Session report to Session 1.3: Basic biomedical research, preclinical drug development

Chairs
Vera Regitz-Zagrosek (Germany) and Claudine Junien (France)

Active participants
Jean Francois Arnal (France), Sandra Brunelleschi (Italy), Ilaria Campesi (Italy), Susanna Hofmann (Germany), Andrea Isidori (Italy), Karolina Kubickiene (Sweden), Gregor Majdic (Slovenia), Walter Malorni (Italy), Stefano Occhioni (Italy), Puck ten Kate (The Netherlands), Hanna Tinel (Germany)

Rapporteur: Ute Seeland (Germany)

Contribution of partners - fields where reliable knowledge on sex differences in basic biomedical research already exists.
Vera Regitz-Zagrosek opened the session with an overview on topics suggested by the participants. **Susanna Hofmann** focused on “Lipids and Diabetes”. Females are more protected from cardiovascular diseases before menopause but this is not the case when they have diabetes. Research work on lipoprotein metabolism has shown that HDL has different functions and it is not only a question of the level. HDL has a direct effect on skeletal muscle glucose metabolism. HDL can have different molecular structures. Modulating HDL may be developed into a novel therapeutic approach improving diabetes in women. Function of HDL seems to be different in males and females and may have an effect on vascular contractility. This is an open field with promising new data.
Vera Regitz-Zagrosek presented data provided by **Susan Howlett** from Canada concerning the question if we really do have male/female differences at the single cell level? She measured **calcium sparks in isolated myocytes**. She found more calcium sparks in the male hearts than in the female hearts. This is a fundamental difference between the sexes because calcium is needed for the contractility as well as for metabolism. Ovarectomized female mice show more calcium sparks. In respect to calcium metabolism they are closer to the males than to the females. Thus also estrogens affect the behaviour of cardiomyocytes.
The effects of estrogen stimulation on the heart and the role of estradiol in male heart was the next important topic in this session. Vera Regitz-Zagrosek gave an example of gene expression profiles, showing up- and downregulation of genes in human heart samples treated ex-vivo with estradiol. These experiments done by **George Kararigas** from Germany show different effects of estradiol on the heart in both sexes due to gene regulation. Estradiol stimulation decreased contractility in male hearts without significant affecting it in females. Older males with high estrogen levels, reaching similar levels as females, have more cardiovascular events. Many effects are mediated by the conversion from testosterone to estradiol in adipose tissue with impact on different tissues like vessels, bones and visceral fat cells in men. **Jean Francois Arnal from France** discussed the importance of sex hormones in different organ systems. Sex hormones and sex hormone receptors are active in both sexes. Can we manipulate estrogens in that way that we can use it for therapeutic approaches in males and females? One example for antagonism of the estradiol-receptor is breast cancer. Furthermore, he mentioned that substitution of estrogens in postmenopausal women is not optimal and more studies on the effects of estrogen receptors at the molecular level are needed. The role of testosterone in males is another issue. Vera Regitz-Zagrosek added that **abdominal aortic aneurysm** is an example for clinical application in this field. Rupture affects more men than women. The question is if testosterone is involved? She presented data from Lisa Cassis showing that male rats treated with testosterone or the castration of male mice is able to reverse the negative fact of dilatation and prevent rupture of the aneurysm.
Another point deals with the recognition that **whole-genome analyses are male biased. Jeanette Erdmann** from Germany is one expert in this field. The genetic mutations in the genomes of several
A thousand people have been analysed. Important data on genetic risks have been produced at high costs. Several reports on single gene mutations increasing the risk of disease are published without any comment about the sex of the donor of the cells these polymorphisms are analysed in. In order to obtain homogeneous groups frequently males have been selected and the X chromosome has not been analysed with the consequence that these results are male biased. Linda Liu (Stanford University, Human Genetics 2012) looked at some famous polymorphism with respect to the sex. For example there is a known polymorphism being associated with the risk of coronary artery disease. She found that this is true for men but not for women. In case of Crohn’s disease genetic association for a well known SNP exists in women, but not in men. Similar findings have been reported for rheumatoid arthritis and type I diabetes. Thus, even in basic science we have to ask very critically for which sex they are valid. Ilaria Campesi from Italy stressed the fact that smoking has more negative effects in women than in men. Why is cigarette smoking more harmful in women than in men? Sex differences at the molecular level are known e.g. in DNA methylation and inflammatory response of cells. Sandra Brunelleschi from Italy added their experimental findings that tobacco smoke affects PPAR-y receptors in a sex specific way. The anti-inflammatory potential is different e.g. in never smokers and ex-smokers with coronary artery disease with respect to release of cytokines and reactive oxygen species. Another aspect is that the molecular mechanisms at cellular level show that female and male cells have different reactivity to stress.

Walter Malorni from Italy focussed on sex differences in cells. In particular, on the role of cell sex in determining cell survival or death, including apoptosis or autophagy. This is a key mechanism of great relevance in the pathogenesis of a plethora of human diseases such as cancer, cardiovascular and degenerative diseases. In vitro cell models should be used before testing a drug in animals. For this purpose cell lines must be characterized in respect to the sex. This is a problem in cancer research as well. Many groups are still working with cell lines without knowing the sex of their cells. Journals should require a statement on the sex of the cells used for experiments.

Gregor Majdic from Slovenia added to include effects of sex chromosomes as a topic for the workshop. Sex chromosomes could have important impact not only as an additional variable together with sex hormones but also independently during prepubertal and postmenopausal life periods.

Claudine Junien from France pointed out that the 4 pillars of “developmental origins of health and disease” (DOHaD): 1) developmental plasticity 2) long term effects 3) transmission to subsequent generations 4) all showing sexual dimorphism are under the control of epigenetics processes hence stressing the importance of this research field in regard to sex differences. Obesity, cardiovascular disease, diabetes are the products of gene-environmental interactions and have their roots in infancy or childhood. Researchers should be encouraged to focus on detecting the earliest aberrations in molecular and biochemical pathways that lead to disease later in life. Risk factors of the father can be transmitted to the next generation as well. Karolina Kublickiene from Sweden added to this topic the fact that genetic variability is important to know. This knowledge could contribute to more successful individualized drug development. There are a lot of unanswered questions: How is susceptibility to e.g. obesity programmed? What are the relevant environments? When are these periods of susceptibility? What are the specific effects of the developmental exposure on e.g obesity in the female and male offspring?

Vera Regitz-Zagrosek closed the session by pointing to a video by David Page (https://www.youtube.com/watch?v=nQcgD5DpVIQ). David Page is a geneticist and the director of the Whitehead Institute for Biomedical Research (Cambridge, MA, USA).
Best practice examples
- In FP 6, considering sex was required in all research projects. This led to the fact that in a number of studies male and female rodents were included in equal numbers. In one study, an European Consortium (Eugenheart) tested a drug in 400 mice including male and female. Only male mice showed improvement in survival.
- In Germany, DFG is funding a research group on sex differences in myocardial hypertrophy coordinated by Charité (FG 1054). 7 different groups work together to analyse sex differences in different animal and cell culture models.

Additional approaches
The fact is that differences in sexual dimorphism from one cell type to another exist, influenced during ontogeny, in response to different environments, physiopathological stages and with aging. Looking only at a specific cell type may lead to overlook and miss important cell differences.

The general problem is that the analysis of sex differences increases costs for animal studies. Therefore, experiments should first be performed in cell lines and sex specific effects should be determined. Thereafter, one of the two sexes or both should be selected for further study. Journals should require information on the sex of cells and animals under investigation.

Implications for WS planning
Title: “Sex and gender aspects in basic biomedical research”, Venue: Berlin, Date: February 2015?
- Topics: Suggestions from the participants for workshop topics: Lipid levels and diabetes, myocardial contractility and hypertrophy, genome analysis, cell death, developmental origin of health & disease and methodology were mentioned. Use of stem cells for novel therapies.
- Special audiences to be invited: Representatives of pharmaceutical Industry, research organizations (e.g. DZHK, ICN), EU governing bodies (in research), Journal editors (List: Cardiovascular Research, Basic Research in Cardiology, European Heart Journal, OSSD Sex and Gender Women’s Health Collaborative, Journal of Molecular Medicine...)
- Funding agencies: DFG, BMBF, ANR,
- Specific approaches for communication
- Workshop: TBD
- Chairs: Vera Regitz Zagrosek, Claudine Junien