HE002

The Association Analysis of Bisphenol A (BPA)-Responsive Genes and Dysregulated Genes in Autism Spectrum Disorder

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Abstract

Autism spectrum disorder (ASD) is a group of neurodevelopmental disorder characterized by deficits in 2 symptoms which are social and communication impairment and repetitive behaviors together with limited interests with the prevalence of 1 in 59 children in the United States. The exact cause of this disorder is still unclear but there is accumulating evidence that the susceptibility of ASD is influenced by the combination of multiple genes and environmental factors. Bisphenol A (BPA) is an endocrine disrupting chemical that has been associated with ASD. Recent studies have reported that children with ASD have increased BPA levels in the blood and the urine. However, it is still unclear whether BPA can alter the expression of genes associated with ASD. In this study, we conducted a series of bioinformatic analyses using CU-DREAMx program and pathway analysis to predict the association of BPA-responsive genes and ASD, and also predict biological functions impacted by BPA exposure. We found that BPA-responsive genes (i.e. *SOD1*, *MEF2C*, and *GNAS*) are significantly associated with ASD and ASD-related neurological functions. These genes may serve as good candidates for studying the effect of BPA exposure at the molecular level and its role in ASD susceptibility in the future.

Keywords: autism spectrum disorder, bisphenol A, endocrine-disrupting chemical, bioinformatics, CU-DREAMx

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1. Introduction

Autism spectrum disorder (ASD) is an early-onset neurodevelopmental disorder with abnormalities in two main functions: impairment in social interaction and communication, and repetitive behaviors and/or restricted interests. The prevalence of ASD recently reported by The Centers for Disease Control and Prevention (CDC) is 1 in 59 children in the United States (Baio, Wiggins et al. 2018). Although there is supporting evidence that some of the ASD cases may be related to genetic factor, the majority of ASD cases are idiopathic with unidentified genetic cause. The risk of ASD is thought to be influenced by gene-environment interactions including epigenetic mechanisms, including DNA methylation (Nguyen, Rauch et al. 2010, Moosa, Shu et al. 2018, Saeliw, Tangsuwansri et al. 2018) and histone modifications (LaSalle 2013, Sun, Poschmann et al. 2016), and exposure to environmental chemicals. Recent studies have revealed that ASD risk is associated with certain environmental pollutants, including endocrine-disrupting chemicals (EDCs) (Miodovnik, Engel et al. 2011), lead(Adams, Audhya et al. 2013), mercury(Geier, Audhya et al. 2010), and pesticides(Roberts, English et al. 2007). EDCs are a group of chemicals widely used in many plastic products. The structure of such chemicals are similar to steroid hormones including estrogen, androgen, and thyroid hormone. BPA is the one of the EDCs mostly used in polycarbonate plastics and thought to disrupt hormone regulatory system in animals and humans. Once ingested, BPA passes through placenta and blood-brain barrier (Sun, Nakashima et al. 2002, Nishikawa, Iwano et al. 2010). Several recent studies have reported that BPA affects brain development and results in behaviors linked to ASD (Tando, Itoh et al. 2007, Xu, Zhang et al. 2010, Wolstenholme, Edwards et al. 2012, Jardim, Sartori et al. 2017, Kumar and Thakur 2017). ASD children have been reported to exhibit increased levels of BPA in the blood and the urine (Stein, Schluter et al. 2015, Kardas, Bayram et al. 2016, Kondolot, Ozmert et al. 2016). However, whether BPA can alter the expression of genes associated with ASD is unclear.In this study, we therefore sought to determine the association of BPA and ASD at the molecular level by using a series of bioinformatic analyses. Moreover, we predicted biological functions and pathways associated with BPA-responsive genes to determine whether they are associated with ASD-related functions.

2. Objectives

1. To predict the association of BPA-responsive genes and ASD using CU-DREAMx bioinformatic software.

2. To predictbiological functions and networks ofBPA-responsive genes that are related to ASD.

3. Methods

Collection of BPA-responsive genes and ASD candidate genes

To obtain the list of BPA-responsive genes, we searched NCBI GEO Data Sets database (https://www.ncbi.nlm.nih.gov/gds/) using "Bisphenol-A" as a search entry to find gene expressionstudies associated with BPA. All BPA studies which were conducted in cell lines, primary cells, or animals were used in this analysis. ASD candidate genes were collected from SFARI database (https://gene.sfari.org/database/human-gene/).

Association analysis of BPA-responsive genes and ASDcandidate genes

The Connection Up and Down Regulation Expression Analysis of Microarrays extension (CU-DREAMx) software is a bioinformatictool used for identification of differentially expressed genes and for association analysis (Termglinchan, Wanichnopparat et al. 2013). To predict whether BPA can cause changes in the expression of genes associated with ASD, CU-DREAMx program was used in this study for identifying genes that were significantly disrupted by BPA exposure and also for determining the association between the list of BPA-responsive genes and the list of ASD candidate genes. The gene expression data from each study obtained from NCBI GEO Data Sets was reanalyzed by CU-DREAMx program to identify up- or down-regulated genes in BPA treatment group compared to controls. P-values of less than 0.05 were considered as significantly differentially expressed. The list of significantly differentially expressed genes was then further overlapped with the list of ASD candidate genes obtained from SFARI database using Pearson's chi-squared test. P-values of less than 0.05 were considered as significantly associated.

Prediction of biological functions and pathways

Ingenuity Pathway Analysis (IPA) software(https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis/) was used for predicting biological functions and pathways associated with significantly differentially expressed genes in BPA treatment group that are associated with ASD. Fisher's exact test (p-value < 0.05) was used for determining significant association.

4. Results

BPA-responsive genes were significantly associated with ASDcandidate genes.

To identify genes that are significantly differentially expressed due to BPA exposure, gene expression data previously deposited in NCBI GEO Data Sets database were downloaded and reanalyzed using CU-DREAMx software as described in the Methods section. Seven transcriptome studies were found to meet the criteria and used for subsequent analyses (**Table 1**). Moreover, the list of up- or down-regulated was then overlapped with the list of 881 ASD candidate genes obtained from autism SFARI databases. Out of seven gene expression studies, the list of BPA-responsive genes from four studies (i.e. GSE44387, GSE63852, GSE50527, and GSE86923) were found to be significantly associated with ASD candidate genes (p-value<0.05) (**Table 1**). Interestingly, as many as 327 genes significantly disrupted in BPA exposure were identified to be ASD candidate genes in SFARI database, suggesting that BPA exposure may cause changes in the genes involved in the molecular mechanisms related to ASD.

Table 1. Association analysis between the list of BPA-responsive genes from each gene expression study and the list of ASDcandidate genes identified by SFARI database.

CU-DREAMx software was employed to identify up- and down-regulated genes that were significantly differentially expressed in BPA treatment group compared to control group in each gene expression dataset. Each list was then overlapped with a total of 881 genes identified to be associated with ASD in SFARI database. Pearson's chi-squared test was conducted to determine the association with ASD candidate genes.

Table 1. Association analysis between the list of BPA-responsive genes from each gene expression study and the list of ASDcandidate genes identified by SFARI database.

GEO Datasets	P-values (Chi-Squared Test)	
	Down-Regulated Genes (number of all; number of genes overlapping with SFARI)	Up-Regulated Genes (number of all; number of genes overlapping with SFARI)
GSE5200	6.89E-01 (29; 2)	3.14E-01 (7,851; 424)
GSE44387	6.23E-01 (165; 5)	**5.70E-04* (3,889; 114)**
GSE63852	7.19E-01 (455; 19)	**4.29E-02* (439; 10)**
GSE58642	1.31E-01 (332; 7)	3.12E-01 (110; 6)
GSE50527	**1.51E-03* (577; 39)**	2.79E-01 (684; 34)
GSE58516	4.68E-01 (48; 1)	3.75E-01 (147; 4)
GSE86923	8.09E-02 (2,276; 67)	**3.22E-03* (2,986; 89)**

Biological functions and pathways associated with BPA-responsive genes

To predict biological functions and pathways associated with BPA-responsive genes linked to ASD, the list of 327 BPA-responsive genes identified to be ASD candidate genes were analyzed by Ingenuity Pathway Analysis (IPA) software. The IPA analysis revealed that BPA-responsive genes identified to be ASD candidate genes are significantly associated with "Developmental Disorder" (p-value=5.94E-41-1.42E-04), "Neurological Disease" (p-value=1.24E-33-1.57E-04), and "Hereditary Disorder" (p-value=6.99E-27-1.42E-04) (**Table 2**). One of the top canonical pathways significantly associated withthis set of BPA-responsive genes is "Reelin Signaling in Neuron" (p-value=2.95E-07) which is a molecular signaling highly implicated in ASD (**Table 3**). Moreover, the BPA responsive genes were also predicted to significantly associated with "Autism or intellectual disability" (p-value=5.94E-41), "Autism" (p-value=7.33E-14), "Vocalization" (p-value=2.07E-05), and "Social behavior" (p-value=3.06E-05) (**Table 4**).

Table 2. Top diseases/disorders significantly associated with BPA-responsive genes. P-values were calculated by Fisher's exact test (p-value < 0.05).

Diseases/Disorders	P-values	Number of Genes
Developmental Disorder	5.94E-41-1.42E-04	100
Neurological Disease	1.24E-33-1.57E-04	191
Hereditary Disorder	6.99E-27-1.42E-04	127
Organismal Injury and Abnormalities	6.99E-27-1.54E-04	318
Cancer	7.64E-25-1.54E-04	314

Table 3. Top canonical pathways significantly associated with BPA-responsive genes. P-values were calculated by Fisher's exact test (p-value < 0.05).

Canonical pathways	P-values
CREB Signaling in Neurons	1.70E-08
GABA Receptor Signaling	3.98E-08
Reelin Signaling in Neurons	2.95E-07
Androgen Signaling	3.47E-07
Synaptic Long Term Depression	1.02E-06

Table 4. Top neurological diseases/disorders and neurological functions significantly associated with BPA-responsive genes. P-values were calculated by Fisher's exact test (p-value < 0.05).

Functional Annotation	P-values	Number of Genes
Developmental Disorder		
Autism or intellectual disability	5.94E-41	69
Mental retardation	3.88E-33	59
Familial syndromic intellectual disability	6.24E-21	37
Autism	7.33E-14	18
Susceptibility to autism	2.89E-10	8
Nervous System Development and Function		
Development of central nervous system	3.5E-10	17
Formation of brain	4.54E-08	11
Neuritogenesis	1.03E-06	11
Hearing	7.13E-05	7
Behavior		
Cognition	7.89E-05	8
Vocalization	2.07E-05	4
Social behavior	3.06E-05	4

Gene regulatory network, which is a collection of genes that interact with each other or with other diseases or biological functions, of the BPA-responsive genes were also conducted using IPA software (**Figure 1**). Interestingly, the network shows that BPA-responsive genes are associated with neurological diseases and behaviors related to ASD including "Autism or intellectual disability", "Susceptibility to autism type 18", "Mental retardation", "Cognitive impairment", "Hypoplasia of cerebellum" and "Methylation of DNA" (**Figure 1**). Taken together, these findings suggest that BPA exposure may alter the expression of genes involved in biological functions associated with ASD.

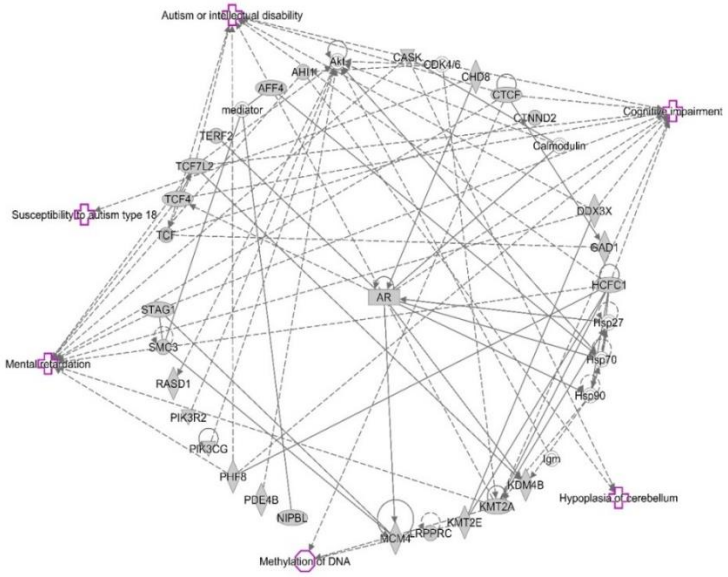

Figure 1. Gene regulatory network of BPA-responsive genes constructed by IPA software.

BPA-responsive genes showed a significant association with dysregulated miRNAs reported in ASD cases.

miRNAs that possibly post-transcriptionally regulate the genes altered by BPA exposure were also predicted by IPA software. Interestingly, BPA-responsive genes were predicted to be regulated by 65 miRNAs (**Table 5**).

Table 5. Top miRNAs predicted to be the regulators of BPA-responsive genes.

miRNAs	P-values	BPA-Responsive Target Genes
miR-199a-3p	1.48E-03	*MET, MTOR*
miR-23a-5p	1.59E-02	*LOC102724788/PRODH*
miR-29a-5p	1.59E-02	*AR*
miR-29b-3p	1.07E-02	*GPR37, HDAC4, RERE*
miR-367-5p	1.59E-02	*AR*
miR-451a	1.04E-02	*BCL2, FBXO33*
miR-519a-3p	3.15E-02	*AR*
miR-93-3p	1.59E-02	*AR*
mir-133	3.15E-02	*RB1CC1*
mir-185	6.62E-03	*AR, EPHB2*

Overlapping of BPA-responsive genes showed candidate genes for further ASD studies

To find BPA-responsive genes overlapping among gene expression studies, we conducted the overlap analysis using the Venn Diagram software (http://bioinformatics.psb.ugent.be/webtools/Venn/) (**Figure 2**). It is noticeable that BPA-responsive genes repeatedly found to be dysregulated by BPA exposure in at least three independent studies are *SOD1, MEF2C,* and *GNAS*. These genes may serve as good candidates for further studies.

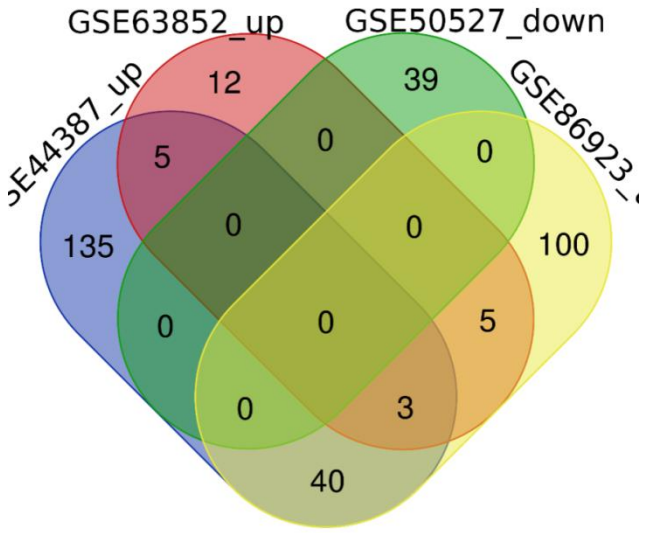

Figure 2. Venn diagram of BPA-responsive genes that are related to ASD.

5. Discussion

This is the first study to demonstrate that exposure to BPA may alter the expression profiles of genes linked to ASD. Using CU-DREAMx bioinformatic analysis of gene expression data from BPA studies in NCBI GEO DataSets database and ASD candidate genes, we found that BPA-responsive genes from four studies were significantly associated with ASD candidate genes. Interestingly, the dysregulated genes found to be reproducibly identified in at least three independent studies are *SOD1, MEF2C,* and *GNAS*. *SOD1* (superoxide dismutase 1) encodes the protein that is responsible for destroying free superoxide radicals in the body. Oxidative stress is found to be associated with ASD etiology and/or susceptibility see reviews in (Kern and Jones 2006, Frustaci, Neri et al. 2012, Rossignol and Frye 2014). A recent study have identified single nucleotide polymorphisms (SNPs) in *SOD1* genes in children with ASD (Kovac, Macedoni Luksic et

al. 2014). *MEF2C* (Myocyte Enhancer Factor 2C) encodes a transcription factor involved in diverse developmental processes, including neurogenesis. Deletion in *MEF2 C*has been reported in individuals with ASD (Novara, Beri et al. 2010). *GNAS*encodesa protein that function as transducers in numerous signaling pathways controlled by G protein-coupled receptors. SNPs and mutation in *GNAS* have been identify in individuals with ASD (Sanders, Murtha et al. 2012). These results suggest that BPA exposure may increase the risk of ASD by causing the dysregulation of these ASD-related genes.

Next, IPA analysis revealed that the list of BPA-responsive genes were associated with **"Reelin signaling pathway".** Reelin protein was found to be impaired in ASD (Zhang, Liu et al. 2002), suggesting that BPA exposure may cause aberrant gene expression profiles involved in pathobiology in ASD. Our gene regulatory network predicted by IPA showed a significant association between the BPA-responsive genes and biological functions related to ASD which are "Autism or intellectual disability", "Susceptibility to autism type 18", "Mental retardation", "Cognitive impairment", "Hypoplasia of cerebellum" and "Methylation of DNA". The cerebellum is known to have functions in motor learning and coordination. Recent studies have reported that ASD cases exhibited the loss of Purkinje cells in the cerebellum (Bailey, Luthert et al. 1998, Palmen, van Engeland et al. 2004). Moreover, cerebellar hypoplasia which is a condition which the patients have a smaller size of cerebellum is also associated with ASD (Courchesne, Yeung-Courchesne et al. 1988). Altered in methylation was also significantly associated with ASD cases (Nguyen, Rauch et al. 2010, Saeliw, Tangsuwansri et al. 2018). Moreover, the prediction of miRNAs regulating these BPA-responsive ASD candidate genes revealed as many as 65 miRNAs. Interestingly, a total 13 miRNAs has been reported in ASD. These miRNAs include miR-23a, miR-367, miR-93, and mir-185 which has been reported to be significantly down-regulated in ASD and miR-133 which was significantly up-regulated (Sarachana, Zhou et al. 2010). This results suggest that BPA exposure may also impair the expression of miRNAs which regulate the expression of several ASD-related genes. The molecular mechanisms through which BPA exerts its effect and impact ASD-related gene expression deserve further investigation.

6. Conclusion

The findings from this study suggest that BPA exposure may lead to changes in the expression of genes and miRNAs associated

with biological functions and pathways associated with ASD. BPA-responsive genes from several transcriptomic studies were significantly associated with ASD candidate genes. Prediction of biological functions associated with BPA-responsive ASD candidate genes revealed ASD-related canonical pathways and neurological functions and disorders including “Reelin signaling pathway”, “autism or intellectual disability”, which are significantly associated with ASD. The role of BPA exposure in the etiology and susceptibility of ASD should be studied further.

7. Acknowledgements

ST is graduate students in the M.Sc. programs in Clinical Biochemistry and Molecular Medicine, Faculty of Allied Health Sciences, Chulalongkorn University. This work is part of ST’s thesis research to be presented in partial fulfillment of the requirements for the M.Sc. degree. This work was partially supported by a Research Grant for New Scholars by The Thailand Research Fund and Office of the Higher Education Commission (MRG6080118) to TS. ST was financially supported by The 90th Anniversary Chulalongkorn University Fund (Ratchadaphieksomphot Endowment Fund: GCUGR1125601056M 53-2), “The Scholarship from the Graduate School, Chulalongkorn University to commemorate the 72nd anniversary of His Majesty King Bhumibhol Adulyadej” and “The Overseas Research Experience Scholarship for Graduate Students from the Graduate School and Faculty of Allied Health Sciences, Chulalongkorn University”.

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