

**Project Summary NOT CONFIDENTIAL**

<b>Title of the project</b> Genetic markers and susceptibility to the effects of endocrine disruptors during mammalian testis development		
<b>Acronym of the project</b> GENDISRUPT		
<b>Type of contract</b> RTD		<b>Total project cost</b> €1.837.804
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**Objectives:**

The mechanisms of action of potential environmental and pharmacological toxic substances acting on humans and organisms present in the environment are not well established. However, it is well known that cells and biological systems are affected by xeno-compounds by alterations of genetic systems at different levels.

Endocrine Disruptors (EDs) are a wide group of compounds, which acting as endocrine deregulators, could generate gene deregulation depending of the genetic background of the organisms.

The reproductive systems have been considered a crucial target of the impact of EDs. Consequently, the analysis of the genetic effects of these compounds on developing gonads has repercussions on the effects upon the individuals and the progeny of future generations by genetic transmission.

The present project intends to analyse, from genetic point of view, the effect on testicular cells of a group of selected endocrine disruptors and the genetic susceptibility to its action.

**Results and Milestones:**

Using microarrays analysis of gene expression, more than 3 million individual gene data have been analysed. This represents an enormous effort of analysis never done before to evaluate comparative gene expression deregulation induce by Endocrine Disruptors (EDs) in developing testis.

-Mono ester phthalate (MEHP) is the EDs that shown the highest level of deregulation.

-MEHP and zearalenone (ZEA) exposure defines specific gene expression signature.

-The highest deregulation affects for all EDs analysed to mice that have been exposed to the different EDs during all life until analysis, (4 weeks postnatal).

-However, MEHP also induce remarkable deregulation when the compound was supplied to mothers during the embryonic time of the in spite of that the animals were not exposed during puberal time.

-In general, Estradiol shown lower effect over gene deregulation in testis than the other EDs used.

-An evident "low dosage effect" is detected in longest period of exposure to MEHP and Lindane

A proteomic profile of the soluble proteins expressed at different stages of mouse testis development has been carried out. 42 proteins were identified. Conspicuous variations in their accumulation (representing up or down-regulation) were detected. Proteins with antioxidant activity were identified in high proportions. In testis exposed to Lindane changes in protein accumulation can be established.

We have demonstrated that, during testis development, a specific pattern of expression of three proteins: flotillin-1, vinculin and vinexin initially associated to cell junctions.

A general conclusion, following these results, is that the effects of different EDs during testis development do not follow the same pathways at the level of gene deregulation. Even more, most of the EDs analysed in this study have a distinctive pattern of gene deregulation from the Estradiol.

The studies on possible effects of ED treatments on mouse testis development also revealed some morphologically visible effects on the seminiferous epithelium, especially higher numbers of apoptotic cells and of diploid spermatids were encountered.

Several genes were found that seemed to change expression upon treatment with ED. Seven of these genes were studied as to the testicular cell types that expressed them and their function in the testis. PP2A,B,beta seems promising as a marker gene that could be used to detect previous exposure to EDs.

The results represent the first indication that exposure to estrogens during embryonic life may have profound effect on germ cell growth and differentiation through their action on gonadal somatic cells and identify involved molecular pathways. Embryo exposure to high levels of estrogens or EDs constitutes a risk for Primordial Germ Cell (PGC) transformation in pluripotent tumorigenic cells. This process can be at the origin of germ cell tumour formation in the testis.

An in vitro assay has been set up to identify and quantify estrogenic activity of E2 and EDs on fetal testis cells after the necessary controls and validation might represent the basis for novel tests for rapid estrogenic and anti-estrogenic activity screenings and to investigate the molecular pathways underlying the effect of these compounds on fetal gonads.

Concerning human studies, nineteen candidate genes were selected to carry out genetic association studies in human male infertility and testicular cancer. 46 Single Nucleotide Polymorphisms (SNPs) were selected to perform these studies. However, only 37 of these SNPs were validated in our population. We designed high throughput genotyping protocols for these 37 polymorphisms.

To perform the genetic association studies we recruited a total of 167 DNA samples from idiopathic infertile men, 1000 DNA samples of people from the general Spanish population, 101 DNA testicular tissue samples from testicular cancer patients, and 7 testicular tissue samples from healthy donors.

We used 28 SNPs within eleven candidate genes to perform these analyses. A total of 13,543 genotypes were obtained.

Our findings support a genetic basis of human male infertility. We have observed genetic association of *ESR1*, *KIT*, *KITLG* and *PTEN* genes with male infertility. These observations are in accordance with the possible existence of male infertility susceptibility factors within these genes in humans.

An important finding is the implication of the *ESR1* gene in male infertility. In fact, this result has been also observed in three independent series from Greece, Japan and Italy.

Multilocus analyses of the *FSHR*, *ESR1*, *ESR2*, *CYP19A1* and *NR1P1* revealed the existence of genetic interaction between these genes and their possible implication in the human male infertility.

We have preliminary evidence for Loss of Heterozygosity in the *PTEN* gene in human testicular cancer. Additional studies of this gene in a larger group of testicular cancer patients will reveal if this observation could be considered as a common event in testicular cancer pathology.

**Benefits and Beneficiaries:**

Different sectors: Environment, Health, Social, Industry and Academic are potential beneficiaries of the results. The results can contribute to evaluate the mechanisms of action of EDs, the impact in human reproductive health and in other mammals. The Social and Economic sectors should regulate the use and production of Endocrine Disruptor compounds.

**Future Actions (if applicable):**

Patents as consequence of validated results are considered. Potential participation in further Programmes of The EC concerning Endocrine Disruption is also considered