



European Gender Medicine Network

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Report from WS 1.3

“Sex Differences in Basic Biomedical Research and Preclinical Drug Development”

Berlin, February 16th and 17th, 2015

Sex Differences in Basic Biomedical Research and Preclinical Drug Development

On February 16th and 17th the third EUGenMed workshop took place in Berlin. 24 experts and stakeholders convened to discuss five major topics:

1. Assemble knowledge about evidence that sex and gender (S&G) play a role in major cellular functions
2. Outline underlying mechanisms like interactions between sex chromosomes and hormones and the epigenetic control of gene regulation
3. Discuss present translational approaches in sex specific medicine
4. Discuss benchmarks for high quality in S&G research and sex differences in drug development
5. Design a structure for a position paper

Day 1 – February 16th

Vera Regitz-Zagrosek, coordinator of the EUGenMed project, opened the workshop and welcomed all participants. The EUGenMed project was briefly introduced in its overall structure and the workshop series explained. The current workshop on “Basic Biomedical Research and Preclinical Drug Development” is to be followed by the workshop 4 on “Sex and Gender in Medicines Regulation and Medical Education” on March 4th, 2015 in Brussels. The project will culminate in a final conference on June 30th 2015 in Brussels. The consortium partner from Maastricht University Ineke Klinge briefly introduced herself.

Sex differences in cellular functions

Walter Malorni focussed on the cytopathology and described the role of “cell sex” (isolated cells with XX or XY chromosomes) and the susceptibility to cell death induction. He suggested the following main features to display a gender disparity at cellular level: 1. the basal redox state, 2. the response to oxidative imbalance, 3. the susceptibility to undergo apoptosis and anoikis and 4. the susceptibility to undergo autophagy. Estrogen receptors (ER) are very important regulators of a plethora of cellular events, including apoptosis and autophagy. Under stress conditions, estrogen rescues female cells from death, whereas it shifts cell death to apoptosis in “male cells”. Another example for sex differences at the cellular level has been reported for neurons. Under starvation conditions, male neurons more readily undergo cell death, whereas neurons from females mobilize fatty acids, accumulate triglycerides, form lipid droplets, and survive longer. The research priority in the field of gender cytopathology and cellular pharmacology should be to understand how XX and XY cells could respond to estrogen receptor signalling. It appears now clear that male and female cells present a different ability to modulate the activity or expression rate of different molecular targets in response to estrogens and this can result in a gender disparity of the adaptive behaviour of cells from males and females.

Elke Dworatzek introduced sex differences in cardiac fibrosis in animals and humans and its association with the development of myocardial hypertrophy and progression to heart failure. Female mouse hearts show significant less cardiac fibrosis compared with males under pressure overload and in the DOCA-salt model. This is strongly correlated with less activation of pro-fibrotic genes in females compared with male hearts. These findings are similar to in clinical settings. E2 decreases collagen I and III mRNA expression in cells from female rats, whereas E2 up-regulated collagen I and III in male cardiac fibroblasts. Furthermore she reported that E2 regulates a network of cardiac fibrosis-associated miRNAs in a sex-specific manner. These data point to a modulating role of E2 and its ER on pro-fibrotic gene expression and signaling in a sex-dimorphic manner.



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Renée Ventura-Clapier described the participation of mitochondria in sex-specific pathologies. Mitochondrial structure and function are tissue specific. ERs are localized in mitochondria of a number of cell types and suggesting an action on mitochondrial DNA transcription and replication. Estrogen increases the expression of mitochondrial proteins from both nuclear and mitochondrial genomes and favour mitochondrial biogenesis, while testosterone inhibits mitochondriogenesis. Mitochondrial diseases are caused by mutations of mitochondrial DNA and thus transmitted by the mother or mutations in the nuclear DNA. Dysfunctional mitochondria can be responsible for a large panel of pathologies including neuromuscular disorders, neuropathies, encephalopathies, metabolic disorders, renal dysfunction and cardiomyopathies. Mitochondria from females have higher resistance to ischemia/reperfusion because they produce less ROS and have higher capacity of antioxidant defences. It appears that mitochondria exhibit marked sex-specificities. Studies are thus necessary for delineating the consequences of mitochondrial sex-specificity in the pathophysiology of chronic diseases and to elaborate new therapeutic interventions.

Sex differences in lipoprotein and glucose metabolism were presented by Susanna Hofmann. Menopause itself causes a loss of musculoskeletal tissue mass and quality, thereby aggravating disease-induced sarcopenia. This additive impairment of muscular function may well contribute to the elevation in cardiovascular disease risk documented in women with diabetes compared to men with diabetes. Changes in mitochondrial bioenergetics are thought to play a key role in muscle function decline, as the mitochondria are the main producers of both cellular energy and free radicals. During the aging process, mitochondria exhibit increases in oxidative stress, decay in mitochondrial DNA, a reduction in some enzyme activities, and alterations in mitochondrial respiration. Her data demonstrate that reduction in circulating HDL and apoAI levels result itself in reduced endurance capacity associated with hyperglycemia and glucose intolerance. Successful completion of her research represents an essential first step toward clinical testing of apoAI as a therapeutic target for decline in muscular strength and metabolic disease. Thus, it has the potential to advance the prevention and treatment of metabolic and cardiovascular disease.

Mechanisms contributing to sex differences

The next session discussed potential mechanisms underlying sex differences at the cellular level. Example for interactions between sex chromosomes and hormones were presented by Gregor Majdic and Tom Carpenter. Some very recent evidence suggests the involvement of the epigenome in sexual differentiation. Two mouse genetic models have provided some insights into the action of sex chromosomes. One is the four core genotype (FCG) model, with the translocation of Sry gene on an autosome, what results in two extra genotypes in addition to WT females (XX) and males (XY), namely males with two X chromosomes (XX males) and females with X and Y chromosomes (XY females). Studies with this model have convincingly shown influence of sex chromosomes on sex differences in behaviors such as addictive behaviors, alcohol abuse, aggressive, parental and female sexual behavior, nociception and some social behaviors. Furthermore, studies with these two mice models have shown sex differences in the brain that are independent of sex hormones. SF-1 KO mice are especially good model not just directly to test the influence of sex chromosomes on certain behaviors, but also to more precisely examine critical periods for organizational effects of different sex steroid hormones. Gregor Majdic pointed out that more efforts should be invested into studies of sex/gender differences in the brain, if we want to better understand differences between sexes in pathogenesis of different psychiatric and neurodegenerative disorders, and to develop better treatments, specifically adjusted to men and women. Tom Carpenter working together with Rebecca M Reynolds presented data of the hypothalamic-pituitary-adrenal (HPA) axis. Their work demonstrated that activity of the HPA axis is ‘programmed’ in utero: overexposure of the developing foetus to excess glucocorticoids is associated with low birth weight and



increased reactivity of the HPA axis with associated adverse health including cardiovascular risk factors, cardiovascular disease and poorer cognitive function. Available data are consistent with the female placenta increasing its permeability to maternal glucocorticoids following maternal stress, thus increasing the level of glucocorticoid exposure to the unborn baby, whereas the male placenta does not. The observations suggesting there is a sex difference in both the effects of maternal stress and in the placental handling of glucocorticoid hormones.

Funding perspectives – Lecture by Ineke Klinge

Ineke Klinge gave an overview about her work in the last years concerning gender equality at a public health and political level. Gendered innovations in science was one of the projects providing a homepage with information on health, medicine and engineering, developed together with the Stanford University. NIH and Horizon 2020 funding programs support the integration of S&G into basic and clinical research and claiming to analyse “gender” in animals as well. Furthermore these calls support training for biomedical researchers on research methods how to integrate S&G aspects into their research work. One possibility is to apply for work packages with extra funding to invest money for e.g. more animals needed to prove the hypothesis not only in a male but also in a female genetic background.

At the end of the lecture Ineke Klinge asked the participants to contribute to the next Scoping Paper for the Horizon 2020 Societal Challenge “health, demographic, change and well-being” to add sex and gender specific aspects. Vera Regitz-Zagrosek and Claudine Junien collected the suggestions and contributed to the paper. The scoping paper will be used as guidance for developing the content of the Horizon 2020 Work Programme for 2016-2017.

Sex differences in gene regulation

The impact of genome wide association studies (GWAS) and the role of the X chromosome on sex specific research was discussed with the expert in this field Jeanette Erdmann. In some instances, the identification of single nucleotide polymorphisms (SNPs) in genomic regions, which point to potential disease-related genes, led to the identification of new therapeutic targets. GWAS findings mainly concern autosomal chromosomes and rarely the X chromosome. Indeed, the X chromosome is commonly excluded from GWAS analyses despite being assayed for a limited number of SNPs by all current GWAS microarray platforms. We have to consider that the signal intensities obtained from standard array genotyping platforms are lower for males, who carry one allele, than for females, who carry two alleles. This needs to be adequately addressed in the genotype-calling step and has consequences for genotype imputation and association analyses. Second, we should consider the process of X chromosomal inactivation. To overcome the hurdles of X chromosomal analyses, Erdmann and König as well as others have established pipelines for analyzing X chromosomal data within a standard GWAS. By selecting specific algorithms and parameter settings, the analysis of X chromosomal SNPs is manageable and gives new clues as to the genetics of complex diseases.

Participants were informed by Claudine Junien and Christin Mieth about epigenetic processes involved in sexual dimorphisms described for various diseases in mammals. Including DNA methylation, non-coding RNAs and histone modifications, epigenetic regulation is essentially involved in X-chromosome inactivation as well as imprinting in men and rat. So far, most studies examining sex differences in epigenetics focused on single epigenetic marks in context of gene expression in a certain tissue and mouse strain. However, for future experiments, big advantage could be taken from data integration, putting together different layers of epigenetics, DNA and histone modifications, gene expression and protein abundance. Sex differences in gene expression have been shown to be highly tissue-specific. Furthermore, epigenetic marks change along with gene expression profiles during aging. Especially in women, hormone



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levels change a lot during adult life affecting the transcriptional program of various tissues and leading to strong sex differences in the frequency and progression of many diseases.

Georgios Kararigas was talking about the heart as a target organ of hormonal and other paracrine factors that in turn modulate its transcriptome in different conditions, along with its physiological and pathophysiological properties. Gonadal hormones exert sex-specific effects on gene expression in cardiac tissue and cells. The degree of sexual dimorphism in the cardiac transcriptome during the development and progression of heart failure is still incompletely understood and a topic of intense investigation. In pressure overload-induced hypertrophy, the response of the cardiac transcriptome differs significantly between men and women. In particular, the male heart increases fibrosis and inflammatory pathways, while it decreases energy-producing processes. In contrast, the female heart induces energy production, while it suppresses fibrosis-related and inflammatory processes. Further studies have demonstrated that sex contributes significantly to changes in global cardiac gene expression in models of myocardial infarction or dietary manipulation.

Translational approaches in sex specific medicine

Jean Francois Arnal mentioned the approaches for optimization and modulation of the estrogen receptor function and structure with potentially greatly improved safety profile for hormonal treatment of menopause and for oral contraception. He talked about future perspectives and explained the development of the forth estrogen generation (Estretrol, E4) with no/little ERalpha membrane effects and a safe approach concerning breast cancer and other side effects in case of estrogen treatment. Sandra Brunelleschi discussed the causal role of cigarette smoking on cell responsiveness and the deep impact on the immune system, starting with the influence of in utero exposure to tobacco smoke and increase in DNA-methylation higher in male infants. She found a significant sex difference, a higher PPAR- γ expression in CAD females compared to CAD males and that tobacco smoke profoundly affects PPAR- γ expression. Karolina Kublickiene talked about pregnancy complications and the sex dependent increased risk for CVD in adulthood of growth restricted babies. Research focus should be on endothelial dysfunction, resistance artery structure and myogenic autoregulation in these women.

Day 2 – February 17th

The next day aims for high quality in S&G research and sex differences in drug development were discussed. The discussion revealed the aim of a novel mechanistic understanding focussing on gender specific physiology and pathophysiological sexual dimorphisms. This is the rationale to study both sexes and link the findings to the diseases. Sex and gender specific phenotypes of a disease will be discovered and sex-specific drugs developed.

Anna Maria D`Ursi and Daniela Fliegner gave an overview about sex differences in drug design and how drug development works in pharmaceutical industry. It is necessary to understand the sex-specific differences in the pathophysiology and pharmacology to provide optimal treatment, which includes drug discovery, development and application. The new technical possibilities to study the “omics” including interactomics and metabolomics help to select sex specific targets. Pharmacodynamic aspects should be considered more intense in sex specific drug design. Targets are “drugable,” when they can be addressed by small molecules, proteins, antibodies, or maybe in the future, gene therapy with small interfering RNAs. There are concerns about the cost of requiring applicants for grants to use male and female animals or cells in preclinical research. On the other hand there should also be concerns on the costs of not taking sex into account: these include failed clinical trials, misdiagnosis and inappropriate therapies for women, and omission of fundamental biological principles. In the era of personalized medicine, there is a need for development of therapies specifically addressing medical needs of pre- and postmenopausal women as well as men. Hot topics are diastolic heart failure, atrial fibrillation and for risk factors such as diabetes.



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One best practice example was presented by Elisa Giannetta on male patients. Lower testosterone and higher E2 levels correlate with increased risk of CVD and CV mortality. Testosterone replacement therapy (TRT) in hypogonadism moderates metabolic components associated with CV risk. Androgen exerts a hypertrophic effect on cardiac myocytes via a direct androgen receptor (AR)-mediated pathway, and androgens could induce cardiac hypertrophy, while castration and flutamide (an AR antagonist) remarkably reduce cardiac hypertrophy and fibrosis. In addition, androgens modulate male cardiac performance by regulating the functional expression of L-type calcium channels in cardiac myocytes. Androgens regulate phosphodiesterase-5 (PDE5) expression and functional activity also in cardiac tissue and PDE5 is overexpressed in cardiac hypertrophy and in ischemic cardiomyopathy. cGMP-specific phosphodiesterase 5 (PDE5) inhibitors have provided beneficial cardioprotection against a broad range of heart diseases in experimental and clinical studies. A recent analysis suggests that the PDE5 inhibitor sildenafil ameliorates female cardiac failure caused by $G\alpha_q$ overexpression or pressure overload through an estrogen-dependent mechanism.

The editorial perspective

A particular pleasure was to welcome Karin Sipido, the Editor-in-chief for Cardiovascular Research - the International Basic Science Journal of the European Society of Cardiology (ESC). She gave a great talk with the title: “A changing landscapes in cardiovascular research”. The translational gap from basic research to clinical use is always a matter of concern.

Conclusion and outlook

After this final input, tasks and work assignments were distributed and the concept of the position paper was discussed. Participants are willing to share their expertise and are welcome to give their input to a draft of the position paper prepared by Vera Regitz-Zagrosek and Ute Seeland.

The rationale of the paper is the question: Why do we need sex and gender (S&G) in basic cardiovascular research and how shall we implement it? Participants agreed to the following objective after intense and fruitful discussion. CV drug development is getting more and more difficult and costly, so we need new approaches and should replace the “one size fits all” philosophy by targeted, gender-based, approaches that will lead to an improved and S&G sensitive understanding.

The paper should provide the results of this workshop following the aims given at the top. It will first provide the evidence for relevant sex and gender differences and that these sex differences are important for clinical outcomes.

This part will be followed by a description of possible underlying mechanisms like the interaction between sex chromosomes and sex hormones with respect to sex differences in gene regulation depending on genetic variants and epigenetic processes. Examples for translational approaches should be mentioned like the development of anti-arrhythmic drugs based on proteomic results concerning sex differences of ion channel expression leading to arrhythmias. Furthermore participants made reference to the progress in developing modified estrogen receptor drugs (SERMs) and the sex specific effects of PDE 5 inhibitors.

The discussants agreed that a methodology part should follow to include typical approaches to the biology of sex differences and discuss advantages and potential pitfalls. It should discuss the use of primary cells and cell lines of both sexes for in vitro experiments, use of animals of both sexes in disease models, transgene- or knock-out animal models genetically unique to sex, use the four core genotype (FCG) mice to provide insights into the action of sex chromosomes and perform micro array sequencing, RNA sequencing and GWAS with respect to both sexes. Limitations of these ideal suggestions as well as taking animal models to study gender aspects will complete this part of the paper.



What do we need in future was the last question of this day and has been answered by the project partners Vera Regitz-Zagrosek and Ineke Klinge. The vision is to act together with the International Society of Gendermedicine (IGM), the Canadian Heart Research Centre (CHRC) and the American Organization for the Study of Sex Differences (OSSD) with the aim to integrate representatives into policy and clarify our opinion.

Vera Regitz-Zagrosek and Ute Seeland thanked all participants for their relevant and insightful comments, which have made this short and intense workshop a great success with relevant outputs to be expected within the next months that will significantly contribute to the roadmap for the implementation of sex and gender in biomedical research and practice.

Ute Seeland
Co-coordinator of the WS1.3

Figure: Discussion round during workshop 1.3



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