

Prenatal Methamphetamine Exposure could Cause Chronic Abnormalities in Transcriptomic Pattern; an Extended Follow-up Cohort Study

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Research article

Keywords: prenatal methamphetamine exposure, gene expression, microarray, follow up

Posted Date: January 15th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-144191/v1>

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Abstract

Background

The high rate of methamphetamine abuse among young adults and women of childbearing age makes it imperative to clarify the long-term effects of Methamphetamine exposure on the offspring. Behavioral and cognitive problems had reported in children with parental Methamphetamine exposure (PME). The present study aimed to assess the acute and chronic effects of PME in molecular regulations and gene expression profiles of children during their first years of life.

Results

All subjects were recruited before birth, and sampling was conducted from the first ten days of birth, twelve months, twenty months and thirty-six months of age. Finally, 2658 children with PME and 3573 normal children had been finished the follow-up. RNA extraction was operated from blood samples and gene expression profiling was conducted by using the Affymetrix GeneChip Human Genome U133 plus 2.0 Array Platform. Gene expression data were confirmed by Real-time PCR. Gene expression profiling during thirty-six months showed several constant mRNA level alterations in children with PME compared with normal. These genes are involved in several gene ontology and pathways involved with the immune system, neuronal functions and bioenergetic metabolism. It seems that Methamphetamine abuse before and during the pregnancy period may affect the expression profile of children, and these changes could be remain years after birth. Affected genes have some similarities to the gene expression patterns of addiction, psychiatric disorders, neurodevelopmental disabilities and immune deficiencies.

Conclusion

Findings may shed light on the molecular effects of prenatal methamphetamine exposure and may lead to new psychological and somatic caring protocols for these children based on their potential abnormalities.

Background

Methamphetamine is a psychostimulant, neurotoxic drug that causes chronically psychological dependence in the abuser. Methamphetamine (MA) abuse is a global problem, and its abusers are more than cocaine and opiates combined (1). Gender analysis showed that female first-time users of methamphetamine are more than male first-time users (2). On the other hand, only less than fifty percent of those women are seeking treatment. In the United States, about seven percents of MA dependents that were referred to clinics for treatment were pregnant women (3).

Previous studies about the effects of prenatal methamphetamine exposure on child development showed an increase in gestational size at birth and decreased length through three years. Deficiency in movement and arousal along with increased stress signs in the newborn period and poor grasping ability at one to

three years old children were reported(4). Further studies in the age of three to five years have been shed light on attentional issues such as inhibitory control, aggressive behavior, and adjustment issues that could be related to abnormalities in the development of executive function in prenatal methamphetamine exposure children(5, 6).

Acute and chronic methamphetamine abusers show severe behavioral problems, including addictive behaviors such as craving, salience, and psychotic symptoms such as aggressiveness, hallucination, and delusion. Also, individuals with methamphetamine dependence have a higher risk of schizophrenia (7). While it is approved that methamphetamine is a highly addictive substance with widespread and constant effects in the central nervous system (CNS), its molecular mechanisms on adult and fetus are not clarified. Methamphetamine increased blood-brain barrier (BBB) permeability by rearrangement of F-actin cytoskeleton and reduction of tight junction (TJ) proteins (8). Mouse model assessments were reported that increased total DNA methylation in the mPFC could be associated with mPFC malfunction and long-term cognitive decline (9).

While there is a lack of information about molecular mechanisms of methamphetamine and its effects on the development of CNS, neuroimaging studies provide valuable evidence about the role of prenatal methamphetamine exposure in functional and structural changes in the brain (10). Methamphetamine abuse in adult persons could decrease the metabolism in the insula and frontal cortex and an increase in basal ganglia (11). However, diffusion tensor imaging (DTI) studies have reported that prenatal exposure to methamphetamine is more associated with white matter microstructure alterations (12). Alteration such as diffusion measures in white matter connections and reduction of fractional anisotropy (FA) in several connections between the striatum and midbrain (12, 13).

Based on some studies, mood difficulties, cognitive deficiencies, and chronic behavioral abnormalities are increased in PME children in childhood and maybe adolescence (14). Early detection of vulnerable children and psychological caring may prevent severe social and psychiatric problems in the next generation. That is why a general pattern of all alterations caused by prenatal methamphetamine exposure in molecular, neuroanatomical, and behavioral levels is highly needed (14).

The present study aimed to comprehensively evaluate the acute (or early) and chronically (or long-standing) effects of prenatal methamphetamine exposure in gene expression profile, behavior, and neuronal activities in the brain. We followed up a large sample size of children with prenatal methamphetamine exposure from date of birth to thirty-six months of age by using a functional genome-wide profiling approach.

Results

Demographic and clinical data of subjects are available in table 2.

DNA microarray results

Initial filtering with MAS 5.0 based on significance, fold change, and the effect size was showed 1234 genes differently expressed (626 up-regulated genes,608 down-regulated genes) in PME infants vs. normal infants. In twelve months sampling 1103 genes (572 up-regulated genes,531down-regulated genes), in twenty-four months sampling 976 genes (561 up-regulated genes,415 down-regulated genes), and in thirty-six months sampling 1006 genes (628 up-regulated genes,378 down-regulated genes) were differently expressed in PME children vs. normal children. Final results were calculated after FDR correction ($p < 0.01$), the ANOVA, and post hoc analysis ($p < 0.01$) along with Bonferroni multiple-comparison correction. Details list of differentially expressed genes in microarray in all comparisons between PME groups and normal subjects have been presented at supplementary data. Total gene expression differentiation in each sampling during the follow up are shown in figure 1.

DAVID and KEGG analysis

In order to reveal the molecular and cellular functional characteristics and pathways affected by PME, all of the DEGs were subjected to enriched gene ontology calculations, pathway analysis by KEGG pathways and DAVID, and findings were presented in table 3, table 4 and figure 2 respectively.

Potential biomarker prediction analysis

QPCR confirmation was conducted on 17 DEGs to validate changes in gene expression from the microarray analysis. Genes were selected based on fold changes, p-value, intensity threshold and KNN classification algorithm. Real-time PCR confirmations in these significantly altered genes were showed the same alterations in the same directions with those observed in the microarray. All genes in this list were showed the same direction in expression in all follow-up samplings. The comparison of the fold change obtained from qPCR and microarray determined a significant correlation between techniques ($P < 0.0001$, $r = 0.874$) that shows confirmations by qPCR indicated a strong reproducibility of genes expression results. Results of biomarker assessments showed a shortlist of candidate genes with high sustainability during the follow-up, which was determined in table 5 and figure 3. Primers of analyzed genes in qPCR presented in table 6.

Discussion

Substance abuse during pregnancy is a pandemic proportion worldwide. Primarily the effects of prenatal cigarette use on the fetus were first identified in the 60's decade, and then effects of alcohol, opiate, and the effects of several illicit drugs have been revealed. Recently studies have focused on the effects of prenatal exposure to CNS stimulant drugs (14, 17). Although there are several reports about the molecular, cognitive and brain structure of methamphetamine abuse, there is a great lack of information about PMEs. Few animal model reports studied the PME effects on molecular pathways in the brain (18,19,20). In addition, some psychological studies evaluated the cognitive effects of PME in children at the age of six or seven or adolescence (21,22). To our knowledge, the present study is the first gene

expression analysis of PME children and also is the first follow-up study of PMEs for a long term of a period and in a cohort big sample size. Especially the long term follow-up may help to separate the acute and temporary alterations of meth toxicity such as hepatotoxicity from chronic and constant changes such as neurodevelopment or immunodeficiency.

Gene ontology assessments

Genome expression profiling on methamphetamine treated animal model studies showed changes in critical genes for brain functions, including neuronal plasticity, mitochondrial energy metabolism and immune response (23). METH treatment induction on mitochondrial energy metabolism and associated glutamate receptor alterations as a neurotoxic response in the brain (including the amygdala, prefrontal cortex, hippocampus and striatum) of rats accompanied with a significant behavioral sensitization had reported (24,25). The present study may suggest that PME may induce chronic bioenergetic deregulations in children that, in turn, could increase the possibility of severe psychiatric disorders caused by mitochondrial dysfunction. Mitochondrial energy metabolism provides energy for brain functions and mitochondrial dysfunction are biomarkers for severe psychiatric disorders such as schizophrenia, bipolar disorder, and major depression disorders (26,27) especially complex I of the respiratory chain that is highly affected by PME are known to be associated with psychosis and number of cognitive dysfunctions in schizophrenia (28). Neuron projection, which is the most affected cellular component during the follow up is any process extending from a neural cell, such as axons or dendrites and is related to addiction tendency and stress problems and changes in brain circuitry (29,30). [Synaptic transmission](#) and [transmission of nerve impulse](#) are two biological processes that are affected in all samplings during the 36 months in PMEs. [Synaptic transmission](#) is essential for normal cognitive functions such as learning and memory (31). Dysfunction in [synaptic transmission](#) and [transmission of nerve impulses](#) had reported in a number of psychiatric disorders such as schizophrenia and autism(32,33). Findings may explain the learning and cognitive problem (34,35) and even anatomical changes in the brain of PMEs, such as reduced caudate and thalamus volume (36).

Pathway analysis

Probably the major and sever [Primary immunodeficiency](#) is the most worrying effect of PME observed in present study. METH treatment may cause an inflammatory response that plays a potential role in METH-induced neuronal injury and d regulation of cellular immune responses (37,38,39). METH also could increase blood-brain barrier (BBB) permeability that may explain similarities between PMEs and methamphetamine abusers(8), but taking together with [primary immunodeficiency](#) could increase the risk of several CNS infections in PMEs. There is comprehensive literature about methamphetamine role in immune deficiency and pathogenesis of [Human immunodeficiency virus](#) (HIV) (40,41). Immunodeficiency caused by methamphetamine can get [escalated](#) when accompanied by mitochondrial damage and increased levels of oxidative stress (42). By the way, [primary immunodeficiency](#) diseases (PIDs) are a

group of more than 300 different heterogeneous disorders, and many PIDs patients are diagnosed late. Due to poor prognosis, many cases suffer from complications by the burden of chronic infections, irretrievable end-organ damage, or even death before the definitive diagnosis (43,44). Results revealed that PME are born at risk of lethal immunodeficiency disorders. But the bright side is that the targeted prognosis of PME may lead to timely diagnosis and appropriate treatment of those PME who may affect by PIDs. Recent epidemiological cohort studies showed that methamphetamine abuse increases the risk for developing Parkinson's disease (PD) and there are similar **neurodegenerative processes** in PIDs and Meth abusers that target the nigrostriatal system (45,46.). In addition, **Parkinson's disease** pathway is mostly involved with dopaminergic and GABAergic pathways(47). Addiction tendency, number of psychiatric disorders including schizophrenia and attention deficit hyperactivity disorder (ADHD) and behavioral abnormalities such as anxiety and low-stress resilience are associated with overexpression of dopamine and GABA receptors, dopamine synthesis genes and downexpression of genes involved in the degradation of dopamine (48). Constant and severe deregulation of Parkinson related genes in PME provide evidence that prenatal methamphetamine exposure can cause long-lasting disabilities or degeneration of dopaminergic cell bodies, and it may increase the risk of PD in PME as well as Meth abusers. Also, these potential risks for PD can be detected in neonates.

The number of deregulations caused by methamphetamine, including reactive oxygen species activation and mitochondrial dysfunctions may lead to death receptor and ER pathways of apoptosis (49,50). Methamphetamine-induced apoptosis pathways are mostly mitochondria-mediated (51), but also the footprint of the mTOR signaling pathway were detected (52,53). Several genes from both mitochondrial and mTOR were differentially expressed in PME. mTOR pathway is also associated with autistic-like behaviors and locomotor activity problems (54).

Potential biomarkers

Nine genes have met the criteria of biomarker selection in all three samplings. These genes were related to dopamine signaling, transcription factors, and immune system regulations. Also, the deregulation of four genes was getting worse during PME life and had met the criteria in twelve months and twenty-four months' samplings and remained deregulated in thirty-six months' samplings. These genes were related to synaptogenesis, memory formation, and dopamine degradations.

Dopaminergic pathway dysfunction

Methamphetamine (METH) is an illicit toxic psychostimulant that is widely abused. Its toxic effects depend on the release of excessive levels of dopamine (DA) that activates striatal DA receptors (55). Psychostimulant can produce robust rewarding effects through modulating the dopaminergic synapse, dopamine re-uptake and dopamine-regulated downstream signals. Five genes of the biomarker list are involved in the dopamine pathway, dopamine receptors (D1, D2, D3), brain-derived neurotrophic factor (BDNF) were overexpressed and MAOA down expressed. BDNF is involved in regulating the expression of

dopamine receptors, especially in methamphetamine abuse and triggers behavioral sensitization, and MAOA are involved in the degradation of dopamine and its downregulation are related to violent behavior (56,57).

The similarity of expression pattern in PME and methamphetamine abuse in adults may support the evidence that methamphetamine can pass through the blood-brain barrier of infants. The up-regulation of dopamine receptors was reduced after twelve months but still significantly high during the next 24 months. About BDNF up-regulation was reduced after 12 months but chronically increased again. These patterns of dopamine pathway over function are well known in several psychiatric disorders such as schizophrenia and bipolar disorder (58). MAOA down-regulation started from first sampling but met criteria of significance at the twelve months sampling and remains almost constantly during the next 24 months. Findings may suggest that PME children could be at high risk for psychiatric disorders, also may explain the stress and violence problem of PMEs (4,5,59,60).

Immune system deregulation

Immune system abnormalities in PMEs are the most significant findings of the present study. By the way, the exact genes related to the immune system that meet the criteria of biomarkers are all involved in neurodevelopmental disorders, especially autism spectrum disorder(ASD). (Imbalances in cytokines level such as IL-1B, IL-6, IL-4, IFN- γ , and TGF-B had reported in ASD. Cytokines are mediators of immunological activity and an increase in infection and toxic exposures. Cytokines participate in normal neural development and function, and abnormal cytokine activity caused by meth neurotoxic effects may lead to several neurological dysfunctions. It may explain some of the similar symptoms of ASDs and PMEs, such as language impairments and lack of communication (4,5,61).

Synaptogenesis abnormalities

Another shared biomarkers between PMEs and ASDs were SHANK2 and DLG4 genes that are related to synaptogenesis. Lack of function in SHANK2 which is detected in late of follow up (at 24 and 36 months sampling) may cause ASD-like behaviours such as reduced social interaction and social communication and repetitive behaviors (62,63,64).

Transcription factors alterations

Four transcription factors that are well known in the development of the central nervous system were revealed in the PMEs biomarker list, CREB1, DLG4, MECP2, EGR2, and FOXP2. These genes are essential in memory formation, language function, and cognition. Dysfunction of these genes is involved in several psychiatric disorders such as schizophrenia, bipolar disorder, and depression. Findings may suggest that

PMEs are vulnerable to affect by psychiatric disorders as well as syndromic and heterogeneous disabilities in executive functions such as memory abnormalities and language impairments (65,66,67).

Hepatotoxicity

CYP2E1 was the only gene that appeared in the list of biomarkers just in the first time of samplings. CYP2E1 is an essential protein for [cytochrome P450](#) mixed-unction oxidase system that is involved in the metabolism and degradation of drugs, toxic environmental chemicals, and carcinogens along with several basic metabolic reactions such as fatty acid oxidations, ethanol, and glucose metabolism(68). It is reported that inflammatory cytokines such as (IL)-1beta inhibit the CYP2E1 expression that may cause metabolic abnormalities (69). It seems that abnormal activity of the immune system in PMEs could cause acute but not constant effects on xenobiotic metabolism and several digestive pathways related to glucose and lipids in PME infants. But it may be reregulated during the years.

Conclusion

Findings may suggest that PMEs are facing several psychological like psychiatric disorders and addiction and somatic problems such as metabolic or immunodeficiency disease during their lifetime. The severity of these problem could be different subject to subject and could be related to time and dosage of exposure. Finding out the nature of these health concern along with early detection of affected pathways may lead to better and targeted preventive health behaviors. Besides mRNA expression patterns, the underlying molecular regulatory networks like DNA methylation alterations, miRNAs and lncRNAs are also important to understand molecular alterations and potential dysfunctions in response to different kinds of METH exposure especially prenatal exposure. It may explain some of complexity that were detected in expression pattern during our follow-up and show the importance of future follow-up studies in different ages, including children at the age of school, after the synaptic pruning period, adolescent and even adulthood. Psychological testings along with molecular evaluations in next years of subjects' life may help to better understanding of PME effects on brain functions.

Material And Methods

Subject selection

Participants were recruited from a national cohort project of methamphetamine addiction (NCPM) in Iran, with over twenty thousand methamphetamine-dependent subjects. Infants were referred from female subjects of NCPM to PME group based on voluntary maternal self-report of methamphetamine abuse during pregnancy, confirmed by positive meconium screen, and gas chromatography or mass spectroscopy confirmation. PME infants and mothers were matched to a normal group for maternal race, age of mother and father in pregnancy time, birth-weight category (<1500 g, 1500–2500 g, >2500 g), health care insurance situation and education in comparison of infant-mother pairs. Maternal and

neonatal characteristics were examined for significant differences between the PME group and the normal group (Table 1). No difference was found in the baseline and demographic characteristics between groups.

Mothers were interviewed for sociodemographic and prenatal substance use information. Study assessments and sampling were conducted five times when infants were 1, 12, 24, 30, and 36 months of age. Mothers with less than twenty years of age, with any records of opiates or opioid, derived medication, lysergic acid diethylamide, phencyclidine, cocaine abuse during the pregnancy, any records of severe emotional disorders, low cognitive functioning, or current or history of psychosis and major depressive disorder or usage of antipsychotic drugs were excluded from the study. Children (in infant period) with exclusion criteria including critical illness, multiple gestations, life-threatening congenital anomaly, chromosomal abnormality associated with mental or neurological deficiency, overt clinical evidence of intrauterine infection, or a sibling previously enrolled in the study. The occurrence of any severe somatic or psychological disease or changes in lifestyle such as immigration, adaptation, or parents' divorce during the thirty-six months of follow-up were lead to the exclusion of subjects from the study. Maternal use of alcohol and tobacco and smoking during pregnancy was considered as background variables in both PME and normal comparison groups. Heavy methamphetamine exposure was defined as maternal use at least three days per week across pregnancy. The pattern of use, according to trimester and were showed in Table 2. Indicated overall decline and quitting methamphetamine use throughout the pregnancy. The level of exposure to other drugs of abuse was calculated as cigarettes per day, ounces of absolute alcohol per day.

At the beginning of the study, 3560 PME infants, including 2217 boys and 1343 girls and 4120 normal infants, including 2252 boys and 1868 girls, were recruited. At the end of the 36 months follow up in the PME group, only 2658 children, including 1287 boys and 930 girls and in normal children group, 3573 children including 2064 boys, and 1509 girls remained in the project and were participated in all terms of sampling and testing. Only these 2658 children in the PME group and 3573 normal children were analyzed for the study, and other children were excluded from the project. Written consent form filled by all mothers after explaining the aim of study for each subject in private. The study approved by the local ethical committee based on Helsinki declaration obligations.

Blood sampling and Microarray Analysis

Blood samples (5 ml, PAXgene vacutainer blood RNA-tubes; PreAnalytiX, Hombrechtikon, Switzerland) were collected between 10.00 and 11.00 AM. Total RNA was extracted from peripheral blood samples immediately after sampling by column Purification kit (GeneJET™ RNA Purification Kit#K0732, Thermo scientific, Latvia). RNA quality and integrity examined by Agilent 2100 Bioanalyzer (Agilent Technologies) before the beginning of the microarray process. Gene expression profiling analyses were conducted by using the Affymetrix GeneChip Human Genome U133 plus 2.0 Array Platform containing probes representing 39,000 genes. Preparation of labeled and fragmented aRNA targets, hybridization, and

scanning was carried out according to the manufacturer's protocol (Affymetrix Santa Clara, CA). A hundred nanograms of total RNA for each sample was processed using the GeneChip 3' IVT Express Kit. RNA was reverse transcribed and converted to double-stranded cDNA prior to biotin labeling during in vitro transcription. Then fifty micrograms of labeled aRNA were fragmented, and quality control (QC) was carried out using the Agilent Bioanalyzer. Fragmented aRNA was hybridized on GeneChip Human Genome U133 Plus 2.0 arrays for sixteen hours at 45°C. Finally, arrays were washed and stained by using the GeneChip Hybridization, Wash, and Stain Kit on the GeneChip Fluidics Station 450. Then chips were scanned using the Affymetrix GeneChip Scanner 3000, and all arrays passed the QC criteria examination.

The GeneChip analysis was performed in Genesis 2.0 (Gene Logic Inc.) and with Microarray Analysis Suite (MAS) 5.0, Data Mining Tool 2.0, and Microarray Database software (available at <http://www.affymetrix.com>). All of the genes represented on the GeneChip were globally normalized and scaled to a signal intensity of a hundred. Genes that passed MAS 5.0 criteria for false-positive results reduce included to further assessment. Expression data were analyzed using Genesis 2.0 (GeneLogic Inc., Gaithersburg, MD, USA) and DAVID software (Strand Genomics, Redwood City, CA, USA). Gene expression values were floored to 1, and then log₂ transformed. Analysis of variance test (ANOVA) was performed for each gene to identify significant gene expression alteration. Genes with statistical significance were subjected to a post hoc t-test for evaluation of contrasts groups one by one for identification of differences between groups. Two criteria were used in all subjects to determine whether a gene was differentially expressed; Fold change greater than 2 and a P value less than 0.05. Then P values adjusted for multiple testing correction.

Pathways and gene ontology analysis

Functional enrichment was conducted on differentially expressed data used for all comparisons. For functional annotation, gene ontology analysis was performed for differentially expressed genes with enrichment algorithms integrated into the online Database for Annotation, Visualization, and Integrated Discovery (DAVID 6.8) (14, 15). Kyoto encyclopedia of genes and genomic (KEGG) pathway enrichment tool used for the differentially expressed genes (DEGs) and calculated the enrichment factor (EF) for each term or pathway. The P values were calculated via hypergeometric tests and go with a correction. Only those GO terms or KEGG pathways with enrichment of corrected $P < 0.05$ and $EF > 2.0$ were included.

Biomarker assessment

Detection of most reliable and potential predictor biomarkers and footprint of prenatal methamphetamine exposure effects that could be potentially used for clinical and prognostic aspects are highly desired. Microarray data had analyzed by using Bioconductor and GeneFilter package from R programming language for the removal of low-intensity features. The GeneFilter package was used to filter and select genes from the microarray dataset according to a variety of different filtering mechanisms. Also, the k-nearest neighbor (KNN) classification was performed using leave-one-out cross-validation. The function

returns the predicted classifications as its returned value. To the exclusion of any false positive and false negative results DEGs that were meet criteria (Fold change > 2, multiple comparisons corrected P-value < 0.05 and intensity threshold > 2000) were selected for confirmation with qPCR and classification conducted based on previous studies (16).

Confirmation by quantitative Real-Time PCR

For confirmation of array data's reliability, mRNA levels of significantly altered genes (fold change>2 and p<0.05) were quantified by quantitative RT-PCR in all subjects. Blood sampling, RNA extraction, and cDNA synthesis repeated with the same method used for DNA microarray. Primers for all selected genes designed by "oligo7" software and blasted on the NCBI website to check the specificity. Quantitative PCR was performed by using SYBR green (Thermo Scientific Maxima SYBR Green/ROX qPCR Master Mix (2X) #K0221, Fermentas, Latvia). Triplicate method performed for Quantitative Real-time PCR by using the [7900HT Fast Real-Time PCR System](#) (Applied Biosystems, Foster City, CA, USA). GAPDH used for normalization as an endogenous reference gene.

Statistical analysis

Statistical analysis analyzed by SPSS, version 26. Descriptive data are expressed as mean \pm SD (range), and the level of statistical significance was set at $P < 0.01$. Compliance with normal distribution for continuous variables was assessed via the Kolmogorov-Smirnov test. One way ANOVA analysis was used for statistical differences in multiple group comparisons. Pearson correlation test was used for the evaluation of relations between gene expression data and clinical results. Pearson correlation analysis was conducted to determine the relationship between variables. Bonferroni correction was used for multiple comparisons corrections. Body mass index (BMI), RNA quality and concentration, cDNA synthesis quality, plates/runs of qPCR, and primer efficiency were added as covariates, and persistence of the significant difference in the main effect between groups was assessed by ANCOVA to control any potential confusion.

Abbreviations

PME: Parental methamphetamine exposure.

MA: Methamphetamine.

CNS: Central nervous system.

BBB: Blood brain barrier.

TJ: Tight junction.

DTI: Diffusion tensor imaging.

FA: Fractional anisotropy.

HIV: [Human immunodeficiency virus](#).

PIDs: [Primary immunodeficiency diseases](#).

PD: Parkinson's disease.

ADHD: Attention deficit hyperactivity disorder.

METH: Methamphetamine.

DA: Dopamine.

BDNF: Brain derived neurotrophic factor.

ASD: Autism spectrum disorder.

NCPM: National cohort project of methamphetamine addiction.

QC: Quality control.

MAS: Microarray analysis suite.

KEGG: Kyoto encyclopedia of genes and genomic.

DEGs: Differentially expressed genes.

EF: Enrichment factor.

KNN: k-nearest neighbor.

BMI: Body mass index.

Declarations

Availability of data and materials

All data are fully available without restriction in Arvin Gene Company data base.

Acknowledgements:

Present study was funded by Arvin Gene Company. We would like to thanks from all participants of study and their families and health care staffs.

Funding

The study were funded by neuroimaging genetic laboratory of Arvin Gene Copmany.

Contributions

Arvin Haghighatfard is the head of the research team and he participated in study design, laboratory procedure, data analysis and manuscript writting. Soha Seifollahi and Pegah Rajabi, participated in laboratory procedure and data analysis and prepration of parts of manuscript. Niloofar Rahmani and Rojin Ghannad zadeh participated in laboratory procedure.

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Ethics declarations

All process of study performed based on Helsinki declaration obligations. Written consent form filled by all mothers after explaining the aim of study for each subject in private. The study approved by the central ethical committee of Islamic Azad university under number of IR-IAU-3466012.

Consent for publications

All authors are informed and consent about the submission of manuscript and order of authors.

Competing interests

The authors declare that they have no competing interests.

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Tables

Due to technical limitations, tables are only available as a download in the Supplemental Files section.

Figures

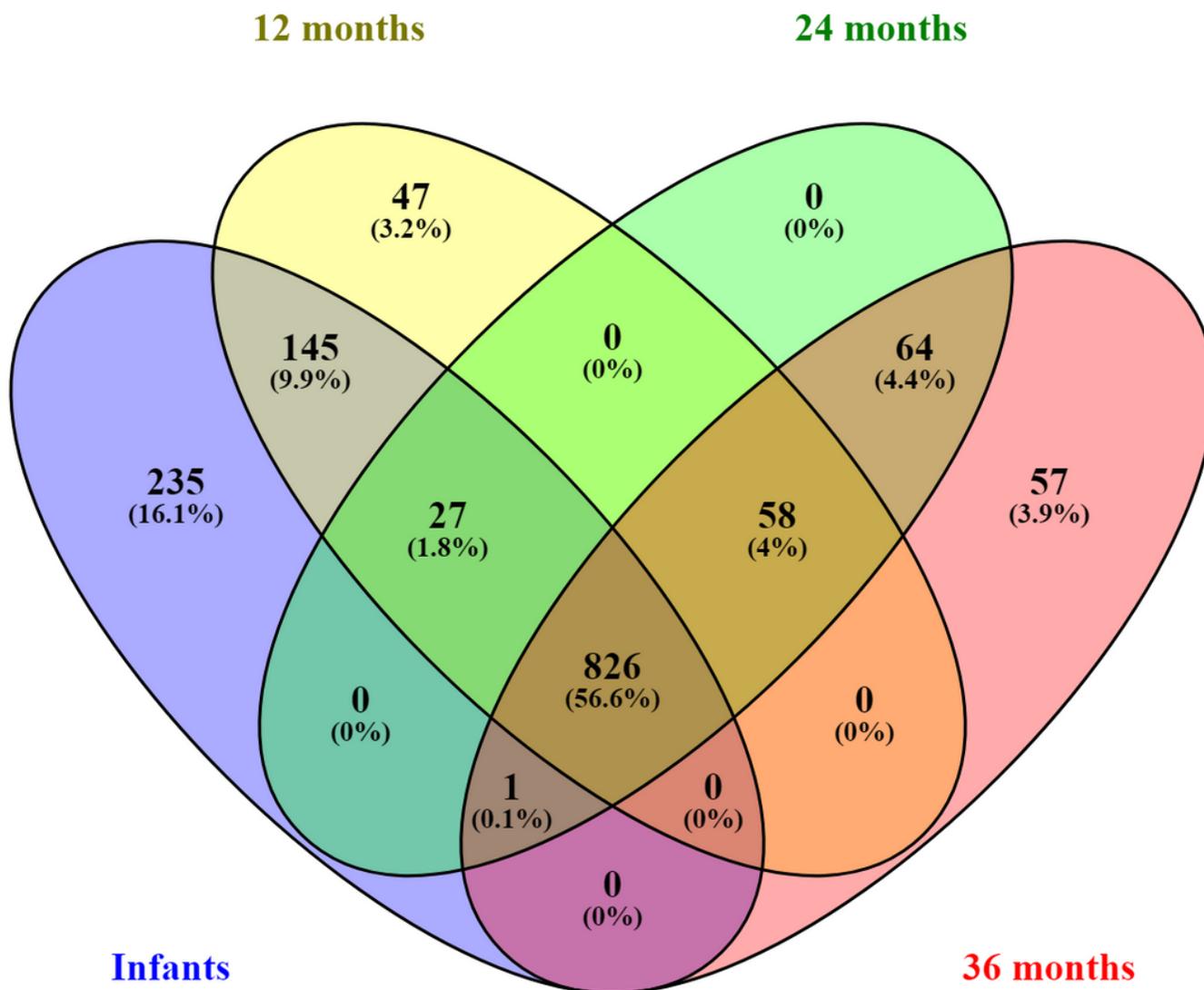


Figure 1

DEGs in response to prenatal meth exposure. Venn diagram showed the common set of DEGs in subjects with prenatal METH exposure during the follow up. None of shared genes showed different directionality

of expression changes .

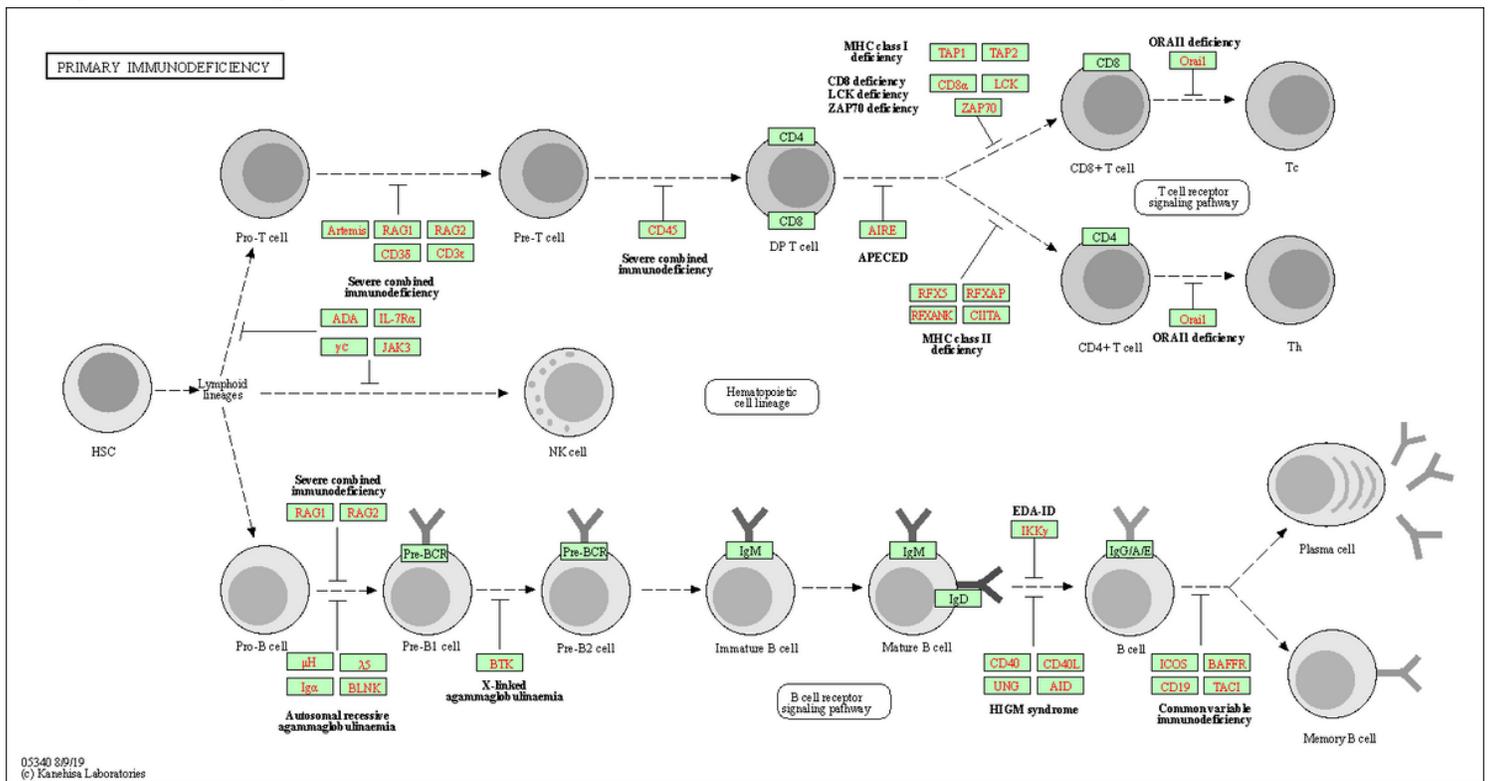


Figure 2

KEGG pathway analysis. The most significantly related pathway which were involved during the whole follow up was primary immunodeficiency. Image is from Kyoto encyclopedia of gene and genome. Red genes were differentially expressed in all four samplings in PME children compared to normal children.

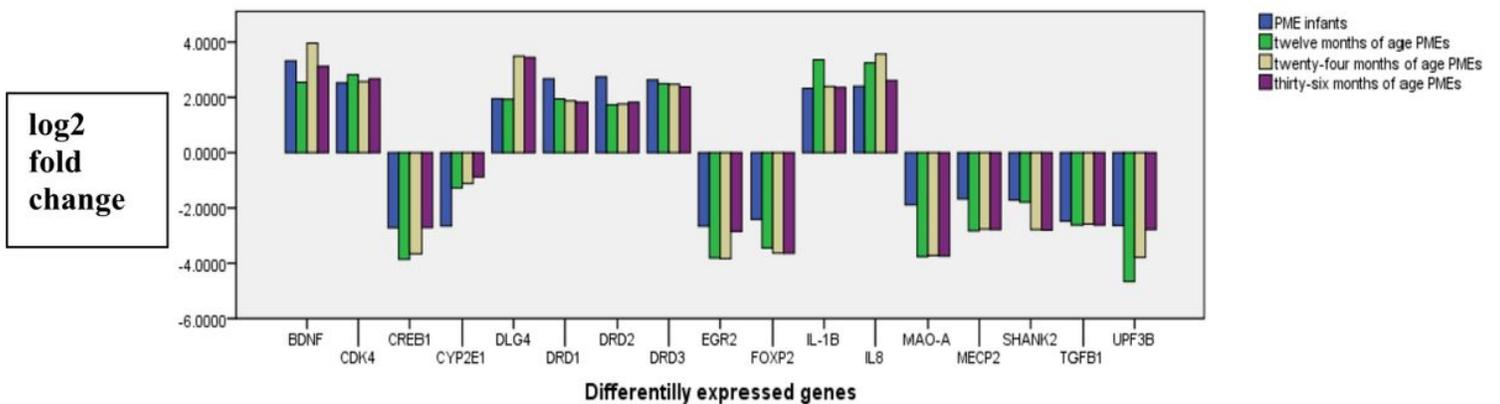


Figure 3

Potential biomarkers log2 fold change. Significant gene expression changes identified through qPCR in PME children during four time samplings. Expression of normal children presumed as one and revealed as black line. Fold changes of PMEs groups calculated in compare with normal children from matched age group. Blue bars is for infant PMEs, red bars is for twelve months of age PMEs, green bars is for twenty four months of age PMEs and yellow bars is for thirty six months of age PMEs.

Supplementary Files

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