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## Biomarkers Linking PCB Exposure and Obesity<sup>‡</sup>

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### Abstract

Recently the prevalence of obesity has increased dramatically across much of the world. Obesity, as a complex, multifactorial disease, and its health consequences probably result from the interplay of environmental, genetic, and behavioral factors. Several lines of evidence support the theory that obesity is programmed during early development and that environmental exposures can play a key role. We therefore hypothesize that the current epidemic might associated with the influence of chemical exposures upon genetically controlled developmental pathways, leading to metabolic disorders. Some environmental chemicals, such as PCBs and pesticide residues, are widespread in food, drinking water, soil, and they exert multiple effects including estrogenic on cellular processes; some have been shown to affect the development of obesity, insulin resistance, type 2 diabetes, and metabolic syndrome. To bring these lines of evidence together and address an important health problem, this narrative review has been primarily designed to address PCBs exposures that have linked with human disease, obesity in particular, and to assess the effects of PCBs on gene expression in a highly-exposed population. The results strongly suggest that further research into the specific mechanisms of PCBs-associated diseases is warranted.

### Keywords

Biomarkers; environmental exposures; gene expression; obesity; PCBs

## INTRODUCTION

Overweight and obesity around the world are becoming epidemic over the past decades, which results into other |co-morbidities deaths. Around 3.4 million adults die each year as a result of being overweight or obese [1–3]. In USA, based on the report from May 2014 by

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### CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

CDC, 69.0% of adults age 20 years and over are overweight, including obesity, and 18.4% children (12–19 years) are obese (<http://www.cdc.gov/nchs/fastats/obesity-overweight.htm>), and even higher than Japan and South Korea (4% each) [4]. Population studies revealed that high-caloric diet and lifestyle changes set in motion this towards epidemic [5]. The behavioral and genetic factors does not suffice enough to make this an epidemic, therefore this genetic susceptibility have to represent heritable variation upon which environmental factors exert their influences. There is growing evidence that perturbations of central endocrine regulatory systems by the endocrine disrupting chemicals (EDCs; e.g. dioxins, PCBs, Organochlorine pesticides) exposure during early gestation may contribute to the development of obesity in later life [6–17]. The field of EDCs was declared a high research priority by the WHO in 2010 [18]. The far reaching effects of such EDCs in developing endocrine-related diseases among children have now been observed worldwide, as well as in the United States [19].

Polychlorinated biphenyls (PCBs) are one of the key components of persistent organic pollutants (POPs). They are essentially man made synthetic chemical mixtures [20], which have been widely used for industrial purpose over the 50 years. Despite their ban in production since 1979 in the U.S. and other developed countries, due to problems with improper disposal and chemical stability, they remain ubiquitous in the environment as contaminants. This chemical class has been responsible for some of the world's major environmental poisonings, e.g., Yushu poisoning in 1968, Japan, and the Yu-chen incident in Taiwan [21–23], and a PCB/dioxin incident in Belgium 1999 [24, 25]. Prolonged exposures to PCBs have been associated with the development of different diseases and disorders, e.g., cardiovascular, reproductive, hearing impairments, endocrine disruption, metabolic disorders, including Type 2 diabetes, and obesity [26–36].

PCBs have been used as a strategic probe compound to develop the knowledge base of persistent organic pollutants (POP) exposure, because: i) PCBs are one of the key constituents of POP all over the world; ii) bioaccumulation of some congeners from the industrial PCB mixtures imitates the persistency of POP, while others bioaccumulate and are capable of evading detoxification systems of the body; iii) bioaccumulation of PCBs measured in human serum in diverse study populations are typically the same across populations; iv) availability of comparative information on environmental concentration and body burdens; v) the structural similarity with other toxic compounds like dioxins; vi) the similarity in spatial structure and congener profile with the well characterized polybrominated diphenyl ethers (PBDE); vii) the extensive experimental work on PCBs both *in vitro*, *in cellule* and *in vivo* provides abundant information on structure-activity associations; and viii) PCBs exhibit a wide range of functional complexities typical of other POP exposures. However, a persistent gap in knowledge of the exact mechanistic role and the detailed toxicogenomics of PCBs that explain the major disease risks observed in epidemiological investigations.

Concomitant with recent advances in the understanding of PCBs toxicity, the era of genomics have stirred the increasing interests in the use of genes (either singly or in genetic panels) and genetic pathways as biomarkers in epidemiological research on the health effects of PCBs. In this review, we particularly aim to critically present the epidemiological

evidence linking environmental exposures to PCBs and obesity. To add value to this approach, we describe different molecular-based approaches that could clarify these relationships, and future therapeutic approaches towards its prevention and management.

## STATUS OF OBESITY IN THE U.S. AND GLOBALLY

Obesity and overweight (BMI>25 or higher according to American Diabetic Association) and its associated metabolic syndrome (i.e. visceral obesity, dyslipidemia, hyperglycemia, and hypertension) are among non-communicable diseases and/or disorders, which has increased noticeably worldwide [37]. The overweight adults now outnumbered the undernourished [38]. The obesity has almost tripled among children and adolescents in USA (Source: <http://www.cdc.gov/obesity/data/childhood.html>). According to Dr. William H. Dietz, Head of the CDC's Obesity Program in a recent "Weight of the Nation" conference (<http://www.cdc.gov/Features/WeightoftheNation/>) said "*Regardless which is correct, we still have a very serious problem*". Several presenters also predicted that 42% of American adults will be obese by 2030.

## THE RELATIONSHIP BETWEEN ENVIRONMENTAL EXPOSURES, GENE EXPRESSION, AND DISEASE DEVELOPMENT: A SYSTEMATIC REVIEW

### Background

Regardless of international treaty and ban on organochlorine pesticides (OCs), PCBs, PCDDs, and PCDFs, PCBs still exists in the environment and food chains [39–42]. Once exposed, we bioaccumulate these lipophilic compounds in our fatty tissues where they persists for years, as they are hardly metabolized [43]. The epidemiological studies revealed that Americans, Europeans, and Asians have historically accumulated significant body burdens of POPs, including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 2,2',4,4',5,5'-hexachlorobiphenyl (PCB153), coplanar PCBs (PCB congeners 77, 81, 126, and 169), *p,p'*-diphenyldichloroethene (DDE), oxychlorane, and *trans*-nonachlor over the past few decades, [44–47]. One hypothesis is that even with the decreasing concentration over time, due to their long retention in our body, the pre-and post-natal exposure to such endocrine disrupting chemicals results in a possible higher susceptibility to obesity by altering the genetic programming during developmental phase, which is now established and well reviewed [48–59].

### Methods

To bring the diverse literature together regarding PCB exposures and their effects on gene expression and obesity, we conducted a systematic review. We used PubMed for our overall basic search for articles published in English from 1990 to current date. We did not include older studies, i.e. from the era that preceded the genomic revolution. PubMed was therefore systematically searched for publications by means of the following terms relating to exposure: "Chemical Exposures", "Organochlorine exposures" "Obesity", "Obesogens", "Endocrine disruption", "PCB", and "gene expression" in all possible combinations. This search yielded 2241 articles, of which 124 (between 2000-current) were used in this review article, after excluding those that did not focus on the topic, or could not be located as full

text. For this review, we only consider the published materials. We organized the results of the review around two major topics: PCBs and obesity, and PCBs and genomic biomarkers.

## **ENDOCRINE DISRUPTING CHEMICALS (PCBS EXPOSURES) AND DEVELOPMENT OF OBESITY: PROOF OF CONCEPT**

Endocrine disrupting chemicals (EDCs) – including PCBs - are exogenous compounds that can imbalance the hormonal regulation and endocrine system, thus causing deleterious health effects [60–63]. These can, thereby also interfering the production, release, metabolism, and elimination of, or can imitate the occurrence of, natural hormones [64]. Several mechanisms are plausible. EDCs have been shown to alter the differentiation of adipocytes [65, 66]. EDCs are capable of mediating changes in methylation patterns and regulate epigenetic encoding in cells, and epigenetic program control by such compounds are now well documented [67–70]. Oxidative stresses are now also considered [31, 71]. A number of genes, viz. *CYP1A1*, *CYP1A2*, *CYP2D6*, involved in antioxidant and detoxification pathways, have the potential functions as biomarkers of risk from EDC exposures [37, 72].

There is consensus that obesity is responsible for the global burden of chronic non-communicable diseases including type 2 diabetes and coronary heart disease [37, 73]. The growing evidences also support the hypothesis that EDCs play an important role towards metabolic dysfunction that make the obesity epidemic [51, 52, 74–84], and early exposure could contribute towards development of obesity later in life [11–16]. *Obesogens* can be defined functionally as chemical mediators that improperly control and accelerate lipid buildup and adipogenesis [85–88]

PCBs are often included in the category of EDCs. Studies supporting the association between the development of metabolic diseases and PCBs exposures are growing [89]. Early life exposure (*in utero*) to these compounds have shown to develop metabolic syndrome, high blood pressure, elevated triglycerides, and glucose intolerance and were having close association with obesity [79, 90–100]. Associations between overweight and PCB concentrations were stronger in girls compared to boys [101]. The NHANES 1999–2002 study showed an association between waist circumferences and BMI in subjects with detectable levels of POPs, making the chemicals plausible contributors to the obesity epidemic [92]. This also hold true for the adults, where a clear evidence for associations with the highly chlorinated PCBs and OC pesticide with existence or development of obesity [51, 79, 102, 103].

## **WHY THE DEVELOPMENT OF BIOMARKERS OF DISEASE DEVELOPMENT IS IMPORTANT**

During last decade, after mapping the whole human genome, the biomedical research is now focused on finding biological markers (Biomarkers), so that early prevention can be made, before the actual symptom arise. Therefore, developing a rich set of biomarkers for monitoring early health effects in the life course is becoming more important [104]. The importance of gene-environment (GxE) interactions lies not only in a better understanding

of the complex interplay of genetic and environmental risk factors in complex biological pathways relevant to human disease phenotypes; the identification of GxE can also influence risk prediction and identify subgroups of individuals that are most genetically susceptible to common environmental exposures.

Some of the recent conceptual and applied frame works, e.g., Biomarkers Definitions Working Group in 2001 [105]; ANGELO (Analysis Grid for Environments Linked to Obesity) [106] have recommended enhanced precision on selecting biomarkers that should reflect biological processes under any therapeutic and/or external responses and understanding the obesogenicity of environments, respectively, as a practical tool for prioritizing environmental elements for research and intervention. These strategies are also supported by COST action B15 group of European Cooperation in the Field of Scientific and Technical Research [107], and also through the effort of the INTARESE ([www.intarese.org](http://www.intarese.org)) and ENVIRISK (<http://www.envirisk.nilu.no>) projects under the 6<sup>th</sup> Framework Program for Research of the EU for biomarker discovery and validation [108]. By applying this framework, the exploratory analyses from 3 community studies from northeastern United States have shown how communities are doing with a change in a physical environment and food [109].

## PCBs EXPOSURES IN HUMAN AND EARLY DISEASE BIOMARKERS: A GENOMIC APPROACH

### Prologue: Environmental Contamination

In Slovakia (Eastern Europe), inappropriate dumping from the Chemko factory resulted in the release of PCB-containing effluent into the Laborec River, and as a consequence its sediment became contaminated and remains so today [110]. This area is considered to be among the worst contaminated by PCBs. Our own pioneering recent gene expression studies with an exposed population in Slovakia, as well as in a human PBMC *in vitro* model [32], have revealed potential future risks of disease and disorder development that corroborate our prior epidemiological investigations of PCB exposure [33–35]. Microarrays were done to capture the differential gene expression along with Ingenuity Pathway Analysis (*IPA*) to evaluate the functional associations of the genes and their pathways in 45-month old children (from a “mother-child study cohort) in Slovakia, who are heavily exposed to PCBs [26]. A highly significant 162 differentially expressed genes between high and low PCB groups were identified [34]. The results suggested that key biochemical and disease pathways may have been affected, consistent with other studies, and some of the altered gene expressions that can act as signature biomarkers of PCB exposures [33–35, 111].

Studies in Slovakia have previously established that the PCB exposure negatively impacted the exposed Slovak population in many ways: (a) their *birth outcomes*, i.e. boys were more susceptible than girls to growth restrictions [26]; (b) *poor neurodevelopment*, in which the results indicated an association of PCBs with decreased Mental Development Index (MDI), and reduced levels of thyroid hormone [27, 28]; *hearing impairments*, wherein the results showed that PCB exposures were associated with harmful effects on the outer hair cells of the cochlea [29]; *endocrine disruption*, characterized by an increase in the prevalence of

several subclinical and overt thyroid and metabolic disorders [30, 112]; and *diabetes*, showing that increasing serum concentrations of individual POPs considerably increased the prevalence of pre-diabetics indicators [10].

Various attempts have been made to establish the biomarkers that can reflect the specific toxic effects based upon the body burden of exposure to chemicals, e.g., Toxic Equivalency Factors (TEFs), [113–115]. Some studies have also targeted some specific genes and their transcriptomic changes in relation to such environmental exposures [33, 116–119]. In the context of the Slovak PCB exposure scenario, our efforts were focused on this biomarker approach, as we learned that other approaches, such as subject recall of exposure and of disease onset, were beset by multiple sources of uncertainty and misclassification. Could a biomarker approach help to reduce some of the uncertainty?.

### The Biomarker-based Approach

In an effort to achieve the goals of developing more accurate tools to measure the effects of PCB exposures, gene expression and relevant disease pathway analysis were conducted among infants who had attained 45-months of age in the Slovak study population, from an existing mother-child birth cohort. In a mechanistic approach, isolated human PBMC cells were exposed to a mixture of five human equivalence PCBs congeners (PCB-118, 138, 153, 170, 180) at the levels found in the Slovak exposed population, and independently with two structurally different congeners (PCB 153 and PCB 138) for a period of 48 hours. High-throughput qRT-PCR [Taqman Low Density Array (TLDA)] was performed to further validate the selected differentially expressed genes (signature biomarkers), in human population-based studies (see technical details in 32–35, and workflow in Fig. 1).

### Results Obtained Through Genes and Pathways Exploration

Several genes emerged as predictive for the disease pathways' processes, viz. *Leptin Receptor (LEPR)*, *Ras-Related Associated With Diabetes (RRAD)*, *Aryl hydrocarbon receptor nuclear translocator (ARNT)*, and *Paraoxonase 1 (PON1)* among highly exposed subjects. We have also been able to identify genes that were significant at the specific pathway level leading to childhood obesity. Among the top canonical pathways, *Insulin Receptor signaling*, *Type 1 and Type 2 Diabetes Mellitus Signaling*, and *Maturity-Onset Diabetes of Young (MODY) signaling* (Fig. 2) were most relevant in terms of their association with disease development, e.g., endocrine system disorders, and metabolic diseases. Further comprehensive analysis of our gene list together with the IPA knowledge base revealed more canonical pathways, viz., *Mitochondrial Dysfunction*, *Cardiac – Adrenergic signaling*, *Thrombin signaling*, *Atherosclerosis signaling*, and *Cardiac Hypertrophy signaling*, that were shown to be variably expressed with a potential effects on cardiovascular disease under the PCB exposure scenario.

### Validation Studies

No validated disease biomarkers exist for environmental stressors (e.g. PCB in this review). However, preliminary progress has been made to identify some signature biomarkers related to disease outcomes both *in vitro*, and in the Slovak PCB-exposed population [32–35]: these genomic classifiers need further refining and validation before than can be accepted as

robust and stable markers. It is always a central question if the genes we selected from our microarray gene expression studies hold true in the actual exposure scenario (in the population). The critical questions remain in their purpose, accuracy, sensitivity, stability, and more importantly, the specificity to the specific disease of our interest (Obesity here). Given the premise that multiple genes are involved in a disease process, we aimed at the identification (followed by validation) of those different gene panels from the high-risk subjects through a small population validation study.

Subjects for our TLDA-based validation study belong to a well defined “mother-child cohort” enrolled in the ‘Slovak PCB Effects on Early Child Development Study’ between 2001 and 2004 [26]. These children live in the Michalovce area, highly contaminated by PCB from a chemical manufacturing plant. For our study, we selected the subjects (n=71; Male=30, Female=41) solely based on their PCBs concentration (>75 percentile) from our earlier studies [34, 35]; the subjects were devoid of any clinical symptoms of PCB toxicity. The subject with the minimum level of exposure served, as the control with the expression of the gene(s) (up-/down-regulated) determined over TLDA platform. Furthermore, their complete gene expression analysis was done already [34, 35]. To categorize the relative changes in gene expression, we used TLDA cards to assess gene expression for 14 genes in both in *in vitro* and population samples, as well as IPA knowledge base. In this study, the TLDA card was configured into a 14 genes set (including triplicate samples per assay within the TLDA cards). The TLDA data were analyzed by SDS Ver. 2.4 software (ABI, CA). Our laboratory results corroborated with the small population validation by high throughput assay (see procedural details in 22); specially, viz., *LEPR* [33], *RRAD*, and *ARNT* (Fig. 3) for metabolic disorders.

### Summary of Findings

Our results suggest that the assessment of changes in gene expression, combined with a cell-based assay system, is an useful tool for studying the effects of toxicants, and will empower us to study the process of development of diseases towards understanding the potential health risk from PCBs. We have been able to select some of the putative signature biomarker genes (focused genes of interest) (Table 1) through our PCB-Gene expression studies in connection with clinical outcomes, e.g., metabolic disorders and obesity. Once these genes (signature biomarkers) are validated in population based studies, it may help to elucidate the mechanisms of action, in which researchers and clinicians are enabled to devise the ways for better therapeutic management of PCB-induced disease and disorder development. The work would lead us to assess the extent of health risk of an individual in cases of exposure of PCBs or structurally related chemicals in the upcoming days.

Once our basic premises are shown to hold true in other exposed populations, we envision the future downstream application of such biomarkers either through a custom made genotyping assay with single or multiple probes, which would provide information on susceptibility or predisposition to disease for an individual, or by developing a high-throughput QRT-PCR based screening (HTS) kit for PCBs exposure and disease risk profiling (Fig. 4).

## Other Approaches

Increasing evidence suggests that a variety of chemicals have impacted epigenetic processes, allowing the developmental environment to modulate gene transcription [120]. Future advances in the development of epigenetic biomarkers has great potential to advance the understanding of mechanisms by which PCBs can exert their effects significantly into the future, i.e. months, years, or even decades after the initial exposure.

In this context of epigenetics, current research now shifts the focus from intervention (cure) to prevention (early diagnosis) during the critical developmental window, where we adjust our metabolic and homeostatic structures to suit the anticipated extrauterine environment [95]. Thus, if the hypothesis proves true, preventing exposure to environmental chemicals during vulnerable windows in development would help to prevent obesity. So, it is important to understand the mechanisms that are involved epigenetic changes that contributed to obesity as downstream effectors of chemical exposures. Information is pouring in recognizing the epigenetic changes induced by (or inducing) obesity, with candidate and genome-wide approaches [68–70, 121, 122]. These anomalous global epigenetic changes may induce the obesogenic expression patterns [123]. Researchers also acknowledged the value of epigenetics that revealed plausible mechanism of environmental interaction that can set off disease state [124, 125].

In the post genomic era, particularly involving the transcriptomics, proteomics, metabolomics, and the field of nutrigenomics [5], integrating those “omics” information may be helpful in the detection of such multilayer interactions. Without knowing all the factors that involved in these intricate pathways, it is complicated to build up screening tests for most diseases and disorders. Our ongoing effort with PCB-exposed population attempts to identify disease specific genes and genotypes associated with for early-onset of PCB-induced diseases, integrating multiple data types (gene expression, disease development, epigenetic variation, specifically variation in DNA methylation). The over-riding aim of this approach is to extend our continuing present investigation to obtain genetic information (through molecular genotyping) with respect to specific exposure that can be assessed as susceptibility markers of disease. This will help us to foresee early disease development before the clinical symptoms arise, and will have an immense impact on the children who are being exposed to PCBs, so that an early intervention can be predicted. These approaches reside in its ability to use non-invasive gene expression tools along with SNP analysis to study the early pathogenesis of disorders, and to help us to develop early interventions for chronic diseases affected by PCBs exposures.

## CONCLUSION

Obesity is resistant to many forms of treatment; thus, an improved understanding of its etiology may be critical for developing new prevention strategies. Using relevant gene associations and pathways predictive for the specific diseases, our group has identified a number of candidate genes that require further study and confirmation. This type of approach will be highly beneficial to prognostic approaches to disease prevention, before clinical symptoms arise. It is time to develop biomarkers of risk for exposed populations so that prediction of long-term effects of chemical exposures can be made using genomic



classifiers. Future prevention efforts would benefit from this new knowledge in two ways: first, through broader public and medical awareness of the importance of avoiding or reducing exposures, while recognizing that exposure reduction may be difficult to achieve given the ubiquity of chemical pollutants in our environment; and second, by increased understanding that the genetically-based sensitivity of some people in the population to even small amounts of exposure means that they may require increased medical surveillance to anticipate and detect preclinical disease and intervene before more serious, chronic conditions ensue.

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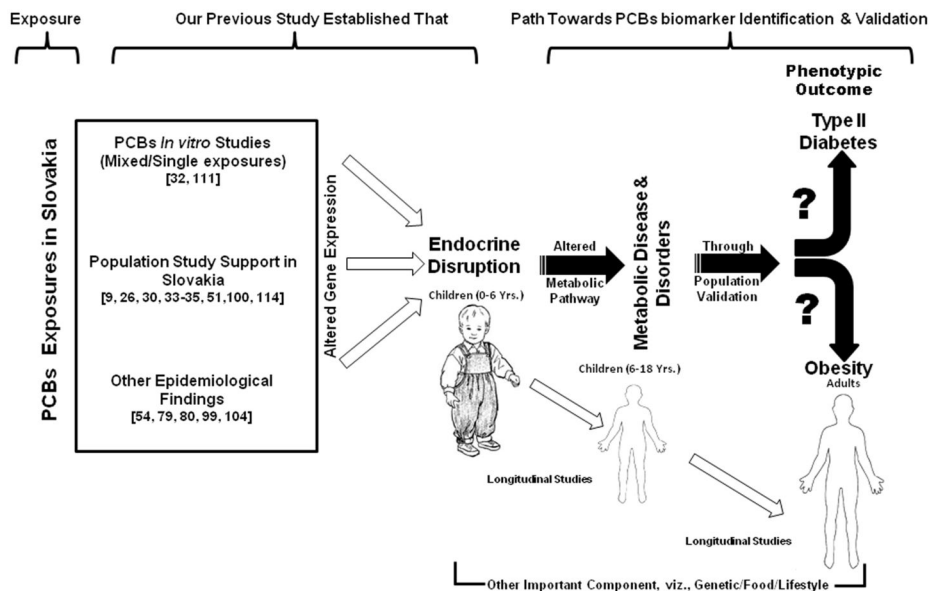
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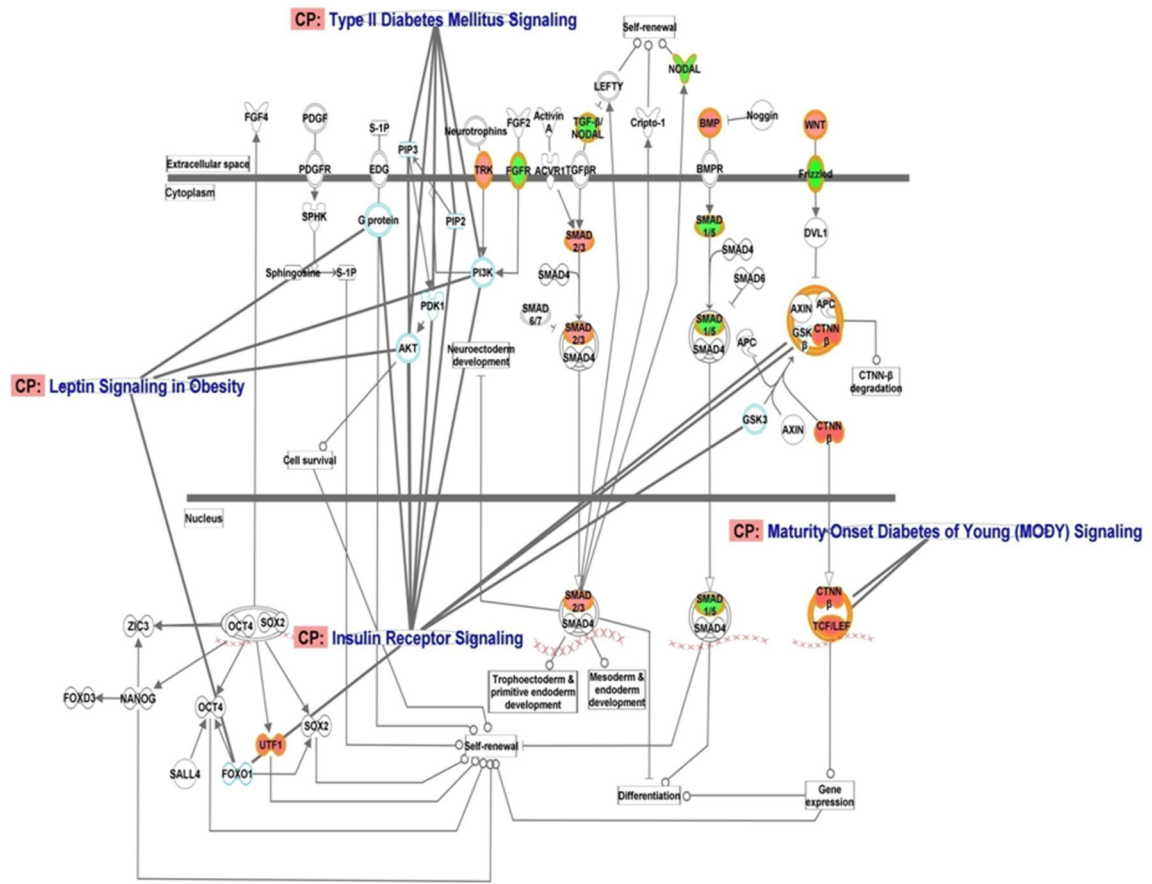
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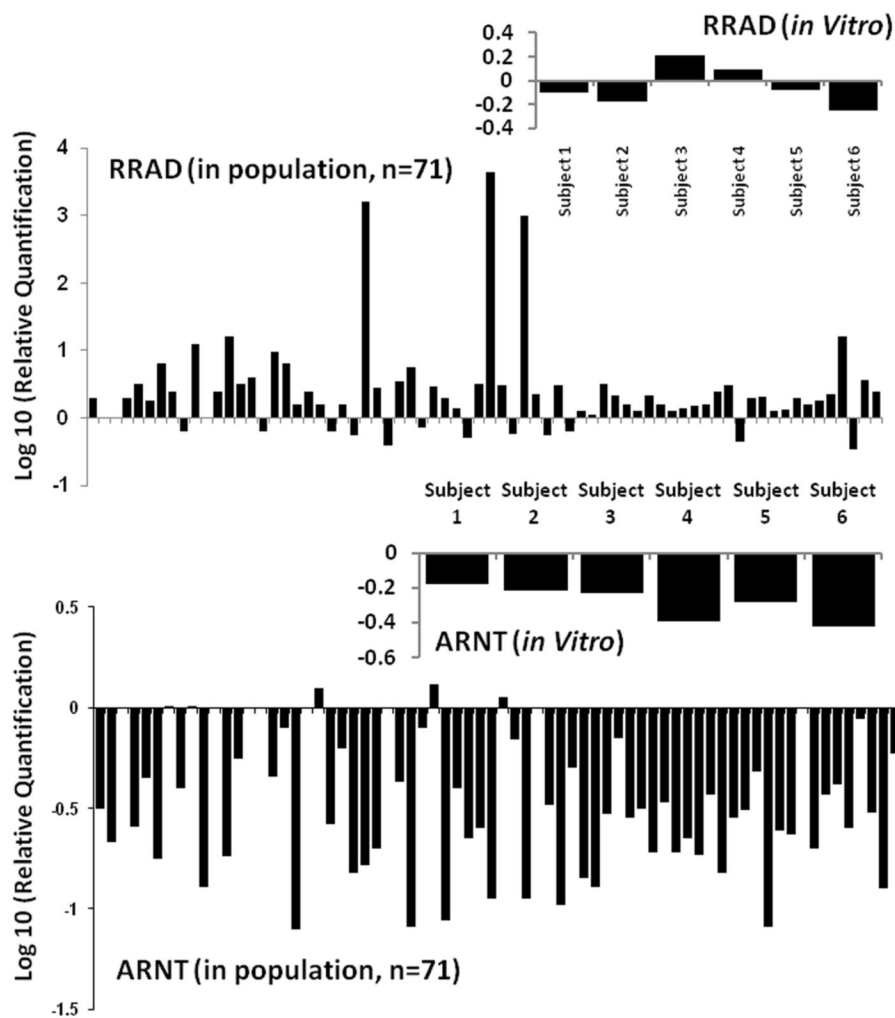




**Fig. 1.** The work flow illustrates the concepts outlining the evolution of our hypothesis and the main steps towards validation of metabolic disease risk in assessing the future disease risks in the vulnerable exposed population. This includes the global gene expression of the PCBs exposed population connecting the reported end points of particular phenotypes (metabolic disorders like obesity and type 2 diabetes) comparing the same disease in formation with public and shared database. The longitudinal studies are important to see the gene expression level over time and that not due to the ontogeny. Strong statistical data interpretation for exposure predisposition combined with Meta data analysis of the candidate genes is also recommended.

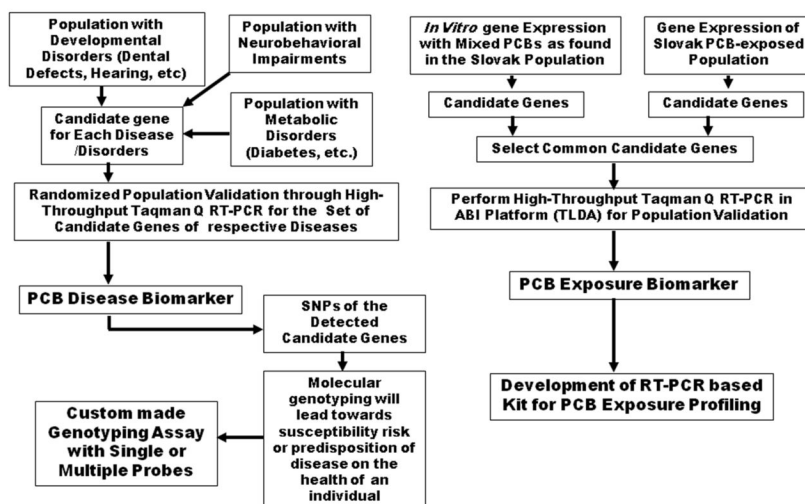


**Fig. 2.** Differentially expressed genes in the important signaling pathway and their connectivity while emphasized into metabolic disease and disorders in the 45 month PCBs exposed population in Slovakia between genes expressed (with 1.5 fold change, t-test,  $p < 0.05$ ) in a cellular level with some downstream effects, e.g.; *Gene expression, Differentiation, Cell survival*, etc. Geometric figures in red denote up-regulated genes and those are green indicate down-regulation. Genes in the top networks were allowed to grow our pathway with the direct/indirect relationship from the IPA knowledge base with the stringent filter, experimentally observed, those who were only from human study. Canonical pathways (functions/signaling; **CP**) viz., *Insulin Receptor signaling, Type 1 and Type 2 Diabetes Mellitus Signaling, Maturity-Onset Diabetes of Young (MODY) signaling* that are highly represented are shown within the box. Genes in uncolored notes were not identified as differentially expressed in our experiment and were integrated into computational generated networks based on evidence stored in the *IPA* knowledge base.



**Fig. 3.** Quantitative Real-time PCR (qRT-PCR) validation of the selected genes (ARNT and RRAD) by Taqman Low Density Array (TLDA) in ABI platform (7900HT Fast Real-Time PCR System) after analyzed by SDS RQ Manager Version 1.2.1. The relative quantification of the genes showing up/down-regulation among the subjects in a short population (n=71) validation. The other panels (*in inset*) with the respective genes represent the relative quantification of the genes with the same gene transcript that has been used in *in vitro* studies (n=6). The relative quantification is calculated in contrast to calibrator samples, i.e.; no-exposure in *in vitro* studies and the subjects with no/background PCBs exposures in the population studies.

## Future Application of PCBs Biomarker



**Fig. 4.**

Our envision on the future application of PCBs biomarkers, if validated through large-scale population, is represented here. It will use non-invasive gene expression tools to study the early pathogenesis of the disease, before the clinical symptoms arise. There is an even greater need to change our focus from treating diseases after they are detected to prevention. The immediate applications of our findings might include using microarrays to generate unique gene expression profiles (*fingerprints* or *signatures*) that would also provide markers through high-throughput assays that are more sensitive and predictive. This will not only identify the disease probability at an early stage, even as early as at the time of birth, but also able to monitor the disease stages in progression over time. This will help us to develop diagnosis assay products for multiple chronic diseases, where few or no products are available on transcriptional gene expression profiling towards practical interference, which we can expect the greatest impact, in terms of timely interventions that improve health, in coming years.

**Table 1**

Our focused gene(s) of interest that have been selected for signature (putative) biomarkers under PCBs exposures with their relative expression (un/down-regulation) and the possible connection with the disease and disorders development.

| Gene Symbol Development | Regulation (Primary)   | Molecular events/Disease/Disorder                                 |
|-------------------------|------------------------|---|
| ITGB1                   | Under-expressed        | Cardiovascular Disease Pathways                                   |
| <i>PON1</i>             | <i>Over-expressed</i>  | <i>Oxidative Stress</i>   |
| <i>ARNT</i>             | <i>Under-expressed</i> | <i>Insulin secretion and glucose Tolerance to Type 2 diabetes</i> |
| CYP2D6                  | Over-expressed         | Poor Neurobehavioral Development                                  |
| <i>LEPR</i>             | <i>Under-expressed</i> | <i>Obesity</i>  |
| LRP12                   | Under-expressed        | Tumor suppressor – related to cancer                              |
| BCL2                    | Under-expressed        | Apoptotic Death – related to cancer                               |
| TP53                    | Under-regulated        | Tumor suppressor – Cancer Signaling                               |
| MYC                     | Under-expressed        | Cell Cycle/Cell Death – cancer                                    |
| TRAP1                   | Under-expressed        | Signal Transduction   |
| <i>RRAD</i>             | <i>Over-expressed</i>  | <i>Associated with Diabetes</i>                                   |

Genes in *bold/italics* in common both in *in vitro* and population-based studies that were also been validated over TLDA platform.