Comparative and Functional Analysis of Testis-Specific Genes

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The testis is the special male gonad responsible for spermatogenesis and steroidogenesis with complex gene expressions. Characterizing and comparing the testis-specific genes in different species can reveal key genes related to testis specific functions and provide supplementary information for study of human testis function. We screened testis-specific genes from Unigene libraries, total 317, 449 and 147 testis-specific genes were identified for human, mouse and rat, respectively. Ten from thirteen selected human testis-specific genes were validated exclusively expressed in the testis by reverse transcription polymerase chain reaction (RT-PCR). Systematic bioinformatics analysis showed that specific genes were mainly related to spermatogenesis and testis development process with significant Glycolysis and Pyruvate metabolism. Enrichment functions were discussed.

Key words testis; specific; digital differential display

The testes produce the male gametes and the male sexual hormones which include all the processes involved in the production of gametes and the enzymatic reactions leading to the production of male steroid hormones.¹⁾ These complicated processes of spermatogenesis and steroidogenesis reflected the complex gene expression in the testis.^{2,3)} Many genes which were crucial for spermatogenesis and male fertility are often exclusively expressed in male germ cells. Some testis-specific genes have been screened including FAM46D,⁴⁾ Adam31,⁵⁾ PDHA2,⁶⁾ TEX101 and SPATA19,⁷⁾ etc. which have been proven to play critical roles in the process of spermatogenesis and steroidogenesis.

Various approaches have been used to analyze these tissue-exclusively expressed genes including suppression subtractive hybridization (SSH),⁸⁾ differential display reverse transcription-polymerase chain reaction (RT-PCR),⁹⁾ microarray,¹⁰⁾ and whole genome approach analysis.¹¹⁾ The UniGene database (<http://www.ncbi.nlm.nih.gov/unigene>) is a collection of sequences of individual clones from the cDNA libraries of various organs. The sequences are organized into clusters and each cluster is likely to contain sequence information of a single gene. Furthermore, the UniGene database includes information of various tissues, developmental, and diseases stages, and also provides valuable information about the putative tissue distribution of gene expression. Using the digital differential display (DDD) tool at the NCBI (<http://www.ncbi.nlm.nih.gov/UniGene/ddd.cgi>), some novel or specific genes had been identified.^{12,13)} Among various species, the highest number of expressed sequence tag (EST) sequences is available for the human and mouse, and most of knowledge of human testis physiology and function was obtained from research of homolog genes to mouse or rat.¹⁴⁾ Recently, we have mined the human, mouse and rat testis-specific gene expression patterns between testis and other organs in the UniGene library (www.ncbi.nlm.nih.gov/unigene/info_ddd.html) by digital differential display tool, and have made a comprehensive comparative and functional analysis of the results. This well-characterized database of testis-specific genes should serve as a source of reference for future research and clinical application.

MATERIALS AND METHODS

Digital Differential Display (DDD) Analysis of Testis-Specific Genes The DDD tool (http://www.ncbi.nlm.nih.gov/unigene/info_ddd.html) was performed to screen the testis-specific genes in human, mouse and rat between testis libraries and 18 other organ libraries. DDD tool takes advantage of UniGene by comparing the number of times that sequences from different libraries were assigned to a particular UniGene cluster. The testis and 18 other organs libraries used in the study were summarized and compared by DDD tool (Table 1). Subsequently, the genes with zero hits in the collected libraries (18 organs) from statistically significant difference results were designated as testis-specific genes.

Bioinformatics Analysis The DAVID tools 6.7 (<http://david.abcc.ncifcrf.gov/>) was used to analyze the differential

Table 1. Summary of Human, Mouse and Rat Libraries Used in This Study

	Human		Mouse		Rat	
	Libraries	ESTs	Libraries	ESTs	Libraries	ESTs
Testis	5	140944	7	67542	2	11732
Other organs						
Adipose	2	9945	1	3135	1	4913
Bladder	1	8220	1	1597	0	0
Bone	1	6209	1	5439	0	0
Brain	11	179504	7	40381	6	20907
Colon	7	29584	1	5219	3	5458
Heart	4	19926	8	67542	4	31600
Kidney	6	49568	10	54164	4	24601
Liver	11	72628	6	25033	3	59093
Lung	7	46559	7	40355	2	8065
Muscle	2	6424	2	3031	1	2429
Ovary	4	10862	2	6847	1	4443
Pancreas	1	4308	6	22020	1	16173
Placenta	12	71806	7	21182	2	8475
Prostate	5	63083	0	0	1	9076
Spleen	2	36689	3	46301	1	3629
Stomach	11	36759	4	18360	0	0
Thymus	4	72916	8	93976	0	0
Uterus	5	58541	0	0	0	0
Total	96	783531	74	454582	30	198862

Libraries from 18 organs against testis libraries were used in the digital differential display (DDD) program.

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Table 2. Lists of Primers Used in RT-PCR to Confirm Gene Transcripts of Testis-Specific Genes

Unigene	Forward primers (5'-3')	Reverse primers (5'-3')	Production (bp)
Hs.126780	TCAACGAGCAATACGAGCAG	GGAGGTGGGAGCGGATAA	489
Hs.472491	CCGTGCGATCGTCTGTTC	GCCTTCCACCCTTTCACC	321
Hs.490715	CCCGAGTGATACACGATG	AGGATGCGGTGGCTGATT	187
Hs.632402	CCTTTCTATCTCCCTCA	CTCTTGCTCCAATCTCCT	200
Hs.149030	TTTGCCCGAACCCGATTA	GCCGTTTAGCCTCACCAC	337
Hs.97542	AGATTGACTGCTGGGATG	GTGCCTCTGGTATGGTA	270
Hs.41101	GACATTGCCATTCTATC	AGTTACCGTCCAAGTTT	190
Hs.126838	CTTAGGTGACCGTGTGG	ATGGCAGTGATGTTTATTGT	352
Hs.177959	CATTGGCAAAGAAGTAG	AACCCAAGTATGAAGAAC	345
Hs.525769	CTGCCTGGTGGCTGATAG	TGGGCTGAGACAGACATCG	334
Hs.497034	CTGGTCTATCGACAAC	AAGCCAAATCCAATCAC	354
Mm.436562, Rn.64496 homolog to human <i>Gapdhs</i>	ACCCACTCGTCCATCTTCG	TCCCGTTTCACTTGTCTCG	151
Mm.143889, Rn.92321 homolog to human <i>ADAM5</i>	ATTACCATAGGGAAAGCC	TGAGTCCTGAACATACGC	197
β -Actin	GCTGTCACCTTACCCTTCC	GCTGTCACCTTACCCTTCC	268

Thirteen genes were selected randomly to confirm its expressions in different human organs.

functional annotations including molecular functions, Biological processes, cellular component, KEGG pathway and functional Clustering. Background was *Homo sapiens*, *Mus musculus* and *Rattus norvegicus*, Fisher Exact test is used to determine significant results.

RT-PCR Analysis Eleven human genes (Hs.126780, Hs.472491, Hs.490715, Hs.632402, Hs.149030, Hs.97542, Hs.41101, Hs.126838, Hs.177959, Hs.525769, Hs.497034) and two homolog genes (Mm.436562 and Rn.64496 Mm homolog to human gene *Gapdhs*, 143889 and Rn.92321 homolog to human gene *ADAM5*) were selected for testis-specific validation (Table 2). Forward and reverse primers for the genes were designed using primer premier 5.0 Software (PREMIER Biosoft International) on the basis of sequences published in GenBank. Gene expression was examined by RT-PCR in the 12 organs (testis, heart, liver, spleen, lung, kidney, stomach, muscle, adipose, placenta, ovary and colon) from accident victims (27–32 years old) who had died in car accidents and were collected while artificial circulation maintained organs assigned for transplantation.¹⁵⁾ All procedures were approved by the Ethics Committee of Yu-Huang-Ding Hospital.

RESULTS

Characteristic of Testis-Specific Genes Comparison of expressed sequence tags (ESTs) between testis and 18 other organs was showed in Table 1. 140944 human testis ESTs (5 libraries) and 783531 ESTs from other organs (95 libraries) were used to screen 317 human testis-specific genes including 38 unknown genes. 67542 mouse testis ESTs (7 libraries) and 454582 other organs ESTs (74 libraries) were used to screen 449 mouse testis-specific genes including 45 unknown genes. 11732 rat testis ESTs (2 libraries) and 198862 other organ ESTs (30 libraries) were used to screen 147 rat testis-specific genes including 8 unknown genes (Table 3).

Twenty specific genes were co-expressed in human, mouse and rat testis, which included five genes common in the human sperm proteome¹⁶⁾ (Table 4, Fig. 1). Comparison of human testis-specific genes with human sperm proteome showed 33 specific genes overlapped in the two database

(Table 5).

Functional Analysis The testis-specific genes (Unigene IDs) were imported into DAVID Function Annotation lists. Total 282, 442 and 140 Unigene IDs were converted into DAVID IDs, respectively of the human, mouse and rat specific genes. Common statistically significant functions including molecular functions, biological processes, KEGG pathway and functional clustering were displayed in Fig. 2. Results showed that specific genes were mainly related to spermatogenesis and development process with significant Glycolysis and Pyruvate metabolism.

RT-PCR Analysis Analysis of selected thirteen genes by RT-PCR confirmed that ten genes including two homolog genes were specifically expressed in the testis tissues. Hs.490715 was also weakly expressed in ovary, Hs.632402 was also detected in muscle, and Hs.149030 was expressed in liver and spleen (Fig. 3).

DISCUSSION

In the present study, eighteen organs libraries were pooled to compare with testis libraries of human, mouse and rat, respectively. The designation of testis-specific genes was only given if the gene had zero hits in the collected libraries (18 organs) from statistically significant difference results generated by DDD tool. The design guaranteed the significance of the results as much as possible. However, the results showed low overlap among human, mouse and rat testis-specific genes, this phenomenon maybe partially due to the number disparities between libraries and partially due to differential gene expressions among species or alternative splicing which included 30% in human testis ESTs.¹⁷⁾ Comparative analysis of human testis-specific genes in this study with Tang's report,¹⁸⁾ only 76 overlapped genes were found. Low overlap maybe mainly due to database update which generally takes less than one month and more rigorous screening methods we used. Comparison of 139 (47%) unknown genes in TANG' report and 38 (11%) unknown genes in present study indicated the current database were annotated more detailed. Baker reported 976 from 1053 proteins detected in spermatozoa demonstrated EST expressions in the testis. Conversely,

Table 3. Lists of Unigene IDs of Human, Mouse and Rat Testis-Specific Genes

Unigene IDs of 317 human testis-specific genes								
Hs.107944	Hs.131098	Hs.213307	Hs.367764	Hs.456833	Hs.558087	Hs.643584	Hs.666581	Hs.98394
Hs.111850	Hs.131179	Hs.220558	Hs.367959	Hs.459153	Hs.558560	Hs.644625	Hs.671096	Hs.98586
Hs.112645	Hs.131361	Hs.223581	Hs.369473	Hs.459534	Hs.558748	Hs.645245	Hs.675821	Hs.98712
Hs.112651	Hs.131469	Hs.226805	Hs.369765	Hs.472491	Hs.567414	Hs.645542	Hs.67639	Hs.98848
Hs.112680	Hs.131615	Hs.236635	Hs.371738	Hs.475243	Hs.567642	Hs.646421	Hs.680123	Hs.99161
Hs.112743	Hs.131654	Hs.240053	Hs.375039	Hs.481369	Hs.567674	Hs.646533	Hs.689473	
Hs.113290	Hs.131824	Hs.245476	Hs.376008	Hs.482520	Hs.567774	Hs.646574	Hs.689547	
Hs.113291	Hs.133095	Hs.247868	Hs.376505	Hs.487409	Hs.568279	Hs.647077	Hs.694285	
Hs.115429	Hs.136555	Hs.2633	Hs.378893	Hs.490715	Hs.568772	Hs.648235	Hs.701555	
Hs.115460	Hs.13861	Hs.274285	Hs.381087	Hs.491734	Hs.568804	Hs.648270	Hs.707861	
Hs.118205	Hs.142003	Hs.283011	Hs.388827	Hs.491885	Hs.571446	Hs.648337	Hs.708818	
Hs.119207	Hs.143519	Hs.283096	Hs.388841	Hs.494981	Hs.572187	Hs.652956	Hs.709890	
Hs.120316	Hs.145601	Hs.283764	Hs.388874	Hs.497034	Hs.572495	Hs.654377	Hs.711267	
Hs.120319	Hs.146544	Hs.298312	Hs.390221	Hs.510812	Hs.575609	Hs.655170	Hs.712915	
Hs.120364	Hs.146614	Hs.302028	Hs.398196	Hs.511849	Hs.584289	Hs.657342	Hs.714939	
Hs.120391	Hs.147276	Hs.303923	Hs.405628	Hs.511983	Hs.585048	Hs.658505	Hs.716685	
Hs.120831	Hs.147816	Hs.304020	Hs.405659	Hs.514820	Hs.585464	Hs.658630	Hs.719419	
Hs.121021	Hs.148250	Hs.307052	Hs.406696	Hs.517091	Hs.587755	Hs.659889	Hs.720893	
Hs.121084	Hs.149030	Hs.307086	Hs.408336	Hs.518957	Hs.591011	Hs.660607	Hs.721200	
Hs.121200	Hs.151167	Hs.307999	Hs.411101	Hs.519507	Hs.591296	Hs.660774	Hs.721231	
Hs.121438	Hs.155942	Hs.320892	Hs.411239	Hs.519941	Hs.591738	Hs.661266	Hs.721828	
Hs.121494	Hs.159043	Hs.324335	Hs.412818	Hs.520554	Hs.591941	Hs.662013	Hs.721999	
Hs.122511	Hs.160594	Hs.326528	Hs.414648	Hs.522093	Hs.592008	Hs.662199	Hs.722049	
Hs.122986	Hs.169222	Hs.333132	Hs.424594	Hs.524331	Hs.592128	Hs.662205	Hs.722203	
Hs.123530	Hs.169797	Hs.335788	Hs.431161	Hs.524406	Hs.620508	Hs.662212	Hs.722222	
Hs.123536	Hs.177959	Hs.343487	Hs.433336	Hs.525666	Hs.629246	Hs.662213	Hs.722843	
Hs.125251	Hs.179646	Hs.343631	Hs.434715	Hs.525769	Hs.629498	Hs.662219	Hs.723454	
Hs.126035	Hs.186363	Hs.348263	Hs.435371	Hs.528454	Hs.630533	Hs.662288	Hs.79363	
Hs.126730	Hs.189183	Hs.348618	Hs.437006	Hs.528847	Hs.631533	Hs.662304	Hs.82921	
Hs.126780	Hs.189184	Hs.348700	Hs.437141	Hs.529965	Hs.631621	Hs.662373	Hs.97335	
Hs.126838	Hs.190267	Hs.349758	Hs.439998	Hs.530358	Hs.631667	Hs.662737	Hs.97408	
Hs.127882	Hs.192090	Hs.350507	Hs.444230	Hs.534390	Hs.631931	Hs.662766	Hs.97501	
Hs.128069	Hs.194610	Hs.350671	Hs.444236	Hs.535004	Hs.632070	Hs.663128	Hs.97542	
Hs.128574	Hs.197354	Hs.351068	Hs.447948	Hs.535216	Hs.632233	Hs.664011	Hs.97633	
Hs.129293	Hs.197801	Hs.351815	Hs.448583	Hs.535454	Hs.632402	Hs.664098	Hs.97714	
Hs.129435	Hs.2042	Hs.357567	Hs.448884	Hs.535667	Hs.632574	Hs.664194	Hs.97726	
Hs.130672	Hs.209206	Hs.363054	Hs.453629	Hs.535746	Hs.632635	Hs.665663	Hs.97933	
Hs.130722	Hs.209542	Hs.367245	Hs.454647	Hs.537348	Hs.637057	Hs.666095	Hs.98008	
Hs.130949	Hs.210377	Hs.367727	Hs.454835	Hs.539964	Hs.641085	Hs.666099	Hs.98025	
Unigene IDs of 449 mouse testis-specific genes								
Mm.189660	Mm.189390	Mm.251434	Mm.287421	Mm.33648	Mm.422869	Mm.45373	Mm.478333	Mm.7411
Mm.10725	Mm.189648	Mm.251601	Mm.287783	Mm.33683	Mm.423014	Mm.45377	Mm.478493	Mm.74465
Mm.107718	Mm.189668	Mm.252110	Mm.291521	Mm.338103	Mm.425024	Mm.45442	Mm.4815	Mm.75060
Mm.107918	Mm.189696	Mm.252733	Mm.291723	Mm.33811	Mm.425335	Mm.45462	Mm.4872	Mm.75066
Mm.108054	Mm.195732	Mm.252738	Mm.291732	Mm.33864	Mm.425687	Mm.45611	Mm.50087	Mm.76826
Mm.108632	Mm.195831	Mm.252761	Mm.291887	Mm.339192	Mm.425989	Mm.45615	Mm.50108	Mm.76859
Mm.108729	Mm.196288	Mm.252823	Mm.292326	Mm.34166	Mm.425994	Mm.45820	Mm.50361	Mm.78341
Mm.116705	Mm.196313	Mm.255732	Mm.293018	Mm.341880	Mm.42733	Mm.45824	Mm.50808	Mm.78368
Mm.116803	Mm.196315	Mm.261400	Mm.293026	Mm.347413	Mm.431297	Mm.45833	Mm.5168	Mm.78551
Mm.116871	Mm.204664	Mm.26189	Mm.293080	Mm.348215	Mm.432087	Mm.458917	Mm.52511	Mm.78900
Mm.116957	Mm.206798	Mm.263615	Mm.29625	Mm.34841	Mm.432764	Mm.460742	Mm.52550	Mm.78965
Mm.120271	Mm.21005	Mm.263642	Mm.296649	Mm.349600	Mm.436562	Mm.46102	Mm.55591	Mm.79031
Mm.121570	Mm.212777	Mm.263706	Mm.29788	Mm.35644	Mm.436668	Mm.46106	Mm.55734	Mm.79070
Mm.12743	Mm.213909	Mm.263708	Mm.297977	Mm.358754	Mm.436785	Mm.46114	Mm.55827	Mm.79072
Mm.128134	Mm.214178	Mm.264645	Mm.30482	Mm.360540	Mm.437189	Mm.46117	Mm.55848	Mm.79114
Mm.12817	Mm.214599	Mm.264651	Mm.30494	Mm.371643	Mm.439708	Mm.46124	Mm.55870	Mm.79179
Mm.1296	Mm.217475	Mm.266280	Mm.307436	Mm.373292	Mm.439725	Mm.46125	Mm.55891	Mm.80075
Mm.140115	Mm.229259	Mm.2688	Mm.308822	Mm.373573	Mm.439815	Mm.46129	Mm.55982	Mm.8019
Mm.1404	Mm.23166	Mm.268996	Mm.30958	Mm.379188	Mm.439819	Mm.46132	Mm.56404	Mm.8122
Mm.141235	Mm.232593	Mm.269223	Mm.30959	Mm.37953	Mm.439983	Mm.46136	Mm.56430	Mm.8149
Mm.141275	Mm.23361	Mm.26987	Mm.310201	Mm.380214	Mm.440041	Mm.46140	Mm.56514	Mm.82775
Mm.141324	Mm.23377	Mm.26988	Mm.314688	Mm.380836	Mm.440232	Mm.46144	Mm.60362	Mm.82820
Mm.141383	Mm.23385	Mm.27023	Mm.31700	Mm.382859	Mm.440412	Mm.46158	Mm.60688	Mm.84331
Mm.141451	Mm.23397	Mm.27027	Mm.31708	Mm.383221	Mm.440422	Mm.46159	Mm.60690	Mm.84467
Mm.141471	Mm.23401	Mm.270295	Mm.318133	Mm.383770	Mm.440432	Mm.46166	Mm.60992	Mm.84502
Mm.142735	Mm.235024	Mm.271255	Mm.320172	Mm.384235	Mm.440453	Mm.46167	Mm.61131	Mm.84522
Mm.143764	Mm.23509	Mm.271285	Mm.320289	Mm.386790	Mm.440554	Mm.46170	Mm.61148	Mm.84567

Table 3. Continued

Unigene IDs of 317 human testis-specific genes								
Mm.143802	Mm.23510	Mm.272119	Mm.32231	Mm.386795	Mm.440760	Mm.46175	Mm.61206	Mm.84725
Mm.143889	Mm.23515	Mm.272226	Mm.32242	Mm.386810	Mm.441372	Mm.46405	Mm.61243	Mm.84774
Mm.1453	Mm.23516	Mm.272519	Mm.325325	Mm.386907	Mm.441493	Mm.46470	Mm.61330	Mm.85045
Mm.148848	Mm.23520	Mm.272720	Mm.325769	Mm.387525	Mm.441494	Mm.467143	Mm.6211	Mm.8637
Mm.1498	Mm.23522	Mm.272723	Mm.329132	Mm.389085	Mm.441500	Mm.467144	Mm.65550	Mm.87067
Mm.154333	Mm.23534	Mm.272746	Mm.329435	Mm.389164	Mm.441963	Mm.467152	Mm.660	Mm.87320
Mm.157323	Mm.23547	Mm.272766	Mm.330943	Mm.389509	Mm.442295	Mm.467157	Mm.66794	Mm.87321
Mm.158148	Mm.235535	Mm.272794	Mm.331893	Mm.389676	Mm.443238	Mm.467163	Mm.66894	Mm.87325
Mm.158494	Mm.235716	Mm.272843	Mm.332291	Mm.389950	Mm.443268	Mm.467176	Mm.66952	Mm.87331
Mm.159193	Mm.23887	Mm.27287	Mm.332570	Mm.390691	Mm.443270	Mm.467190	Mm.67234	Mm.87352
Mm.159404	Mm.239376	Mm.272871	Mm.332809	Mm.390750	Mm.443272	Mm.467193	Mm.67300	Mm.87357
Mm.159949	Mm.243849	Mm.273301	Mm.333010	Mm.390785	Mm.443285	Mm.467210	Mm.67595	Mm.87365
Mm.160088	Mm.24454	Mm.273316	Mm.333542	Mm.391200	Mm.443287	Mm.4688	Mm.67628	Mm.87382
Mm.160106	Mm.246123	Mm.273327	Mm.334092	Mm.391427	Mm.443291	Mm.473053	Mm.67665	Mm.87419
Mm.160714	Mm.248902	Mm.275677	Mm.334107	Mm.391486	Mm.443311	Mm.473700	Mm.67677	Mm.87446
Mm.16563	Mm.249912	Mm.276086	Mm.334425	Mm.391543	Mm.444289	Mm.475127	Mm.69388	Mm.87448
Mm.173395	Mm.250430	Mm.276332	Mm.334503	Mm.392402	Mm.444291	Mm.475210	Mm.717	Mm.87624
Mm.17413	Mm.250432	Mm.276558	Mm.335431	Mm.393774	Mm.444650	Mm.475302	Mm.71906	Mm.87748
Mm.17621	Mm.250447	Mm.276780	Mm.335525	Mm.394644	Mm.444671	Mm.476887	Mm.72491	Mm.89197
Mm.17649	Mm.250465	Mm.279725	Mm.335855	Mm.398302	Mm.444704	Mm.477010	Mm.72959	Mm.89943
Mm.178707	Mm.250837	Mm.279939	Mm.33629	Mm.415388	Mm.44896	Mm.477086	Mm.72970	Mm.9493
Mm.179050	Mm.251303	Mm.280878	Mm.33631	Mm.4223	Mm.45302	Mm.477599	Mm.73205	Mm.9500
Mm.179144	Mm.251409	Mm.282330	Mm.33646	Mm.422673	Mm.45306	Mm.4776	Mm.73222	
Unigene IDs of 147 rat testis-specific genes								
Rn.10423	Rn.122594	Rn.126314	Rn.155649	Rn.18131	Rn.208235	Rn.27474	Rn.50288	Rn.66649
Rn.105169	Rn.122663	Rn.127514	Rn.159914	Rn.187184	Rn.208286	Rn.27738	Rn.50442	Rn.67823
Rn.107052	Rn.122669	Rn.136809	Rn.159999	Rn.195918	Rn.208808	Rn.27773	Rn.50944	Rn.68598
Rn.108313	Rn.122802	Rn.136989	Rn.160028	Rn.198212	Rn.209597	Rn.3253	Rn.51329	Rn.81074
Rn.108510	Rn.122806	Rn.137100	Rn.160149	Rn.198417	Rn.209823	Rn.33200	Rn.51791	Rn.83796
Rn.108545	Rn.123031	Rn.137247	Rn.160929	Rn.198431	Rn.210834	Rn.34654	Rn.51883	Rn.83901
Rn.108612	Rn.123117	Rn.137540	Rn.160960	Rn.198484	Rn.210888	Rn.38091	Rn.53634	Rn.8467
Rn.109395	Rn.123156	Rn.137580	Rn.161900	Rn.198886	Rn.212099	Rn.42917	Rn.54001	Rn.92321
Rn.109532	Rn.123187	Rn.138402	Rn.162390	Rn.198897	Rn.214118	Rn.42918	Rn.55448	Rn.92436
Rn.110654	Rn.123202	Rn.138828	Rn.162573	Rn.199021	Rn.215004	Rn.43509	Rn.57137	Rn.95004
Rn.11126	Rn.123222	Rn.144145	Rn.162717	Rn.201753	Rn.215007	Rn.45038	Rn.57304	Rn.96432
Rn.111442	Rn.123227	Rn.145908	Rn.162868	Rn.202679	Rn.215015	Rn.45119	Rn.58091	
Rn.111731	Rn.123299	Rn.146844	Rn.163190	Rn.202733	Rn.215017	Rn.46772	Rn.59403	
Rn.113616	Rn.123316	Rn.150800	Rn.163425	Rn.205065	Rn.215025	Rn.47152	Rn.60723	
Rn.113868	Rn.123383	Rn.154599	Rn.170874	Rn.205660	Rn.217373	Rn.47921	Rn.63585	
Rn.114052	Rn.123420	Rn.155129	Rn.172256	Rn.206234	Rn.219514	Rn.48706	Rn.6427	
Rn.119400	Rn.125706	Rn.155462	Rn.172733	Rn.20672	Rn.26693	Rn.50006	Rn.64496	

Total of 317, 449 and 147 Unigenes were identified through the program mentioned above.

Table 4. Common Testis-Specific Genes in Human, Mouse and Rat Testis

Unigene	Gene name	Gene description	Species
Hs.98008; Mm.61206; Rn.108510	*GK2	Glycerol kinase 2	Human, mouse, rat
Hs.115460; Mm.348215; Rn.109532	CCIN	Calicin	Human, mouse, rat
Hs.131361; Mm.4223; Rn.11126	PDHA2	Pyruvate dehydrogenase alpha 2	Human, mouse, rat
Hs.226805; Mm.67677; Rn.113616	KIF2B	Kinesin family member 2B	Human, mouse, rat
Hs.236635; Mm.30958; Rn.119400	*ACTRT2	Actin-related protein T2	Human, mouse, rat
Hs.131098; Mm.87624; Rn.123117	FAM71F1	Family with sequence similarity 71, member F1	Human, mouse, rat
Hs.335788; Mm.307436; Rn.123420	ALS2CR11	Amyotrophic lateral sclerosis 2 chromosome region, candidate 11	Human, mouse, rat
Hs.186363; Mm.23361; Rn.136809	SPERT	Spermatid associated	Human, mouse, rat
Hs.120319; Mm.272843; Rn.137540	NT5C1B	5'-Nucleotidase, cytosolic 1B	Human, mouse, rat
Hs.41101; Mm.296649; Rn.150800	CCDC110	Coiled-coil domain containing 110	Human, mouse, rat
Hs.518957; Mm.8122; Rn.162573	ADAD1	Adenosine deaminase domain containing 1	Human, mouse, rat
Hs.307052; Mm.475210; Rn.208286	*LDHAL6B	Lactate dehydrogenase A-like 6B	Human, mouse, rat
Hs.487409; Mm.441372; Rn.210888	PAPOLB	Poly polymerase beta	Human, mouse, rat
Hs.647077; Mm.141471; Rn.215017	GALNTL5	UDP-N-acetyl-alpha-D-galactosamine : polypeptide N-acetylgalactosaminyltransferase-like 5	Human, mouse, rat
Hs.437141; Mm.386907; Rn.50442	CCDC63	Coiled-coil domain containing 63	Human, mouse, rat
Hs.367727; Mm.478333; Rn.50944	*PGK2	Phosphoglycerate kinase 2	Human, mouse, rat
Hs.129293; Mm.61148; Rn.59403	*FAM71A	Family with sequence similarity 71, member A	Human, mouse, rat
Hs.534390; Mm.251434; Rn.63585	ACTL7B	Actin-like 7B	Human, mouse, rat
Hs.189184; Mm.334092; Rn.66649	UBQLN3	Ubiquilin 3	Human, mouse, rat
Hs.629246; Mm.7411; Rn.81074	HDGFL1	Hepatoma derived growth factor-like 1	Human, mouse, rat

Table 4. Continued

Unigene	Gene name	Gene description	Species
Hs.665663; Mm.121570	MARCH10	Membrane-associated ring finger 10	Human, mouse
Hs.2042; Mm.1296	CRISP2	Cysteine-rich secretory protein 2	Human, mouse
Hs.131654; Mm.141324	DMRTB1	DMRT-like family B with proline-rich C-terminal, 1	Human, mouse
Hs.97633; Mm.1498	*AKAP4	A kinase anchor protein 4	Human, mouse
Hs.209206; Mm.154333	*ACTL9	Actin-like 9	Human, mouse
Hs.303923; Mm.159193	TKTL2	Transketolase-like 2	Human, mouse
Hs.67639; Mm.159949	*NUP210L	Nucleoporin 210 kDa-like	Human, mouse
Hs.567774; Mm.160714	CCDC83	Coiled-coil domain containing 83	Human, mouse
Hs.654377; Mm.16563	*LDHC	Lactate dehydrogenase C	Human, mouse
Hs.302028; Mm.173395	DPEP3	Dipeptidase 3	Human, mouse
Hs.298312; Mm.179050	HORMAD1	HORMA domain containing 1	Human, mouse
Hs.472491; Mm.189660	OXCT2	3-Oxoacid CoA transferase 2	Human, mouse
Hs.122511; Mm.195831	CETN1	Centrin, EF-hand protein, 1	Human, mouse
Hs.121021; Mm.196313	SPATA3	Spermatogenesis associated 3	Human, mouse
Hs.304020; Mm.196315	CALR3	Calreticulin 3	Human, mouse
Hs.663128; Mm.23515	*ROPN1B	Roppin, raphilin associated protein 1B	Human, mouse
Hs.414648; Mm.23534	*TEKT3	Tektin 3	Human, mouse
Hs.112743; Mm.243849	SYCP1	Synaptonemal complex protein 1	Human, mouse
Hs.405659; Mm.272720	PIWIL1	Piwi-like 1	Human, mouse
Hs.723454; Mm.279939	*TXNDC3	Thioredoxin domain containing 3	Human, mouse
Hs.169797; Mm.293080	BOLL	Bol, boule-like	Human, mouse
Hs.147816; Mm.30494	MDH1B	Malate dehydrogenase 1B, NAD	Human, mouse
Hs.123530; Mm.30959	*ACTL7A	Actin-like 7A	Human, mouse
Hs.444236; Mm.330943	SPATA16	Spermatogenesis associated 16	Human, mouse
Hs.367764; Mm.33646	POM121L1P	POM121 membrane glycoprotein-like 1 pseudogene	Human, mouse
Hs.2633; Mm.37953	DSG1	Desmoglein 1	Human, mouse
Hs.454835; Mm.440232	TTLL10	Tubulin tyrosine ligase-like family, member 10	Human, mouse
Hs.491885; Mm.45615	DNAJC5B	DnaJ homolog, subfamily C, member 5 beta	Human, mouse
Hs.151167; Mm.46125	*PPP3R2	Protein phosphatase 3, regulatory subunit B, beta isoform	Human, mouse
Hs.121494; Mm.4688	*SPAM1	Sperm adhesion molecule 1	Human, mouse
Hs.210377; Mm.477086	CCDC38	Coiled-coil domain containing 38	Human, mouse
Hs.169222; Mm.4776	ACRV1	Acrosomal vesicle protein 1	Human, mouse
Hs.97542; Mm.50808	PLCZ1	Phospholipase C, zeta 1	Human, mouse
Hs.412818; Mm.56514	FAM71D	Family with sequence similarity 71, member D	Human, mouse
Hs.378893; Mm.61243	TTC29	Tetratricopeptide repeat domain 29	Human, mouse
Hs.388827; Mm.79070	RFX4	Regulatory factor X, 4	Human, mouse
Hs.398196; Mm.84725	MYCBPAP	MYCBP associated protein	Human, mouse
Hs.490715; Mm.87352	RNF32	Ring finger protein 32	Human, mouse
Hs.126780; Mm.87382	SEPT12	Septin 12	Human, mouse
Hs.388841; Mm.9500	*ZPBP	Zona pellucida binding protein	Human, mouse
Hs.320892; Mm.66952	Adcy10	Adenylate cyclase 10	Human, mouse
Hs.411239; Rn.160028	*KLKBL4	Plasma kallikrein-like protein 4	Human, rat
Hs.307086; Rn.162868	FSCB	Fibrous sheath CABYR binding protein	Human, rat
Hs.350671; Rn.209597	TSGA10IP	Testis-specific, 10 interacting protein	Human, rat
Hs.644625; Rn.215025	LRR1Q3	Leucine-rich repeats and IQ motif containing 3	Human, rat
Hs.435371; Rn.33200	*TCP11	T-complex 11 homolog	Human, rat
Hs.177959; Rn.42917	ADAM2	ADAM metalloproteinase domain 2	Human, rat
Hs.98848; Rn.42918	ADAM3A	ADAM metalloproteinase domain 3A	Human, rat
Hs.367245; Rn.51329	ZPBP2	Zona pellucida binding protein 2	Human, rat
Hs.240053; Rn.68598	GSG1	Germ cell associated 1	Human, rat
Hs.655170; Rn.95004	SPAG6	Sperm associated antigen 6	Human, rat
Mm.143889; Rn.92321	Adam5	A disintegrin and metalloproteinase domain 5	Mouse, rat
Mm.17413; Rn.219514	Fam178b	Family with sequence similarity 178, member B	Mouse, rat
Mm.189668; Rn.122594	Ubqln1	Ubiquilin-like	Mouse, rat
Mm.250465; Rn.26693	Lrrc46	Leucine rich repeat containing 46	Mouse, rat
Mm.255732; Rn.122669	*Txndc2	Thioredoxin domain containing 2	Mouse, rat
Mm.263615; Rn.123383	*Spesp1	Sperm equatorial segment protein 1	Mouse, rat
Mm.269223; Rn.170874	Adam32	A disintegrin and metalloproteinase domain 32	Mouse, rat
Mm.270295; Rn.123187	Tuba3b	Tubulin, alpha 3B	Mouse, rat
Mm.282330; Rn.27474	*Tekt4	Tektin 4	Mouse, rat
Mm.29788; Rn.47152	Slfn1l	Schlafen like 1	Mouse, rat
Mm.310201; Rn.163190	Tssk2	Testis-specific serine kinase 2	Mouse, rat
Mm.31708; Rn.3253	Fscn3	Fascin homolog 3, actin-bundling protein, testicular	Mouse, rat
Mm.432087; Rn.138402	Tcp10b	T-complex protein 10b	Mouse, rat
Mm.436562; Rn.64496	*Gapdhs	Glyceraldehyde-3-phosphate dehydrogenase, spermatogenic	Mouse, rat
Mm.439725; Rn.114052	Tssk1	Testis-specific serine kinase 1	Mouse, rat
Mm.442295; Rn.172733	Fbx113	F-box and leucine-rich repeat protein 13	Mouse, rat
Mm.52511; Rn.51791	Atp8b3	ATPase, class I, type 8B, member 3	Mouse, rat
Mm.61330; Rn.201753	Gtf2a1l	General transcription factor IIA, 1-like	Mouse, rat
Mm.67300; Rn.107052	Ankrd36	Ankyrin repeat domain 36	Mouse, rat
Mm.87067; Rn.27773	*Spata18	Spermatogenesis associated 18	Mouse, rat
Mm.87748; Rn.38091	*Akap3	A kinase anchor protein 3	Mouse, rat

Total of 92 genes were overlapped in human, mouse and rat testis (see Fig. 1), asterisk (*) indicated common genes in the human sperm proteome (ref. 16).

33 human testis-specific genes in this study were present in human sperm proteome which maybe play critical role in sperm biology. Integrated testis-specific genes from different species would provide supplementary information for insight into testicular physiology and functions.

RT-PCR analysis of thirteen randomly selected human genes showed that ten genes were exclusively expressed in the human testis, and three were weakly expressed in other organs which maybe have alternative splicing¹⁹⁾ or have too lower expression levels in other organs to be detected in transcriptome. Due to the limitation of tissue source, only 12 or-

gans from 18 organs were validated, but these results could also prove the authenticity of the results. Interestingly, two homolog genes to human from mouse and rat were not screened in the human ESTs, but exclusively expressed in human testis by RT-PCR detection. The results indicated that some homolog genes to human from mouse and rat could provide complementary information for human testis studies and contribute to a more comprehensive human testis-specific gene profiles.

All testis-specific genes were performed gene-enrichment and functional annotation analysis through DAVID tools, a comprehensive analysis of the results showed that human, mouse and rat testis-specific genes have some common functional enrichments which were mainly related to spermatogenesis and development process with significant Glycolysis and Pyruvate metabolism. Testicular cells are different from other somatic cell which has special missions (spermatogenesis and steroidogenesis). Therefore, testis-specific genes of different species also have features in common.

Interestingly, differential enrichment analysis of protein domains through "PIR_SUPERFAMILY" revealed some significant superfamilies related to sperm function and fertilization potentially. Septin 12 and 14 were significantly expressed in testis which belongs to a family of polymerizing guanosine 5'-triphosphate (GTP)-binding proteins that are required for many cellular functions, such as membrane compartmentalization, vesicular trafficking, mitosis and cytoskeletal remodeling,²⁰⁾ for example, septin12 is critical for

Fig. 1. Overlap of Testis-Specific Genes among Human, Mouse and Rat

The overlap of human (317), mouse (449) and rat (147) testis-specific genes are shown. The information of common genes in the three group are shown in Table 4.

Table 5. Thirty Three Genes Overlapped in Human Testis-Specific and Human Sperm Gene Database

Unigene	Gene name	Gene description	Chromosome
Hs.121494	SPAM1	Sperm adhesion molecule 1	7
Hs.123530	ACTL7A	Actin-like 7A	9
Hs.126838	ADAM29	ADAM metalloproteinase domain 29	4
Hs.129293	FAM71A	Family with sequence similarity 71, member A	1
Hs.143519	TEKT5	Tektin 5	16
Hs.149030	SLC25A31	Solute carrier family 25, member 31	4
Hs.151167	PPP3R2	Protein phosphatase 3, regulatory subunit B, beta isoform	9
Hs.155942	TRPM1	Transient receptor potential cation channel, subfamily M, member 1	15
Hs.179646	TMCO5A	Transmembrane and coiled-coil domains 5A	15
Hs.209206	ACTL9	Actin-like 9	19
Hs.236635	ACTRT2	Actin-related protein T2	1
Hs.307052	LDHAL6B	Lactate dehydrogenase A-like 6B	15
Hs.348618	TRIML1	Tripartite motif family-like 1	4
Hs.367727	PGK2	Phosphoglycerate kinase 2	6
Hs.388841	ZPBP	Zona pellucida binding protein	7
Hs.411239	KLKBL4	Plasma kallikrein-like protein 4	16
Hs.414648	TEKT3	Tektin 3	17
Hs.435371	TCP11	T-complex 11 homolog	6
Hs.511983	CABYR	Calcium binding tyrosine-phosphorylation regulated	18
Hs.522093	C9orf144B	Hypothetical protein LOC259308	9
Hs.525769	C15orf55	Chromosome 15 open reading frame 55	15
Hs.591011	FAM71C	Family with sequence similarity 71, member C	12
Hs.643584	IGSF10	Immunoglobulin superfamily, member 10	3
Hs.654377	LDHC	Lactate dehydrogenase C	11
Hs.662219	ATP1A4	ATPase, Na ⁺ /K ⁺ transporting, alpha 4 polypeptide	1
Hs.663128	ROPN1B	Ropporin, rhophilin associated protein 1B	3
Hs.666099	FAM71B	Family with sequence similarity 71, member B	5
Hs.67639	NUP210L	Nucleoporin 210 kDa-like	1
Hs.701555	TSSK1B	Testis-specific serine kinase 1B	5
Hs.723454	TXNDC3	Thioredoxin domain containing 3	7
Hs.97633	AKAP4	A kinase anchor protein 4	X
Hs.98008	GK2	Glycerol kinase 2	4
Hs.98712	TXNDC2	Thioredoxin domain containing 2	18

Human sperm gene database were referenced from human sperm proteome (ref. 16).

Fig. 2. Common Enrichment Differential Functions among Human, Mouse and Rat Testis-Specific Genes Analysis through DAVID

(A) PIR keywords; (B) KEGG pathways; (C) molecular functions; (D) biology processes. Vertical coordinates represent the p values. Horizontal ordinate represent statistically significant functions.

Fig. 3. RT-PCR Results of Thirteen Selected Testis-Specific Genes in Different Human Organs

mRNA extraction from 12 human organs were used to identify testis-specific genes expression; β -actin was the positive control; water instead of DNA was the negative control.

spermiogenesis.²¹⁾ Tektin family had been verified to be present in sperm flagella which may be involved in flagellar stability and sperm motility.²²⁾ In this study, four tektin genes were found including Tekt2 (Rat), Tekt5 (human), Tekt3 (human and mouse) and tekt4 (mouse and rat). A Disintegrin and Metalloprotease (ADAM) family members were expressed in male reproductive tissues which were involved in fertilization process.²³⁾ Some ADAMs in human (3 genes), mouse (9 genes) and rat (5 gene) were detected specifically in the testis, three ADAM genes were validated by RT-PCR.

These ADAMs genes maybe play important role in the sperm maturation process.

In conclusion, we present a systematic mapping of testis-specific genes from human, mouse and rat unigene libraries, the combination of testis-specific genes should serve as a source of reference for future research and clinical application. Further studies are warranted to substantiate the expression of mouse and rat homologue genes in human testis. Such studies will advance our understanding of testis physiology and function, also facilitate studies of sperm maturation.

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