Reconstruction and Analysis of Regulatory Gene Networks Involving Human Genes Associated with Main Forms of Pathozoospermia

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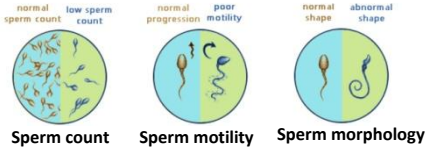
Motivation and Aim: **A study of the molecular-genetic mechanisms, which predispose to a decline in the male reproductive potential (spermatogenic failure) is an actual problem of reproductive biology. In clinical andrology and reproductive medicine for evaluating male fertility, the analysis of ejaculate is used. A pathological condition called pathozoopermia is diagnosed if sperm parameters are impaired. To reveal regulatory interactions between genes associated with pathozoospermia, we reconstructed gene regulatory network involving genes harboring allelic variants associated with pathozoospermia.**

Methods and Algorithms (part 1):

Keywords denoting specific forms of pathozoospermia:

1) non-obstructive azoospermia;
2) cryptozoospermia;
3) oligozoospermia;
4) severe oligozoospermia;
5) asthenozoospermia;
6) teratozoospermia;
7) oligoasthenoteratozoospermia ;
8) oligoasthenozoospermia;
9) oligoteratozoospermia;
10) globozoospermia



ANDSystem - computer tool for the automated extraction of knowledge from the texts

Manual verification

88 human genes associated with specific forms of pathozoospermia

Methods and Algorithms (part 2):

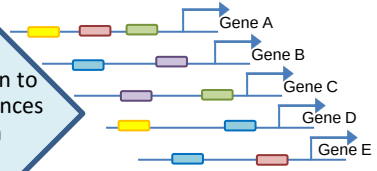
88 human genes associated pathozoospermia

Genes encoding transcription factors:
WT1, AHR, NR0B1, ETV5, AR, NFE2L2, SOX5, AHRR, YBX2, SOX6, DMRT1, NPAS2, SOHLH1

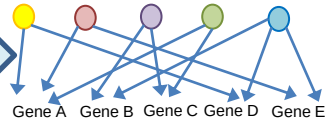
BioMartTool
- web-based tool for extraction of DNA sequences from genomic databases

Promoter regions (500 bp and 1000 bp)

MoLoTool
- web application to scan DNA sequences for transcription factor binding sites



Cytoscape
- software platform for visualizing networks

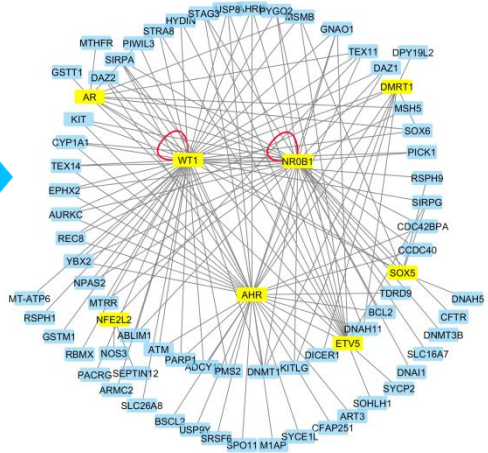


Transcription factor binding sites for WT1, AHR, NR0B1, ETV5, AR, DMRT1, SOX5, and NFE2L2 identified at significance threshold $p < 10^{-4}$

Transcription regulatory network

Results: Networks constructed based on the analysis of 500 bp and 1000 bp promoter regions

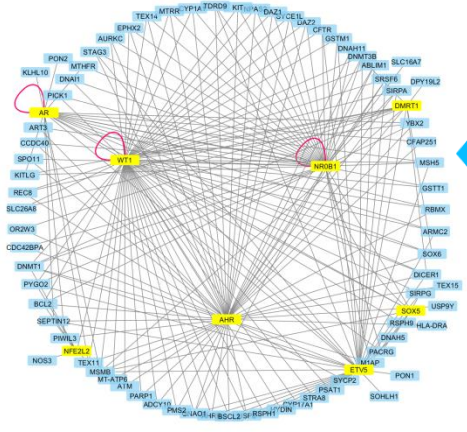
When 500 bp promoter regions were analyzed, the network contained 79 nodes, and 147 edges.



We revealed three regulatory self-loops involving transcription factors **WT1**, **NR0B1**/DAX1, and **AR**.

Target genes

Genes encoding transcription factors

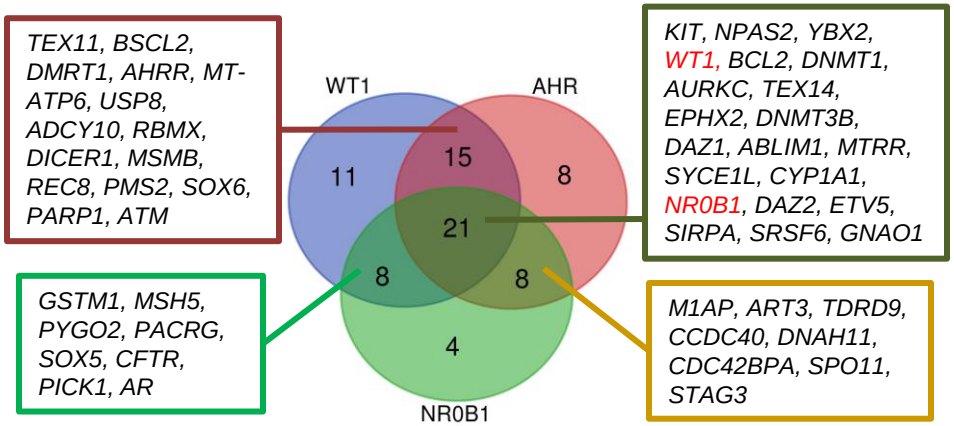


When 1000 bp promoter regions were analyzed, the network contained 84 nodes, and 223 edges.

Results: Ranking transcription factors according to the number of target genes in the networks

Transcription factor	Network based on analysis of			
	500 bp promoter regions		1000 bp promoter regions	
	Number of binding sites	Number of target genes	Number of binding sites	Number of target genes
WT1	67	41	103	55
AHR	62	39	100	52
NR0B1	51	32	77	41
ETV5	18	13	45	33
AR	10	8	24	18
DMRT1	7	7	11	10
SOX5	4	4	7	7
NFE2L2	3	3	7	7

The greatest number of target genes had **WT1** (41 for 500 bp analyzed, and 55 for 1000 bp analyzed) . **AHR** had 39 and 52 target genes, and **NR0B1**/DAX1 had 32 and 41 targets.



VENN diagram representing the number of target genes for transcription factors **WT1**, **AHR**, and **NR0B1**/DAX1. A callout rectangles coming from intersections of the circles show the genes which belong to all three or two categories.

Conclusion: Using MoLoTool we revealed potential target genes for eight transcription factors and reconstructed transcriptional regulatory networks involving 79 and 84 human genes associated with pathozoopermia. We identified three key regulatory transcription factors that had the greatest number of target genes: **WT1**, **AHR**, and **NR0B1**/DAX1. Our findings are in good agreement with results obtained in mice, indicating that **WT1** is critical for spermatogenesis via regulation of Sertoli cell polarity via Wnt signaling pathway [Wang X.N. et al 2013]. We propose to keep in mind genes encoding these transcription factors as most promising candidates for investigating the genetic factors predisposing to pathozoospermia.

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