Bulletin of Environmental Contamination and Toxicology
https://doi.org/10.1007/s00128-021-03249-w

Effects of Low-dose Mercury Exposure in Newborns on mRNA Expression Profiles

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Received: 22 February 2021 / Accepted: 21 April 2021

Abstract

This study was designed to investigate the molecular mechanism of mercury (Hg) toxicity in the newborns by mRNA sequencing (mRNA-seq). A questionnaire survey, routine blood parameters of pregnant women, and umbilical cord blood (UCB) of newborns were collected. The median (25th percentile, 75th percentile) of total Hg (THg) concentrations in UCB of newborns was 3.63 (2.50, 6.19) μg/L. A total of 504 differentially expressed genes of mRNA were revealed between the case and control group, including 456 upregulated and 48 downregulated genes. The Gene Ontology (GO) analysis showed that differentially expressed genes were primarily involved in mitophagy, hemoglobin complex, and oxygen carrier activity. Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis demonstrated that the most differentially expressed genes were annotated in Huntington's disease, Parkinson's disease, and Alzheimer's disease. The qRT-PCR was used to validate the results of mRNA-seq. Low-dose Hg exposure could increase blood NE# and WBC in the pregnant women. This study provides scientific evidences on mechanism of Hg toxicity in newborns.

Keywords Newborns · Low-dose Hg exposure · Umbilical cord blood · mRNA · Differentially expressed genes

Mercury (Hg) is an toxic environmental pollutant, and methylmercury (MeHg) is one of the most toxic compound (Dos Santos et al. 2016). MeHg can cause extensive damage to human health, primarily neurotoxicity (Bjørklund et al. 2017), which manifests predominantly as learning memory impairment and creeping paralysis (Clarkson et al. 2003). Pregnant women and fetuses are incredibly vulnerable populations to low-dose Hg exposure. The liquid solubility and short-chain hydrocarbyl structures of MeHg enable the toxin to form high-affinity binding with hemoglobin in the placentas and fetuses in pregnant women, enabling the accumulation of the MeHg in fetuses (Cordier et al. 2002). The average of total Hg (THg) concentrations in umbilical cord blood (UCB) was about 1.72 times that of pregnant women (Davidson et al. 2004). The newborns can develop evident nervous system impairments due to Hg exposure, even though the mother without obvious symptoms (Strain et al. 2008). The mechanism of Hg toxicity to newborns is complex and still unclear. Previous studies focused on single genes or pathways to elucidate the mechanism of Hg toxicity. The interactions and regulatory relationships between genes cannot be fully understood (Yaginuma et al. 2010, Dantzing. 2003). Most studies were conducted on animal models with high-dose Hg exposure. The results may not be applicable to pregnant women and newborns with long term low-dose Hg exposure. The transcriptome sequencing (Yang et al. 2016), especially mRNA sequencing (mRNA-seq), can study gene functions and structures from the overall level and disclose specific biological processes and molecular mechanisms correlated with disease. The molecular mechanism of Hg toxicity can be studied quickly and efficiently at the gene level through mRNA-seq. This study was designed to evaluate Hg exposure in newborns, and screen the differentially expressed genes by mRNA-seq.

The Wanshan Hg Mining Area (WMMA) is located in Tongren City, Guizhou Province, and it is the third-largest Hg mine in the world. It is a typical region to study Hg environmental pollution. Previous study evaluated blood

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Published online: 04 May 2021

heavy metals levels (Mn, Fe, Cu, Zn, As, Cd, Hg, Pb) in the population of the WMMA, and found that Hg exposure was the main issue (Li et al. 2016). In WMMA, blood MeHg concentrations in residents ranged from 2.20 to 9.36 μg/L (Li et al. 2015). The United States Environmental Protection Agency (USEPA) states that acceptable Hg limits in blood is 5.8 μg/L (USEPA 1997). The observed blood MeHg concentrations indicated the health risks of Hg exposure in Wanshan population.

This study was designed to evaluate Hg exposure in the newborns in the Wanshan area and differential expressions of mRNA. The overall expression modes of mRNA were investigated and analyzed. The obtained results can provide scientific evidences for the molecular mechanism of Hg-induced toxicity in newborns.

Materials and Methods

A questionnaire survey was conducted on pregnant women to obtain the information on height, weight, dietary habits, etc. The routine blood parameters of pregnant women were collected in the clinical laboratory of People's Hospital, Wanshan County. A total of 114 UCB samples were collected. All pregnant women were local permanent residents with living in the study area for more than one year. Exclusion criteria were (a) pregnant women who have communication difficulties and could not participate in the investigation; (b) pregnant women with hypertension, heart disease, liver disease, and kidney; and (c) pregnant women with infectious diseases, such as "Acquired Immune Deficiency Syndrome (AIDS), hepatitis-B, and syphilis". All respondents signed informed consent before the study. This project was approved by the Ethics Committee of Human Body Test of Guizhou Medical University (Approval No. 2020-75).

After delivery, two tubes of UCB were collected (4.5 mL), which were divided into a 2 mL EDTA-K2 anticoagulant tube and a 2.5 mL PAXgene blood sampling tube. The two tubes were inverted immediately for 8–10 times to mix evenly. All samples were stored under freezing conditions (−80℃) and then brought back to the laboratory. The whole blood in the EDTA-K2 anticoagulant tube was oxidized using BrCl, reduced using $SnCl_2$, enriched in a gold tube, and determined THg concentrations by atomic fluorescence spectrometry (Tekran 2500). The recoveries of THg concentrations in the certified reference materials (Seronorm Whole Blood L-2, Norway) averaged at 95.7 %. The average of relative percentage difference was 3.47 % for THg concentrations in blood duplicate samples. We divided the study population into the case and control groups according to the monitored THg concentrations in the UCB samples. We selected three samples in each group for mRNA sequencing by Shanghai Ouyi Biomedical Technology Co, Ltd. The constructed library was used for sequencing on the Illumina sequencer after passing Agilent 2100 Bioanalyzer's quality inspection. Fragments Per Kb Per Million Reads (FPKM) values represent the expression of protein-coding genes, and they can be used to calculate each gene's expression level. The calculation formula of FPKM is:

$$FPKM(A) = \frac{The\ number\ of\ fragments\ compared\ to\ gene\ A}{The\ total\ fragments\ of\ all\ genes\ were\ compared \times The\ length\ of\ gene\ A} \times 10^9$$

To compare the differential expressions of mRNA genes, the critical values were: set as $p\text{Value} < 0.05$, and the difference magnification is $|\log_2\text{FoldChange}| > 1$. The overall distribution situations of differential expressed genes were understood by drawing a volcano map. The unknown data features were analyzed by drawing an unsupervised hierarchical clustering analysis diagram.

The mRNA analysis was performed based on the original data gained by bioinformatics. The mRNA transcript sequences were compared to Swiss-Prot (http://www.gpmaw.com/html/swiss-prot.html) and KAAS (http://www.genome.jp/tools/kaas/) databases, thus getting Gene Ontology (GO) annotation information of each mRNA transcript and analyzing the Kyoto Encyclopedia of Genes and Genomes (KEGG) signal pathway database.

The quantitative real time polymerase chain reaction (qRT-PCR) was used validate the expression of mRNAs screened by mRNA-seq. Total RNA was extracted from blood using miRNeasy Serum/Plasma Kit (TransGen Biotech, Beijing, China) according to the manufacturer's specifications. qRT-PCR was performed using the PerfectStart™ Green qPCR SuperMix ((TransGen Biotech, Beijing, China) to quantify mRNA levels. All primers used for qRT-PCR were obtained from TsingKe Company (TsingKe, Beijing, China). qRT-PCR was performed using LightCycler® 480 II Real-time PCR (Roche, Swiss) in a 10 μL reaction mixture. The cycling conditions were: 94°C for 30 s, followed by 45 cycles of 94°C for 5 s, 60°C for 30 s. Each sample was run in triplicate for analysis. The expression levels of mRNAs were normalized to ACTB (TsingKe, Beijing, China), and were calculated using the $2^{-\Delta\Delta Ct}$ method (Zhang et al. 2018).

Statistical analyses were performed using SPSS for Windows 25.0, and the normal distribution of data was tested using the Kolmogorov-Smirnov test. Chi-square test and Mann-Whitney U test were used to analyze the differences in demographic and clinical characteristics. THg concentrations in the UCB samples were described by median (25th

percentile, 75th percentile). Spearman rank correlation was used to analyze the relationship between THg in UCB and routine blood parameters of pregnant women. We divided the study population into four groups according to the THg concentrations in the UCB samples: Q1 ≤ P_{25}; Q2: P_{25}-P_{50}; Q3: P_{50}-P_{75}; Q4 ≥ P_{75}. Kruskal-Wallis H test was used to compare the routine blood parameters of pregnant women between four groups. Values of $p < 0.05$ indicated that the difference was statistically significant.

Results and discussions

The basic characteristics of all subjects are shown in Table 1. No significant difference was found in age, weight, height, smoking habit, alcohol use, and whitening products use between different groups.

The median (25th percentile, 75th percentile) of THg concentrations in UCB of newborns in the Wanshan area was 3.63 (2.50, 6.19) μg/L (n = 114). The minimum and maximum of THg concentrations were 0.53 and 29.4 μg/L, respectively. Among the THg concentrations in UCB, 29.0 % exceeded 5.8 μg/L recommended by USEPA. Overall, the Hg exposure in newborns of WMMA indicated a low level over the world, but long term low-dose Hg exposure still cause certain health risks in newborns.

The volcanic diagram of differential genes (Fig. 1) depicted 504 differentially expressed genes screened in the case group, relative to the control group, including 456 upregulated genes and 48 downregulated genes. The unsupervised hierarchical clustering analysis graph showed that genes in one cluster might have similar biological functions.

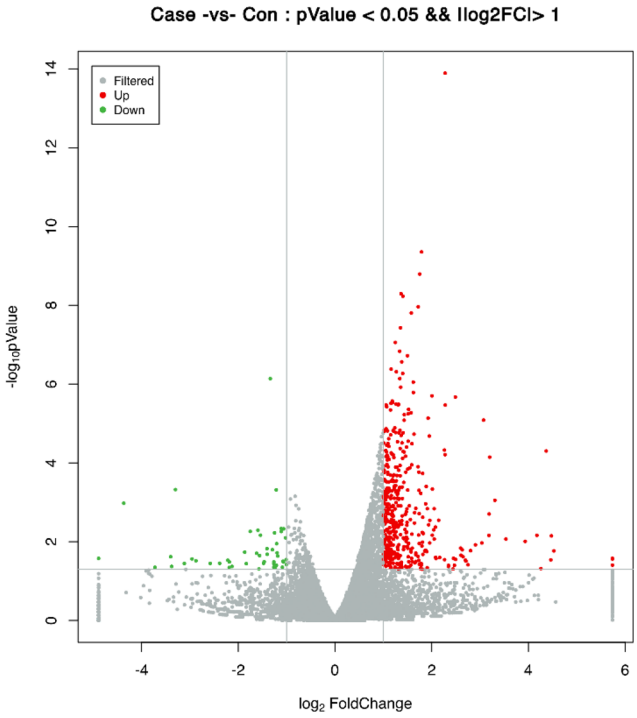

Fig. 1 Volcanic distribution map of differentially expressed mRNA. Notes: Grey is non-differentially expressed genes. The red is significantly upregulated genes, and the green is significantly downregulated genes. The X-axis is log_2FoldChange, and the Y-axis is -$log_{10}p$Value

The case group's mRNA mode was significantly different from that of the control group (Fig. 2).

The GO analysis showed that the case group had 2142 GO terms of differentially expressed genes compared with the control group, which include 1346 GO terms of biological

Table 1 Basic characteristics of all subjects

	mRNA-seq		Whole population		
	Case	Con	Case	Con	*p*
n	3	3	57	57	–
Age (mean ± SD)	31.7 ± 1.3	30.3 ± 1.5	25.8 ± 3.8	25.9 ± 5.6	0.532[b]
Weight (mean ± SD)	66.7 ± 3.1	66.0 ± 2.0	68.6 ± 9.2	67.2 ± 9.9	0.370[b]
Height (mean ± SD)	156.7 ± 5.7	155.3 ± 4	155.8 ± 4.9	155.5 ± 5.3	0.925[b]
Smoking					
Yes	0	0	6	5	0.751[a]
No	3	3	51	52	
Alcohol use					
Yes	0	0	5	7	0.542[a]
No	3	3	52	50	
Whitening products use					
Yes	0	0	9	12	0.469[a]
No	3	3	48	45	

[a] Two-tailed Chi-square test

[b] Independent sample Mann-Whitney U test

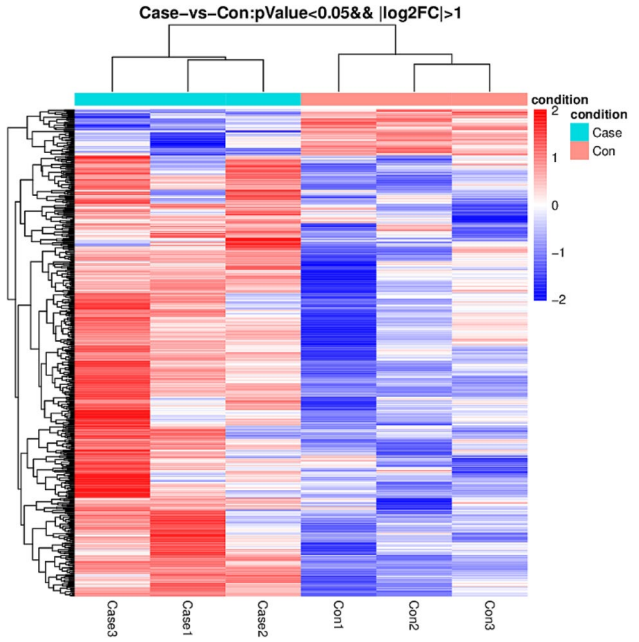

Fig. 2 Clustering diagram of differentially expressed mRNA. Notes: The X-axis is samples name, and the Y-axis is genes. The red is relatively high-expressed protein-coding genes, and the blue is relatively low-expressed protein-coding genes. Color gradation shows gene expression changes from the relatively low level (blue) to the relatively high level (red)

processes (62.8 %), 331 GO terms of cellular components (15.4 %), and 465 molecular functions (21.7 %), respectively. The functions of mitophagy, hemoglobin complex, and oxygen carrier activity changed significantly (Fig. 3), confirming that low-dose Hg exposure influences mitochondrial functions and hemoglobin contents in newborns.

The KEGG pathway analysis revealed 41 significant correlations between the case and control groups (pValue < 0.05). The most differentially expressed genes were associated with pathways of Huntington's disease, Parkinson's disease, and Alzheimer's disease (Fig. 4), which indicated that low-dose Hg exposure can interfere pathways related to neurodegeneration.

Consideration of the differences of gene functions and expression data enabled differentially expressed protein-encoding genes to be screened. The hemoglobin subunit gamma2 (HBG2) is upregulated significantly in the case group, while CD177 glycoproteins are significantly downregulated. The expressions of the two candidate mRNAs were measured using qRT-PCR. These results were consistent with those of the mRNA sequence (Fig. 5). As a member of the globin family, HBG2 is in the fetal hemoglobin subgroup and is closely inter-related with fetal hemoglobin expression (Lux et al. 2019; Metais et al. 2019; Perry et al. 2008). CD177 is an adhesion molecule that can exist in endothelial cells. Blood platelets combine with various adhesion molecules such as PECAM-1 and CD31 of blood platelet endothelial cells on the surface of circulating leukocytes, enabling the neutrophile granulocyte adhesion as well

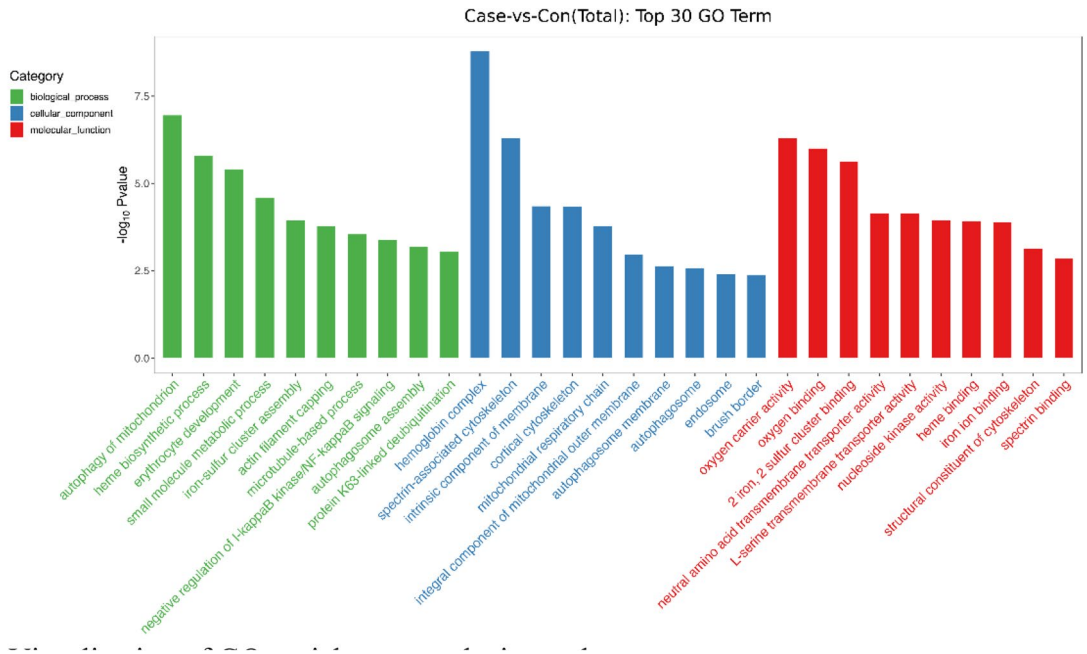

Fig. 3 Visualization of GO enrichment analysis results. Notes: The X-axis depicts the GO Term, and the Y-axis the -$\log_{10}p$Value. The three colors represent three different components of GO

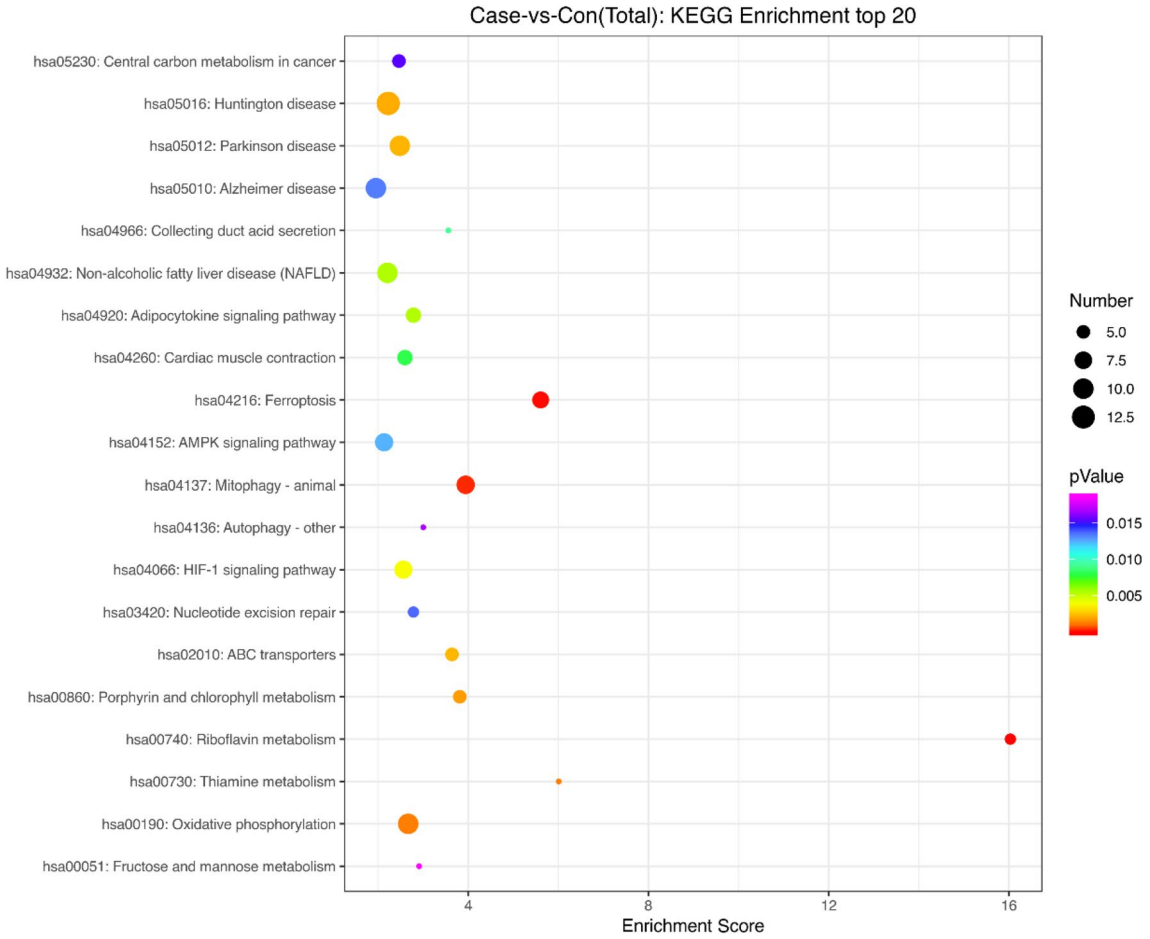

Fig. 4 Top20 bubble graph from KEGG enrichment analysis. Notes: The X-axis is the enrichment score, and the larger bubble term contains more differential protein-encoding genes. Bubble colors vary incrementally from purple to blue, green, and finally red. The smaller *p*Value indicates a larger significance

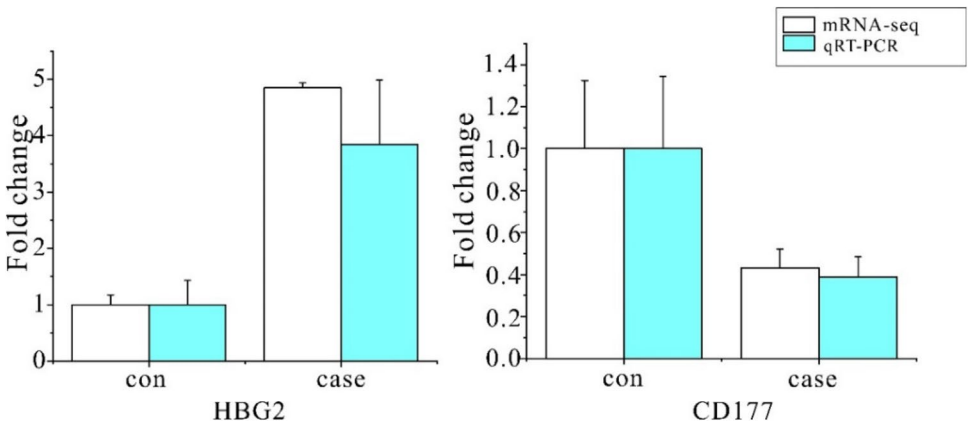

Fig. 5 Comparison of the results obtained by qRT-PCR and mRNA-seq

as the migration of neutrophile granulocytes (Wolff et al. 2003). Previous studies have confirmed that CD177 genes are associated with the immune syndromes and hematopoiesis activities (Huang et al. 2019; Abd El-Wahed et al. 2018).

The correlation coefficients (r_s) of THg concentrations in UCB with platelet (PLT), neutrophil (NE#), and white blood cell (WBC) were 0.240, 0.228, and 0.243, respectively. The THg concentrations in UCB showed significant positive correlations with PLT, NE#, and WBC ($p< 0.05$). The PLT,

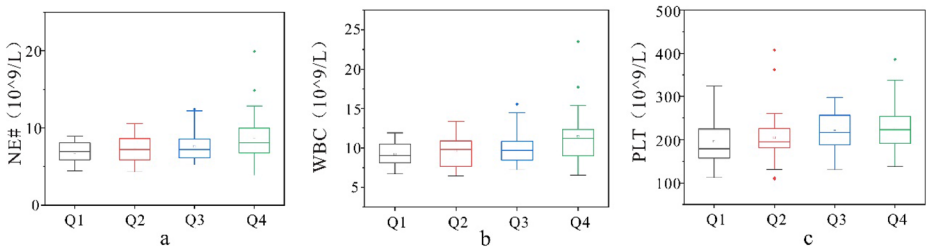

Fig. 6 Box diagram of routine blood parameters. Notes: The X-axis is the quartiles of THg concentrations in the UCB samples: Q1 ≤ P_{25}; Q2: P_{25}-P_{50}; Q3: P_{50}-P_{75}; Q4 ≥ P_{75}

NE#, and WBC were compared between four groups with different THg concentrations in UCB. The fourth quartile (Q4) showed significantly higher NE# and WBC than the first quartile (Q1) ($p< 0.05$), indicating that Hg exposure does increase NE# and WBC (Fig. 6a and b). The glycoprotein CD177 is associated with neutrophile granulocytes, and low-dose Hg exposure can influence immunity and hematopoiesis in pregnant women and newborns by regulating the expression of CD177 genes.

Hg is extremely neurotoxic. Low-dose Hg exposure in pregnant women and newborns can cause inattention and memory impairment. The molecular biology of Hg-induced neurotoxicity has recently investigated. This study evaluated THg concentrations in UCB of newborns, and mRNA-seq data showed that low-dose Hg exposure can influence mitochondrial functions. One hypothesis for this observation is that Hg exposure might decrease the electron transport chain activity of the mitochondria's enzyme complex, thus decreasing stresses (Krishna Chandran et al. 2019). Additionally, low-dose Hg exposure can cause mitochondria damages, which further triggers mitophagy. Mitophagy primarily refers to the autophagy process of selectively removing residual or damaged mitochondria, playing an essential role in regulating mitochondria in cells and maintaining the normal function of mitochondria (Lemasters. 2005). Mitophagy promotes the initiation and development of neurodegenerative diseases (Sheng. 2014), such as Huntington's disease (Wong et al. 2014), Alzheimer's disease (Moreira et al. 2014) and Parkinson's disease (Batlevi et al. 2011).

Erythrocyte development and hemoglobin complex formation were also implicated within the differential genes highlighted between the control and case groups. In particular, HBG2 genes are significantly upregulated, indicating that low-dose Hg exposure might influence fetal hemoglobin content by regulating the expression of HBG2 genes. Low-dose Hg exposure also altered in vivo NE# and WBC by regulating the expression of CD177 genes, thus influencing the pregnant women's and newborns' immunity and hematopoiesis.

In summary, the Hg exposure in newborns from Wanshan area indicated low level over the world, but long term low-dose Hg exposure still cause certain health risks in newborns. Differential gene expression profiles showed that Hg exposure mainly affected the functions of autophagy of mitochondrion and oxidative stress. The main related pathways were similar with those involved in neurodegenerative diseases such as Huntington disease, Parkinson disease and Alzheimer disease. In addition, low-dose Hg exposure can alter genes in immune-related signaling pathway, which may affect the immune function in pregnant women and newborns. These research results provide new insights on the mechanisms in newborns experiencing chronic, low-dose Hg exposure, and provide scientific advices for risk control of human Hg exposure in Hg polluted areas.

Acknowledgements This study is funded by the National Natural Science Foundation of China (U1812403), Key Laboratory of Environmental Pollution Monitoring and Disease Control, Ministry of Education, Guizhou Medical University (GMU-2020-HJZ-04).

References

Abd El-Wahed MR, Gawish HH, Ghobashy AA, Ismail NE (2018) CD177 expression in β-thalassemia patients in Zagazig University. Egyptian J Haematol 43:25–31. https://doi.org/10.4103/ejh.34.17

Batlevi Y, Albert R, Spada L (2011) Mitochondrial autophagy in neural function, neurodegenerative disease, neuron cell death, and aging. Neurobiol Dis 43:46–51. https://doi.org/10.1016/j.nbd.2010.09.009

Bjørklund G, Aaseth J, Ajsuvakova OP, Nikonorov AA, Skalny AV, Skalnaya MG, Tinkov AA (2017) Molecular interaction between mercury and selenium in neurotoxicity. Coord Chem Rev 332:30–37. https://doi.org/10.1016/j.ccr.2016.10.009

Clarkson TW, Magos L, Myers GJ (2003) The toxicology of mercury — Current exposures and clinical manifestations. N Engl J Med 349:1731–1737. https://doi.org/10.1056/NEJMra022471

Cordier S, Garel M, Mandereau L, Morcel H, Doineau P, Gosme-Seguret S, Josse D, White R, Amiel-Tison C (2002) Neurodevelopmental investigations among methylmercury exposed children in French Guiana. Environ Res Sect 89:1–11. https://doi.org/10.1006/enrs.2002.4349

Dantzig PI (2003) A New Cutaneous Sign of Mercury Poisoning. J Am Acad Dermatol 49:1109–1111. https://doi.org/10.1016/s0190-9622(03)02485-x

Davidson PW, Myers GJ, Weiss B (2004) Mercury exposure and child development outcomes. Pediatrics 113:1023–1029

Dos Santos AA, Hort MA, Culbreth M, Lopez-Granero C, Farina M, Rocha JBT, Aschner M (2016) Methylmercury and brain development: A review of recent literature. J Trace Elem Med Biol 38:99–107

Huang YH, Lo MH, Cai XY, Liu SF, Kuo HC (2019) Increase expression of CD177 in Kawasaki disease. Pediatric Rheumatology Online J 17:13. https://doi.org/10.1186/s12969-019-0315-8

Krishna Chandran AM, Christina H, Das S, Mumbrekar KD, Satish Rao BS (2019) Neuroprotective role of naringenin against methylmercury induced cognitive impairment and mitochondrial damage in a mouse model. Environ Toxicol Pharmacol 71:103224. https://doi.org/10.1016/j.etap.2019.103224

Lemasters JJ (2005) Perspective-Selective mitochondrial autophagy, or mitophagy, as a targeted defense against oxidative stress, mitochondrial dysfunction, and aging. Rejuvenation Res 8:3–5. https://doi.org/10.1089/rej.2005.8.3

Li P, Feng XB, Chan HM, Zhang XF, Du BY (2015) Human body burden and dietary methylmercury intake: the relationship in a rice-consuming population. Environ Sci Technol 49:9682–9689. https://doi.org/10.1021/acs.est.5b00195

Li P, Li YF, Feng XB (2016) Mercury and selenium interactions in human blood in the Wanshan mercury mining area, China. Sci Total Environ 573:376–381. https://doi.org/10.1016/j.scitotenv.2016.08.098

Lux CT, Pattabhi S, Berger M, Nourigat C, Flowers DA, Negre O, Humbert Oet al (2019) Talen-mediated gene editing of HBG in human hematopoietic stem cells leads to therapeutic fetal hemoglobin induction. Mol Therapy - Methods Clini Develop 12:175–183. https://doi.org/10.1016/j.omtm.2018.12.008

Metais JY, Doerfler PA, Mayuranathan T, Bauer DE, Fowler SC, Hsieh MM, Katta V et al (2019) Genome editing of HBG1 and HBG2 to induce fetal hemoglobin. Blood Advances 3:3379–3392. https://doi.org/10.1182/bloodadvances.2019000820

Moreira PI, Siedlak SL, Wang XL, Santos MS, Oliveira CR, Tabaton M, Nunomura A et al (2014) Increased autophagic degradation of mitochondria in Alzheimer disease. Autophagy 3:614–615. https://doi.org/10.4161/auto.4872

Perry RT, Gearhart DA, Wiener HW, Harrell LE, Barton JC, Kutlar A, Kutlar F et al (2008) Hemoglobin binding to Aβ and HBG2 SNP association suggest a role in Alzheimer's disease. Neurobiol Aging 29:185–193. https://doi.org/10.1016/j.neurobiolaging.2006.10.017

Sheng ZH (2014) Mitochondrial trafficking and anchoring in neurons: New insight and implications. J Cell Biol 204:1087–1098. https://doi.org/10.1083/jcb.201312123

Strain JJ, Davidson PW, Bonham MP, Duffy EM, Stokes-Riner A, Thurston SW, Wallace JMW et al (2008) Associations of maternal long chain polyunsaturated fatty acids, methylmercury, and infant development in the seychelles child development nutrition study. Neurotoxicology 29:776–782. https://doi.org/10.1016/j.neuro.2008.06.002

USEPA (1997) Mercury study report to the congress (Volume V): health effects of mercury and mercury compounds [J]. Washington: United states Environmental Protection Agency, 1997

Wolff J, Brendel C, Fink L, Bohle RM, Kissel K, Bux J (2003) Lack of NB1 Gp (CD177/HNA-2a) gene transcription in NB1 GP$^-$neutrophils from NB1 GP–Expressing individuals and association of low expression with NB1 gene polymorphisms. Blood 102:731–733. https://doi.org/10.1182/blood-2002-09-2831

Wong YC, Holzbaur ELF (2014) The regulation of autophagosome dynamics by Huntingtin and HAP1 is disrupted by expression of Mutant Huntingtin, leading to Defective Cargo Degradation. Neuroscience 34:1293–1305. https://doi.org/10.1523/JNEUROSCI.1870-13.2014

Yaginuma-Sakurai K, Murata K, Shimada M, Nakai K, Kurokawa N, Kameo S, Satoh H (2010) Intervention study on cardiac autonomic nervous effects of methylmercury from seafood. Neurotoxicol Teratol 32:240–245. https://doi.org/10.1016/j.ntt.2009.08.009

Yang Y, Yu H, Li H, Wang A (2016) Transcriptome profiling of grass carp (ctenopharyngodon idellus) infected with Aeromonas hydrophila. Fish Shellfish Immunol 51:329–336. https://doi.org/10.1016/j.fsi.2016.02.035

Zhang J, Jiang Y, Han X, Roy M, Liu W, Zhao X, Liu J (2018) Differential expression profles and functional analysis of plasma miRNAs associated with chronic myeloid leukemia phases. Future Oncol 15(7):763–776. https://doi.org/10.2217/fon-2018-0741