

Original Research Article

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Characterization of Micro RNA Signature in Peripheral Blood of Schizophrenia Patients using μ Paraflo™ miRNA Microarray Assay

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ABSTRACT

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Development of biomarkers for psychiatric disease still remains challenging. Recently, numerous alterations in the expression of microRNAs (miRNAs) in peripheral blood, serum and post-mortem brain tissue have been linked to schizophrenia. Additionally, it is known that miRNAs can inactivate a target mRNA sequence through binding to its 3'UTR region. Thus, miRNAs might not only serve as prognostic biomarkers for diagnosis or prognosis of pathological conditions like schizophrenia, but could also actively modulate gene function. Therefore, the aim of this study was to test whether specific miRNA display differential expression in patients with schizophrenia vs. healthy controls by using miRNA microarray assay. Venous blood samples of 33 affected patients and 25 healthy controls were collected and the quantification of microRNAs was established using miRNA microarray assay. Moreover, we searched for potential validated target genes for each of the differentially expressed miRNAs that might play a role in the regulation of schizophrenia susceptibility genes.

Introduction

As one of the most common psychopathological disorders, schizophrenia is considered to be a disease with an extensive social impact. Although symptoms and diagnostic criteria of this mental condition are well defined, the etiology of schizophrenia still remains incompletely elucidated. Both environmental and genetic

factors are reported to play important role in schizophrenia development. Heritability of schizophrenia has already been described and its genetic background has been clearly established. Hersen and Beidal report higher co-occurrence of the disease among monozygotic in comparison with dizygotic twins (Herson *et al.*, 2011). Additionally

several genome-wide association studies have revealed the involvement of many different loci in schizophrenia pathology (Mirnics *et al.*, 2000). Abnormal gene expression connected with the disease has also been described by many groups (Perkins *et al.*, 2007). MicroRNAs (miRNAs) can be considered as master regulators of essential cellular processes (Bartel, 2009; Sun *et al.*, 2010) and could therefore be affected by disease processes. Profiling of miRNA is being undertaken in the search for potential biomarkers in a variety of diseases. Increasing evidence shows that miRNAs take part in many biological processes, including cell proliferation, differentiation, migration, and apoptosis (Bartel, 2009). Thus, changes in the levels of expression of either miRNA could have long-term consequences, making it plausible for them to play a role in the etiology of schizophrenia.

Materials and Methods

Participants

The study design and the Inform Consent Form (ICF) were approved by the Ethics Committee of Plovdiv Medical University. The Institutional Review Board of the university approved the use of the samples for this study. Thirty schizophrenic patients from State Psychiatric Hospital - Pazardjik, Bulgaria were recruited after obtaining a written informed consent. All the patients were interviewed by certified Mini-International Neuropsychiatric Interview by a certified psychiatrist rater to evaluate diagnosis of schizophrenia on Diagnostic and Statistical Manual of Mental Disorders fourth text revised edition (DSM IV TR) criteria and to exclude any mental disorder in the controls. The control group included 25 healthy subjects matched by gender and age. Main inclusion criteria was that the

participants did not receive any medication before blood sampling for at least 2 weeks. People with other chronic medical conditions and current somatic/neurologic diseases, alcohol or drug abuse/dependency were excluded.

Blood collection and RNA extraction

An aliquot of whole peripheral blood (2.5 ml) for each subject (schizophrenia and healthy controls) was collected directly into a PAXgene blood RNA tubes (PreAnalytiX) and stored at room temperature for minimum of 4 hours and then frozen at -20°C, prior to total RNA extraction. Total RNA was isolated using PAXgene blood miRNA kit (PreAnalytiX), according to the manufacturer's protocol. Assessment of absorbance ratio of 260/280 nm revealed that all RNA samples are with sufficient quality (1.9 - 2.1) for further analysis, according to the service requirements. Pooled samples were created by adding an equivalent amount of total RNA from each individual sample to final concentration of 5 µg RNA samples. Pooled RNA samples were precipitated according to the service requirements, each pooled RNA sample was mixed with 1/10th volume of 3M NaOAc, pH 5.2 and 3 volume 100% ethanol, to the final volume of 400 µl. Aliquots of pooled RNAs were frozen at -80°C and shipped on dry ice. RNA integrity of pooled samples was assessed by agarose gel electrophoresis and checked by Agilent 2100 Bioanalyzer.

MicroRNA Expression Profiling (LC Science)

The µParaflo™ miRNA microarray assay was performed using a service provider (LC Sciences, Houston, TX) with a proprietary microfluidic array based on the Sanger miRBase v18.0 database (<http://www.sanger.ac.uk/Software/>

Rfam/mirna), designed to detect 1898 unique mature human miRNA sequences. MiRNA microarray analysis was carried out to determine differential expression in blood miRNAs between pooled samples of healthy individuals and pooled RNA samples derived from patients with schizophrenia. Fluorescence images were collected using a GenePix 4000B laser scanner (Molecular Device, Sunnyvale, CA) and digitized using Array-Pro image analysis software (Media Cybernetics, Bethesda, MD).

Target gene analysis of differentially expressed microRNAs in schizophrenia patients

In order to study the potential modulation in gene expression that may be associated with specific miRNAs changes identified in our study, miRNA validated target studies were carried out using the publically available database, miRWalk available at (<http://mirwalk.uni-hd.de>). The validated targets module hosted experimentally verified miRNA interaction information associated with target genes were used.

Results and Discussion

Analysis of miRNA microarray assay (LC Sciences)

In order to study the potential significance of certain miRNAs in schizophrenia, we analyzed global miRNA expression profiles in pooled peripheral blood samples from 30 schizophrenia patients and 25 healthy controls using miRNA microarray for mature human miRNAs. To assess the reproducibility of the microarray technique, two replicates from the same preparation were used for each of the two microarray hybridizations conducted in this research. In order to statistically estimate significance of every observed change in microRNA

expression, a two sample independent t test was performed. Comparison between the micro-RNA expression levels in these pooled samples identified 19 miRNAs with significant change in expression ($p < 0.05$), the majority (13) of which showed increased expression in schizophrenia patients. Among them, 4 showed fold change greater than 2.5 compared with the controls. 11 micro-RNAs appeared to be significantly downregulated ($p < 0.05$) in schizophrenia patients in comparison with healthy controls. These notable alterations in regulation of the respective microRNAs suggest that they may play important role in the pathogenesis of schizophrenia. Moreover the heat map, which was generated to visualize the results of hierarchical clustering, clearly shows the distinction between schizophrenia and control samples. (Table 1). The cluster graph represents all microRNAs that displayed statistically significant change with p value less than 0.01. The clustering analysis revealed microRNAs (such as hsa-miR-4668-5p and hsa-miR-421) with very similar patterns of expression, despite the fact that their genes occupy very distant genome loci.

High-throughput miRNA expression analysis of 1898 unique annotated mature human miRNA sequences (from miRBase 18.0) revealed a set of 19 differentially expressed miRNAs. 13 of which was up-regulated and 6 down regulated, respectively ($p < 0,05$) Significantly downregulated molecules includes, miR-183-5p, ($\log_2 - 0,65$, $p=0,026$), miR-3173-5p, ($\log_2 - 0,85$, $p=0,029$), miR-125b-5p, ($\log_2 - 0,58$, $p=0,033$), miR-192-5p, ($\log_2 - 0,47$, $p=0,033$), miR-1976, ($\log_2 - 0,42$, $p= 0,033$), miR-4701-3p, ($\log_2 - 0,54$, $p=0,044$). A trend towards increased expression (up-regulation) showed miR-4311, ($\log_2 - 0,96$, $p=0,014$), miR-4668-5p, ($\log_2 - 0,38$, $p=0,022$), miR-421, ($\log_2 - 0,30$, $p=0,022$), miR-4498, ($\log_2 - 0,98$, $p=0,027$), miR-

4691-3p, ($\log_2 - 1,50$, $p=0,029$), miR-3185, ($\log_2 - 1,01$, $p=0,035$), miR-4728-5p, ($\log_2 - 0,70$, $p=0,037$), miR-3184-5p, ($\log_2 - 2,54$, $p=0,039$), miR-4479, ($\log_2 - 0,32$, $p=0,041$), miR-30b-3p, ($\log_2 - 1,26$, $p=0,041$), miR-5010-5p, ($\log_2 - 1,45$, $p=0,041$), miR-492, ($\log_2 - 1,97$, $p=0,042$) and miR-30e-3p, ($\log_2 - 1,13$, $p=0,047$).

Target Analysis of Schizophrenia-Associated microRNAs

Validated target genes of differentially expressed microRNAs identified by miRNA microarray analysis are shown in Table 1.

Abnormal miRNA expression has widely been reported in numerous human diseases. A series of recent studies have shown that miRNAs in whole blood serum and plasma are considered to be a biomarkers for several diseases (Scholer *et al.*, 2010). Several recent reports have confirmed the principle that the peripheral blood is a potentially useful source of diagnostic biomarkers for neuropsychiatric diseases and disorders (Gardiner *et al.*, 2012; Lai *et al.*, 2011; Mellios *et al.*, 2012; Ivanov *et al.* 2015). Some of them have indicated the usefulness of using whole blood for miRNA expression studies and have demonstrated altered expression of blood and brain miRNAs involved in brain plasticity and maturation associated to schizophrenia (Gardiner *et al.*, 2012; Lai *et al.*, 2011; Mellios *et al.*, 2012). All these studies suggest that whole blood cells could reflect, or in part share, the molecular phenotype of neural cells and thus could be used in studies of psychiatric disorders. This study demonstrates the use of miRNA signatures as an important advance in schizophrenia research. Elucidating miRNA dysregulation in schizophrenia will promote understanding of

the contribution of miRNAs in neurocognitive, behavioral and other common disorders that demonstrate differential miRNA expression.

Recently, global analysis of the expression profile of micro RNA from peripheral blood mononuclear cells (PBMC) of 112 patients with schizophrenia and 76 non-psychiatric controls conducted by Gardiner and colleagues revealed a significant dysregulation of micro RNA expression in schizophrenia. In their study, the authors found 83 micro RNA molecules that showed statistically significant decreased expression in the group of schizophrenia patients. In addition, some of the differentially expressed micro RNA molecules (~ 20%) are transcribed from a single imprinting locus, maternally expressed in the DLK1-DIO3 region on chromosome 14q32 (Gardiner, 2011). All this suggests the possible involvement of major locus-specific genetic or epigenetic control mechanisms associated with this disorder.

The micro RNA molecules, identified by us with a similar change in expression and mapped in single loci would be intriguing subject for clarification of the relationship between their expression and localization into the genome (locus-specific expression).

In order to assess in detail the biological consequences of the observed changes Gardiner *et al.* also analyze the predicted target genes of the differentially expressed micro RNA molecules. They found that the target genes are involved in biological pathways directly related to nerve function by axons guidance, regulation of actin cytoskeleton, long-term potentiation, long-term depression, adhesion (focal adhesions) and neurotrophins (Gardiner, 2011).

Table.1 List of validated target genes of dysregulated miRNAs

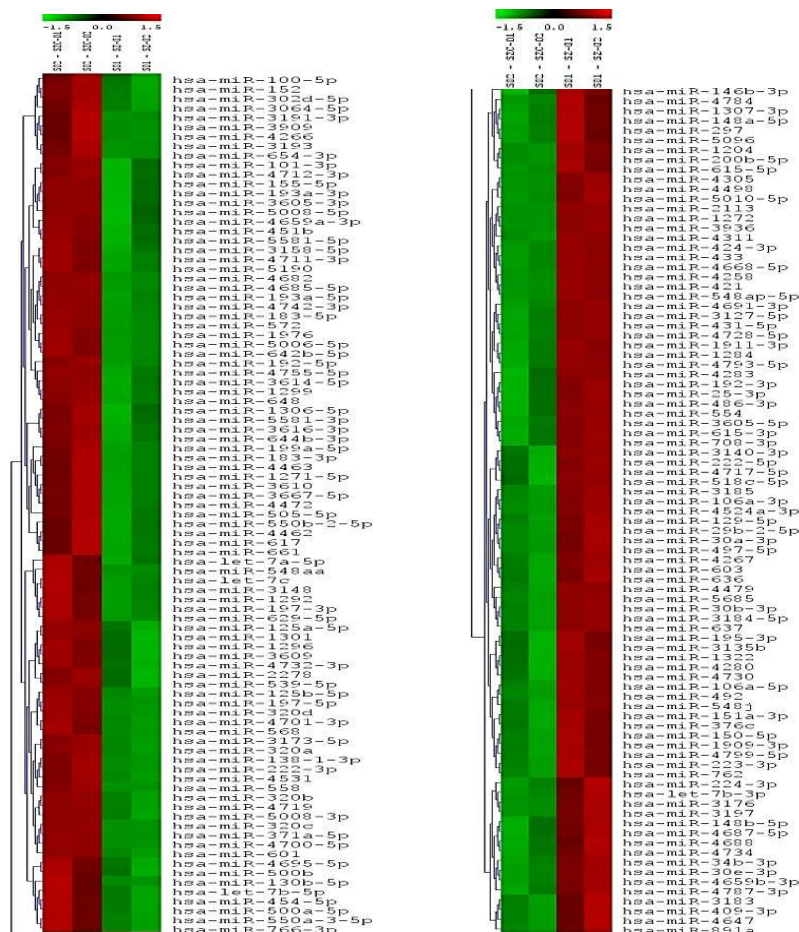
MiRNAs	Dysregulation	Validated target genes
miR-183-5p	Downregulated	<p>AAMP, ABCF1, ARHGDI, ASNS, AUH, CCND1, CAPNS1, CCNB1, SLC31A1, CR1, CS, CSNK2A1, DAP, DMWD, DOCK1, DTYMK, EGR1, FAT2, FEN1, FOXO1, FNTB, GAS1, GATA6, KAT2A, GLO1, GLUL, GSPT1, GSR, HARS, HCFC1, HINT1, HLA-A, HNRNPL, HOXA5, HSPA1B, HSP90AA1, FOXN2, IDH2, IGF1R, FOXK2, INSIG1, ITGA5, ITGB1, ITPKB, KIF2A, KIF5C, MCM4, MSH2, TRIM37, MYO10, HNRNPM, NEO1, NOTCH2, P4HB, PDE8A, PLOD2, POLH, PPP3R1, PSEN1, PSMD13, PTPN9, PTPN11, RAB5B, RALGDS, RCN2, REV3L, RPLP0, SRSF2, SRSF2, SFSWAP, SLC2A3, SLC5A3, SNRPD3, ST13, CCT3, TRO, UBPI, UCHL3, UQCRC1, EZR, BRPF1, ARHGEF5, PTP4A2, TTF2, ITGA8, API5, DGAT1, TIMELESS, BTRC, BTAFI, USP8, RPL23, BAG4, AKAP12, TRAF4, KIAA0430, PHF14, KNTC1, ARHGAP32, EIF4A3, SERTAD2, PLEKHM1, PDCD6, PREB, TRIM28, RNF41, CTDSPL, CEPT1, GNB2L1, LYPLA1, MYBBP1A, HYOU1, TXNIP, SRSF10, USP19, STIP1, C14orf1, MTF2, RBM34, MYCBP2, PPRC1, ERC1, TNRC6B, FAM175B, ZC3H4, USP22, LARP1, OPN3, TMEM245, NIPBL, UBXN7, GNL3, PDCD4, HIPK2, SENP1, RDH11, LARS, NUB1, RC3H2, OTUD4, KLHL24, TRIT1, COMMD8, RIF1, LAPTM4B, SMEK1, TSR1, SERTAD4, C10orf2, C15orf39, RTN4, THOC2, CNOT6, MTUS1, NUFIP2, ARHGAP21, MKL1, ZFAT, ABHD17C, ELAC2, FKBPL, TUT1, BCL11B, WNK1, UBE2Z, STK33, TMEM108, GCC1, ZFH4, PEAK1, RPAP2, FAM188A, TUBB1, GUCD1, STK40, ARFGAP2, TNRC18, FAM104A, MASTL, ABCC10, FAM110B, LENG8, NAA30, ZC3H18, CYB5D2, IZUMO2, TPRG1L, C5orf24, KDELC2, C18orf25, KLHL23, SH3D19, NHSL2, WDR53, SHISA2, ZBTB34</p>
miR-192-5p	Downregulated	<p>ACADSB, ACVR2A, ACVR2B, ACVR2B, ADRBK2, AGL, ALCAM, ALCAM, ALDH9A1, ANG, APC, XIAP, TRIM23, ASPH, ATF1, ATP1B1, BARD1, BARD1, BCL2, BGLAP, BICD1, BLM, BMPR2, DST, BRCA1, BRCA2, BTC, BUB1B, BYSL, CA8, CAMK4, CASP7, CCNE1, ENTPD3, CDC20, CDC25A, CDKN1B, CDKN2A, CDKN2D, CDKN3, CENPA, CENPE, CENPF, CFL2, RCBTB2, CHM, CHML, CLIC1, CLK1, CREBL2, CRK, CTH, CYP24A1, DBT, DDOST, DDX3X, DDX3X, COCH, DHCR24, SLC26A2, HBEGF, DYRK1A, E2F5, ECT2, PHC2, EEF1A2, EFNB2, EGR1, EGR1, EIF2S1, EML1, EPS8, ERCC3, ERCC3, ERCC4, ERCC4, EXTL2, ACSL1, FBN1, FEN1, GPC4, FGF2, FOS, CENPI, GATM, GCH1, GNRH1, GPR19, GPR37, GRIA1, MSH6, HADH, HAS3, HMGCS1, HMMR, FOXA1, HOXA10, HOXA13, HOXB9, HRH1, HSPA2, ICAM3, ID1, ID2, ID4, IDI1, IFRD1, IGF1R, IL1R1, IL1RAP, IL6ST, IL7, IL15, IMPA1, INSIG1, IRAK1, ITGAV, ITGB1, KCNS3, KIF5B, KIF5C, KPNA5, LIMS1, LIPA, FADS1, LMAN1, LMNB1, LOXL2, MAD2L1, MAN2A2, MCAM, MCM3, MCM3, MCM6, DNAJB9, MDM4, MEIS2, MAP3K1, MFI2, MKI67, MSN, MSN, MT1F, MT1X, MXI1, MYB, PPP1R12A, NAB1, NBN, NCBP1, NEK1, NFIB, NFYA, NKX3-1, NSF, NUCB2, ODC1, OPA1, ORC1, OVOL1, PAFAH1B1, PARN, PAWR, PCDH7, PDE2A, PET112, CDK14, PGM3, PIM1, PIM1, PLAGL2, PLAUG, PLOD1, PLS3, UBL3, PODXL, POU2F1, PPARG, PPAT, PPM1A, PPP1CA, PPP1CB, PRIM1, PRKAA2, PRKAR1A, MAPK9, DNAJC3, PRNP, PRPS2, LGMN, PTPRE, PTS, RAD1, PURA, RAB1A, RAB2A, RAB2A, RAB3B, RAB27A, RAD21, RAD51, RAD51, RAP1GAP, RB1, RB1, RBL2, RECQL, RET, RFC4, RNASE4, RNASEL, RNF6, RPS6KB1, RRM1, ATXN7, SCD, CCL3L1, TRAPPC2, SGCB, STIL,</p>

		<p>SLC1A4, SLC7A2, SLC9A5, SMARCA2, SMARCB1, SNRPD1, SOAT1, SP4, SPARC, SPTBN1, SRD5A1, SS18, STK3, MED22, TBL1X, TCEB3, HNF1B, TCF7, TDG, THBD, THOP1, KLF10, TMPO, TOP1, TPM4, HSP90B1, TRPC1, TTK, TTPA, UBE2D1, UBE2D2, UBE2V2, UGT8, UNG, UPP1, UQCRC2, USP1, UVRAG, TRPV1, WHSC1, WNT3, XK, XPA, XPO1, ZBTB14, ZNF45, ZNF85, ZNF165, ZNF184, ZNF200, ZNF215, ZNF226, ZNF230, LRP8, PXDN, MANF, DEK, ALDH5A1, EPM2A, BRD3, STAM, CUL5, NCOA3, CDC7, FZD1, FZD4, FZD7, PIP4K2B, EEA1, STX7, STX7, CUL3, TTF2, RANBP3, PKP4, ENC1, CDC14A, DEGS1, TMEFF1, B3GALNT1, B3GALNT1, ABCC3, TNFSF13, PABPC4, CD164, CD164, SAP30, HESX1, IQGAP1, KAT2B, FUBP1, AP1S2, HERC2, MPZL1, SPAG9, USP14, NMI, SLC16A6, SYNGR1, SLC16A7, LRRFIP1, LRRFIP2, DLG5, STK17B, CD83, MMP20, NREP, TRIP13, ZNHIT3, B4GALT6, ARHGAP29, FAM189A2, TJP2, ABCG2, EIF2AK3, AKAP7, KIF23, ATP6V1G1, KIF20B, ARHGEF10, USP34, DLGAP5, MTSS1, ARHGAP11A, GAB2, EPM2AIP1, PPP1R26, PSMD6, SEC24D, FAM20B, KBTBD11, KIF14, AMMECR1, HS3ST3B1, ZBTB33, SH2B3, HCN4, TROAP, SMC4, ACTR2, KIF20A, CTR1B, ARL4C, ZNF263, AKAP9, LHFP, ZNF443, MFSD10, TRAIP, ZNF267, CCNO, TFG, ABCA8, CORO2B, ANAPC10, ST3GAL6, TUBGCP3, C1D, C1D, ZBTB18, SEC23B, SYNCRIP, SEMA4D, SEMA4D, CIB2, EAF1, LAMTOR5, SLC19A2, ARFGEF1, SMC2, ATP5L, KHDRBS3, CTCF, TNFSF13B, POLQ, TCFL5, PLK4, PHTF1, ZMYND11, ZBTB6, ZNF273, NMU, AP3M2, RAB40B, CKAP4, TMED10, PAPD7, CPSF6, TOPBP1, DNAJB4, PRSS23, PRDM4, IBADH, CIT, FGFR1OP, PTP4A3, ERLIN2, AKAP11, RASSF8, SEC63, USP18, ZNF652, ZFP30, RHOBTB3, COBLL1, RAB11FIP2, ICK, ENPP4, INPP5F, ANKRD6, CLSTN1, RUFY3, WDR47, NCBP2, ELL2, SCMHI, LIMCH1, FNBP1, ENDOD1, EMC1, SNX13, PASK, OTUD3, TMEM194A, FKBP15, SATB2, RPGRIP1L, NCAPH, DICER1, SIRT1, TARDBP, ABCB10, NPTXR, LEPROTL1, MACF1, GLTSCR1L, ZNF346, ARL2BP, RABGAP1, POLA2, SLC7A11, TMEM2, SH3BP4, PRKD3, CADM1, PITPNB, BCL2L13, PANX1, PANX1, DSTYK, RAD54B, DNAJB5, PARM1, ANAPC15, SAMHD1, TPGS2, LRIG1, TRNL1, APPL1, ARHGEF26, TOR1AIP1, C10orf137, PHF19, FBXW2, PITPNC1, FBXO25, FBXO5, FBXO4, FBXO3, PCOLCE2, NUFIP1, NPHP3, AGO2, SLC39A1, DIMT1, PDLIM3, KCNMB4, TOR1B, PPP2R3B, SLCO3A1, FLVCR1, NOB1, MRPL13, ATAD2, RACGAP1, GRHL1, TFCP2L1, SENP1, SNX10, PSMC3IP, GPSM2, UBIAD1, PIK3R4, CUZD1, IRX4, COPS7A, RNF141, CDON, MTERFD1, METTL9, NAA20, IRAK4, RNFT1, CKLF, PLCE1, NIN, ZDHHC2, CCDC174, RSRC1, THEM6, CRLF3, GULP1, PTPLAD1, DTL, LARS, TAF9B, CCZ1, TMEM66, NLK, RAB23, CMPK1, RAB8B, SIX4, SIX4, COQ3, BRWD1, SETD4, C21orf91, ANLN, WDR44, MRPL50, MTMR12, NDFIP2, FAM63B, RSBN1, EPDR1, NDE1, ERCC6L, VPS13C, BIVM, BCOR, UHRF1BP1, WHSC1L1, CMTM6, PARP16, PIGX, CLN6, GID8, CDCA4, UACA, CXorf57, TRIM68, LARP1B, RFWD3, CEP55, FAIM, RIC8B, BBS7, FANCI, NEIL3, NUDT15, PHF10, UBE2W, MIS18BP1, AGPAT5, MNS1, SLC39A9, CHDH, HJURP, MCM10, CSGALNACT2, CHST12, HES6, IL17RB, ZNF823, ENOSF1, BMP2K, RNPC3, FERMT1, G2E3, TBC1D22B, DEPDC1, CHD7, SLC48A1, KDM4D, RADIL, ARHGEF40, FAM178A, IQCC, N4BP2, C1orf112, TMEM30A, ZNF701, TDP1, SAYSD1, MBNL3, DCP1A, EAF2, WWC3, PPP2R2D, RCC2, NKRF, SLC25A40, SERTAD4, CTPS2, EMC7, PAK6, ARNTL2, NT5M, STOX2, KIF15, CHPT1, CTNBP1, CCDC47, CASC5, ENTPD7, PHTF2, ATP10D, ZNF248, GPR126, NHSL1, CLK4, KIAA1143, MKL2, KIDINS220, MIB1, KLHL42, NCEH1, STAMBPL1, KLHL8, HCN3, FANCM, DENND1A, IGDCC4, ZFYVE28, TRIB3, GATAD1, PTBP2,</p>
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		<p>CREBZF, INIP, ELOVL5, AASDHPPT, SPCS3, BCORL1, CLSPN, PERP, PERP, CENPK, MOAP1, SLC39A8, NOD2, TOR3A, PAPD5, NFKBIZ, ZMAT3, MPP5, NUCKS1, CCDC14, FNDC3B, ELOVL1, PLEKHG2, REEP1, SOWAHC, WNK1, ZBTB10, OTUB2, MIS12, DDX50, DDX54, KCTD15, PRRG4, C2orf49, RNF26, ZXDC, HAUS3, RNF219, OGFRL1, SCR3, CCDC121, RPAP3, HSPBAP1, PLEKHF2, GALNT12, E2F8, CLIP4, FBXO31, VASH2, ZMYM1, TMEM180, BORA, RPAP2, ATAD5, MAP6D1, CNTNAP3, L2HGDH, ERMP1, PHF17, WDR76, DSN1, RMI1, WWC2, CCDC15, PIF1, CCDC176, ASRGL1, DBF4B, NAA50, C2orf44, KLHL15, TRAF3IP3, DDHD1, DUSP16, SGPP1, APOLD1, FAM83D, CAB39L, MAP1LC3B, C6orf62, LOC81691, VANGL1, SPRY4, ZNF611, KIF18A, NUF2, DNAL1, UCK1, ATG10, ROPN1L, GSG2, BRIP1, B3GNT5, KATNAL1, RAB6C, CEP78, TMEM164, MEX3B, LRRC8C, ANKRD32, TCHP, PRADC1, PHF6, TMEM246, PCGF5, HOOK3, KIAA1841, RPL43, FAXC, HIATL1, FAM126A, PSRC1, LMNB2, C1orf198, ATAD1, TMTC4, FAM136A, SLC35B4, FAM222A, ALG10, SERAC1, PRPF38A, CGNL1, UBASH3B, CEP19, ARHGAP19, USP45, KIAA1671, EAF1, ZIC5, LRRC1, KIAA1731, FHDC1, ZC3H12C, ANKRD13A, LMLN, EFCAB11, C5orf30, TICRR, LYRM7, BTF3L4, C12orf65, SLC25A51, MTDH, TIFA, TMEM169, TEX30, PIGM, PIGM, TBC1D31, CADPS2, FAM71E1, GLCCI1, TMEM106A, TMEM200A, OSBPL6, OSBPL8, OSBPL10, C1QTNF3, ATG4A, KCTD12, DIS3L, LRRC58, LYSMD3, GINM1, RBP7, LEAP2, SFR1, ZNF641, NEDD1, NIPA1, C16orf46, NOXO1, GABPB2, CCSAP, CMPK2, LYPD6, AHS2, FBXO36, DNAJC19, DCBLD2, C4orf32, EMB, PRRC1, AFAP1L1, HINT3, FAM199X, SPIN4, ZSWIM3, KDELC2, PRICKLE1, ALG10B, MIPOL1, CEP128, NRG4, EME1, SAMD13, SLC35F3, LSM14B, CKAP2L, GAREML, SEPT10, ZNF385B, FAM132B, KLHL23, SGOL2, SLC16A14, NIPAL1, BTNL9, SAMD3, ESCO2, CLVS1, C9orf163, FAM120AOS, KIAA1958, USP54, SLC35G1, TMTC2, TMTC3, GPR180, ZFP1, ZNF519, C18orf54, ZNF320, ZNF114, IFNLR1, SASS6, FAM171B, SGMS2, FUT11, ANO6, ACSF3, C1orf86, TMEM17, DHFRL1, RDM1, CCDC111, C8orf47, ZNF449, USP12, C10orf35, CCNY, RTKN2, HYLS1, TMEM136, SKA1, OTUD1, CCDC7, SPATA13, KDM1B, PXDC1, CCZ1B, RSN1L, STXBP4, SLC25A30, CERS6, MSRB3, EHBPL1, MCM9, C8orf46, ZDHHC23, PCSK9, NPNT, CNEP1R1, ASPM, GAS2L3, LC46A3, GPR137C, C17orf58, ZADH2, ZNF780A, RIMKLA, CCDC150, FAM126B, EOGT, CYP4V2, LIN9, TRIM59, MMAB, CHSY3, GOLGA6A, TMC3, CCDC18, KIF24, GEN1, TICAM2, FAM101B, FAM111B, LHFPL4, C5orf34, CA13, NHLRC3, C1orf53, VGLL3, ZBTB34, ZNF704, FAM110C, C16orf52</p>
miR30e-3p	Upregulated	<p>LNPEP, TRIM37, NFKBIA, SERPINE2, PPP2R2B, RBBP7, RPS3, SGSH, YWHAE, CLSTN1, TBC1D9B, UBXN1, DGCR8, FAM47E</p>
miR30b-3p	Upregulated	<p>RORC, MYBBP1A, RNPS1, CXXC4</p>
miR421	Upregulated	<p>SLC25A6, RHOB, RND3, ATM, ATP6V0C, ZFP36L2, CASP2, CEBPB, COPA, DDX3X, DFFB, DUSP3, EEF1A1, EIF4A1, EIF4G2, FAT2, NR6A1, GCSH, GRK6, NR3C1, HADHA, HTT, HSP90AA1, INPP4A, ITPK1, LBR, LIMS1, CD46, SEPT2, NPM1, PHKA1, POLR2D, PPP2CB, PRCC, MAPK8, MAPK8, PSME1, RANBP2, RBBP7, TRIM27, RPL18A, RPS10, RPS18,</p>

		SRSF7, SLIT1, SRP9, SRPR, ZXDA, RASAL1, AP3B1, USO1, VAPA, TTC37, CEP57, KIAA0100, DDX39A, DDX39A, TUBA1B, SLC9A6, CAP1, LEPREL4, MTHFD2, PPARGC1A, MSL3, CDC37, DDX20, ATXN2L, MLXIP, CNOT1, PHLPP2, NCDN, RHOBTB2, ZC3H7B, DNAJC16, SMCHD1, PPP1R13B, SPECC1L, LARS2, CBX7, ACOT9, ZMYND8, FKBP8, NIPBL, RAB26, NSL1, FAM32A, AGO1, AFF4, EIF3K, LMCD1, RPL26L1, DNAJC27, AMZ2, RNF138, PQLC2, GID8, ATG2B, RIF1, MIS18BP1, LIN7C, COPRS, ZNF280C, NDC1, DOK4, TSR1, CMAS, DUS3L, CDC42SE2, RALGAPB, SENP7, ISY1, FAM160B1, CREBZF, NCAPG, PCNXL4, MRPL38, GID4, AHNAK, TMEM109, CTC1, FBXO11, RAB11FIP1, CD276, PRR7, GFOD2, FAM172A, PARP10, RAB2B, ZNF30, MCFD2, RASL10B, NXPE3, SFXN1, PAXBP1, FAM122A, FAM168B, GAREML, CREBRF, OTUD1, SREK1IP1, RGMB, ZNF678, RBMXL1, HIST2H4B
miR125b-5p	Downregulated	ABL1, ACLY, ADM, PARP1, AKT1, XIAP, ARF3, ATP5B, BACH1, BAK1, BAK1, BCL2, BCL3, PRDM1, BMPR1B, CBF3, CBF3, CCNE1, CDKN2A, CEBPA, CEBPG, CNGB1, COX7C, CREBBP, MAPK14, CYP24A1, E2F2, E2F3, E2F3, EEF1A1, EIF4EBP1, EIF5, EMD, EPHA7, ERBB2, ERBB2, ERBB2, ERBB3, ETF1, ETS1, EXTL3, FAT1, FBN1, FGFR2, FUS, GABRB3, GLI1, MKNK2, GRIN2A, GRINA, HDGF, HK2, HMGN1, HMGA1, HNRNPK, HOXA13, HSPA1B, HSPD1, IGF2, IRF4, KCNS3, KPNB1, KRT7, LIF, LSS, LTA4H, MMP13, ABCC1, MTF1, MUC1, NME2, NPM1, NRD1, NTRK3, PAM, PCBP2, PIGF, PKM, PMAIP1, MED1, PPAT, PRKAG1, PSMB1, RAF1, RANBP2, RNH1, RPL3, RPL29, RPL35A, RPLP0, RPS3A, RPS6KA1, RPS7, RPS12, RPS28, SORT1, S100A8, ATXN1, SDHB, SEL1L, SHMT1, SLC7A1, SLC19A1, SLC25A1, SMARCD2, SMO, SMO, SNRPB, SSR3, STAT3, SUPT6H, TDG, PRDX2, TEF, TNFAIP3, TP53, TP53, TP53, UNG, VCL, VDAC1, VDR, ZNF212, KMT2D, FXR1, HMGA2, TAF15, FZD4, IRS4, KHSRP, STC2, DGAT1, B4GALT3, PABPC4, SGPL1, MTMR3, SLC7A6, USP8, MTMR4, SLC16A4, AURKB, MFHAS1, SLC9A3R2, RASAL2, PTGES, ZNF592, NUP93, EDEM1, STARD8, RNF144A, MATR3, DHX38, IP6K1, KIAA0141, CEP170, UBAP2L, THRAP3, HUWE1, RASGRP1, SUGP2, ABCC4, RGS19, BCKDK, SMYD5, NEBL, RNASEH2A, SMC2, ARID3B, MAP3K2, NES, HBS1L, ZMYND11, SEC24A, ILVBL, ADRM1, AKAP2, LYPLA2, CASC3, CASC3, R3HDM2, BTBD3, SPEN, EMC1, TNRC6B, PLXND1, NUP205, GANAB, TBC1D1, SIK2, PDS5A, EXOC7, FAM208A, CRTCL, FRAT2, RYBP, TARDBP, SRRM2, NNT, CARHSP1, HSPBP1, TMEM2, BCL2L13, THUMPD3, ZDHHC5, ULK3, TIMM10, PABPC1, NPTN, BBC3, NDOR1, RRP7A, NKIRAS2, STRN3, PSAT1, MYEF2, MRTO4, PDZD11, CD320, KLF13, CHMP3, LUC7L3, ZFYVE1, PTOV1, CHPF2, MTRF1L, MTMR12, BCOR, NSUN2, C1orf109, C17orf80, RFW3, UBA6, THUMPD1, NDC1, WDR11, ZNF395, PCDHGB2, MEPCE, C15orf39, SMIM8, PPM1H, CGN, CTDSP1, FAM60A, UNKL, RMND5A, WNK1, DDX50, DYNC2H1, SMC6, LIN28A, EHMT1, PIP4K2C, ESRP2, MYO19, ABTB1, FBXO38, CSRNP2, SPRTN, C2orf88, ACSS1, PLEKHA8, LTV1, FBXL20, TNKS1BP1, LBX2, THAP3, BMF, DHX57, FMNL3, TP53INP1, SLC35A4, LACTB, GPRIN1, CCDC124, FAM199X, SAMD10, RIMS4, E2F7, CMTM4, LSM14B, FAM91A1, ZNF483, QSOX2, RHOV, TMEM9, PLA2G4F, CDRT4, C19orf54, HKR1, MMAB, KIF24, GALNT18, LIN28B, HIST2H2BF, NRARP, TIMM23

Fig.1 Differentially expressed microRNAs identified by miRNA microarray analysis in whole blood samples from SZ patients versus control subjects.



In a similar study, Lai *et al.* (Lai *et al.* 2011) identified specific expression profile of seven micro RNA molecules in an initial cohort of 30 patients with schizophrenia and 30 controls. 6 of the micro RNA molecules were with increased expression (micro RNA-34a, micro RNA-449a, micro RNA-564, micro RNA-548d, 572 micro-RNA and micro RNA 652) and one - micro RNA 432 with decreased expression. These results were confirmed in an extended subsequently cohort of 60 patients with schizophrenia and 30 controls.

Based on our results it can be assumed that changes in the expression levels of micro RNA molecules contribute to complex

global changes in gene expression, that underlie the pathophysiology of schizophrenia. In this study we observed that micro RNA expression profile from peripheral blood is dysregulated in the patients diagnosed with the disorder. At the molecular level, our results can be explained by one or more of the following options: a) reduction of transcriptional and epigenetic repression of primary micro RNA transcripts b) changes in levels of Argonaute and homologous proteins, c) changes in micro RNA Processing (e.g., from DICER1, etc.), or d) an increased half-life of the micro RNA molecules.

However, it remains to be determined if the

miRNA dysregulation described here is also present in brain tissue and therefore playing a key role in the neuropsychiatric phenotype. Finally, the crucial challenge will be to identify and validate the target genes that are affected by those microRNAs that are found dysregulated in schizophrenia and to characterize the involved pathways in a much more comprehensive manner in order to improve our understanding of how alterations in microRNA mediated genetic networks can contribute to the pathophysiology of the disorder. MiR-let-7d suppresses neural stem cell proliferation by reducing TLX expression in neural stem cells, which may be a novel strategy for identifying potential interventions in relevant neurological diseases (Zhao *et al.*, 2013).

References

- Bartel, D.P. 2009. MicroRNAs: target recognition and regulatory functions. *Cell*, 136: 215–233.
- Gardiner, E., Beveridge, N.J., Wu, J.Q., Carr, V., Scott, R.J., Tooney, P.A., Cairns, M.J. 2011. Imprinted DLK1-DIO3 region of 14q32 defines a schizophrenia-associated miRNA signature in peripheral blood mononuclear cells. *Mol. Psychiatry*, 17: 827-40.
- Herson, M. 2011. Etiological considerations Adult psychopathology and diagnosis John Wiley & Sons ISBN 9781118138847.
- Ivanov, H., Stoyanova, V., Popov, N., Vachev, T. Biodiscovery 2015. Blood-Based Gene Expression in children with Autism spectrum disorder; 17: 2; DOI: 10.7750/BioDiscovery. 2015. 17.2
- Lai, C.Y., Yu, S.L., Hsieh, M.H., Chen, C.H., Chen, H.Y., *et al.* 2011. MicroRNA expression aberration as potential peripheral blood biomarkers for schizophrenia. *PLoS One*, 6: e21635.
- Mellios, N., Sur, M. 2012. The Emerging Role of microRNAs in Schizophrenia and Autism Spectrum Disorders. *Front Psychiatry*, 3: 39.
- Mirnic Károly, Frank, A., Middleton, Adriana Marquez, David, A., Lewis. Molecular Characterization of Schizophrenia Viewed by Microarray Analysis of Gene Expression in Prefrontal Cortex. 2000 Pat Levitt DOI: [http://dx.doi.org/10.1016/S0896-6273\(00\)00085-4](http://dx.doi.org/10.1016/S0896-6273(00)00085-4).
- Perkins, D.O., Jeffries, C.D., Jarskog, L.F., *et al.* 2007. microRNA expression in the prefrontal cortex of individuals with schizophrenia and schizoaffective disorder. *Genome Biol.*, 8(2):R27. doi:10.1186/gb-2007-8-2-r27
- Scholer, N., Langer, C., Dohner, H., Buske, C., Kuchenbauer, F. 2010. Serum microRNAs as a novel class of biomarkers: a comprehensive review of the literature. *Exp. Hematol.*, 38(12): 1126–1130. doi: 10.1016/j.exphem.2010.10.004 PMID: 20977925.
- Zhao, C., Sun, G., Ye, P., Li, S., Shi, Y. 2013. MicroRNA let-7d regulates the TLX/microRNA-9 cascade to control neural cell fate and neurogenesis. *Sci. Rep.*, 3: 1329. doi: 10.1038/srep01329 PMID: 23435502.

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