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ALZHIEMER MYSTERY AND Y CHROMOSOME

NEHA GUPTA*[1] AND VARUN JAISWAL[2]

1 School of Biotechnology, Shoolini University, Solan, Himachal Pradesh, India
2 School of Electrical and Computer Science Engineering, Shoolini University, Solan, Himachal Pradesh, India

ABSTRACT

Alzheimer Disease (AD) is a neurodegenerative disorder with unknown etiology even after 110 years of its discovery. Etiology of AD is different in male and female and female brain is more susceptible to AD but underlying molecular mechanism is unknown. Current study aims to find Y chromosome genes associated with AD incidence. Analysis of genes differentially expressed in male and female AD samples may be helpful to identify causes of AD etiology. Genes encoding cell adhesion molecules, protocadherin (PCDH11Y) and neuroligin (NLGN4Y), were found to be involved in AD. Beside protein coding genes, antisense RNA and pseudogene were also differentially expressed. This is the first study focusing Y chromosome transcriptome in context to the AD disease. Differentially expressed genes were suggested to be associated with AD in previous studies justifies the current approach. These genes if explored more will aid in understanding of AD and design better therapeutics.

KEYWORDS: ***Alzheimer, neuroligin, transcriptome, sex, differential***

NEHA GUPTA

School of Biotechnology, Shoolini University, Solan, Himachal Pradesh, India

Corresponding Author

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INTRODUCTION

Alzheimer, prime cause of dementia, even after a century of its discovery has no cure. With highly complex etiology AD stands unfathomable to the scientific community all across the globe. It is an irreversible, progressive disorder which is characterized by gradual loss of cognitive functions including behaviour, memory, thinking and recognition. In 2015, throughout the world, approximately 46.8 million people were living with dementia. It is speculated to reach a score of 131.5 millions by 2050 and going to be a global epidemic by then.[1] Total estimated worldwide cost of dementia in 2018 will be US$ 1 trillion.[2] It is the third killer among elderly population in the United States and also is listed in top ten causes of death. The two most identifying pathological features of this disease include presence of senile plaque deposits and neurofibrillary tangles (NFTs). Senile plaques are the accumulation of amyloid beta (Aβ) peptide inside the neurons, whereas, NFTs are the aggregated protein consisting of microtubule associated phosphorylated tau protein in the extracellular spaces.[3] Since various causative factors are linked to AD it is christened as a multifactorial disease. Major risk factors include advancing age, gender, lifestyle, family history, ApoE allele e4, environmental factors, smoking, high cholesterol, high blood pressure, diabetes and obesity. Sex and gender has gathered interest as one of the significant risk factor for developing AD apart from age.[4] Women comprises of almost two-thirds of American seniors living with AD [2]. A recent study have added to possible gender difference in risk of developing AD by finding that brain's cognitive reserve is reduced in women.[5] Sex hormones and genetic differences (X and Y chromosomes), play significant role in sexual dimorphism of human brain affecting neurovascular development, neurogenesis and synaptogenesis.[6] According to a meta-analysis of 13 population studies from United States, Europe, and Asia women are at significantly greater risk of developing AD.[7] Sex has been observed to play a significant role in AD pathology and disease progression also women and men are differently affected by AD.[8] Many studies have put forward new evidence supporting that women's brains are more vulnerable than men's brains to AD at Alzheimer's Association International Conference 2015.[9] Estrogen has potential to contrast the neurodegenerative processes that characterizes AD.[10] In humans estrogen replacement therapy has been found to have neurologic benefits which include reversal of estrogen deficiency-induced memory dysfunction and reduced risk of Alzheimer's disease.[11] Neuroprotective role of estrogen has also been ascertained by preventing mitochondrial damage.[12] Their exist a dire need to study various factors associated with AD in order to have better knowledge about it's etiology and pathogenesis. How sex and gender affects AD etiology needs to be studied more and demands attention as little is known about this disease. Why females are more susceptible to AD is not clear. Gender biasness of AD offers plethora of opportunities in order to unravel the Alzheimer mystery. NGS has many advantages over other sequencing technologies and best for differential gene expression studies. The current study aims to explore gene targets associated with human Y chromosome in order to answer the hypothesis that AD affects men and women differently. This study aims to explore Y chromosome genes differentially expressed in AD samples from different regions of brain. It would be helpful in the better understanding of the disease and exploring therapeutic targets for AD.

MATERIAL AND METHODS

Data collection

Y chromosome genes were listed as Y genes (Table1 Supplementary Data). Sequences and coordinates of these genes were extracted from National Centre for Biotechnology Information (NCBI). For expression analysis whole transcriptome data of AD reported in literature was taken.[13] The transcriptome sequence (SRA027308.2) was retrieved from Sequence Read Archive (SRA) of NCBI (Table 1). The six samples of frontal, temporal and total brain tissue of both AD and healthy brains were taken for the study.

Expression Analysis of sex associated genes in AD and control samples

Whole transcriptome analysis pipeline (Figure. 1) was used to analyse the expression of Y chromosome associated genes in RNA Seq data (Table 1). Galaxy NGS platform[14] was used for RNA-Seq and further differential gene expression analysis.

Fastq manipulation and Quality Check

FASTQ Groomer version 1.0.4 was used to convert RNA-Seq reads into fastqsanger format.[15] FastQC read quality reports (Version 0.65) and poses quality control checks.[16] The results obtained from QC analyses were used to determine whether or not the data can be further processed.

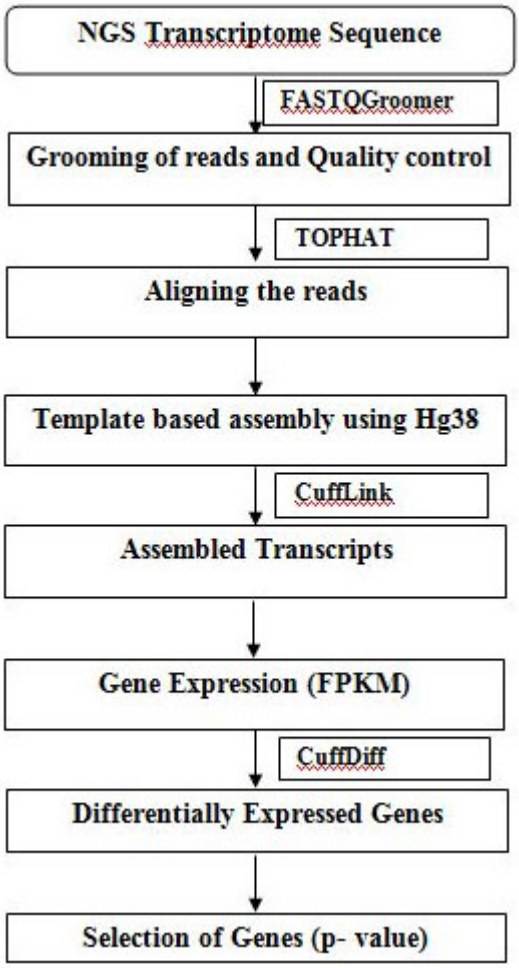

Figure 1
Whole transcriptome analysis pipeline

Table 1
AD transcriptome data

SRA ID	Library Name	Symbol
SRR085725	normal human whole brain	WN
SRR087416	Human Alzheimer's disease total brain mRNA	WD
SRR085473	polyA mRNA from Alzheimer's Disease brain temporal lobe	TD
SRR085471	Human normal brain temporal lobe mRNA	TN
SRR085726	Alzheimer's disease brain frontal lobe	FD
SRR085474	normal frontal lobe	FN

Transcriptome data comprising control and diseased samples from different brain region

Mapping of RNA-Seq reads and assembly of transcripts

Tophat Gapped-read mapper for RNA-Seq data version 2.1.0 was used to map RNA-Seq reads by aligning them to reference human genome (Hg38). Mapped files were processed by Cufflink (Galaxy Version 2.2.1.0).[17] The software assembles transcript to the reference genome (Hg38) and examines the abundance of transcripts. The abundance was calculated with respect to FPKM (Fragments per Kilobase of exon model per million Mapped reads) estimates for RNA-Seq data.

Differential Expression analysis of genes in AD samples

This classified each transcript as known or novel. Then Cuffdiff algorithm was used to calculate differential gene expression (DGE) in the samples. It was used to perform three pairwise comparisons of expression between normal and diseased samples from temporal, frontal and total brain regions. Relative abundance of transcripts was calculated as FPKM value. Expression (abundance) value of each gene was calculated as summation of FPKM values of all the associated transcripts. Self developed perl script was used to find DGE of sex associated genes in the RNA-Seq analysis output. The p-value cut-off was kept at < 0.05. Genes, up-regulated and down-regulated in the samples taken for the study were tabulated.

RESULT AND DISCUSSION

Susceptibility to neurodegenerative disorders, brain cognition ability, brain structure and behaviour sex and gender differences have been reported.[18-20] Total 529 genes encoded by Y chromosome were listed as Y

chromosome associated genes (Table1 Supplementary Data). These genes were taken for further analysis (Figure. 1).

RNA Seq Analysis

RNA-Seq pipeline of next generation sequencing (NGS) was used and finally high mapping percentage (more than 95%) in all six SRA datasets was achieved. To assess the quality of mapping of reads to the reference genome (Hg38) by reference based assembly the mapping percentage was compared to earlier studies (Table 2). Tophat mapped RNA Seq reads to the reference genome with 96-98% of mapping.

Table 2
RNA Seq reads mapped to Hg38 by TopHat

	FN	FD	TN	TD	WD	WN
Total reads	15772947	15228832	15256752	14227702	14720816	13442077
Mapped reads	15276636	15276636	14809336	13560187	14253449	12972477
%Mapping	96.9	96.2	97.1	95.3	96.8	96.5

percentage of reads mapped uniquely to reference genome in transcriptome data.
F- frontal lobe , T – temporal lobe, W- whole brain, N- normal, D- diseased

Differentially expressed genes

DGE of sex-associated genes in RNA-Seq data of AD samples was analysed. On the basis of p-value significance ($p<0.05$) in Y chromosome 5 genes were DGE in frontal lobe and 3 genes were DGE in whole brain AD samples. Genes found to be differentially expressed on the basis of p-value significance ($p<0.05$) in frontal lobe and whole brain are depicted in Figure 2 and 3.

Differentially Expressed genes in frontal lobe

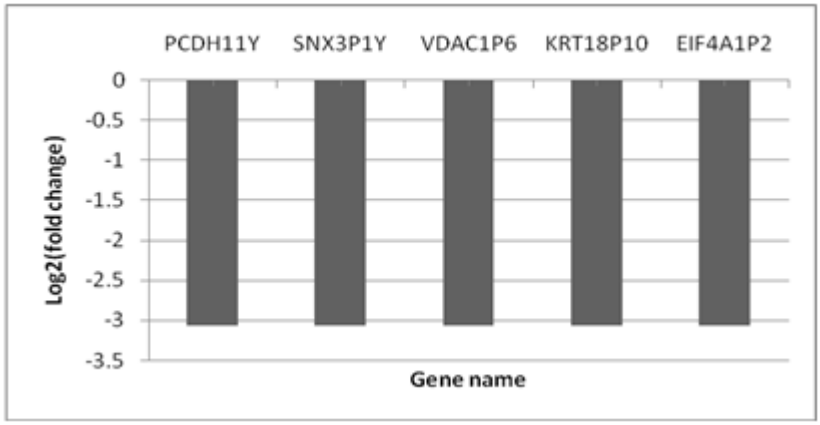

Figure 2
Genes differentially expressed in AD frontal lobe (P< 0.05)

Differentially Expressed genes in whole brain

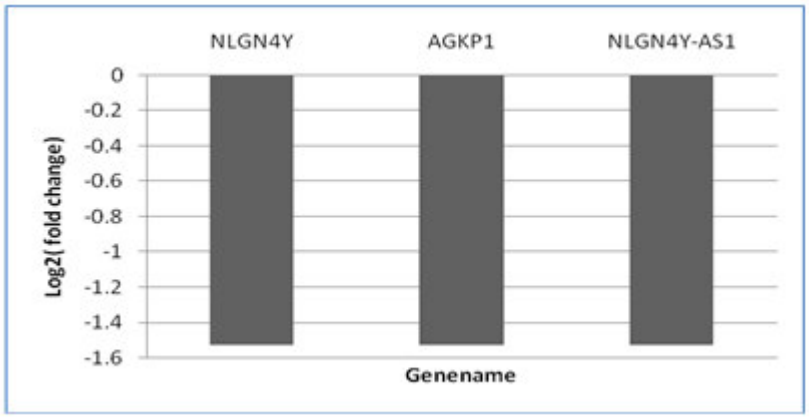

Figure 3
Genes differentially expressed in AD whole brain

In temporal lobe Taxilin gamma psuedogene, Y linked (*TXLNGY*) was found to be differentially expressed with infinite fold change ($p<0.09$). In all the AD samples only two protein coding genes, *PCDH11Y* and *NLGN4Y*,

were DGE. Most of the genes were down-regulated suggesting repressed transcriptional activity of AD brain which was also supported by earlier studies.[13] PCDH11Y gene encodes transmembrane cell adhesion molecules of the δ1-protocadherin family[21] expressed predominantly in the brain. This protein is thought to play a role in cell-cell recognition during development of the central nervous system[22]. PCDH are neuronal cell-surface proteins that bind in a homophilic manner to each other and form symmetric intercellular junctions. The role of neuronal cell adhesion molecules including neuroligins (NLGN) and protocadherins (PCDH)—in psychiatric disorders was recently evidenced by [Jamain et al., 2003, Bray et al., 2002]. Presence of neurological defects in individuals with sex chromosome aneuploidy, Klinefelter's syndrome[23] and Turner's syndrome[24] supports the idea that genes having locus on sex chromosome have significant effect on brain functions like language and cognition[25] and structure[26]. Loss of synapses in brain neural circuits is one of the earliest signs of AD[27]. Many studies have stated repercussion of Aβ induced toxicity in AD to the synapse loss.[28,29] AD pathogenesis leads to dysfunction of synaptic cell adhesion molecules and it has been reported that APP has significant role in maintaining synaptic morphology and plasticity.[30] Neuroligin4 Y linked (NLGN4Y) was found to be down-regulated in AD samples. Role of neurexin (NRXN)-binding ligand i.e neuroligins have been ascertained in mammalian brain. Neuroligin comprises of post-synaptic transmembrane proteins and out of those *NLGN4Y* is the gene located on the Y chromosome[31] involved in neurodegenerative disorders. Neuroligins (NLGN) are the transmembrane proteins that possess domains for cholinesterase, lipase a carboxylesterase activity but lack one or more of the residues that are essential for catalytic activity. NRXN-NLGN interaction leads to formation of synapse and signal transduction. In mice, knockout studies of NRXN-NLGN adhesion complex, behaviour anomalous to autism in humans was observed[32]. These studies support the current findings that expression of NLGN4Y is reduced in AD. Among the DGE only two genes were protein coding genes and remaining belongs to the category of psuedogene, anti sense RNA (Figure. 2 and 3). Noticeable number of these non- protein coding genes (non-pcg) is DGE stating their significance in disease progression. Majority of studies ignore these genes over the protein coding counter parts thereby little is known about these genes with respect to AD. These non protein coding genes need to be explore more to know how these operate at cellular and molecular level in order to have promising therapeutics and treatment for AD.[33] Also antisense transcripts and micro RNA are studied for their role in gene expression regulation in AD samples and their potential for future treatment of AD.[34, 35]

CONCLUSION

This was the first ever Y chromosome based study carried out on Alzheimer transcriptome. Genes found to be differentially expressed in AD samples were found to have significant role in AD etiology and suggests studies focussing on these molecules and genes i.e. *NLGN4Y* are suggested. Loss of neurons and dysfunction of synapse is found to be responsible for AD etiology which implicates role of neural cell adhesion molecules in neurological disorders. Understanding how these molecules are responsible for neuronal functioning and signaling would be highly intriguing and help in deciphering the AD mystery. These genes should be further explored for their detailed role in Alzheimer's and could be the promising therapeutic targets for the disease.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Against Dementia GA. G8 Dementia Summit. UK; 2013.
2. Association As. 2016 Alzheimer's disease facts and figures. Alzheimer's & Dementia. 2016;12(4):459-509.
3. Bertram L, Lill CM, Tanzi RE. The genetics of Alzheimer disease: back to the future. Neuron. 2010;68(2):270-81.
4. Viña J, Lloret A. Why women have more Alzheimer's disease than men: gender and mitochondrial toxicity of amyloid-β peptide. J Alzheimers Dis2010;20(S2):527-33.
5. Laws KR, Irvine K, Gale TM. Sex differences in cognitive impairment in Alzheimer's Disease. World J Psychiatry 2016;6(1):54.
6. Nugent BM, Tobet SA, Lara HE, Lucion AB, Wilson ME, Recabarren SE, et al. Hormonal programming across the lifespan. Horm Metab Res. 2012;44(08):577-86.
7. Gao S, Hendrie HC, Hall KS, Hui S. The relationships between age, sex, and the incidence of dementia and Alzheimer disease: a meta-analysis. Arch Gen Psychiatry1998;55(9):809-15.
8. Carter CL, Resnick EM, Mallampalli M, Kalbarczyk A. Sex and gender differences in Alzheimer's disease: recommendations for future researchJ Womens Health. 2012;21(10):1018-23.
9. Mason V. Alzheimer's Association International Conference on Alzheimer's Disease 2015 (AAIC 2015);2015
July 18-23; Washington, DC, USA: Drugs of today (Barcelona, Spain: 1998); 2015.p. 447-52.
10. Musicco M. Gender differences in the occurrence of Alzheimer's disease. Funct Neurol 2009;24(2):89.
11. Brinton RD. Cellular and molecular mechanisms of estrogen regulation of memory function and neuroprotection against Alzheimer's disease: recent insights and remaining challenges. Learn Mem. 2001;8(3):121-33.
12. Mielke MM, Vemuri P, Rocca WA. Clinical epidemiology of Alzheimer's disease: assessing sex and gender differences. Clin Epidemiol. 2014;6:37-48.

13. Twine NA, Janitz K, Wilkins MR, Janitz M. Whole transcriptome sequencing reveals gene expression and splicing differences in brain regions affected by Alzheimer's disease. PloS one. 2011;6(1):e16266.
14. Giardine B, Riemer C, Hardison RC, Burhans R, Elnitski L, Shah P, et al. Galaxy: a platform for interactive large-scale genome analysis. Genome Res. 2005;15(10):1451-5.
15. Blankenberg D, Kuster GV, Coraor N, Ananda G, Lazarus R, Mangan M, et al. Galaxy: a web-based genome analysis tool for experimentalists. Curr Protoc Mol Biol2010;19(10):1-21.
16. Andrews S. FastQC: A quality control tool for high throughput sequence data. Reference Source. 2010.
17. Trapnell C, Roberts A, Goff L, Pertea G, Kim D, Kelley DR, et al. Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nat Protoc. 2012;7(3):562-78.
18. Cosgrove KP, Mazure CM, Staley JK. Evolving knowledge of sex differences in brain structure, function,and chemistry. Biological psychiatry. 2007;62(8):847-55.
19. Cahill L. Why sex matters for neuroscience. Nat Rev Neurosci2006;7(6):477-84.
20. Trabzuni D, Ramasamy A, Imran S, Walker R, Smith C, Weale ME, et al. Widespread sex differences in gene expression and splicing in the adult human brain. Nat Commun. 2013;4.
21. Priddle TH, Crow TJ. Protocadherin 11X/Y a human-specific gene pair: an immunohistochemical survey of fetal and adult brains. Cereb Cortex2013;23(8):1933-41.
22. Durand CM, Kappeler C, Betancur C, Delorme R, Quach H, Goubran-Botros H, et al. Expression and genetic variability of PCDH11Y, a gene specific to Homo sapiens and candidate for susceptibility to psychiatric disorders. American Journal of Medical Genetics Part B: Neuropsychiatr Genet. 2006;141(1):67-70.
23. Visootsak J, Graham JM. Klinefelter syndrome and other sex chromosomal aneuploidies. Orphanet J Rare Dis 2006;1(1):42.
24. Kesler SR, Haberecht MF, Menon V, Warsofsky IS, Dyer-Friedman J, Neely EK, et al. Functional neuroanatomy of spatial orientation processing in Turner syndrome. Cereb Cortex2004;14(2):174-80.
25. Itti E, Gaw Gonzalo IT, Boone KB, Geschwind DH, Berman N, Pawlikowska-Haddal A, et al. Functional neuroimaging provides evidence of anomalous cerebral laterality in adults with Klinefelter's syndrome. Ann Neurol. 2003;54(5):669-73.
26. Itti E, Gaw Gonzalo I, Pawlikowska-Haddal A, Boone K, Mlikotic A, Itti L, et al. The structural brain correlates of cognitive deficits in adults with Klinefelter's syndrome. J Clin Endocrinol Metab 2006;91(4):1423-7.
27. Scheff SW, Price DA. Synaptic pathology in Alzheimer's disease: a review of ultrastructural studies. Neurobiol Aging2003;24(8):1029-46.
28. Wilcox KC, Lacor PN, Pitt J, Klein WL. Aβ oligomer-induced synapse degeneration in Alzheimer's disease. Cell Mol Neurobiol. 2011;31(6):939-48.
29. Pham E, Crews L, Ubhi K, Hansen L, Adame A, Cartier A, et al. Progressive accumulation of amyloid-β oligomers in Alzheimer's disease and in amyloid precursor protein transgenic mice is accompanied by selective alterations in synaptic scaffold proteins. FEBS journal. 2010;277(14):3051-67.
30. Klevanski M, Herrmann U, Weyer SW, Fol R, Cartier N, Wolfer DP, et al. The APP intracellular domain is required for normal synaptic morphology, synaptic plasticity, and hippocampus-dependent behavior. J Neurosci 2015;35(49):16018-33.
31. Jamain S, Quach H, Betancur C, Råstam M, Colineaux C, Gillberg IC, et al. Mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4 are associated with autism. Nature genetics. 2003;34(1):27-9.
32. Südhof TC. Neuroligins and neurexins link synaptic function to cognitive disease. Nature. 2008;455(7215):903-11.
33. Luo Q, Chen Y. Long noncoding RNAs and Alzheimer's disease. Clinical Interventions in Aging. 2016;11:867.
34. Guo J-H, Cheng H-P, Yu L, Zhao S. Natural antisense transcripts of Alzheimer's disease associated genes:Short communication. DNA Sequence. 2006;17(2):170-3.
35. Esteller M. Non-coding RNAs in human disease. Nat Rev Genet2011;12(12):861-74.

Reviewers of this article

Prof.Tek Chand Bhalla

UGC-BSR Faculty Fellow, Department of Biotechnology, Himachal Pradesh University, Summer Hill, Shimla, India.

Prof. Srawan Kumar G.Y

Associate Professor,Nalanda Institute of Pharmaceutical Sciences, Sattenapalli, Guntur, Andrapradesh, India

Prof.Dr.K.Suriaprabha

Asst. Editor , International Journal of Pharma and Bio sciences.

Prof.P.Muthuprasanna

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