

## Conference Paper

# Chromosomal Microarray Analysis on Intergenerational Effects of Oligomeric Proanthocyanidins Against Bisphenol-A Induced Brain Deformities

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**Abstract**

Oligomeric Proanthocyanidins (OPC) is a type of polyphenolic compound which have been demonstrated to have anti-cancer, anti-oxidant, anti-inflammatory and anti-mutagenic properties that may have the potential to reduce intergenerational effect of BPA towards DNA. This study was conducted to determine the effect of OPC on the DNA damage of BPA-induced rats using microarray CGH Chromosome Karyotyping. Adult Male *Sprague Dawley* rats were divided into six groups which are Normal, BPA, OPC10, OPC20, BPA+OPC10 and BPA+OPC20. The administration of BPA and/or OPC were conducted for 21 days using oral gavage before being mated with female rats of the same age at 1:1 mating ratio. Once the female rats were confirmed pregnant, the male rats were decapitated and their blood were collected for chromosome microarray analysis. The male offspring (F<sub>1</sub> generation) were allowed to grow until 10 weeks old and their blood were also collected for chromosome microarray analysis. BPA group had a deletion of Vomeronasal receptor genes in which the deletion magnitude increased from P to F<sub>1</sub> generation may affect the ability of the rats to detect chemosensory cues during neurobehavioral test. The amplification of Major Histocompatibility complex (MHC) class I gene in BPA+OPC20 group may aid in a better performance during hippocampal-dependent memory tests. These results suggested that OPC could be a potential agent in reducing the intergenerational effect of BPA. Current finding may enrich our understanding particularly in the possible mechanism of OPC on BPA-induced memory impairment

**Keywords:** Bisphenol-A, Brain, microarray CGH Chromosome Karyotyping, Oligomeric Proanthocyanidins, Intergenerational

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## 1. Introduction

Environment has the ability to alter the genetic composition not only in humans but also in other species [1]. A wide range of exogenous contaminants being used as pesticides, fertilisers, heavy metals and industrial gases which can cause epigenetic diseases, where most of the studies that had been conducted, revolves around the epigenetic mechanism via DNA methylation. Epigenetics can be defined as the change in the expression of gene caused by the alteration in the DNA by epigenetic marks and not because of the change in the DNA sequences itself [2]. The epigenetic marks can be passed down from one generation to another which can cause a stable change in the gene expression of the descendants, a condition known as transgenerational epigenetic inheritance. This causes the descendants who are unexposed to the contaminant to experience changes in their gene expression which is inherited from their progenitors that are exposed to those contaminants [3].

Endocrine disrupting chemicals such as Bisphenol-A (BPA) are among component being recently studied by researchers. BPA is a type of chemical monomer usually found in polycarbonate plastics, canned container and plastic bottles which can leach and enter the human body [4]. These substances can cause epigenetic inheritance due to its ubiquitous presence in the environment in which their structures and mechanisms resemble those of natural hormones (estrogen) in human body. Oligomeric proanthocyanidins is a polyphenolic compound which is usually found in various plants and fruits [5]. Proanthocyanidins is also known as procyanidin and belongs to tannin, a polyphenolic compound in which it is categorised under condensed tannin since it arises from the polymerisation of flavan-3-ol or also called flavanol monomers (catechin and epicatechin) [6]. We previously demonstrated that BPA may hamper the novel object discrimination test while the administration of OPC may ameliorate the effect of BPA in memorising the previously encountered object in Novel Object Discrimination (NOD) test. This study was conducted to determine the effect of OPC supplementation on the DNA damage of BPA-induced intergenerational rats using chromosome karyotyping and provide an extension in the possible explanation behind NOD test result from our previous study.

Microarray comparative genomic hybridisation (microarray CGH) is a cytogenomic assessment tool that allows for the detection of microdeletions and microduplication in genes which are often neglected during conventional metaphasic chromosome karyotyping. Microarray CGH is versatile since it enables the screening of the whole genome at the same time. Various studies supported that microarray CGH caters the need for

a fast and accurate detection of birth defects among pregnant women and prenatal diagnosis of cognitive impairment, mental retardation and developmental abnormalities [7]. The ease of this technique lies on the microarray chip at which short DNA segments (i.e. probe) had been mapped on the chip [8]. DNA test sample and reference sample are tagged using different fluorescent dye colours and hybridised accordingly to the probe on the chip [9]. A microarray scanner, equipped with specialised software determines the ratio of fluorescence intensity which indicates the number of copies of a DNA sequence in the test sample as compared to the reference sample [8, 10].

## 2. Methods

### 2.1. Animals

Male *Sprague Dawley* rats at 8 weeks old with a total of 36 (P generation) were bred in the animal holding room, Faculty of Health Sciences, UiTM Puncak Alam, exposed to equal light and dark cycle and were fed *ad libitum*, underwent acclimatisation for 1 week. The rats were randomly divided into six groups and being administered with BPA and different concentrations of OPC.

### 2.2. Experimental Design

Normal group was administered with distilled water and BPA group was administered with 200 mg/kg b.wt. BPA (11). OPC10 group and OPC20 group were supplemented with 10 µg/kg b.wt. and 20 µg/kg b.wt. OPC, respectively. While for BPA+OPC10 and BPA+OPC20 groups, the rats were supplemented with 200 mg/kg b.wt. of BPA along with 10 µg/kg b.wt. and 20 µg/kg b.wt. of OPC, respectively. The administration of BPA and OPC were conducted for 21 days [11]. All experimental procedures were conformed to the guidelines by the Committee on Animal Research and Ethics of Universiti Teknologi MARA (UiTM CARE: 169/2017).

After 21 days, the male rats were mated with adult female rats of the same age in a timely manner at 1:1 mating ratio. After the females were confirmed to be pregnant by examining the copulation plug and increase in their body weight, they were individually housed until the dams gave birth while the males were decapitated and their blood were collected for chromosome microarray analysis. The litters were allowed to grow until 10 weeks old and only six male adult offspring (F<sub>1</sub> generation) were pulled to make up each group (total F<sub>1</sub> generation = 36 male rats). The male offspring were not

being administered with BPA and OPC. At 10 weeks old, the male offspring ( $F_1$  generation) were decapitated and their blood were collected for chromosome microarray analysis. One blood sample from each group for P and  $F_1$  generations were utilized for chromosome microarray analysis.

### 2.3. Chromosome Microarray Analysis

The first step for chromosome microarray analysis is genomic DNA extraction in which DNeasy Blood & Tissue Kit (Qiagen, USA) was used to isolate genomic DNA from 2 ml blood tissue collected from both P and  $F_1$  rat generations. Genomic DNA quantity and quality were assessed in terms of its concentration and purity by using NanoDrop ND-1000 UV-VIS Spectrophotometer. The genomic DNA was prepared, labelled, purified and hybridised on the SurePrint G3 Rat CGH Microarray 4X180K slides (Agilent Technologies, USA) which followed the manufacturer's protocol. The microarray slides were scanned using the Agilent SureScan Microarray Scanner. After the completion of microarray scanning, the features were extracted and analysed. Feature extraction is when the data was extracted from the scanned microarray image (.tif) and translated into log ratios, which allows for the identification of chromosome aberrations in the samples. Agilent Cytogenomics Software was used to analyse the results and generate feature extraction files, Quality Control (QC) and aberration reports. Data shown is in  $\text{Log}_2$  Ratio where the experimental sample was normalised with reference data and compared with Normal group. Amplification and deletion of gene region were detected by probes. Amplification occurs when  $\text{Log}_2$  Ratio reached +0.58 which indicates an addition of 1 copy of chromosomal region while deletion occurs when  $\text{Log}_2$  Ratio reached -1 which indicates a deletion of 1 copy of chromosomal region.

## 3. Results

### 3.1. Chromosome Microarray Analysis

Table 1 and Table 2 summarises the list of chromosome aberrations detected across groups as compared to the Normal group. The aberrations that were listed occurred in both P and  $F_1$  generations. Based on the result obtained, BPA group had a deletion of *V1rj6* and *V1rj4* (Figure 1, top) in which the deletion magnitude increased from P to  $F_1$  generation. The deletion involving the same gene regions also occurred in OPC20 group (Figure 1, bottom) but it was subtle.

Amplification of chromosomal region involving Major Histocompatibility Complex type 1 genes occurred in BPA+OPC20 group (Figure 2). Other type of gene that was amplified is Olfactory receptor genes which were found in OPC10 (Figure 3, top), OPC20 (Figure 3, middle) and BPA + OPC20 groups (Figure 3, bottom). These aberrations can be found in both P and F<sub>1</sub> generations.

TABLE 1: List of chromosomal region containing genes expressing deletion versus Normal group.

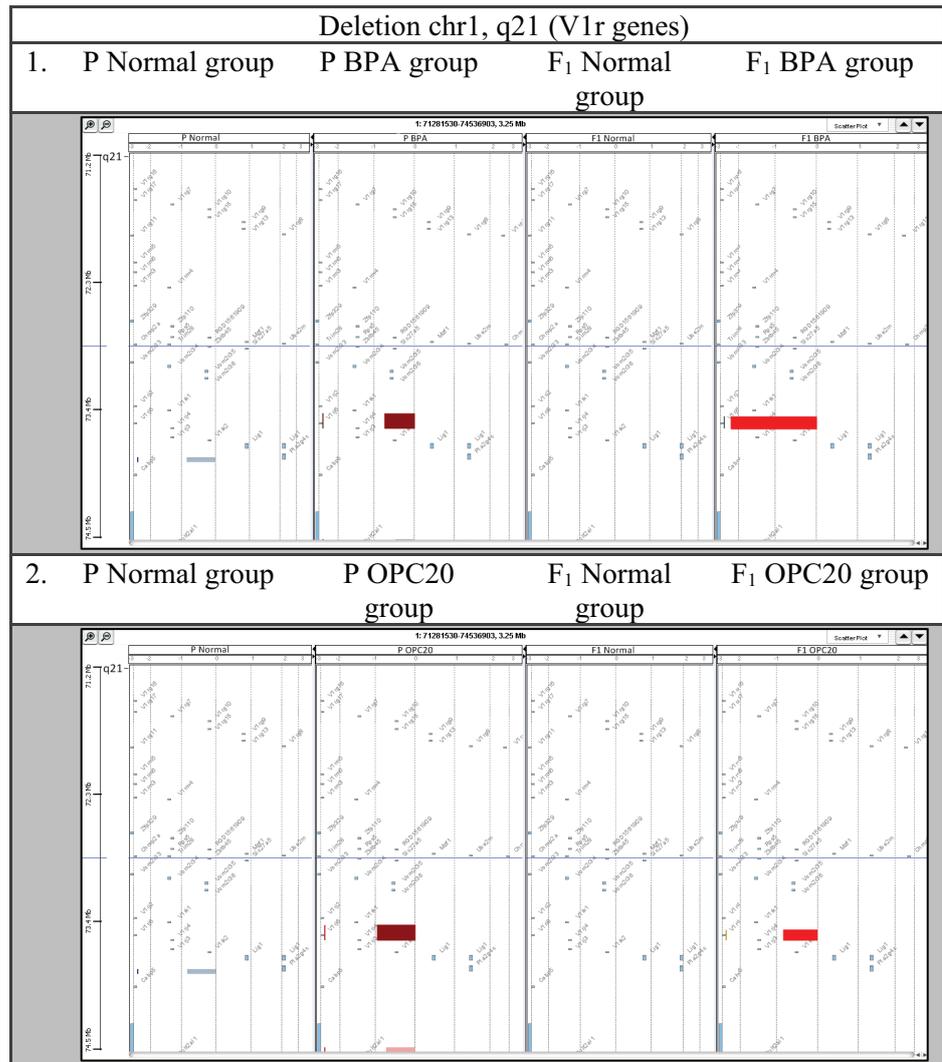
	Gene symbol	Gene description	Group	Magnitude of Deletion vs. Normal group (log <sub>2</sub> ratio)		Location	
				P generation	F <sub>1</sub> generation	Chromosome	Cytoband
1.	<i>V1rj6</i> ,	Vomeronasal receptor genes	BPA	-0.73	-2.38	chr1	q21
	<i>V1rj4</i>		OPC20	-1.00	-0.82		

TABLE 2: List of chromosomal region containing genes expressing amplification versus Normal group

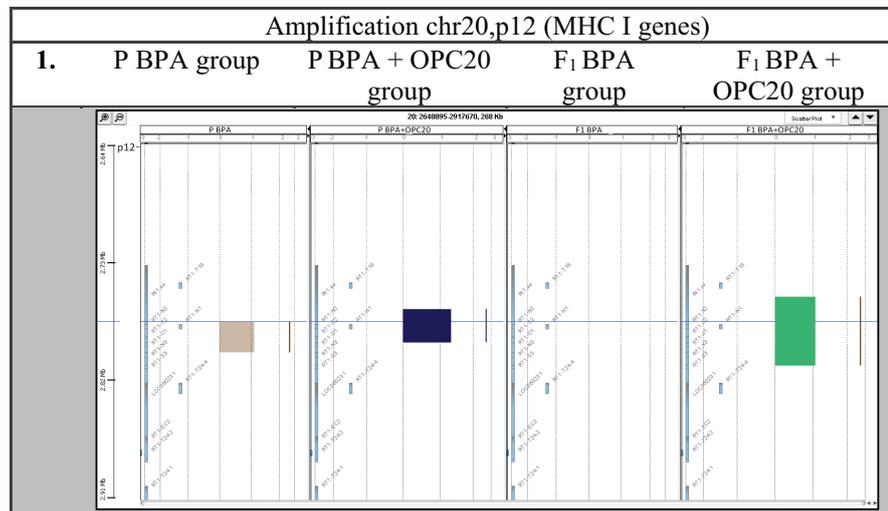
	Gene symbol	Gene description	Group	Magnitude of Amplification vs. Normal group (in log <sub>2</sub> ratio)		Location	
				P generation	F1 generation	Chromosome	Cytoband
1.	<i>RT1.aa</i> , <i>RT1-N2</i> , <i>RT1-N1</i> , <i>RT1-S2</i>	Major Histocompatibility complex (MHC) class I genes	BPA+OPC20	1.28	1.14	chr20	p12
2.	<i>Olr886</i> , <i>Olr889</i> , <i>Olr892</i> , <i>Olr894</i> , <i>Olr896</i> , <i>Olr898</i> , <i>Olr901</i>	Olfactory receptor genes	OPC10	0.52	0.47	chr7	q11
			BPA+OPC20	0.50	0.53		
	<i>Olr903</i> , <i>Olr905</i> , <i>Olr906</i>		OPC10	0.52	0.47		
	<i>Olr881</i> , <i>Olr910</i> , <i>Olr883</i>		BPA+OPC20	0.50	0.53		
	<i>Olr1358</i>		OPC20	1.38	1.58	chr10	q12

## 4. Discussion

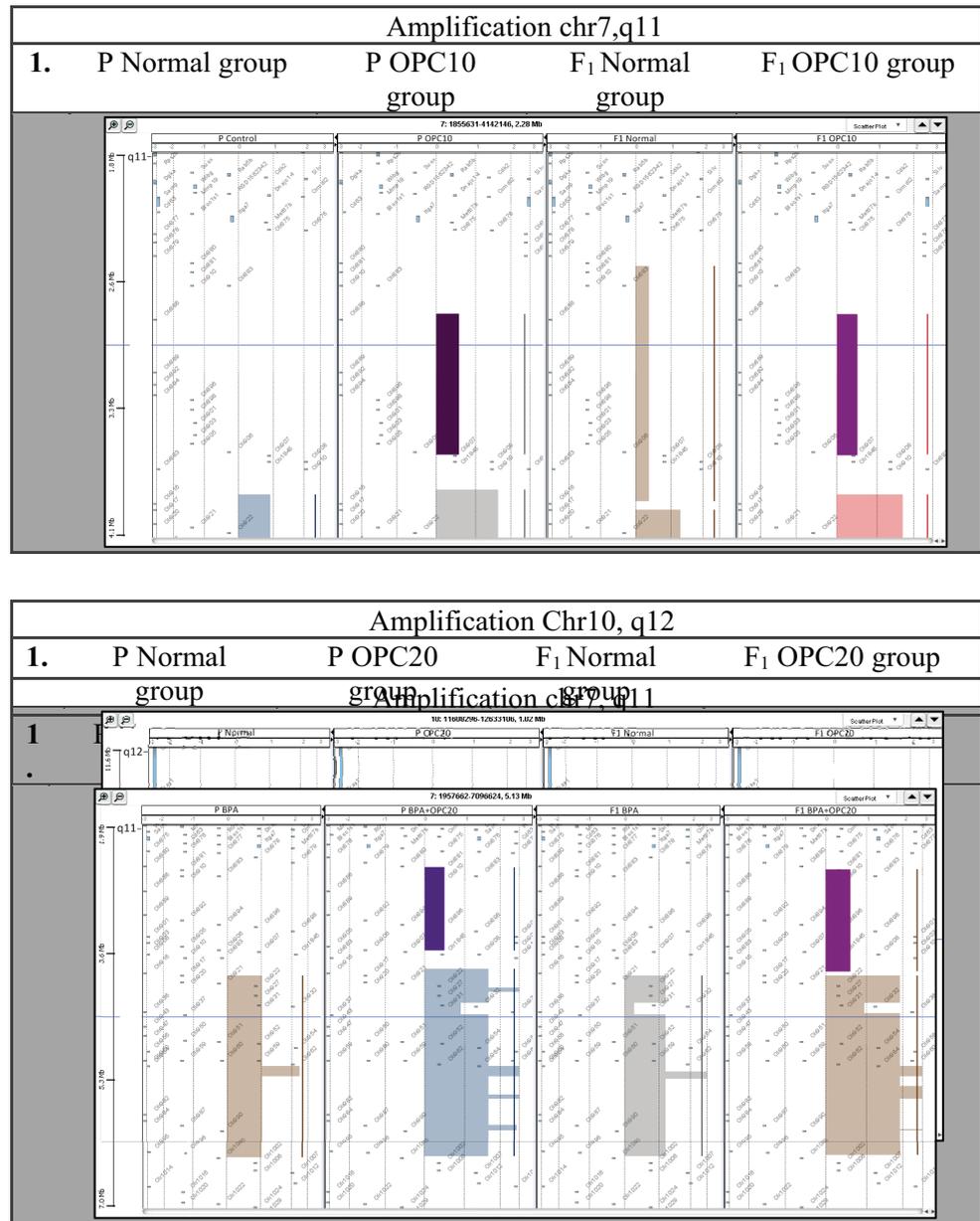
In this study, microarray CGH test was conducted on the blood samples of rats to determine the effect of OPC supplementation on the DNA damage of BPA-induced intergenerational rats using chromosome karyotyping and provide an elaborate explanation behind NOD test result from our previous study.



**Figure 1:** Deletion at chromosome 1, q21 involving vomeronasal V1r-type receptor genes for BPA and OPC20 groups. The height of box = magnitude, width = number of copies.



**Figure 2:** Amplification at chromosome 20, p12 (Major histocompatibility genes) in BPA + OPC20 group. The height of box = magnitude, width = number of copies.



**Figure 3:** Amplification involving Olfactory receptor genes at chromosome 10 q12, chromosome 7 q11. The height of box = magnitude, width = number of copies.

The deletion of gene region which occurred was involving Vomeronasal V1r-type receptor gene. Vomeronasal V1r-type receptor gene is important for the detection of odorant cues using vomeronasal organs which is located at the mammalian nose and allows for the detection of pheromones, a vital substance for social cues [12]. The deletion of Vomeronasal V1r-type gene in BPA group was parallel with no significant difference between familiar and novel object during novel object discrimination test ( $p > 0.05$ ). Ibarra-Soria *et al* [13] stated that Vomeronasal V1r-type receptor gene plays a major role especially during memory retention test that uses chemosensory cues in order to evaluate the mental cognition. The deletion of such gene may cause by BPA

administration that may affect the ability of the rats to detect chemosensory cues which hampers their performance during neurobehavioral test

The amplification of gene occurred was Major Histocompatibility complex (MHC) class I gene (*Rt1.aa*, *RT1-N2*, *RT1-N1*, *RT1-S2*) in BPA+OPC20 groups for both P and F<sub>1</sub> generations. The amplification of MHC class I gene was parallel with our previous finding for BPA+OPC20 group, where BPA+OPC20 group had a significantly higher discrimination index as compared to the BPA group during Novel Object Discrimination test ( $p < 0.05$ ). MHC class I gene is a type of gene commonly known to be involved in antigen-processing and presentation which ultimately may cause immune system impairment if the gene is poorly regulated [14]. However, additional finding showed that MHC class I gene may has a functional role in synaptic plasticity which is important during memory formation. Nelson [15] showed that MHC class I is responsible in hippocampus-dependent memory since mice which were genetically MHC class- I deficient had an impaired novel object recognition, social and contextual memory. Not only that, Lazarczyk [16] postulated that MHC class-I is important in aging brains in order to maintain its neuronal integrity shown by the decrease in dendritic length and complexity and alterations of the dendritic spine in CA1 pyramidal neurons. Such finding further supports that MHC class I may has an important role in hippocampal synaptic plasticity. The observation in the present study showed that the administration of OPC may results in the amplification of MHC which may led to a better memory performance among BPA-induced rats.

Other gene that was amplified in this study were involving the Olfactory receptor genes which occurred across generations in OPC10, OPC20 and BPA + OPC20 groups. Olfactory receptor genes initiate neuronal response that involves in odour perception. Moreover, this gene is also important in the amplification and transduction of the odourant signal which occurred in a cascade event of enzymes [17]. Besides that, Olfactory receptor gene also provides a pathway for interaction between sensory functions and neurogenesis of the brain as it initiates neuronal response that is involved in odour perception [18]. The amplification of Olfactory receptor gene in OPC10 and OPC20 groups were parallel with their longer novel object exploration time as compared to familiar object exploration time as during NOD test ( $p < 0.05$ ). This finding suggested that OPC may contribute to the amplification of the Olfactory receptor gene and may led to a better performance among OPC-treated rats during novel object discrimination test.

## 5. Conclusion

The administration of OPC may results in the amplification of gene regions which were MHC type-I and Olfactory receptor gene while BPA administration led to the deletion of Vomeronasal V1r-type gene. The amplification of such genes may aid in a better performance during hippocampal-dependent memory tests such as NOD test. OPC could be a potential agent in reducing the intergenerational effect of BPA These results may be useful for further studies particularly in the possible mechanism of OPC on BPA-induced memory impairment. However, extended studies should be done especially in gene expression of these regions to elucidate a clearer perspective on the mechanism of OPC.

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