid arthritis in which TNF blockers seem to affect the level of sex hormones in the RA synovial tissue before they have any influence on the hormonal serum levels as well as locally increased estrogens may exert activating effects on synovial cell proliferation, including macrophages and fibroblasts.
In this regard, recent studies suggested that men have better responses to treatments with biologic agents than women, and other studies indicate that male gender is a major predictor of remission in early RA. In conclusion, further, larger and well designed studies are needed to improve knowledge of gender differences in autoimmunity disease pathogenesis and treatment response.

NOTES

Gaseous transmission and gender difference

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Hydrogen sulphide (H_2S) is an endogenous gaseous mediator produced from L-cysteine by two different enzymes: cystathionine β-synthetase (CBS) predominantly present in central nervous system (CNS), and cystathionine γ-lyase (CSE) predominantly localised in cardiovascular network (Levonen et al., 2000; Yap et al., 2000; Yang et al., 2008). We have recently demonstrated that the non genomic vasorelaxant effect of androgenic hormone testosterone (T) is mediated by H_2S production in rat aorta (Bucci et al., 2008). Aim of this study was to perform a pharmacological dissection on androgenic versus estrogenic steroids on H_2S biosynthesis in rat aorta, in order to define if there is a different profile on vascular reactivity gender-related. H_2S production was determined by performing a colorimetric assay on homogenates of rat aortic tissues incubated with T (10 μM), stanozolol, an anabolic steroid with poor androgenic activity (ST, 10 and 100μM), 17-beta oestradiol ES (10μM), and progesterone (PS, 100μM) for 15 or 30 minutes. Incubation of aortic tissues with T significantly increased H_2S production (5.73±1.13 vs. 3±035 nmoles/mg of protein/min, $n=11$, $p<0.05$, T and vehicle, respectively) while both ES and PS did not affect H_2S production at all time points tested. Similarly ST did not modify H_2S production suggesting that the action of T on H_2S biosynthesis is related on its androgenic activity and not to anabolic one. In order to assess the involvement of androgen receptor in T-induced H_2S production, aortic tissues were incubated with nilutamide (nil 10 μM), an androgen receptor antagonist, and then stimulated with T. In presence of nil, a significant reduction of T-induced H_2S production occurred (2.34±0.30 vs 5.73±1.13 nmoles/mg protein/min, $n=5$ $p<0.05$), suggesting a role of androgenic receptor in T-induced H_2S production. These data suggest a specific role for T in H_2S biosynthesis in vascular district, more likely dependent on its interaction with androgen receptor.

NOTES

The basis of sex-gender differences in hypertension

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Hypertension defined by the National Heart Lung and Blood Institute (United States) as systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mmHg. For survey purposes the definition also includes taking antihypertensive medication, or being told twice by a physician or other professional that one has hypertension.[1] Based on these definitions, the prevalence of hypertension in men exceeds that of women until the age of 45 years, is similar between the sexes between the ages of 45-65 years and is greater in women compared to men after the age of 65 years. Although treatment rates for hypertension in women 50-79 years of age average about 63%, hypertension treatment control to blood pressures <140/90 mmHg was only 29% in women over the age of 70 years. Why do these differences exist between men and women in prevalence of hypertension with age and how can treatment efficacy be improved in older women? The answer to these questions may lie in a better understanding of the etiology of the disease or even at a more basic level understanding differences in control of peripheral resistance between males and females. Many of the animals models used to study hypertension demonstrate a sex disparity in development of hypertension between males and females. Most subsequent studies historically have focused on the etiology in males (most effected sex) with little regard to factors which might be protective in females (least effected). Therapeutic approaches then focused on mechanisms to reduce hypertension in males. Blood pressure is the result of action of three control systems: the autonomic nervous system, the vascular endothelium and renin-angiotensin system. In males, genes associated with the sex determining region on the Y chromosome (Sry) are associated with development of hypertension in genetic models of hypertension(9). This region encodes for tyrosine hydroxylase a key enzyme required in production of the adrenergic neurotransmitter norepinephrine. However, estrogen modulates activity, disposition and transport of norepinephrine in the brain and peripheral nerve endings (2, 7, 8). In young men, sympathetic nerve activity correlates positively with total peripheral resistance. However, this relationship does not exist in young women suggesting that mechanisms associated that autonomic regulation of vascular tone is

fundamentally different between the sexes(6). Whether this difference reflects estrogenic regulation of vasodilatory endothelium-derive factors such as nitric oxide, vasoconstrictor factors such as endothelin 1 or regulation of angiotensin converting enzyme remains to be evaluated in a systematic way. In estrogen modulates all of these aforementioned mechanisms in isolated cell systems and experimental animals(5). Contribution of other steroids hormones such as testosterone, progesterone or corticosteroids related to stress are not considered in diagnosis or treatment of hypertension in humans. In addition to hormonal transitions associated with menopause, some women may carry a life-long risk for hypertension following a hypertensive episode with pregnancy (3,4). The etiology of these pregnancy related events such as pre-eclampsia may be multifactorial and are just now being evaluated in animal models for the disease. More information is needed into stratification of causes of hypertension in women. In the future, practice guidelines for treatment of hypertension may be stratified by sex, hormonal status and/or pregnancy history to improve control of blood pressure in both men and women as they age.

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NOTES

Sex differences in cerebral ischemia

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Stroke is the third leading cause of death in the U.S. and the most common cause of disability [1-3]. The economic burden of stroke is increasing, making the prevention and treatment of stroke a critical public health issue. Sex differences in stroke are increasingly recognized [1-3]. Female rats and mice of many different inbred and outbred strains sustain less tissue damage from an equivalent insult after focal or global cerebral ischemia [4-6]. Rodent strains engineered for expression of diseases that are important clinical risk factors for stroke, including non-insulin dependent diabetes and hypertension, have shown that females are less sensitive to a controlled ischemic insult than males [4-6]. Most of this "male-sensitivity" pattern has been attributed to exposure to ovarian hormones, particularly estrogen (E2). In rodent models, young females have smaller infarcts than males, and this effect that is reversed by ovariectomy and restored with E2 replacement. Estrogens are also neuroprotective in males and in middle aged animals [4]. However, randomized clinical trials of post-menopausal hormone replacement therapy have demonstrated an increased stroke risk in treated women [7]. This failure of translation may be secondary to a number of factors including incorrect dosing, estrogen formulation, route of administration or timing of estrogen replacement. *Alternatively*, E2 may not be the sole contributor to ischemic sexual dimorphism. Emerging data have established that tissue damage and functional outcome after experimental brain injury are shaped by ***biologic sex in addition to exposure to reproductive hormones*** [6]. Data from cell culture models and *in vivo* studies of neonatal and adult animals indicate that mechanisms of cell death differ in males and females even in the absence of hormones [8-16]. Clinical evidence that suggests intrinsic sex differences play a role in the response to stroke comes from the extreme ends of the lifespan – in the very young and in the elderly. In the perinatal, neonatal and childhood population, males are at higher risk of both hemorrhagic and ischemic stroke. A sex disparity in *outcomes* after ischemic injury also exists, with female infants faring better than males [17]. Considering that sex

hormone levels are low in both females and males at this age, the discrepancy in outcomes may be influenced by sex-specific hormone-independent factors or by early "organizational" effects of gonadal steroid exposure.

Sex differences in molecular cell death: We have made considerable progress in our understanding of the cell death pathways activated by an ischemic insult in the adult brain. We have shown that the cell death mechanisms activated by ischemia are sex dependent [9, 10]. In males stimulation of neuronal nitric oxide synthase (nNOS) generates the potent oxidant peroxynitrite, a major initiator of oxidative and nitrosative DNA damage. DNA repair is initiated by poly-ADP-ribose polymerase (PARP-1) but the subsequent energy failure and by-products of PARP activation trigger the release of apoptosis inducing factor (AIF) from the mitochondria leading to *caspase-independent* cell death. Over the past five years we have used a variety of methods (genetic and pharmacological) to interfere with the sequential activation of the nNOS/PARP/AIF pathway. In each case, despite equivalent reduction in the activation of PARP and ischemia-induced AIF translocation between the sexes*, only males benefit* [9, 10, 13]. Therefore, the nNOS-PARP pathway of ischemic cell death is sexually dimorphic in brain, mediating cell death in males and cell survival in females. Similar results are seen in neonatal models and in ovariectomized animals [8], suggesting independence from the activational effects of hormones.

In contrast, we have found that females are exquisitely sensitive to caspase induced cell death [14, 16]. Stroke-induced caspase activation is higher in adult females, caspase inhibition differentially protects females, and this protection is unrelated to serum estrogen levels at the time of stroke. One potential mechanism underlying the differential sensitivity of the female brain to caspase activation is translational repression of X-linked inhibitor of apoptosis protein (XIAP) by micro RNAs. XIAP is the most potent endogenous inhibitor of caspases and is differentially regulated by sex. Stroke induced a significant increase in miR-23a, a microRNA that binds XIAP, in males but significantly reduced miR-23a in females. This was seen in *both* gonadally intact and ovariectomized females, suggesting that this is an E2-independent mechanism of cell death regulation. As both XIAP and AIF are on the X chromosome we hypothesize that these two sex-specific cell death pathways diverge early in development, either from chromosomal factors or early organizational effects of hormones. The translational importance of these sex differences is still not known. However the possibility exists that men and women

may respond differently to neuroprotective agents and this should be taken into account in the design of clinical trials. A brief summary of our new unpublished findings on miR-23a will be highlighted.

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NOTES

Gender Aspects in Heart Failure

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Heart failure (HF) is a common cause of cardiovascular death and carries a poor prognosis in both genders. Risk factors and myocardial adaptations in HF in men and women are different. Women have more frequently diastolic HF, associated with the major risk factors diabetes and hypertension and men more frequently systolic HF due to coronary artery disease. Female hearts develop a more favorable physiological form of myocardial remodeling than male hearts. In contrast, under stress, male hearts develop more easily pathological hypertrophy with dilatation and poor systolic function than female hearts. This may be related to sex specific gene regulation, epigenetics, sex hormones, estrogens and testosterone. HF management differs between both sexes, with underdiagnosis and undertreatment and less use of invasive therapies in women. Nevertheless, women frequently have better outcomes than men or respond better to treatment. Gender research will contribute directly to patient-oriented benefit by suggesting clinical protocols.

N O T E S

Coronary heart disease in women

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Coronary heart disease (CHD) is the most common cause of death amongst women, who experience more complications after acute myocardial infarction (AMI) than men [1]. Recent advances in the field of cardiovascular medicine have not led to significant drops in case-fatality rates for women, compared to the dramatic reductions achieved for men [2]. Although a lower intensity of care may be, in part, related to a differential clinical history, symptom profile and acuity of presentation, under-recognition of this condition in women may also be contributory to worsening outcome, especially in women with an established diagnosis of ischaemic heart disease or AMI [3].
Supplementary evidence has observed significant delays in health-care-seeking behavior, less intensive resource use patterns and longer diagnosis times for women than men. For example, available data indicate that women are less likely to be referred for coronary angiography and revascularization procedures than men, and referral tends to occur at a later stage in the disease process [4,5]. Furthermore, pharmacological therapy is hampered by defective evidence, as women are frequently underrepresented in clinical trials and there may be gender differences in therapeutic response [6].
For the sizeable proportion of women presenting for CHD evaluation, traditional disease management approaches that focus on detecting 'critical stenosis' often fail to identify those women who are critically at-risk [7]. Guidelines emphasize the importance of recognizing the full spectrum of cardiovascular disease and thus classify women as being at high risk, intermediate risk, lower risk and optimal risk [8]. New findings support the concept of a multifactorial model, in which sex hormones interact with traditional and conditional risk markers, leading to an increase in the functional expression of atherosclerotic plaque deposition or vascular or metabolic alterations resulting in worsening outcomes for women [3]. Whereas the risk factors are the same in both sexes, gender-specific differences are noted [9].
For example diabetes mellitus is the most important risk factor and CHD mortality is 3- 5 times higher in diabetic compared to non-diabetic women, whereas the risk is 2 -3 times higher in diabetic men [10].

Furthermore, clinical presentation of CHD in women is different than in men. In approximately 60% of cases, the initial presentation of CHD in women is acute myocardial infarction (AMI) or sudden cardiac death and up to half of all women presenting with an AMI report no prior chest pain symptoms [3,11]. After sudden cardiac death, the most common presentation of obstructive coronary heart disease for women is atypical symptoms, such as back pain, dyspnea, nausea/vomiting and weakness [9,12]. Women reported more pain in the jaw and neck than men and they were more likely to describe their chest pain as a feeling of fullness than men [12]. When women underwent to coronary angiography, non-obstructive (i.e., <50% stenosis) CHD was frequently reported [13]. Most women with non-obstructive CHD at coronary angiography continue to experience symptoms that contribute to a poor quality of life and high health-care resources use due to repeated examinations and hospitalizations [14,15]. Although many of these women are diagnosed with 'non cardiac' chest pain, an alternative mechanism for their symptoms is coronary microvascular dysfunction. Differentiation between these mechanisms of chest pain is important, because 'non cardiac' chest pain is not associated with cardiovascular sequelae and may require further medical evaluation and treatment. By contrast, syndrome X, which is thought to be caused by microvascular dysfunction, is associated with inducible metabolic ischaemia [15].
Current limitations on health-care resources emphasize the need for better identification of those women most likely to have coronary artery disease before referral for invasive assessment [16].

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N O T E S

Thrombosis and sex-gender specific aspects

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The study of atherothrombosis is a rapidly evolving field, and significant progress was achieved in various aspects of the disease during the past year. Differences between males and females in symptoms presentation, drugs' response and clinical complications have been well demonstrated (1-10). Management of atherothrombosis disease, however, is generally guided by evidence from trials conducted predominantly in men, with few studies focused on women alone. Epidemiological studies have demonstrated the increased rate of cardiovascular events in women (1-10). All these issues have underlined the urgent need of identification and application of preventive strategies aimed to the control and reduction of athero-thrombotic risk factors through the formulation of risk card in asintomatic women, and therapeutic strategies for the correction of risk factors exposure in sintomatic and asintomatic women. The different "penetrance" of cardiovascular risk factors in women together with their interaction with hormonal therapies plays an important role in the development of atherosclerotic processes at the bases of cardiovascular diseases. Despite many primary and secondary preventive strategies, the pathologies correlated with the atherosclerotic process remain the main causes mortality and morbidity (1-10). Inflammation and endothelial dysfunction have a pivotal role in the pathogenesis of the atherothrombosis and the consequent cardiovascular events. Smoking habit, incorrect eating habits, sedentary life, and, in general, an incorrect lifestyle associated with hypertension or dyslipidemia shifts the risk threshold. Nevertheless, the correction of the lifestyle associated with specific therapeutic strategies aimed to the reduction of risk factors could counteract the pro-atherogenic environment by the correction of the inflammatory process and the endothelial dysfunction.
The presence of genetically determined conditions correlated with alterations of the coagulative profile and endothelial function could interact with environmental components in determining the critical phenotype of high thrombotic risk in women.
Clinical evidences exist evidencing gender differences with respect to the weight of the single traditional risk factors and risk factors correlated with

inflammation (e.g. physical exercise, metabolic status, C reactive protein levels, and pro-inflammatory cytokines/chemokines). It has been hypothesized that these differences may be the direct consequence of the chromosomal differences. The Y chromosome contains the SRY gene which is implicated in the development of hypertension modulating the norepinephrine synthesis (11), while the X chromosome contains the gene coding the androgens receptor. Further genes on the chromosome X codify for enzymes involved in the oxidative stress, in cell survival, apoptosis and fat distribution (12-14). These data indicate that sex chromosomes regulate a wide range of responses influencing the development, progression and clinical complications of atherotrombotic diseases. Furthermore, gender differences in atherotrombotic diseases exposure and therapeutic treatment response may be related to the complete expression of polymorphisms of genes on the chromosome X in males who have only one copy of chromosome X, while, due to the random inactivation of one out of the two X chromosomes in females, the polymorphisms may present with a mosaic phenotype in females (15,16).
By studying global gene expression profiles of peripheral blood as well as genetic polymorphisms in genes involved in response to antiplatelet therapies, and platelet function in acute coronary sindrome patients undergoing percutaneous coronary intervention we identified new mechanisms that characterize the atherothrombotic disease in females. For example, besides several genes on X and Y chromosomes, such as XIST gene regulating the X chromosome inactivation, we found different genes located on autosomal chromosomes that were differentially expressed between females and males. Among these genes we found the ABHD2 (abhydrolase domain containing 2) gene. In 2007, Miyata et al. demonstrated that this gene was expressed in coronary atherosclerotic lesions and was associated with plaque stability.

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NOTES

Gender difference in the effect of angiotensin receptor blocker on cardiovascular disease

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Background and Aim:
The concept of gender difference has been established in the fields of cardiology, in particular, aging and gender relationship could play a pivotal role for the development of cardiovasucular disease. Moreover, gender difference may exist in the treatment of hypertensive patients although there have been few evidences among the large clinical trials. The objective of this study was to subanalyze the Jikei Heart Study (JHS) which demonstrated the addition of angiotensin receptor blocker (ARB), valsartan to conventional treatment significantly prevented cardiovascular events compared with the conventional treatment group (Mochizuki S. et al for the Jikei Heart Study group, Lancet 369;1431-9,2007) .

Methods:
The JHS, a multicenter controlled trial, was performed by a prospective randomized open-labeled, blinded endpoint design. Effect of the treatment were evaluated by sex (1038 women and 2043 men) as hazard ratios with 95% confidence interval (CIs) using Cox regression models adjusted for age, BMI, smoking, dyslipidemia, diabetes, antihypertensives, and statin use at baseline.

Results:
Blood pressure was similarly reduced in both genders in both treatment groups, and these reductions were consistently noted throughout the study. In the combined analysis of the two treatment groups age, ejection fraction by echocardiography, LDL and HDL cholesterol, triglyceride were higher in women than in men. On the other hand, current smokers, diabetes mellitus were higher in men than in women. Trends in greater cardiovascular events occurred in men than women (hazard ratio 1.37(95%CI 1.02-1.85)). Fewer men in the valsartan group had a primary

endpoint (hazard ratio 0.61(95%CI 0.44-0.82), p=0.001), whereas in women, a nonsignificant reduction in the primary endpoint was found in the valsartan group compared with the non-ARB group (hazard ratio 0.64(95% CI 0.39-1.06), p=0.075)

Discussion and Conclusions:
The subanalysis of the Jikei Heart Study demonstrates that valsartan-based treatment in men resulted in ferwer cardiovascular events, whereas, in women at all ages, this effect was not demonstrated. In addition to this, such a valsartan effect was not observed in women less than 55 years of age though small event numbers were seen in women less than 55 years of age. In connection with this, the risk of cardiovascular events in women becomes significant after the age of 55-60 years. This suggests that valsartan therapy might provide a potential benefit for cardiovascular risk reduction in postmenoposal women as well as men. In conclusion, valsartan based treatment added to conventional antihypertensive therapy is more effective than non-ARB treatment on the cardiovascular risk reduction in men but not significantly in women although future studies for low risk patients with hypertention will be needed. (Yoshida H. et al for Jikei heart Study group, J. Hypertens 28;1150-7,2010)

Short summary
Substudy of the Jikei Heart Study analyzed the gender difference in cardiovascular disease risk reduction. A greater incidence of primary endpoint occurred in men versus women. Men in the valsartan group had a significant reduction in the primary endpoint, whereas a nonsignificant effect was found in wemen.

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