Evolutionary study of thymic involution using comparative proteome data in mice

M. A. Japati and C. E. Liaw

Sinarmas World Academy, South Tangerang, Banten, Indonesia

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The atrophy of the thymus is found in most vertebrates, including humans. This process starts shrinking the thymus as early as the age of 1. This process is linked to the decline of free radical (molecules with unpaired electrons) defenses. Atrophy of the thymus happens more rapidly compared to other tissues and is considered as a sequence of stomal catalase deficiency in the context of a highly metabolic environment designed to support the demands of T-cell proliferation. Research led by Howard Petrie, senior study author of Scripps Research Institute, shows that thymic atrophy represents the process of accumulated cellular damage resulting from lifelong exposure to the oxidative byproducts of aerobic metabolism. Our team plans to analyse the mouse thymus genome set at different ages to see which distinct genomes are expressed differently and can lead us to potentially finding the specific cause of thymic involution. Through this, we aim to expand the research on the phenomenon and provide more research focused on how to stop thymic involution.

Keywords: Thymic involution, atrophy, mice, humans, naive T-Cells, ageing

Mentors: C. J. Hayden and P. Holec

Direct correspondence to 24evelynl@swa-jkt.com, pholec@mit.edu

Background

Introduction to the problem

Our immune system is crucial to protect us from harmful substances that enter our bodies. Without it, the chances of death increase and a decrease in the standard of living occurs. The thymus is a small, irregularly shaped gland near the mediastinum. It is part of the lymphatic and endocrine systems. The thymus is responsible for producing progenitor cells that mature to become thymus-derived cells (T-Lymphocytes). The process starts when our hematopoietic stem cells produce lymphoid progenitors (thymocytes) which are then transferred to the thymus to undergo the maturation process to antigen-independent T-cells with their unique markers including TCR, CD3, or CD4. In the thymus, t-cell precursors enter the subcapsular cortical area to the thymic stroma and undergo a process of proliferation and start differentiating which allows them to mature into distinct types of T-cells in our body. T-cells are responsible for killing infected host cells, activating immune cells, and regulating immune responses to pathogens. Through time, our body goes through a process called thymic atrophy which results in the shrinking of the thymus. By the age of 65, the thymus becomes almost non-existent in humans which makes vaccines less effective, creating a higher risk of contracting infectious diseases and in fact, scientists have found a correlation between the higher risk of cancer and thymic atrophy (Palmer et al., 2018).

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electrons) defenses. Atrophy of the thymus happens more rapidly compared to other tissues and is considered as a sequence of stomal catalase deficiency in the context of a highly metabolic environment designed to support the demands of T-cell proliferation. Research led by Howard Petrie, senior study author of Scripps Research Institute, shows that thymic atrophy represents the process of accumulated cellular damage resulting from lifelong exposure to the oxidative byproducts of aerobic metabolism.

The cause of thymic involution

Thymic involution is closely related to the loss of thymic epithelial space (TES) which is where thymopoiesis, the process where thymocytes turn into mature T-cells occurs, but there is, unfortunately, no universally accepted explanation as to why our thymus shrinks. One possibility suggests that this process may be an effect of antagonistic pleiotropy which is when we select genes whose benefits early in life outweigh costs late in life. Though the thymus is in charge of letting our body know which organisms are harmless and which are not, this can eventually lead to an easy target for any microbial parasites which can fool the immune system into thinking that they are harmless.

A new study has also identified a specific cell that may be involved in thymic involution (Sheridan et al., 2017). The research has found a cell named stromal progenitor which readily changes into fat cells and since the thymus increasingly turns into a mass of fat cells over time, there's a possibility that the stromal progenitor is what causes the involution.

Other causes of thymic involution that have been suggested include physiological stress such as pregnancies, fighting off infections, proinflammatory cytokines, hormones, steroids as well as treatments for cancerous cells. This results in compromised host immunity and decreased levels of T-lymphocyte output.

The process of thymic involution

When the thymus gland undergoes involution, thymic architecture becomes disorganized which results in reduced numbers of naive T-lymphocytes output. It can be observed using the Thymic Epithelial Space (TES) or the perivascular space (PVS). In humans specifically, the TES decreases at the start of one year old by 3% until middle-age (Steinmann et al., 1985). Other characteristics often observed during thymic involution include the declining of TEC-associated markers such as the major histocompatibility complex class-II (MHC-II) and also the altered rations of mTECs (medullary thymic epithelial cells) and cTECs (cortical thymic epithelial cells) which are both involved in different stages of thymocyte development. During thymus involution, fat cells build up to cover or replace lost thymic tissue.

Impacts of thymic involution

Thymic involution results in a reduced ability to produce new T-lymphocytes. The decline of immune systems and the production of T-lymphocytes are closely related to the dramatic increase in risks of cancer and infectious disease. Datasets analysed and published in PNAS show that the declining T cell production leads to an increase in disease incidence and assumes that immunogenic cells arise with the same probability at any age (Smith et al., 2019). This also makes vaccine efficacy rates lower in those aged 65 and above compared to those aged 30 to 40.

From an economical point of view, with more diseases and a sick population, people with a low financial status will not be able to look for treatments and therefore, increase the death rate of a country. The sick population is also directly related to the decrease in a country's productivity as they will not be able to work and contribute to the country's economy. The impacts of thymic involution go further than just causing a person to be more susceptible to diseases, it impacts our society economically and socially.

Exploring present research

A study published in the Journal of Immunology (Sutherland et al., 2005) described the effects of sex steroids and thymic involution through analysing prostate cancer patients who routinely underwent sex steroids ablation therapy called the LHRH-A treatment. They've found that after 4 months of treatment using the serum testosterone concentration, there is an increase in the total lymphocytes, T-cells (mostly $CD4^+$), and NK cells. More detailed observations found that there is a drastic increase in the numbers of naive $CD4^+$ T-cells with a p-value less than 0.05. This research shows evidence of sex steroids in the development of the thymus and a potential impact as the cause of thymic involutions.

Our approach and idea

The problem we are trying to solve is thymic involution in humans. We plan to do this by researching mice genes and their effect on thymic atrophy by using "abnormal" T-Test p-values defined by p-values below 0.05. This data report will help support this process by identifying potential genes of interest that may hold significance in the research of thymic involution.

Materials & Methods

Materials

We started this research by exploring the data sets available on the internet that are valuable and relevant to our research. Data sets from the AGEMAP data spreadsheet on thymus were collected and we chose this because of its completeness with mice ranging from different ages, the changes in every cell and it is specified on the thymus. Other than the datasets, we mainly use google sheets to create diagrams and perform our unigene analysis.

Basic T-tests

T-Tests were conducted on females months 1 and 6, females months 16 and 24, males months 1 and 6, and males months 16 and 24. A T-test value below 0.05 was considered as "significant" unigene type. T-tests are a type of inferential statistics which are used to determine if there is a significant difference between the mean of the 2 groups. It is a test often used for hypothesis testing. Through this, we eliminate cell changes expressed which are insignificant or that show no correlation to the possible involution of the thymus. The unigenes for each data set were then collected and a histogram was created to identify whether or not the unigene had recurred throughout the 4 data sets on t-Tests.

With these collected genes that fit the standard criteria for p-value, we monitored the changes in the unigene expression and further identified which of these unigenes had major variations.

Results

Out of the 1688 unigene types that fit the criteria of a T-test with the p-value below 0.05 (the standard) which means that there is a 95% chance that this occurrence is not caused by chance. However, this also means that there is a 5% chance that the specific genes aren't relevant in the contribution of thymic involution.

From all 4 data sets, **7 unigenes recurred thrice** (0.4%) and **154 unigenes recurred twice** (9.1%). No unigenes recurred four times.

Key: Those highlighted in cyan recurred twice *and green recurred* thrice*.*

This table only contains mouse unigenes and their corresponding gene names for those that have recurred twice. Full annotated data set can be downloaded here: https://drive.google.com/file/d/1izMlIEmv76y7myrEBv-MEap8JcDdf18z/view?usp=sharinghttps://drive.google.com/file/d/1izMlIEmv76y7myrEBv-MEap8JcDdf18z/view?usp=sharing

Gene Analysis (based on available data on immune systems, ageing and mortality)

Contrary to all the genes found on an online database, collected by Mouse Genome Informatics, that contained phenotypes and genetic marker data for mortality/ageing and the immune system and recurred thrice in our data collection (gene names highlighted in green), gene type Vamp8, gene type 8430410A17Rik (also known as Hmces) and gene type Ap2s1 were related to premature death, postnatal lethality and/or embryonic lethality (both complete and incomplete).

Vamp8 genes on the immune system are seen to have abnormal mast cells. There is also a significant reduction in beta-hexosaminidase release and serotonin secretion Cathepsin D secretion. Vamp8 genes in mice are also linked with kidney atrophy and increased blood osmolality as well as premature death. No mice in the Mouse Genome Informatics study survived more than 10 months. The 8430410A17Rik gene (also known as Hmces gene) is linked to abnormal epigenetic regulation of gene expression. It also comes with abnormal DNA methylation. Mice with this gene type come with low postnatal weight. It also comes with increased chances of embryonic lethality. The Ap2s1 gene was linked to increasing embryonic lethality. The Ap2s1 gene was also linked to gastrulation failure. It also comes with a decreased embryo size. This gene also results in an enlarged heart in Ap2s1 gene mice.

Among the genes with gene ontology info given by the AGEMAP database, Vamp8, Ap2s1, Uhrf1, and Yif1 came with gene ontology information. The cellular component of the Vamp8 gene is the early endosome. Its cellular component is integral to the membrane. Its molecular function is protein binding and its biological process is vesicle-mediated transport. The cellular component of the Ap2s1 gene is an Assembly polypeptide-2 (AP-2) adaptor complex. Its biological process and molecular function are so far unknown. The cellular component of the Uhrf1 gene is having a nucleus. Its molecular function is DNA binding and transporter activity. Its biological process is cell proliferation and regulation of transcription and transport. The cellular component of the Yif1 gene is that this gene is integral to the membrane.

Conclusion

To conclude our study, there were 1688 unigenes with a t-test p-value below 0.05. Among the 1688 genes

Table 1: *Shows mouse unigenes and their corresponding gene names for those that have recurred twice*

No	Mouse Unigene	Gene Name	No	Mouse Unigene	Gene Name	No	Mouse Unigene	Gene Name
1	Mm.102278	Scamp5	55	Mm.24937	1500001M20Rik	110	Mm.314113	Elovl6
2	Mm.10665	Tex292	56	Mm.252405	Mgl1	111	Mm.315430	2610524G07Rik
3	Mm.10946	Ccdc60	57	Mm.25259	Npr3	112	Mm.32009	5830406J20Rik
4	Mm.11934	Zar1	58	Mm.253403	1810073N04Rik	113	Mm.32012	5730507H05Rik
5	Mm.12459	Ankrd10	59	Mm.255649	Eif4enif1	114	Mm.320469	Pan3
6	Mm.1262	Cyp17a1	60	Mm.25608	1110057K04Rik	115	Mm.321227	Trim21
7	Mm.131118	Mm.131118	61	Mm.258969	Unknown	116	Mm.323997	Rbpms
8	Mm.133851	Mrpl12	62	Mm.259197	Rbm5	117	Mm.328360	4631422O05Rik
9	Mm.1363	Trh	63	Mm.25921	AU015558	118	Mm.330803	Gm1305
10	Mm.137134	Tiam2	64	Mm.259969	BC003322	119	Mm.333594	BC057627
11	Mm.139214	Unknown	65	Mm.260103	D030051D21	120	Mm.333597	Ap2s1
12	Mm.146984	Psmf1	66	Mm.260712	A930025J12Rik	121	Mm.335866	C330019G07Rik
13	Mm.155583	Il1rl2	67	Mm.260786	Rad54l	122	Mm.339755	Fliih
14	Mm.157442	Hgd	68	Mm.26468	4632434I11Rik	123	Mm.34108	Rragd
15	Mm.158361	Phactr4	69	Mm.272551	Cnot7	124	Mm.341747	6330442E10Rik
16	Mm.159681	AW060207	70	Mm.272930	Cog4	125	Mm.344071	1700001M19Rik
17	Mm.16925	Thy28	71	Mm.274492	2410002F23Rik	126	Mm.349603	B130036O03
18	Mm.169673	AI642036	72	Mm.27477	2010100O12Rik	127	Mm.353059	Unknown
19	Mm.172634	C77488	73	Mm.275583	AA409802	128	Mm.353936	Syncrip
20	Mm.182671	Mm.182671	74	Mm.275586	9430095K15Rik	129	Mm.36885	Leng4
21	Mm.182737	Smc1l2	75	Mm.276389	Hmox1	130	Mm.371592	Ubb
22	Mm.1838	Vamp8	76	Mm.279782	Prdx5	131	Mm.371613	D4Wsu43e
23	Mm.193099	Fn1	77	Mm.280544	Ddx52	132	Mm.37581	1700013G20Rik
24	Mm.196290	5830411E10Rik	78	Mm.280559	BC022145	133	Mm.379060	C330027G06Rik
25	Mm.197518	Laptm4b	79	Mm.28209	Perp	134	Mm.379221	1700040I03Rik
26	Mm.20108	1200011D03Rik	80	Mm.283406	2810433K01Rik	135	Mm.379369	Kctd3
27	Mm.206206	AU040320	81	Mm.28437	2700038L12Rik	136	Mm.38055	Es10
28	Mm.209300	Pcyt1a	82	Mm.284770	Lypla3	137	Mm.381181	AI462493
29	Mm.20973	Lgals7	83	Mm.28484	Tspan3	138	Mm.3815	Sdc4
30	Mm.209941	Mtl5	84	Mm.28560	Lyar	139	Mm.38166	2610203C20Rik
31	Mm.210197	9530026P05Rik	85	Mm.286061	Tars	140	Mm.382329	Mm.382329
32	Mm.210447	LOC114601	86	Mm.288693	D14Wsu89e	141	Mm.383308	BC038943
33	Mm.211654	MGC6357	87	Mm.288728	Srpk2	142	Mm.384234	Ube2i
34	Mm.212066	Abi2	89	Mm.288788	Rev3l	143	Mm.386783	Ikbke
35	Mm.21414	Ccs	90	Mm.28924	1810008A14Rik	144	Mm.3996	Chuk
36	Mm.21482	Clns1a	91	Mm.289583	C920003I06	145	Mm.41220	Btbd9
37	Mm.219684	AA939927	92	Mm.289645	Gtl2	146	Mm.41715	5330440M15Rik
38	Mm.22239	Ap1m2	93	Mm.289914	D1Ertd396e	147	Mm.4206	4432417N03Rik
39	Mm.223508	Glis3	94	Mm.290026	Cltb	148	Mm.42196	Uhrf1
40	Mm.227983	1110059P08Rik	95	Mm.290414	Mfn1	149	Mm.43278	Olfm1
41	Mm.22847	Cobl	96	Mm.29071	Nek9	150	Mm.43636	Zfp289
42	Mm.229532	Ednrb	97	Mm.290876	Sod2	151	Mm.44202	Yif1
43	Mm.230853	D330050I23Rik	98	Mm.291005	Mm.291005	152	Mm.49041	0610006K04Rik
44	Mm.233799	Igfbp4	99	Mm.291192	Tmem59	153	Mm.52312	5330438E18Rik
45	Mm.234823	1200015K23Rik	100	Mm.29133	Bub1b	154	Mm.5915	2410012M04Rik
46	Mm.23951	Gmppa	101	Mm.294783	Bclaf1	155	Mm.60224	Mphosph9
47	Mm.241489	5830416A07Rik	102	Mm.295767	Unknown	156	Mm.6390	Grcc2f
48	Mm.245210	Lrig1	103	Mm.297109	Nf2	157	Mm.6710	Rock1
49	Mm.245340	Atrnl1	104	Mm.297371	Pou3f1	158	Mm.795	Csf1
50	Mm.2462	Psmc2	105	Mm.29824	Psa	159	Mm.833	Ap1s1
51	Mm.246241	8430410A17Rik	106	Mm.29845	Suclg1	160	Mm.87513	4932417I16Rik
52	Mm.246952	Xrcc5	107	Mm.29997	Rcn3	161	Mm.9935	Arhgap28
53	Mm.24788	2310047M15Rik	108	Mm.30084	Np15			
54	Mm.248779	4932434G09Rik	109	Mm.30155	Atp6v0c			

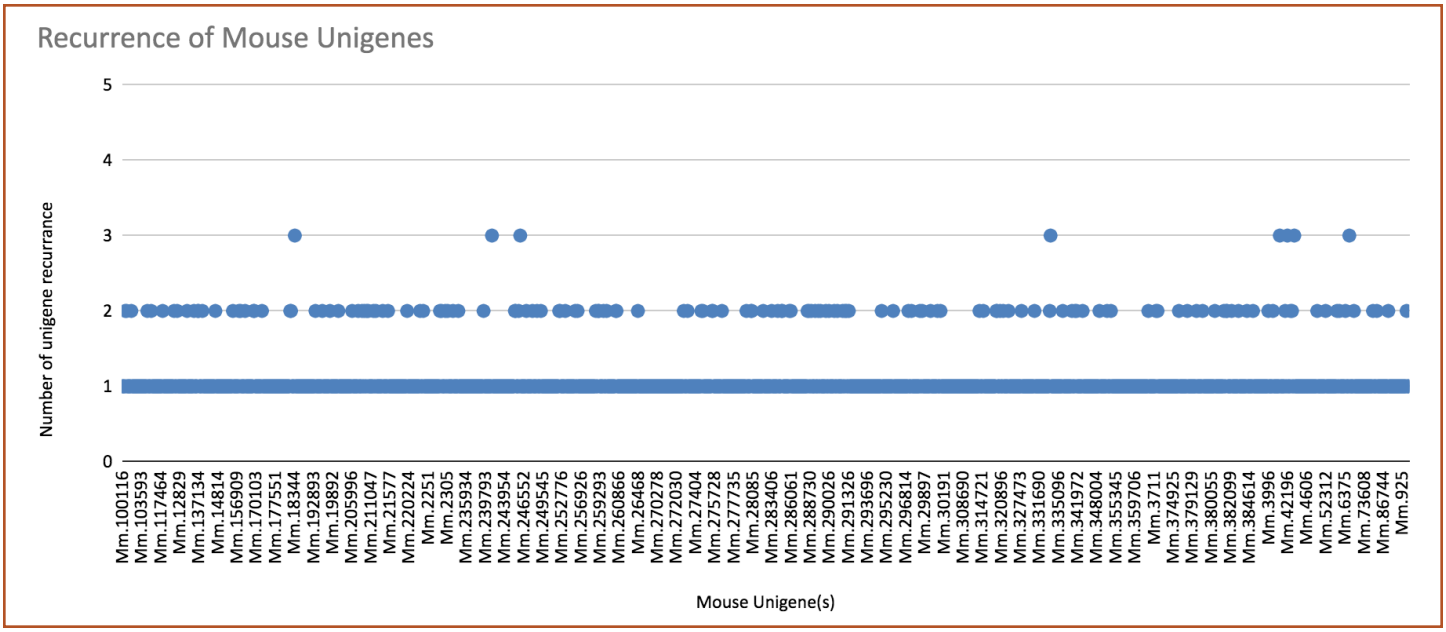

Figure 1: ***Scatter Plot Histogram of Recurrence of Mice Unigenes***

Further Reading: Vamp8; 5830416A07Rik; 8430410A17Rik; Ap2s1; 5330440M15Rik; Uhrf1; Yif1

that spanned across female mouse 1 and 6 months, female mouse 16 and 24 months, male mouse 1 and 6 months, and male mouse 16 and 24 months, 154 unigenes recurred twice and 7 unigenes recurred thrice. No unigenes recurred four times (refer to the scatter plot histogram, Figure 1). Data analysis was done on the unigenes that recurred thrice, which included genomes Vamp8, 5830416A07Rik, 8430410A17Rik, Ap2s1, 5330440M15Rik, Uhrf1, and Yif1. Further findings done on these unigenes/genomes showed that these genomes are also linked with premature death and/or embryonic lethality. However, no clear statement was given by the databases as to how these factors affect the rate of thymic involution.

Discussions

Limitations

As mentioned above, our report is strictly based on the AGEMAP unigene data in mice presented by professors of Stanford University and scientists from the National Institute on Aging at the National Institutes of Health, Baltimore. Although experiments have been done through rodents such as mice, it does not imply that these gene changes apply to humans as human genes are greatly more complex and hard to understand.

Furthermore, experiments in mice were under scrutiny for a few years due to repeated failures of valid data and conclusions. However, in recent years, studies have shown that these data failures are not mainly caused by the mice themselves, but by improper data analysis (Justice & Dhillon, 2016). Therefore, using the unigenes of mice may affect the results, though there is a low chance of this happening.

The purpose of the t-test is to find changes with a p-value less than 0.05 which indicates that there is at least a 95% chance of the changes found being relevant and significant but still a 5% chance of it being irrelevant and completely unrelated. Therefore, this unigene expression is not completely relevant to finding the true genes that may reduce/increase the rate of thymic involution, though it still has a high chance of it being a potential gene that affects the rate of thymic involution.

In addition, the data sets we analysed consist of datasets of mice ranging from 1 month - 24 months. Although this could provide a conclusion, the sample size is still too small to provide a reliable study. To improve this for the next experiment, the data sample size should be increased to improve the reliability of this study.

Benefits

The datasets that were collected were from a reliable website and are collected by professors of Stanford University and scientists from the National Institute on Aging National Institutes of Health Baltimore. It is from

a well-known and reliable source which improves the reliability of this report.

Our report provides possible genes that contribute to the process of thymic involution which will, in the future, help further experiments in streamlining which unigenes should they look for. This will direct the attention of the scientific community into focusing on these genes and help us understand better the process of thymic involution and the causes of this phenomenon affecting most vertebrates.

Next Steps

The unigenes and genomes of mice that have the potential due to its high recurrence (displays commonality between different mice genders and ages) of unigenes/genomes with T-Test p-values below 0.05 show gene "abnormality". These unigenes and their respective genomes can be used in further research or scientific experiments to identify whether this "abnormality" affects thymic involution or the rate of the atrophy of the thymus.

Potential experiments using synthetic biology in ways such as inserting this gene into a bacteria to see the way it affects microorganisms can be used to further explore what the specific gene does to our biological system.

Author Contributions

C.E.L. Contributed to data collection of potential gene mutations/variations in the thymus of mice. Contributed to the abstract, background and contributed to the methodology. M.A.J. Contributed to data tabulation of potential gene mutations/variations in the thymus of mice. Contributed to the abstract, background and contributed to the methodology.

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