Institute of Human Genetics
Polish Academy of Sciences
in Poznan

mgr Agnieszka Malcher

# „Identification of genes critical for spermatogenetic process; an attempt to determine molecular markers of azoospermia”

Doctoral thesis
written under supervision of
prof. dr hab. Maciej Kurpisz
and dr Natalia Rozwadowska
at Department of Reproductive Biology
and Stem Cells at Institute of Human Genetics PAS
in Poznan

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## SUMMARY

Infertility involves about 15% of couples which attempt to conceive and the ‘male factor’ accounts for about half of them. The male infertility may be a reason to a number of irregularities occurring in the spermatogenetic process. The regulation of spermatogenesis depends on the cooperation of hormonal, genetic and environmental factors, therefore dysfunctions in one of these factors can lead to azoospermia (the absence of spermatozoa in ejaculate). The causes of non-obstructive azoospermia can be complex and the current andrological diagnosis is not sufficient. Especially the genetic causes are still not well recognized therefore the main target of the present studies was to identify novel diagnostic and prognostic biomarkers for men with non-obstructive azoospermia through the identification of genes potentially essential for male fertility, genes that determine the degree of spermatogenetic impairment, as well as genes that define the chances of successful treatment of men with azoospermia by therapy with hCG/rFSH.

The study included: (1) comparison of hierarchical clustering (gene expression profile) with histopathological analysis of azoospermic men, (2) comparison of gene expression profiles of normal versus abnormal human testes, (3) comparison of gene expression analysis of two main created subgroups of men with spermatogenetic impairment at early and late differentiation stage, (4) comparison of gene expression profile of men with NOA who responded positively to hormonal therapy to those with no improvement. We analyzed the gene expression profile using Affymetrix Gene chip human 1.0 arrays. The validation was performed at the mRNA level using Real-Time PCR and on an independent set of data derived from the ArrayExpress database, as well as at the protein level by using Western blot and immunohistochemistry. Additionally, there was performed sequencing of selected fragments to determine the alleles.

The comparative analysis of gene expression profiles allowed the identification of 4946 differentially expressed genes ($p<0.05$) between control and infertile group. There were selected 7 genes: *AKAP4*, *UBQLN3*, *SPACA4*, *SPATA3*, *GGN*, *CAPN11*, *FAM71F1* which presented at least 4-fold lower expression level ($p\leq0.005$) in men with azoospermia in comparison to control group. These genes were successfully validated on an independent set of data from ArrayExpress database and/or by Real-time PCR. Moreover, the analysis of the protein products of UBQLN3 and FAM71F1 genes generally confirmed the results obtained at the mRNA level. Furthermore, the global gene expression profile analysis classified the men with NOA on two subgroups depending on degree of spermatogenetic impairment (at

late and early differentiation stages). These analysis allowed the identification of another 7 genes with minimum 5-fold higher expression level ($p < 0.0001$) in men with spermatogenetic failure at late stage compared to those at early stage, i.e. *WBSCR28*, *SPATS1*, *TMEM225*, *FSCN3*, *GTSF1L*, *ADCY10*, *GSG1*. All of these genes have been successfully validated by Real-Time PCR and on an independent set of data from the ArrayExpress database. Western blot analysis, immunohistochemistry and data from *The Human Protein Atlas* database showed that the protein products of these selected genes were mainly observed at the late stage of spermatogenesis, what confirmed the results obtained at the mRNA level.

Whereas, the analysis concerning the men who underwent hormonal therapy allowed the selection of *HLA-DQB1* gene which expression level was significantly increased ($p < 0.05$) in non-responders group. In addition, sequence analysis of this gene showed that men who did not respond to the therapy presented homozygotic alleles - **060301*, **020201*, and **060201*, while those who responded positively to gonadotropin treatment were heterozygotes of DQB1**040201/050201*, **040201/030101*, and **030201/020101*.

There was also compared the testicular gene expression profile in one individual before and after gonadotropin treatment. There could be noticed that genes such as *AKAP1*, *PRM2*, *CLDN1*, *TNP1*, *ODF1*, *PRM1* which play important roles in spermiogenesis were up-regulated (over 2-fold) in the re-biopsied sample.

In summary, the identified biomarkers may be used to create a molecular diagnostic and prognostic platform for the efficient determination of the infertile causes, as well as defining the stage of spermatogenetic impairment and/or the chances for successful treatment of azoospermia.