

# The computational analysis of human testis transcriptome reveals closer ties to pluripotency

MT Anand,

Burra VLS Prasad

IGNOU-I<sup>2</sup>IT Center of Excellence for Advanced Education and Research, Pune, Maharashtra, India

**Address for correspondence:**

Dr. Prasad VLS Burra, School of Biotechnology, IGNOU-I<sup>2</sup>IT Center of Excellence for Advanced Education and Research, P14/1, Rajeev Gandhi Infotech Park, Hinjewadi (Phase-I), Pune - 411057, Maharashtra, India.

E-mail: [prasadb@isquareit.ac.in](mailto:prasadb@isquareit.ac.in)

Received: 23.05.12

Review completed: 25.07.12

Accepted: 28.07.12

## ABSTRACT

**AIMS:** The purpose of this study was to identify the differentially expressed genes (DEG) in human testis and also evaluate the relationship between human testis, human Embryonic Stem Cells (hESC), mouse testis and mouse ESCs (mESC). **SETTINGS AND DESIGN:** It is a prospective analysis designed computationally. Methods and Material: The microarray data for human testis, hESCs, mouse testis and mESCs were obtained from NCBI-GEO and analyzed for identification of DEGs. The results were then compared with mouse testis and extended to ESCs. **STATISTICAL ANALYSIS USED:** Data was analyzed in R using various Bioconductor packages. To identify DEGs, 2-fold cut-off and a False Discovery Rate (FDR) below 0.01 criterions was used. **RESULTS:** A total 2868 transcripts (DEGs) were found to be significantly up-regulated and 2011 transcripts significantly down-regulated in human testis compared to other normal tissues. Of the up-regulated transcripts, 232 transcripts were grouped as unclassified i.e. had unknown annotations at the time of analysis. Gene Ontology (GO) based functional annotation of testis specific DEGs indicate that most of the DEGs (~80%) are involved in various metabolic processes. Pathway analysis shows over-representation of Ubiquitin-mediated proteolysis pathway. A core group of 67 transcripts were found to be common among human testis, mouse testis, hESCs and mESCs. **CONCLUSIONS:** Testis seems to be metabolically very active relative to other normal tissues as indicated by functional annotation. The comparison of human and mouse testis shows conserved functions and pathways involved in both species. Large numbers of genes were found conserved between testis and ESCs suggesting very close expression level relationship between reproductive organs and complex phenomenon such as dedifferentiation and reprogramming.

**KEY WORDS:** ESC, microarray, pluripotency, spermatogenesis, testis

## INTRODUCTION

The Indian Council of Medical Research (ICMR) recently conducted a nationwide study on infertility and places the percentage at 15-18. Of these, in up to 50 percent of cases it is the male factor or the husband who is responsible for the infertility.<sup>[1]</sup> The mammalian testicle is the male gonad and like ovary, testis is one of the very important tissues of both reproductive system and endocrine system. Testis is responsible for number of extraordinary biological processes. Spermatogenesis is one such complex process that occurs in the testis and is dependent on successful testis development and includes mitosis of diploid germ cells, meiosis and morphological differentiation of post-meiotic germ cells.<sup>[2]</sup> Development

of viable spermatids during spermatogenesis and production of androgens such as testosterone are few of the pivotal roles played by testis in fertility and reproduction.

In the recent times high throughput technologies have taken the center stage in biological research. Oligonucleotide based microarray chips such as Affymetrix GeneChips and others enable the simultaneous measurement of the expression levels of tens of thousands of genes from a particular mRNA sample.<sup>[3]</sup> Such high-throughput expression profiling can be used to compare the level of gene expression in clinical conditions in order to identify diagnostic or prognostic biomarkers, classify diseases, monitor response to therapies and understand the mechanisms involved in

### Access this article online

Quick Response Code:



Website:

[www.jhrsonline.org](http://www.jhrsonline.org)

DOI:

10.4103/0974-1208.106339

the genesis of disease processes.<sup>[4]</sup> For these reasons DNA microarrays are considered important tools for discovery in clinical and molecular medicine.

In 2004, Su *et al.*, have analyzed the transcriptomes of many tissues including testis in both human and mouse.<sup>[5]</sup> Although this provided valuable information about the expression of genes in various tissues, these studies did not present comprehensive picture of human testis transcriptome because of limited coverage of probesets on the chips (Affymetrix U95A platform was used in the study which had 12675 probesets).<sup>[6]</sup> Attempts have been made to address this problem using candidate gene approaches employing RT-PCR and differential display. In addition serial analysis of gene expression (SAGE), cDNA libraries were generated from mouse testis and SAGE tags and expressed sequence tags were sequenced in the testis.<sup>[7]</sup> However, these molecular approaches resulted in a small number of genes analyzed in each sample. The current status suggests, analysis the transcriptome generated from recent technologies (Affymetrix HGU133plus2.0 arrays containing 54675 probesets) and improvised algorithms might provide more reliable and valuable additional information about the biological phenomenon.

There have been reports of isolating embryonic stem cell 'like' cells from adult human testis, indicating the potential of testicular cells to develop into multipotent cells when given proper niche.<sup>[8]</sup> It is also known that, testis similar to stem cells gives rise to terminally differentiated spermatocytes. Identifying the genes in testis that are essential for spermatogenesis, endocrine system, gametogenesis, inducing pluripotency and other similar functions may provide the molecular basis of tissue specific regulation, cellular reprogramming in reproductive and developmental biology. This opens a new therapeutic

opportunities relevant to reproductive biology addressing issues such as infertility, impotency among others in males.

In this paper we performed "class comparison" experiments i.e. identification of genes differentially expressed in a normal testis with reference to other samples. The present study is an attempt to understand the relationship between normal testis, other tissues, ESC of Human and Mouse origin.

## MATERIALS AND METHODS

### Microarray data

The expression datasets necessary for comparative analysis were downloaded from NCBI-GEO.<sup>[9]</sup> Further details are furnished in Table 1. The criteria for selecting the datasets: a) each sample should have at least three technical replicates and b) the publication date should not be earlier to 2005.

The reference dataset for human testis was obtained from GEO dataset GSE12034. We could not find similar kind of reference dataset for mouse. We computationally derived a reference dataset by calculating the median expression value of 10 different tissues (adrenal gland, brain, diaphragm, heart, kidney, liver, lung, muscle, pituitary gland, spleen) not including testis (GSE9954 dataset). We selected probesets with an expression value in mouse testis samples that are 2 fold higher and FDR below 0.01.

### Microarray data analysis

The Affymetrix CEL files containing probe intensity data were analyzed in R using Bioconductor packages.<sup>[14]</sup> Quality control analyses were carried out on probe level model (PLM) normalized samples. Normalized Un-scaled standard error (NUSE) boxplots, Relative Log Expression (RLE) plots, correlation between chips were analyzed.<sup>[15-17]</sup>

**Table 1: Datasets used in this article**

Sample details	GEO accession number (status of publication)	Total replicates (replicates considered)	Platform	Comments	Series published on NCBI-GEO
Human testis	GSE7307[unpublished]	6(3)	Affymetrix Human Genome U133 Plus 2.0 Array	Human body index transcriptional profiling	Apr 09, 2007
Human reference	GSE12034 <sup>[10]</sup>	3(3)	Affymetrix Human Genome U133 Plus 2.0 Array	Pool of ten normal human tissues for reference	Jul 09, 2008
Undifferentiated human embryonic stem cells (hESC)	GSE21668 <sup>[11]</sup>	3(3)	Affymetrix Human Genome U133 Plus 2.0 Array	Undifferentiated embryonic stem cells, H9	May 06, 2010
Mouse testis and other ten tissues	GSE9954 <sup>[12]</sup>	36(33)	Affymetrix Mouse Genome 430 2.0 Array	Large-scale analysis of the mouse transcriptome	Dec 20, 2007
Mouse Embryonic Stem Cells (mESC)	GSE13805 <sup>[13]</sup>	3(2)	Affymetrix Mouse Genome 430 2.0 Array	mouse embryonic stem cell	Dec 02, 2009

RNA degradation procedures and other recommended procedures for quality control were employed as explained by Alvord *et al.*<sup>[18]</sup> The normalized signal intensities from testis and reference replicates were visualized by pair wise scatter plots in which individual chips within same group were plotted against each other (within group). The majority of data points representing the signal intensities for both testis and reference chips were highly correlated for all replicates (spearman rank correlation > 0.9).

Probe intensity data were then normalized using Robust Multi-array Averaging (RMA), which generates background adjustments, quantile normalization and summarization of raw scan data in order to produce a measure of mRNA expression levels.<sup>[19]</sup> It has been demonstrated that RMA has superior precision, better

estimation of fold change and provides high specificity and sensitivity when analyzing fold changes to detect DEGs.<sup>[20]</sup> To identify DEGs the Linear Models for Microarray Data (LIMMA) package was used.<sup>[21]</sup> False Discovery Rates (FDR) of pair wise comparisons were calculated using p-values generated from LIMMA.<sup>[22]</sup> DEGs were selected using a 2-fold cut-off and an FDR of <0.01 criterion. GeneAnswers, a Bioconductor package, was used for functional annotation of DEGs.<sup>[23]</sup> The same package was used for pathway analysis and disease ontology.<sup>[24]</sup> Figure 1 shows the flow chart of data analysis.

## RESULTS AND DISCUSSIONS

### Quality assessment of the chips

In a recent study, it is shown that approximately 10

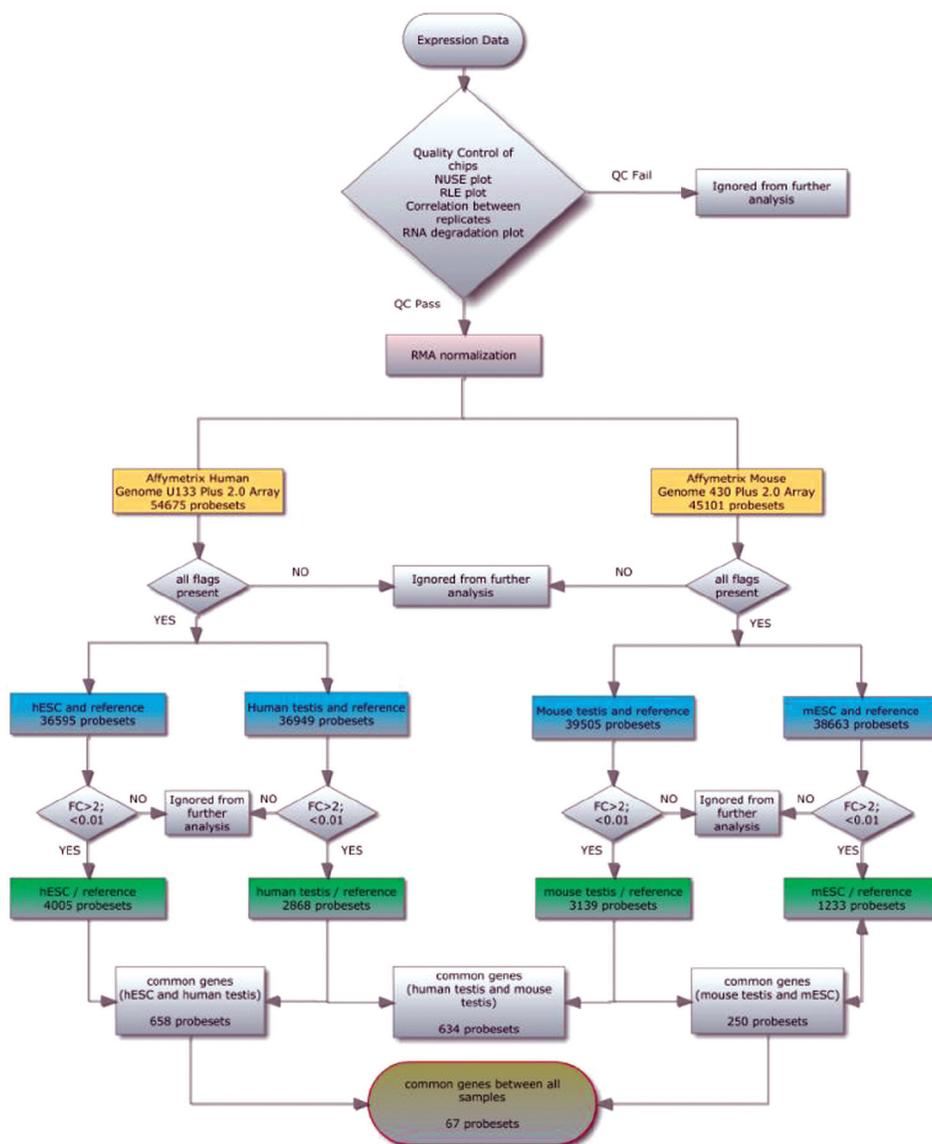


Figure 1: Flowchart of analysis process

percent of the Affymetrix GeneChip data, available in the public domain, is of poor quality, which emphasizes the importance of the quality of the data obtained from chips.<sup>[25]</sup> As yet there are no standard procedures which strictly can be followed in order to contain the artefacts, errors and noise in microarray data. However, after thorough literature survey, we employed the procedures recommended by Alvord *et.al* for quality assessment. NUSE, RLE, RNA degradation and other plots were plotted and analyzed for the quality (provided as additional material). The chips which failed to satisfy the criterion were ignored from further analysis. Currently, quality assessment procedures of microarray data are an active area of research. Hence, some of the quality assessment procedures are questioned for their efficiency. For example, there are no clear guidelines on how large a degradation slope must be in RNA degradation plots to categorize a chip as good or bad chip. Our analysis shows plots that appear to be reasonably parallel indicating good quality chips. In addition to the chip level quality assessment, we also implemented probe level quality check by calling, MAS5 calls procedure to remove all probe sets which were flagged absent. Of 54675 probe sets found on HGU133plus 2.0 array, an average of 68% of probes had presence calls, in both testis and reference samples. The number of DEGs is shown in Table 2.

### DEGs in human testis

The combination of 2 fold cut off and FDR < 0.01 criterion resulted in 2868 transcripts to be differentially up-regulated and 2011 transcripts to be differentially down-regulated between testis and reference. The fold change of DEGs ranged between 2.0 fold (minimum fold change) and 9.0 fold. Protamine-2, a testis specific gene involved in condensing the sperm DNA into highly condensed, stable and inactive complex is expressed 9 fold higher in testis. Other testis specific genes such as Transition Protein-1 (TNP1), Capping protein muscle 2 line-alpha 3(CAP2A3) are also found to have significant fold change (8.63 and 6.97 respectively).

One of the primary queries in the current analysis was to understand the status of cellular reprogramming genes in testicular cells. We examined the expression profiles of a set of known reprogramming genes.<sup>[26]</sup> Interestingly, we observed that ALDH1A1, KLF5, KLF11, SOX30, and SOX5

**Table 2: Number of DEGs in each sample compared with reference**

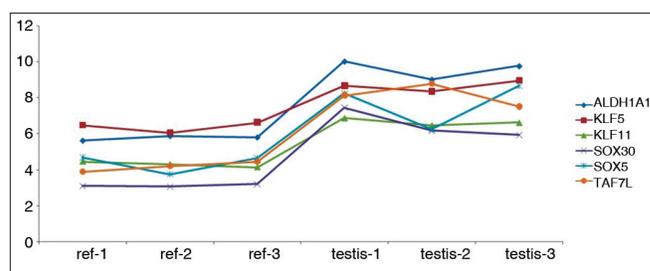
Tissue	Number of DEGs (up-regulated + down-regulated)
Human testis	4879 (2868 + 2011)
Mouse testis	4792 (3139 + 1653)
hESC	6343 (4005 + 2338)
mESC	4038 (3308 + 730)

were significantly up-regulated [Figure 2]. LIN28B, shown to be a marker of undifferentiated ESCs, was found with a fold change of 2.63.<sup>[27]</sup> Also TAF7L, down regulation of which causes oligospermia in men, was identified with a fold change of 4.<sup>[28]</sup>

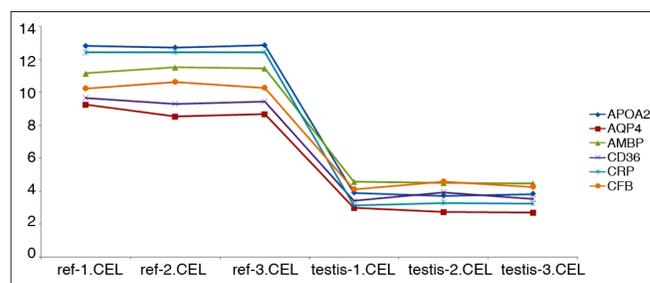
Similarly among down-regulated genes, majority of the genes were related to defence response, immune response and immune response system. This list includes some well known genes such as CD36, AMBP, CRP, APOA2 and AQP4. Expression of some of the selected down-regulated transcripts is as shown Figure 3.

### Functional annotation of genes over-expressed in human testis

Under "Biological Processes" category of Gene Ontology (GO) classification, top over-represented processes were related to various "metabolic processes" (based on number of genes found) [Figure 4]. The sum of genes within the subcategories of every major category may exceed 100% because some transcripts were classified into more than one sub-category in each of the three major categories. It can be construed from annotation results that the human testicular cells are active in development and constantly undergoing active metabolism, consistent with the high energy requirements of spermatogenesis. Further analysis revealed that around 80% of the unique genes were involved in various metabolic processes. The biological



**Figure 2:** Normalized intensity values of specific up-regulated transcripts in all reference and human testis microarray replicates. Note that reference and testis microarrays are labeled as ref-(n) and testis-(n) respectively



**Figure 3:** Normalized intensity values of specific down-regulated transcripts in all reference and human testis microarray replicates. Note that reference and testis microarrays are labeled as ref-(n) and testis-(n) respectively

process - “regulation of metabolic process” was also found to be over-represented correlating with the above “metabolic processes”.

Another biological process - “Regulation of gene expression” was also over-represented, owing to the fact that gene expression is tightly regulated in testis.<sup>[29]</sup> The over-representation of testis specific biological processes such as Gamete generation, Spermatogenesis, Sexual reproduction, male gamete generation was statistically significant ( $P < 0.01$ ).

Spermatogenesis occurring in testicular cells involves production of mature spermatozoa with haploid DNA, involving conformational changes of chromatin. This process is highly dependent on chromatin re-modeling genes and these genes were found to be significantly expressed in the human testis. Genes in this category include DNA methyltransferases (DNMT1, CDHC, and LDHCL6B), ATP dependent remodelling complexes (SMARCA4, SMARCA1, and SMARCA2), Chromatin modifying genes (HMGB4, HMGXB4, HMMR, and HMG5), Histone methyl transferases (EHMT1, SETX, SETDB2, and SETD2) and other chromatin modifying genes (ASH family, ARID2, ASXL1). The in-depth analysis of GO results revealed that most of the genes were distributed among those subcategories indicating active role in growth and development.

Within the Gene Ontology category of “cellular components”, the subcategory – “intracellular components” contained the largest amount of differentially expressed genes in comparison to extracellular components. Cellular components that have abundant transcripts included those associated with cytoplasm and nucleus [Figure 4]. Also a large number of transcripts found to be associated with chromosome (123 transcripts), centrosome (80 transcripts), microtubule (70 transcripts), chromatin (51 transcripts) and cilium (50 transcripts), indicating high activity within nuclear region.

The molecular function subcategories are most notably associated with various “binding” functions such as protein binding, nucleic acid binding, DNA binding and nucleotide binding, maximum of which are representing cell-division and cell-death, accounting for nearly 52% of total genes.

The GO based annotation not only validates our transcriptome analysis, more importantly, it's shedding new light into large number of biological processes (333 GO BP categories in this case) that take place in human testis.

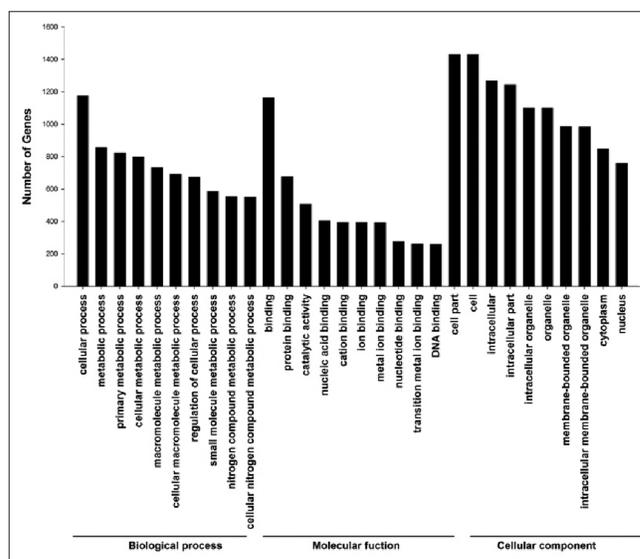
Disease ontology studies shows the up regulation of

diseases specific to testis such as infertility in male, testicular dysfunction, further validating our analysis [Figure 5].

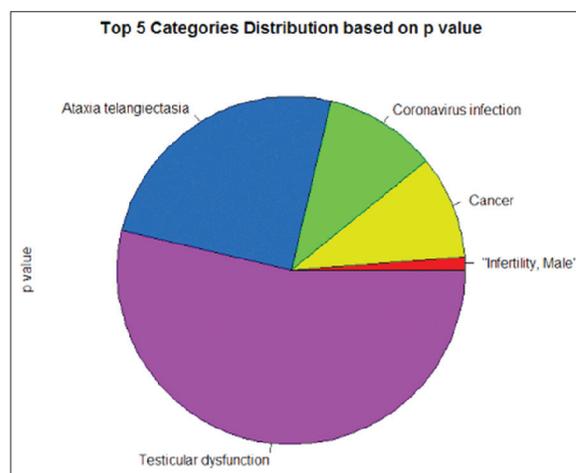
### Pathway analysis

Pathways involved in testis are analyzed using GeneAnswers package. According to our pathway analysis results, 209 genes were assigned to specific pathways. A cluster of genes are found to be expressed within the Ubiquitin mediated proteolysis pathway.

Ubiquitin is a small, highly-conserved regulatory protein which is ubiquitously expressed in eukaryotes. Its most prominent function is to label proteins for proteasomal degradation.<sup>[30]</sup> Ubiquitin and proteosomal subunits are found in human sperm centromere. It undergoes dramatic reduction during spermatid elongation. Ubiquitin also



**Figure 4:** Functional annotation of differentially expressed genes found in human testis with known Gene Ontology annotations



**Figure 5:** Disease ontology of testis up-regulated genes based on P values

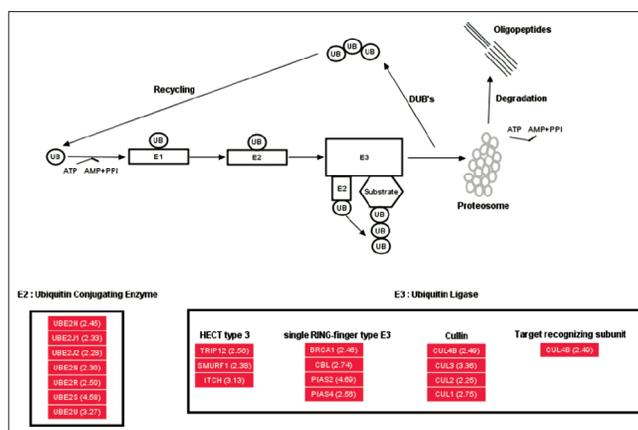
plays important role in replacement of histones with protamines in spermatids, and in promoting maternal inheritance of mitochondrial DNA (mtDNA) in mammals. Defective spermatozoa become surface ubiquitinated during sperm descent down the epididymis.<sup>[31]</sup> A total of 23 genes involved in ubiquitin mediated proteolysis pathways were found to be up regulated. In addition, all three classes of ubiquitin enzymes - E1, E2 and E3 are found to be active in testis. The observations of high activity in metabolic processes perfectly correlates to the function of testis i.e. spermatogenesis.

The higher abundance of E3 class of enzymes, which are known as “ubiquitin protein ligases”, was found followed by E2 class of enzymes, known as “Ubiquitin conjugating enzymes”. A Total of 23 genes of the Ubiquitin mediated proteolysis pathway are differentially expressed with fold change greater than 2. The general descriptions of pathway and genes up-regulated along with corresponding fold change are shown in Figure 6.

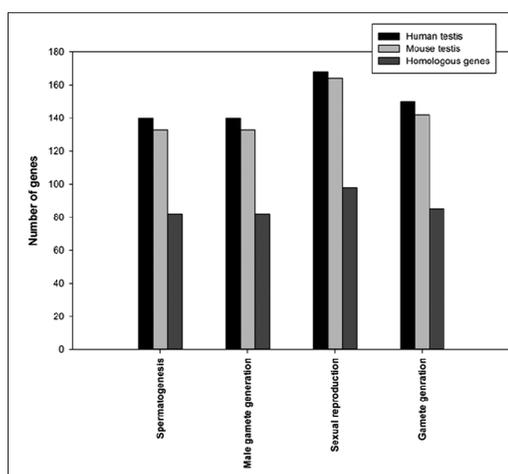
**Comparison of human testis and mouse testis transcriptome**

In order to find the similarity between human and mouse testis, we compared human testis transcriptome with mouse testis transcriptome (further details in Methodology section). Mouse transcriptome had 3139 transcripts up-regulated (FDR<0.01 and fold change>2), against 2868 transcripts in human. We compared differentially up-regulated genes in human and mouse testis and found 634 genes to be common. Functional annotation of these 634 homologous genes reproduced the same results as that of human testis data, with almost same number of genes over-represented in testis specific biological processes such as spermatogenesis, male gamete generation, gamete generation and sexual reproduction [Figure 7]. Top 20 categories according to GO Biological process of the homologous genes are represented by pie chart in Figure 8. Pathway analysis reproduced the same results showing over-representation of genes involved in Ubiquitin mediated proteolysis. The homologous genes of this pathway include Cbl, Pias2, Ubc2j1, Fzr1, Pias4, Trim37, Smurf1, Ubc2s, and Ubc2u. Our analysis shows the conserved function of testicular genes and pathways in mouse and human.

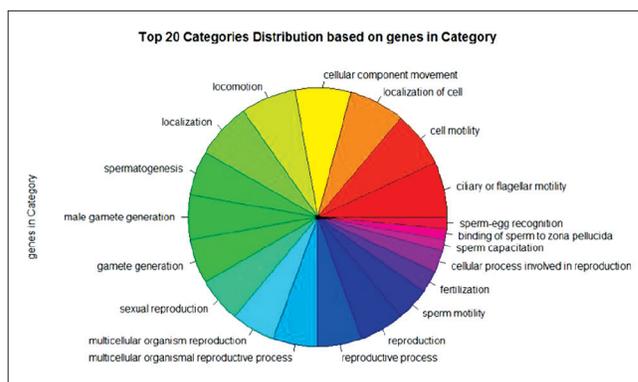
Considering the high degree of similarity between mouse and human, these 634 genes deserve particular attention and must be considered for future candidate gene approach studies related to Spermatogenesis, Oligospermia, Azoospermia and other developmental defects. Furthermore mouse model will continue to provide a platform for functional characterization of highly conserved genes that may bear significance in understanding germ cell formation, spermatogenesis and its associated disorders.



**Figure 6:** Ubiquitin mediated proteolysis pathway. Genes shown in red are up-regulated in human testis



**Figure 7:** Distribution of up-regulated genes in human testis, mouse testis and homologous genes between human and mouse into testis specific categories: spermatogenesis, male gamete generation, sexual reproduction and gamete generation



**Figure 8:** Top 20 categories according to GO Biological processes of homologous genes between human testis and mouse testis

**Comparison between testis and ESC transcriptome**

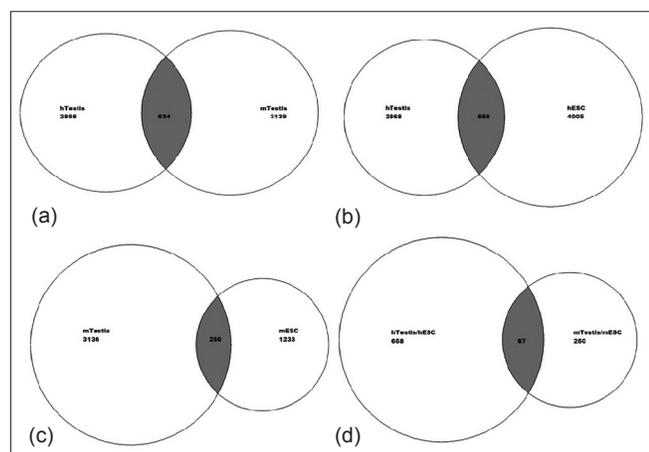
There have been studies on isolation and discovery of molecular signature of spermatogonial stem cells.<sup>[32]</sup> To identify the genes common between testis and stem cells, differentially up-regulated genes from human

testis are compared with the differentially up-regulated genes in stem cells. Our analysis of human ESC (hESC) transcriptome revealed 4005 differentially up-regulated transcripts. On comparing hESC up-regulated genes with human testis data (2868 genes), we found an overlap of 658 transcripts.

Similar procedure was performed on mouse testis transcriptome and mouse ESC (mESC) transcriptome which resulted in 250 common transcripts. To find the set of common genes which are common among testis and ESCs of both the species, we compared the 658 human transcripts and 250 mouse transcripts. A final set of 67 common genes were obtained [Figure 9] (The list of these 67 genes is provided as supporting information). ESCs are characterized by two properties, the ability to self-renew and the ability to differentiate into multiple cell lineages (pluripotency). These properties are associated with different states of chromatin structure, which are established, maintained, and eventually altered by the activities of chromatin remodeling enzymes.<sup>[33]</sup> Among the 67 genes, chromatin and DNA remodeling genes (such as HELLS, MLFIP, NASP, OIP5, SUV39H2) and cell proliferation genes (such as CDK1, MCM7, NASP, STIL, TTK, NUP62) were found in high abundance (around 25%). This strongly suggests similar gene regulation in testis and ESC. The over-representation further suggests the potential of testicular cells to behave like pluripotent cells when given proper niche.

### Comparison between testis and oocyte transcriptome

Similar kind of analyses was done by Kocabas *et.al*, who found 66 unique genes common between human oocyte,

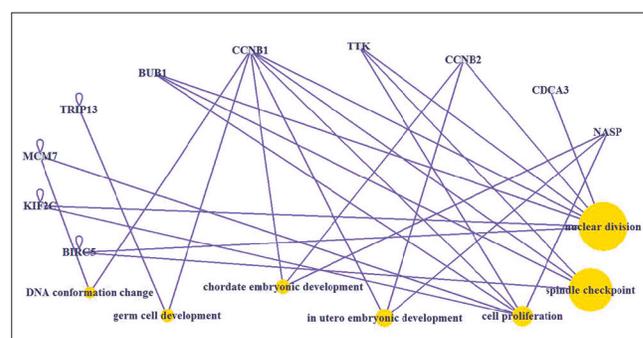


**Figure 9:** Venn diagrams showing the intersection between differentially up-regulated genes in the mouse in the human testis and mouse testis (634 transcripts were found to be common in both species) (a); human testis and hESC (658 transcripts were found to be common in both cell type) (b); mouse testis and mESC (250 transcripts were found to be common in both cell type) (c); human testis/hESC and human testis/mouse testis (67 transcripts were found to be common in all three cell types) (d)

hESCs, mouse oocyte and mESCs.<sup>[10]</sup> In order to narrow down a common gene list, we compared the above 66 unique genes with our set of 67 common genes. This resulted in total of ten genes that are common between testis, oocyte and ESCs in both the species – human and mouse. These genes along with description are shown in Table 3. These genes are involved in various pluripotency related processes as shown in a concept network, generated based upon biological processes in which they are involved in Figure 10.

### CONCLUSION

Our report provides a comprehensive expression baseline of gene transcripts present in *in vivo* human testis. We have identified 2868 transcripts that are highly expressed in human testis, including well known genes such as SOX5, SOX30, KLF5, ALDH1A1, and LIN28B. Functional



**Figure 10:** Gene interaction network of common genes found among testis, ESC and oocyte of both human and mouse. (Node size indicates the number of genes involved in each process)

**Table 3: Genes found common among human testis, mouse testis, hESC, mESC, human oocyte and mouse oocyte**

Gene symbol	Entrez gene ID	Gene title
CCNB1	891	Cyclin B1
NASP	4678	Nuclear autoantigenic sperm protein (histone-binding)
TTK	7272	TTK protein kinase
TRIP13	9319	Thyroid hormone receptor interactor 13
BUB1	699	BUB1 budding uninhibited by benzimidazoles 1 homolog (yeast)
BIRC5	332	Baculoviral IAP repeat-containing 5 (survivin)
MCM7	4176	MCM7 minichromosome maintenance deficient 7 (Saccharomyces cerevisiae)
KIF2C	11004	Kinesin family member 2C
CCNB2	9133	Cyclin B2
ECT2	1894	Epithelial cell-transforming sequence 2 oncogene
CDCA3	83461	Cell division cycle associated 3

annotation of these genes shows over-representation of various metabolic activities, indicating high energy requirements for spermatogenesis. Pathway analysis of up-regulated genes shows over-representation of Ubiquitin mediated proteolysis pathway. We also compared human testis genes with mouse testis to find a large number of homologous genes involved in conserved functions and pathways. More importantly 232 transcripts of testis up-regulated transcripts have unknown functions, arguing for the need for further studies aimed to elucidate the functional role of these genes in human testis.

Also we have identified 67 conserved genes common between testis and ESCs, from both human and mouse species. Such genes may provide missing link between ESC and testicular cells and may serve as genetic resources to identify ESCs that have full potential for differentiation into testicular cells.

Further understanding of the biological roles of these genes may expand our knowledge of spermatogenesis, chromatin remodelling, lineage commitment, pluripotency and endocrine system involved in the production of androgens such as testosterone. Also the practical implications may provide solutions to infertility related disorders.

## REFERENCES

- Poongothai J, Gopenath TS, Manonayaki S. Genetics of human male infertility. *Singapore Med J* 2009;50:336-47.
- Schmidt JA, de Avila JM, McLean DJ. Analysis of gene expression in bovine testis tissue prior to ectopic testis tissue xenografting and during the grafting period. *Biol Reprod* 2007;76:1071-80.
- Schena M, Shalon D, Davis RW, Brown PO. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* 1995;270:467-70.
- Adi L, Tarca RR, Sorin Draghici. Analysis of microarray experiments of gene expression profiling. *Am J Obstet Gynecol* 2006;195:16.
- Su AI, Cooke MP, Ching KA, Hakak Y, Walker JR, Wiltshire T, *et al*. Large-scale analysis of the human and mouse transcriptomes. *Proc Natl Acad Sci U S A* 2002;99:4465-70.
- Hey Y, Pepper SD. Interesting times for microarray expression profiling. *Brief Funct Genomic Proteomic* 2009;8:170-3.
- Divina P, Vlcek C, Strnad P, Paces V, Forejt J. Global transcriptome analysis of the C57BL/6J mouse testis by SAGE: evidence for nonrandom gene order. *BMC Genomics* 2005;6:29.
- Mizrak SC, Chikhovskaya JV, Sadri-Ardekani H, van Daalen S, Korver CM, Hovingh SE, *et al*. Embryonic stem cell-like cells derived from adult human testis. *Hum Reprod* 2010;25:158-67.
- Edgar R, Domrachev M, Lash AE. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. *Nucleic Acids Res* 2002;30:207-10.
- Kocabas AM, Crosby J, Ross PJ, Otu HH, Beyhan Z, Can H, *et al*. The transcriptome of human oocytes. *Proc Natl Acad Sci U S A* 2006;103:14027-32.
- Evsenko D, Zhu Y, Schenke-Layland K, Kuo J, Latour B, Ge S, *et al*. Mapping the first stages of mesoderm commitment during differentiation of human embryonic stem cells. *Proc Natl Acad Sci U S A* 2010;107:13742-7.
- Thorrez L, Van Deun K, Tranchevent LC, Van Lommel L, Engelen K, Marchal K, *et al*. Using ribosomal protein genes as reference: A tale of caution. *PLoS One* 2008;3:e1854.
- Faustino RS, Chiriac A, Niederlander NJ, Nelson TJ, Behfar A, Mishra PK, *et al*. Decoded calreticulin-deficient embryonic stem cell transcriptome resolves latent cardiophenotype. *Stem Cells* 2010;28:1281-91.
- Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, *et al*. Bioconductor: open software development for computational biology and bioinformatics. *Genome Biol* 2004;5:80.
- Bolstad BM, Collin F, Simpson KM, Irizarry RA, Speed TP. Experimental design and low-level analysis of microarray data. *Int Rev Neurobiol* 2004;60:25-58.
- BM B. Low Level Analysis of High-density Oligonucleotide Array Data: Background, Normalization and Summarization [Phd Thesis]. Berkeley University of California; 2004.
- Coussens AK, Wilkinson CR, Hughes IP, Morris CP, van Daal A, Anderson PJ, *et al*. Unravelling the molecular control of calvarial suture fusion in children with craniosynostosis. *BMC Genomics* 2007;8:458.
- Gregory Alvord W, Roayaei JA, Quinones OA, Schneider KT. A microarray analysis for differential gene expression in the soybean genome using Bioconductor and R. *Brief Bioinform* 2007;8:415-31.
- Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, *et al*. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* 2003;4:249-64.
- Lim WK, Wang K, Lefebvre C, Califano A. Comparative analysis of microarray normalization procedures: effects on reverse engineering gene networks. *Bioinformatics* 2007;23:i282-8.
- Smyth GK. Linear models and empirical bayes methods for assessing differential expression in microarray experiments. *Stat Appl Genet Mol Biol* 2004;3:Article3.
- Benjamini Y HY. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J R Stat Soc* 1995;57:12.
- Feng G, Du P, Krett NL, Tessel M, Rosen S, Kibbe WA, *et al*. A collection of bioconductor methods to visualize gene-list annotations. *BMC Res Notes* 2010;3:10.
- Du P, Feng G, Flatow J, Song J, Holko M, Kibbe WA, *et al*. From disease ontology to disease-ontology lite: statistical methods to adapt a general-purpose ontology for the test of gene-ontology associations. *Bioinformatics* 2009;25:i63-8.
- Li M, Reilly C. Assessing the quality of hybridized RNA in Affymetrix GeneChips using linear regression. *J Biomol Tech* 2008;19:122-8.
- Yamanaka S, Takahashi K. Induction of pluripotent stem cells from mouse fibroblast cultures. *Tanpakushitsu Kakusan Koso* 2006;51:2346-51.
- Richards M, Tan SP, Tan JH, Chan WK, Bongso A. The transcriptome profile of human embryonic stem cells as defined by SAGE. *Stem Cells* 2004;22:51-64.
- Cheng Y, Buffone MG, Kouadio M, Goodheart M, Page DC, Gerton GL, *et al*. Abnormal sperm in mice lacking the Taf71 gene. *Mol Cell Biol* 2007;27:2582-9.
- Grimes SR. Testis-specific transcriptional control. *Gene* 2004;343:11-22.
- Cook C, Petrucelli L. A critical evaluation of the ubiquitin-proteasome system in Parkinson's disease. *Biochim Biophys Acta* 2009;1792:664-75.
- Sutovsky P. Ubiquitin-dependent proteolysis in mammalian spermatogenesis, fertilization, and sperm quality control: Killing three birds with one stone. *Microsc Res Tech* 2003;61:15.
- Kokkinaki M, Lee TL, He Z, Jiang J, Golestaneh N, Hofmann MC, *et al*. The molecular signature of spermatogonial stem/progenitor cells in the 6-day-old mouse testis. *Biol Reprod* 2009;80:11.
- Saladi SV, de la Serna IL. ATP dependent chromatin remodeling enzymes in embryonic stem cells. *Stem Cell Rev* 2010;6:62-73.

**How to cite this article:** Anand MT, Prasad BV. The computational analysis of human testis transcriptome reveals closer ties to pluripotency. *J Hum Reprod Sci* 2012;5:266-73.  
**Source of Support:** Nil, **Conflict of Interest:** None declared.