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Bioinformatics Based Analysis of Microarrays in Human Bipolar Disorder

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ABSTRACT: Human brain disorder occurring in less than 30 age group termed bipolar disorder has no available cure or treatment. The diagnosis characterized by manic depression is based on psychopathology which makes it difficult to detect early. Development of biological markers is urgently required accompanied by new treatment approaches. The proteins expressed in the disease vs the normal conditions is a viable approach referred as microarray technology. An attempt has been made to analyse the microarray data using R statistics, to come up with knowledge of proteins expressed in bipolar disorder. The analysis provides probable biomarkers of the disease which could be developed as drug-targets.

KEYWORDS: Bipolar disorder,NCBI-GEO, R SCRIPT, Bioinformatics

I. INTRODUCTION

Bipolar disorder is a brain disorder that causes unusual mood swings. Also known as manic-depressive illness, it affects energy levels, accompanied by inability to carry out day-to-day tasks. Symptoms of bipolar disorder may be severe to moderate, affecting people before the age of 30 [1]. Bipolar disorder occurs approximately equally in both sexes [2]. Disorders are marked by severe mood swings leading to manic or major depressive episodes. Treatment includes modafinil/armodafinil as adjuncts for unipolar and bipolar depression. Though it was earlier considered as an adult-onset disorder, recent findings indicate juvenile occurrence [3]. The most reliable markers for bipolar disorder identification are derived from psychopathology and case histories. There have been no biomarkers discovered that predicts treatment responses for the individual suffering from bipolar disorder [4].The biomarkers must be suitable for identification of neurodevelopmental features that help to evaluate clinical results of mood swings.

II. RELATED WORK

Around the end of the 19th century, the concept of bipolar disorder originated out of Emil Kraepelin's classification of manic depressive insanity[5]. Hippocrates also described bouts of frenzy associated with the manic state in the ancient Egyptians. In 1957, the term bipolar was coined by Leonhard for those patients with manic-depression. Angst and Perris demonstrated in 1966, that the clinical presentation was related to family history and therapeutic response. Significant impairment of functioning of speech and ideas has been observed.

Bipolar disorder symptoms can vary from poor performance at school, poor job, damaged relationships and suicide. Clinical trials have been launched for illnesses of Bipolar Disorder of the Brain in adults and children (https://www.clinicaltrials.gov).Treatment includes the use of Lithium, though the mechanism of action is not certain. However, the effectiveness of lithium therapy on a regular basis has been disputed [1].

Production of cytokines such as IL-6, IL-1β, and TNF-α have been implicated in the bipolar disorder. Elevated levels of circulating IL-18 usually seen in cell mediated immunity have also been observed[6, 7] . Other cytokine mediators include macrophage migration inhibitory factor (MIF) with neurogenic potential [8,9,10].

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III. MICROARRAY ANALYSIS FOR PROTEIN EXPRESSION STUDIES

METHODOLOGY

Expression profiling of human adult postmortem brain tissue from subjects with bipolar disorder and healthy control were carried out from the cortical areas of the brain. Affymetrix HG-U133A GeneChips were scanned with the Agilent GeneArray Scanner. Generated raw data (CEL files) were read into R statistical package. Normalisation was done by using the robust multi-chip average (RMA) method (Irizarray et al, 2003). Later GEO2R tool was used that allows a sophisticated R-based analysis of GEO data. Identification and visualization of differential gene expression was done. GEO2R uses established Bioconductor R packages to transform and analyze GEO data. The statistical parameters with which to perform the analysis were selected and gene annotation done for each gene using Gene Ontology database. P values for False dicovery Rate, logFC and t test was calculated.

Datasets from 82 samples of postmortem brain tissue of patients suffering from bipolar brain disorder were screened. 16 samples were selected from samples with age at death varying from 20 years to 55 years of age. The criteria for selection of postmortem dataset was age at death less than 35 years and were diagnosed with the disorder for at least 10 years. This way classification of data based on those who did not survive for more than 35 years would indicate the severity of the disease. The control group included samples from healthy subjects, whose age at death was also less than 35 years.

IV. SCRIPTS GENERATED IN R

```
# Version info: R 2.14.1, Biobase 2.15.3, GEOquery 2.23.2, limma 3.10.1
# R scripts generated  Fri Jan 23 10:08:52 EST 2015

################################################################
#   Differential expression analysis with limma
library(Biobase)
library(GEOquery)
library(limma)
# load series and platform data from GEO
gset <- getGEO("GSE5392", GSEMatrix =TRUE)
if (length(gset) > 1) idx <- grep("GPL96", attr(gset, "names")) else idx <- 1
gset <- gset[[idx]]
# make proper column names to match toptable
fvarLabels(gset) <- make.names(fvarLabels(gset))
# group names for all samples
sml <-
c("X","X","X","X","X","G0","G0","X","X","X","X","X","X","G0","X","X","X","G0","X","X","X","X","X","X","X","X","X","X","
G0","X","X","X","G1","G1","X","G1","X","X","X","X","X","X","X","X","X","G1","X","X","X","X","X","G1","X","G1","X","X",
"X","X","X","X","X","G0","X","X","X","X","G0","X","X","X","G0","X","X","X","X","X","X","G1","X","X","X","G1");

# eliminate samples marked as "X"
sel <- which(sml != "X")
sml <- sml[sel]
gset <- gset[ ,sel]

# log2 transform
ex <- exprs(gset)
qx <- as.numeric(quantile(ex, c(0., 0.25, 0.5, 0.75, 0.99, 1.0), na.rm=T))
LogC <- (qx[5] > 100) ||
        (qx[6]-qx[1] > 50 && qx[2] > 0) ||
        (qx[2] > 0 && qx[2] < 1 && qx[4] > 1 && qx[4] < 2)
```

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```
if (LogC) { ex[which(ex <= 0)] <- NaN
  exprs(gset) <- log2(ex) }

# set up the data and proceed with analysis
fl <- as.factor(sml)
gset$description <- fl
design <- model.matrix(~ description + 0, gset)
colnames(design) <- levels(fl)
fit <- lmFit(gset, design)
cont.matrix <- makeContrasts(G1-G0, levels=design)
fit2 <- contrasts.fit(fit, cont.matrix)
fit2 <- eBayes(fit2, 0.01)
tT <- topTable(fit2, adjust="fdr", sort.by="B", number=250)

# load NCBI platform annotation
gpl <- annotation(gset)
platf <- getGEO(gpl, AnnotGPL=TRUE)
ncbifd <- data.frame(attr(dataTable(platf), "table"))

# replace original platform annotation
tT <- tT[setdiff(colnames(tT), setdiff(fvarLabels(gset), "ID"))]
tT <- merge(tT, ncbifd, by="ID")
tT <- tT[order(tT$P.Value), ]  # restore correct order

tT <- subset(tT, select=c("ID","adj.P.Val","P.Value","t","B","logFC","Gene.symbol","Gene.title"))
write.table(tT, file=stdout(), row.names=F, sep="\t")

################################################################
#   Boxplot for selected GEO samples
library(Biobase)
library(GEOquery)

# load series and platform data from GEO

gset <- getGEO("GSE5392", GSEMatrix =TRUE)
if (length(gset) > 1) idx <- grep("GPL96", attr(gset, "names")) else idx <- 1
gset <- gset[[idx]]

# group names for all samples in a series
sml <-
c("X","X","X","X","X","G0","G0","X","X","X","X","X","X","G0","X","X","X","G0","X","X","X","X","X","X","X","X","X","X","
G0","X","X","X","G1","G1","X","G1","X","X","X","X","X","X","X","X","X","G1","X","X","X","X","X","G1","X","G1","X","X",
"X","X","X","X","X","G0","X","X","X","X","G0","X","X","X","G0","X","X","X","X","X","X","G1","X","X","X","G1")
   # eliminate samples marked as "X"
sel <- which(sml != "X")
sml <- sml[sel]
gset <- gset[ ,sel]
# order samples by group
ex <- exprs(gset)[ , order(sml)]
sml <- sml[order(sml)]
fl <- as.factor(sml)
labels <- c("Bipolar_lessthan35yrs","control")
```

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```
# set parameters and draw the plot
palette(c("#dfeaf4","#f4dfdf", "#AABBCC"))
dev.new(width=4+dim(gset)[[2]]/5, height=6)
par(mar=c(2+round(max(nchar(sampleNames(gset)))/2),4,2,1))
title <- paste ("GSE5392", '/', annotation(gset), " selected samples", sep ='')
boxplot(ex, boxwex=0.6, notch=T, main=title, outline=FALSE, las=2, col=fl)
legend("topleft", labels, fill=palette(), bty="n")
```

V. RESULTS OF MICROARRAY ANALYSIS

The box plot was calculated in R for the 16 samples , out of which 8 were controls who were healthy subjects (Fig 1). The GSM sample number is shown in the plot. The analysis was further carried out using GEO2R. The top 14 differentially expressed in bipolar disorder is shown in Table1.

Table 1.Top 14 genes differentially expressed in Bipolar disorder

1	ATPase, Na+/K+ transporting	8	selenium binding protein
2	glutathione S-transferase	9	solute carrier family 6-neurotransmitter transporter
3	mitogen-activated protein kinase	10	small nuclear ribonucleoprotein
4	SRY (sex determining region Y)-box 2	11	fatty acid binding protein 3,
5	transmembrane protein 47	12	interleukin 10 receptor
6	inhibitor of DNA binding 4	13	megalencephalic leukoencephalopathy with subcortical cysts
7	ABL proto-oncogene 1	14	sphingosine-1-phosphate lyase

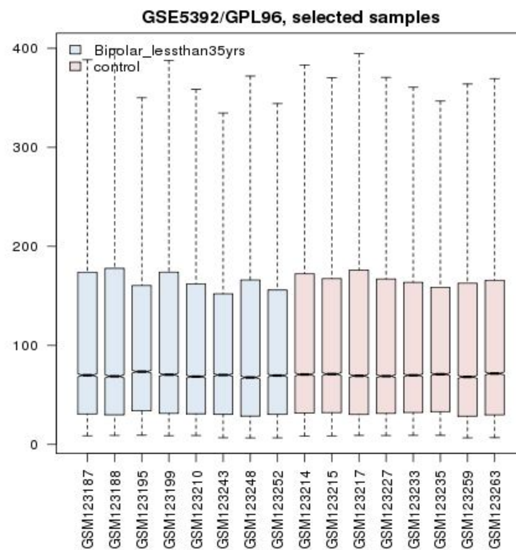

Fig1. Boxplot of 16 samples used in the study

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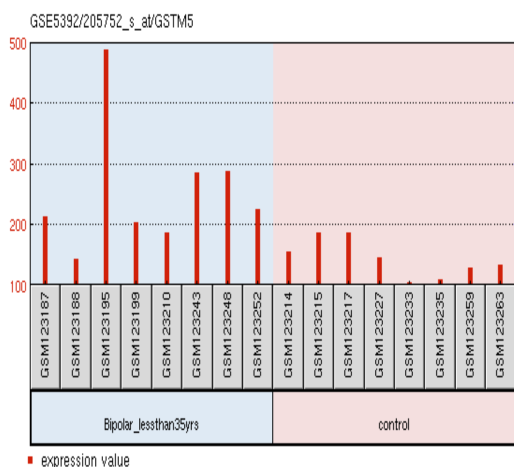

Fig2.Increased expression of ATPase in Bipolar Disorder age group less than 35yrs.

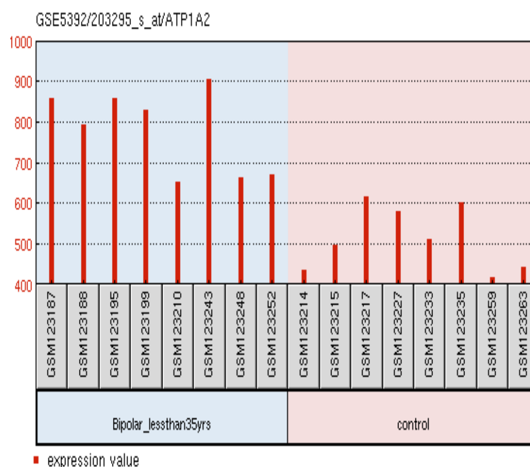

Fig. 3. Glutathione – S -Transferase expression in BD

The results of microarray expression analysis were obtained by clicking on individual result.The expression of sodium-potassium transporting ATPase was found to be increased as depicted in the graph(Fig 2). The expression levels of Glutathione S-transferase having was also found to be increased in patients of bipolar disorder (Fig 3). Both the enzymes, namely ATPases and Glutathione -S- transferases have detoxification function indicating increased synthesis in the disorder as compared to normal subjects. Fig 4. and Fig 5. depicted the reduced expression of IL-10 and corticostatin activity (G protein coupled receptor) respectively.

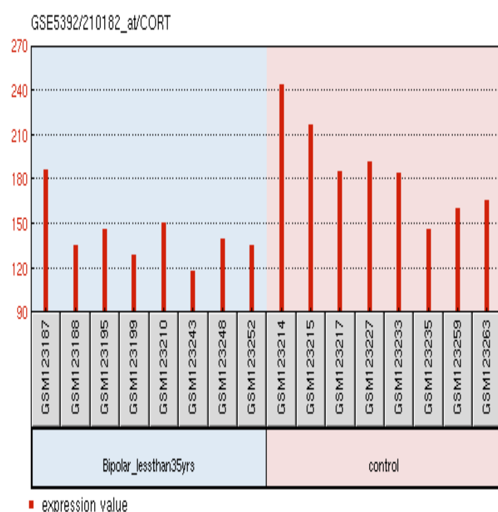

Fig. 4. Reduced expression of IL-10 receptor

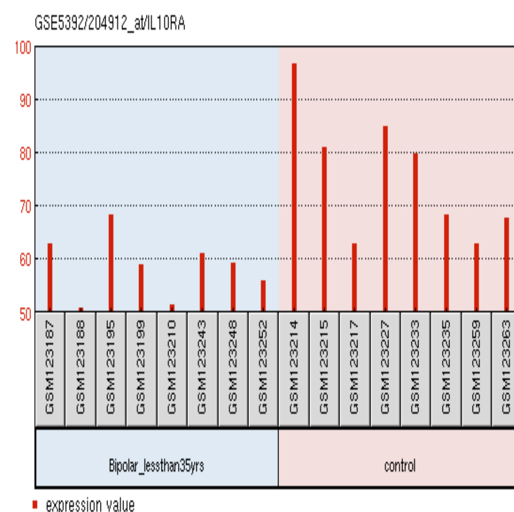

Fig 5. Corticostatin GPCR activity in BD

Small nuclear ribonucleoprotein or snRNP gives specificity to individual introns by "recognizing" the sequences of critical splicing signals. In our result the expression of sn RNP in bipolar disorder is reduced (Fig 6). Megalencephalic leucoencephalopathy subcortical cysts are associated with developmental delay of motor nerves of mild nature and responsible for seizures [11]. The subjects under the present study group had higher levels of proteins associated with Megalencephalic leucoencephalopathy (Fig 7). Thus, it may be concluded that proteins associated with delay in formation of motor nerves and certain detoxification functions were impaired. The involvement of the above mentioned proteins as well as the proteins mentioned in Table 1, are envisage in Bipolar disorder affecting people severely.

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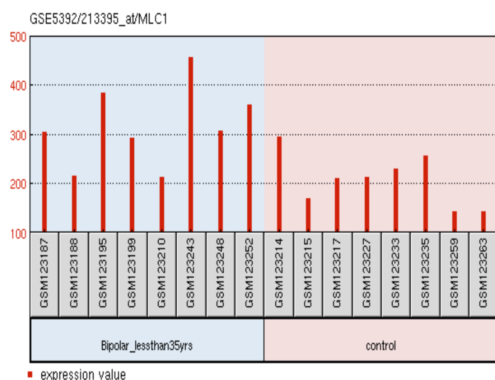

Fig 6.Small nuclear ribonucleoprotein expression in BD

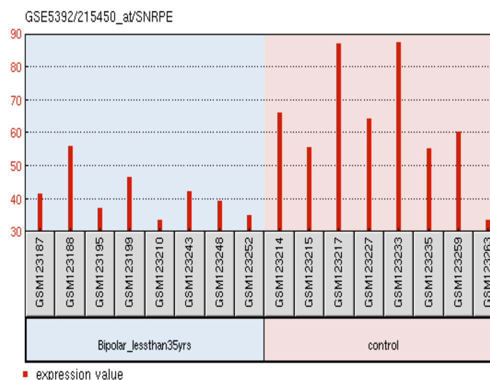

Fig7. Megalencephalic leucoencephalopathy subcortical cysts

VI. CONCLUSION AND FUTURE WORK

With valuable inputs obtained from microarrays, the analysis of proteins most likely expressed in bipolar disorder offers valuable direction for future validation. The analysis was carried out for data sets whose death age was less than 35 years. The profile of samples in the selected age group of below 35 years gives an indication of acute disease, not considering other parameters. The results obtained may be validated for use in laboratory studies for development of biomarkers and develop drugs as target inhibitors.

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BIOGRAPHY

Dr. Rama Adiga is an Assistant Professor at Nitte University Centre for Science Education & Research, Biomedical Science department, Nitte University, Mangalore. Qualified as a Biochemist, she has several papers in Bioinformatics. She has been teaching Biochemistry and Bioinformatics in the university department since 2013. The interdisciplinary nature of research work adds value to research. Her current interests are evolutionary Bioinformatics, clinical Bioinformatics and image processing in the biomedical field.