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ID3, Estrogenic Chemicals, and the Pathogenesis of Tumor-Like Proliferative Vascular Lesions

Vincent E. Avecilla

Florida International University, vavec001@fiu.edu

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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

ID3, ESTROGENIC CHEMICALS, AND THE PATHOGENESIS OF TUMOR -
LIKE PROLIFERATIVE VASCULAR LESIONS

A dissertation submitted in partial fulfillment of

the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

PUBLIC HEALTH

by

Vincent E. Avecilla

2017

To: Dean Tomás R. Guilarte
Robert Stempel College of Public Health & Social Work

This dissertation, written by Vincent E. Avecilla, entitled ID3, Estrogenic Chemicals, and the Pathogenesis of Tumor-Like Proliferative Vascular Lesions, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read the dissertation and recommend that it be approved.

Deodutta Roy

Changwon Yoo

Alok Deoraj

Quentin Felty, Major Professor

Date of Defense: October 27, 2017

The dissertation of Vincent E. Avecilla is approved.

Dean Tomás R. Guilarte
Robert Stempel College of Public Health & Social Work

Andres G. Gil
Vice President for Research and Economic Development
and Dean of the University Graduate School

Florida International University, 2017

DEDICATION

To whomever has a dream or goal in life. If you believe it, you can achieve it. I recall during my 1st year as a doctoral candidate I came across a PhD candidate's memoir about his experience from Massachusetts Institute of Technology (MIT). What this student had written in his memoir but particularly in his epilogue had an everlasting impression on me and was one of many sources of motivation through the ups & downs of graduate school. "So why would anyone spend six or more years doing a PhD when they aren't going to become professors? Everyone has different motivations but one possible answer is that a PhD program provides a safe environment for types of people to push themselves far beyond their mental limits and then emerge stronger as a result. For example, my six years of PhD training have made me wiser, savvier, grittier and more steely, focused, creative, eloquent, perceptive, and professionally effective than I was as a fresh college graduate." – Philip J. Guo

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teacher/mentor to the younger generation of students. Also thank you to Banco De Exitos Ventures for the funding opportunity & flexibility so that I was able to complete my dissertation in a timely manner & be part of the wonderful, stressful, and complicated world of venture capitalism & entrepreneurship. Truly blessed to have been part of a hardworking, driven, & sincerely ambitious team. The guidance and invaluable skills you have instilled in me will be constantly everlasting and has taught me that if an idea will solve a problem, add value to society, & service other people it can be achievable no matter how great the obstacle & setback. Lastly an incredible thank you to both my aunt Dr. Divina Grossman who used to be part of the FIU faculty in the College of Nursing & Health Sciences and Office of Engagement and to Dr. Deodutta Roy, professor and founding chair of the Environmental & Occupational Health Department. Without her initial guidance when deciding to come to FIU for graduate school meeting Dr. Deodutta Roy who introduced me to Dr. Quentin Felty would not have been possible.

ABSTRACT OF DISSERTATION

ID3, ESTROGENIC CHEMICALS, AND THE PATHOGENESIS OF TUMOR - LIKE PROLIFERATIVE VASCULAR LESIONS

Vincent E. Avecilla

Florida International University, 2017

Miami, Florida

Professor Quentin Felty, Major Professor

Tumor-like proliferative vascular lesions manifest in several diseases such as peripheral arterial disease (PAD) and atherosclerosis (AS) after arterial injury. The cause of the vascular cell dysfunction in PAD patients is not known. Our recent novel discovery shows that inhibitor of differentiation 3 (ID3) is highly expressed in intimal lesions of clinical vascular disease samples. The central hypothesis of our study is: estrogenic chemical induced dysregulation of ID3 target genes is involved in the development of vascular disease. NHANES data analysis demonstrated higher geometric levels of all 6 PCB congeners in both PAD diagnosed participants and defined AS participants when compared to the rest of the population. Adjusted models showed association between higher exposure of PCBs, phthalates, BPA, and increased risk of PAD. Furthermore PCB153 was shown to have the highest geometric mean amongst all PCB congeners in both participants diagnosed with PAD and at risk of AS. Gene expression of ID3 & ID3 candidate targets in blood & tissue studies identified ID3 & ID3 candidate target genes as a driver of vascular

disease. Overlapping ID3 & ID3 candidate target genes included: ABCB6, ACP1, BYSL, CAD, CDH15, DCBLD2, DHRS3, DNMT1, ID3, MCM4, and NDUFA7. The ID3 target genes involved in the: focal adhesion pathway were ACTN1, COL1A2, COL3A1, COL6A1, CTNNB1, IBSP, ID3, ITGA8, and MYL2; ECM-receptor interaction were COL1A2, COL3A1, COL6A1, IBSP, ID3, and ITGA8; oxidative phosphorylation pathway ATP5D, ATP5H, ATP6V0B, ATP6V0D1, ATP6V1B2, COX5A, COX7C, COX8A, CYC1, ID3, NDUFA1, NDUFA7, NDUFS4, NDUFV1, NDUFV2; and cell cycle pathway ANAPC10, ATM, CDKN2B, E2F5, MCM3, and MCM4. In summary our results showed an association between exposure to PCBs, phthalates, BPA, and increased risk of PAD and AS, and possible molecular mechanisms of interaction of ID3 target genes and estrogenic chemicals involved in PAD and AS.

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ABBREVIATIONS AND ACRYONYMS

AVM	Arteriovenous malformation
AS	Atherosclerosis
BPA	Bisphenol A
CDC	Center for Disease Control & Prevention
CI	Confidence Interval
E2	Estradiol
ECM	Extracellular Matrix
EDC	Endocrine Disrupting Chemical
GEO	Gene Expression Omnibus
GM	Geometric Mean
GSE	Geometric Standard Error
HLH	Helix-loop-helix
ID1	Inhibitor of Differentiation-1
ID2	Inhibitor of Differentiation-2
ID3	Inhibitor of Differentiation-3
IH	Infantile Hemangioma
IL	Interleukin

IPAH	Idiopathic pulmonary arterial hypertension
LDL	Low density lipoprotein
MetS	Metabolic Syndrome
NHANES	National Health and Nutrition Examination Survey
LAM	Lymphangioleiomyomatosis
OR	Odds Ratio
PAD	Peripheral Arterial Disease
PDR	Proliferative Diabetic Retinopathy
PCB	Polychlorinated Biphenyl
SNP	Single Nucleotide Polymorphism
TNF	Tumor Necrosis Factor
VEGF	Vascular Endothelial Growth Factor
VEGFR	Vascular Endothelial Growth Factor Receptor
VSMC	Vascular Smooth Muscle Cells

CHAPTER I

INTRODUCTION

Systemic & pulmonary vascular biologists have inclined to position their particular research in different ways. This distinction is readily implicit in light of their predominantly different interests. Those that are engaged in research on pulmonary circulation have often centered their attention on pulmonary arterial hypertension, a life threatening condition that affects your arteries which go from your heart to your lungs. Oppositely systemic circulation have traditionally focused on the pathogenesis of atherosclerosis, a common disease that affects large arteries and in which the lipid metabolism features chiefly have been explored in mechanisms in restenosis after angioplasty. These divergent pathways intersect at obliterative and proliferative vascular diseases which commonly disrupt the blood vessel. These two types of vascular diseases certainly have similar elements such as interactions between the vessel wall and various components of blood. Others involve interactions between the smooth muscle of the media & endothelial lining but while exasperating and preserving mechanisms have been established in both, the unique perturbations that initiates these processes remains uncertain.

A class of proteins that have been seen to be involved with these types of vascular diseases are ID proteins (ID1, ID2, ID3, and ID4). Their names relate to their ability to regulate the programming of cell fate determination and differentiation in various types of cells through normal development and adult life

(Lasorella et al., 2014). The ID3 gene was first identified as a serum-inducible immediate early gene in an established murine fibroblastic cell line (Christy et al., 1991). Previous studies have demonstrated its involvement in various biological processes, including: tumor-induced angiogenesis, embryonic neurogenesis, osteogenesis, vascular smooth muscle cell proliferation, T & B cell development, and skeletal muscle differentiation (Atherton et al., 1996; Deed et al., 1996; Melnikova & Christy, 1996; Pan et al., 1999, Lyden et al., 1999; Rivera et al., 2000; Forrest et al., 2004; Maeda et al., 2004). ID3 has been shown to be involved in various types of vascular diseases such as adult kidney injury (AKI) which is involved in loss of capillary function and vascular remodeling in the luteum (CL), a temporary endocrine organ that is involved in the production of high levels of progesterone and moderate levels of estradiol, atherosclerotic disease, and pulmonary hypertension (Lee et al., 2014; Yang et al., 2013; Nio-Kobayashi et al., 2012; Doran et al., 2010). Expression and function of the protein is under several multifaceted layers of regulation and therefore can provide beneficial targets for therapeutic interventions.

Vascular diseases can manifest from a combination of both environmental & molecular determinants. Progression in machine/deep learning and genomic technologies have facilitated investigators to generate hypotheses regarding the integration & contribution of genetic factors at a rapid pace. However, the assessment of specific and numerous environmental pollutants and their interactions with the genome has not. There still lacks methodologies to comprehensively analyze and interpret several multifaceted environmental &

cellular constitutes to disease and human health. One environmental determinant that has been demonstrated to show association with ID3 are chemicals commonly known as estrogenic or endocrine disrupting chemicals (EDCs). These chemicals are synthetic and natural that have the ability to disrupt metabolic pathways by either mimicking or blocking endogenous hormones and modifying hormone function (Roy et al. 1997). Many EDCs are considered highly carcinogenic and include an assortment of chemicals such as industrial compounds, by-products of industrial procedures, chemicals that are used in the manufacturing of plastics, and pesticides. Of specific concern to public health professionals are agents that are persistent in the environment that are hormonally active, greatly lipophilic, amplify within the food chain, and are readily bio-accumulative (Lyche et al., 2009; Meeker et al., 2009).

Since many of these chemicals are used in numerous amounts of consumer products, exposure to EDCs through the general population is prevalent. Human exposure to EDCs may result in different ways such as absorption through the skin, ingestion of contaminated food & water, and airborne inhalation (Balabanic et al., 2001). During the last decade multiple EDC exposure such as polychlorinated biphenyls (PCBs), bisphenol A (BPA), and phthalates have been detected in > 90% urine & blood samples collected from the general population of the United States (Silva et al., 2004; Calafat et al., 2008; Woodruff et al., 2011). EDCs that are persistent today in the environment have shown properties of endocrine disruption. PCBs have demonstrated to have estrogenic and anti-estrogenic properties contingent on the congener. Previously PCB exposure has

been shown to be associated with cardiovascular disease (Perkins et al. 2016). Higher exposure of urinary phthalates furthermore have been demonstrated to be associated with hypertension in childhood (Trasande et al. 2013). Exposure to BPA has demonstrated to result in atherosclerosis, altered cardiac remodeling, and changes in blood pressure in rodents (Gao & Wang, 2014). Although there are vast amount of EDCs in the environment the most extensively studied EDCs include polychlorinated biphenyls, phthalates, and bisphenol A.

The existing disciplines of studying environmental pollutants & molecular determinants of health include epidemiology, molecular/genetic biology, & toxicology which function on population, bioinformatics, and molecular scales, respectively. This dissertation proposes methodology in all of these disciplines emphasizing the framework of environmental - and genomic - wide association studies through the utilization of public databases such as the National Health and Nutrition Examination Survey (NHANES) and NCBI Gene Omnibus Expression (GEO). The overarching goals of this research was to first determine the association between exposure to these endocrine disrupting chemicals in a U.S. population diagnosed with vascular disease and secondly; determine the role of ID3 in vascular-intimal lesion formation and investigate its activity via regulation of cell adhesion, extracellular matrix proteolysis, and cell growth. Documenting both exposure to EDCs among the U.S. population & associations with vascular disease and the role of ID3 in vascular-intimal lesions will help guide our understanding of the complexity of environmental molecular determinants in vascular disease.

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HYPOTHESIS

Estrogenic chemical induced dysregulation of ID3 target genes is involved in the development of vascular disease

SPECIFIC AIMS

Aim 1: Collect environmental exposure data from National Health and Nutrition Examination Survey (NHANES) database and gene expression data from the Gene Expression Omnibus (GEO) database

Aim 2: Investigate the association between exposure to estrogenic chemicals and vascular disease (peripheral arterial disease)

Aim 3: Identify ID3 regulated molecular gene networks

CHAPTER II

REVIEW OF LITERATURE

MANUSCRIPT 1

Contribution of Inhibitor of DNA-binding/Differentiation-3 (ID3) and endocrine disrupting chemicals to pathophysiological aspects of chronic disease

Abstract

The overwhelming increase in the global incidence of obesity and its associated complications such as insulin resistance, atherosclerosis, pulmonary disease, and degenerative disorders including dementia constitutes a serious public health problem. The Inhibitor of DNA binding/Differentiation-3 (ID3), a member of the ID family of transcriptional regulators, has been shown to play a role in adipogenesis and therefore ID3 may influence obesity and metabolic health in response to environmental factors. This review will highlight the current understanding of how ID3 may contribute to complex chronic diseases via metabolic perturbations. Based on the increasing number of reports that suggest chronic exposure to and accumulation of endocrine disrupting chemicals (EDCs) within the human body are associated with metabolic disorders, we will also consider the impact of these chemicals on ID3. Improved understanding of the ID3 pathways by which exposure to EDCs can potentiate complex chronic diseases in populations with metabolic disorders (obesity, metabolic syndrome, and glucose intolerance) will likely provide useful knowledge in the prevention and control of complex chronic diseases associated with exposure to environmental pollutants.

Keywords: endocrine disrupting chemicals, ID3, metabolic disorders, chronic disease

Introduction

Inhibitor of DNA binding/Differentiation-3 is a member of the ID family of helix-loop-helix proteins encoded by an immediate-early gene responsive to mitogenic signals and oxidative stress. ID3 functions as a transcriptional regulator known to prevent stem cell differentiation and promote cell cycle progression. An increasing body of evidence suggests that ID3 may be involved in metabolic perturbations characterized by insulin resistance, hyperglycemia, abdominal obesity, dyslipidemia, and hypertension. Interactions across multiple organ systems that contribute to metabolic perturbations presents a challenge to ongoing research attempting to elucidate biological mechanisms of chronic disease associated with metabolic health. For instance, insulin resistance and systemic low-grade inflammation result from complex interactions between the vasculature, metabolic tissue, and immune cells. With regard to these interactions, it is noteworthy that ID3 plays a significant role in vasculogenesis, energy metabolism, and in the development of the immune system. In the vasculature, ID3 is essential to embryonic vasculogenesis and endothelial cell activation (Sakurai et al. 2004; Felty and Porther. 2008; Eelen et al. 2015). Given that metabolic perturbations are observed in endothelial cells from diseased vasculature (He et al. 2014), ID3 may mediate endothelial dysfunction often found in individuals with metabolic syndrome. In addition to the vasculature, ID3 function spans to metabolic tissue and immune cells. In vivo studies have demonstrated that ID3 mediates high fat diet induced obesity and promotes obesity-induced inflammatory macrophage accumulation (Doran et al. 2008; Cutchins et al. 2012). Thus, we intend to discuss

the current understanding of how ID3 may influence chronic diseases associated with metabolic perturbations. Since adipose tissue is an endocrine organ as well as a metabolic organ, exposure to endocrine disrupting chemicals (EDCs) may also contribute to metabolic perturbations associated with chronic disease. EDCs are mostly synthetic chemicals ubiquitously found in our environment that act by altering hormone action. Estrogenic EDCs such as diethylstilbestrol (DES), bisphenol A (BPA), estrogenic polychlorinated biphenyls (PCBs), and phthalates have been implicated to interfere with metabolic health during critical periods of development and into adulthood. Epidemiological studies have reported associations between exposures to EDCs and metabolic syndrome (Swedenborg et al. 2003; Hatch et al. 2010; Casals-Casas and Desvergne. 2011). Based on our recent findings that showed ID3 dependent endothelial cell activation by exposure to estrogenic PCB congener 153 (Feltz. 2011; Das and Feltz. 2014), we will also discuss how low dose EDC exposure from the environment may potentiate complex chronic disease in populations with metabolic disorders (obesity, metabolic syndrome, and glucose intolerance) via ID3. A better understanding of interactions between ID3 and EDC is critical in deepening our understanding of how environmental factors modify chronic disease risk and health outcomes. Further study in these areas may reveal novel or more effective therapeutic modalities as well as provide prevention and control strategies of complex chronic diseases associated with exposure to EDCs.

Inhibitor of DNA binding/Differentiation-3 (ID3)

Structure and Function. The ID (Inhibitor of DNA binding/Differentiation) family of small proteins consists of four genes (ID1-ID4). The four members of the ID family share extensive amino acid sequence homology (69-78%) within their helix-loop-helix (HLH) domain (Lyden et al. 1999, Yang et al. 2014), but the remaining parts of the proteins are non-related. Experimental studies in genetically engineered mice have revealed the importance of ID3 in embryonic development and cell differentiation. ID3 gene knockout mice are viable, however, they have demonstrated defects in immune cell differentiation (Pan et al. 1999; Lasorella et al. 2014). In contrast, double ID1/ID3 knockout mice showed abnormal vascularization of the brain (Poulsen et al. 2001), neuronal differentiation, and cardiac defects (Fraidenraich et al. 2004) that were embryonically lethal. Resistance to tumor angiogenesis was reported in mice deficient in 1-3 alleles of ID1/ID3 gene knockout combination (Lyden et al. 1999). ID3 is highly expressed in embryonic tissue, but declines as cells differentiate (Lyden et al. 1999). In adult tissues, the expression of ID3 is context specific and tends to be highest in proliferating and undifferentiated cells. ID3 expression has been reported to be induced by diverse stimuli in many cell types (Lim and Wu. 2005).

The ID3 gene was initially identified as a serum-inducible immediate-early gene in mouse fibroblasts that peaks transcriptionally at 1h (Lasorella et al. 2001; Lasorella et al. 2014). Subsequently, ID3 expression has been reported to be biphasic with maximal stimulation at 1h following a second burst at 24h as in the case of tissue regeneration after injury. We and others have shown that ID3

expression is redox sensitive (Felty and Porther. 2008; Mueller et al. 2012) specifically, we have shown that vascular endothelial cells exposed to either 17 β -estradiol (E2) or the estrogenic PCB congener 153 (PCB153) resulted in increased ID3 expression, protein phosphorylation, and endothelial neovascularization. Treatment with reactive oxygen species scavengers inhibited estrogenic chemical induced neovascularization (Das and Felty. 2014; Das and Felty. 2014). Proteasomes reportedly degrade ID3 by an ubiquitin dependent mechanism. The protein half-life of ID3 has been demonstrated to be approximately 20 min in HEK293 cells (Yang and Morrell. 2014). In mammals, ID protein-protein interactions occur via the HLH-motif in which ID proteins dimerize and block the DNA binding activity of basic HLH transcription factors, such as a group of E proteins (E12/E47, E2-2, HEB) encoded by the TCF3, TCF4, and TCF12 gene, respectively. Among these E proteins, ID3 has been most often reported to interact with E12/E47 (Loveys et al. 1996). The E proteins are basic HLH transcription factors that bind to the E-box consensus sequence (CANNTG) in the promoter of target genes. ID3 plays an important role in cell proliferation via its interactions with E proteins. For example, E proteins have been shown to bind the E-box sequence in the promoter of the cyclin dependent kinase inhibitor p21^{Cip1} and activate its transcription (Liu et al. 2004). The level of p21^{Cip1} is elevated in quiescent cells where it acts as a suppressor of cell proliferation (Sherr and Roberts. 1995). In the context of the cell cycle, ID3 promotes cell cycle progression by the inhibition of p21^{Cip1} expression (Forrest and McNamara. 2004). Specifically, ID3 protein-protein interactions with E proteins can disrupt their ability to bind gene promoters and

thereby blocks transcriptional activation by these factors. ID3 has been shown to inhibit E proteins from activating the p21^{Cip1} promoter in proliferating vascular cells (Taylor et al. 2006). Thus, ID3 has been frequently described as a dominant negative inhibitor of E proteins. Although ID proteins have been shown to function as dominant negative transcriptional regulators of E proteins, there may be circumstances by which ID3 acts as a positive transcriptional regulator. ID3 has been shown to regulate the binding of Transcription Factor 3 (TCF3) to the E-box motif in target gene promoters (Langlands et al. 1997). TCF3 has been reported to repress the expression of pluripotency genes OCT4, SOX2, and NANOG that contribute to cell differentiation (Lasorella et al. 2014). Our research has shown that ectopic overexpression of ID3 increased OCT4 and SOX2 expression in endothelial cells as well as resulted in a population of cells that were positive for the molecular stemness signature CD133⁺ VEGFR3⁺ CD34⁺ (Felty et al. 2015). These endothelial stem cells were morphologically differentiated into smooth muscle cells and neuron cells. Based on these lines of evidence, ID3 maintains cells in an undifferentiated or non-committed state by preventing the repression of pluripotency factors by TCF3. Hence, it is also plausible for ID3 to function as a positive regulator of gene transcription. In lieu of a recent report that showed ID3 to modulate genes essential for maintaining genome integrity during cell division (Ji et al. 2011) a dual regulatory role of ID3 in both positive and negative gene transcription expands its influence as shown in Figure 1.1. ID3 protein-protein interactions are not exclusive to E-proteins as ID proteins have also been reported

to bind to proteins that do not contain the HLH motif such as caveolin-1 (Zhang et al. 2007).

Figure 1.1 ID3 transcriptional regulation. Scheme illustrating how ID3 can repress expression of p21 gene (upper panel) or activate gene expression of embryonic transcription factors (lower panel).

ID3 and Metabolic Syndrome (MetS)

There has been increasing evidence that ID3 plays a role in adipogenesis. ID3 through adiponectin is considered to improve β -cell function, circulating lipids, and insulin sensitivity levels (Arita et al. 1999; Steinberg and Kemp. 2009). ID3 inhibits transcriptional activity of E47 in undifferentiated pre-adipocytes (Doran et al. 2008). ID3 negatively inhibits the FAS (fatty acid synthase) promoter via SREBP-1c in adipose tissue. ID3 furthermore plays a role in blood glucose, which if dysregulated can lead to insulin resistance. In human islet cells ID1 and ID3 mRNA levels are increased with addition of glucose (Wice et al. 2001). The induction of ID1 and ID3 expression, insulin secretion, and gene transcription suggests that IDs may play a role in promoting β -cell function (Wice et al. 2001, Lee et al. 2011).

ID3 & Endocrine Disrupting Chemicals Influence Metabolic Perturbation

Metabolic Syndrome (MetS) and its associated complications such as insulin resistance, abdominal obesity, dyslipidemia, and hypertension contribute to chronic diseases including cardiovascular disease (CVD), type 2 diabetes, cancer, and chronic kidney disease (CKD). Some studies have shown the prevalence of MetS in the United States at approximately 34% of the adult population (Ford et al. 2002). MetS is an illness of energy consumption and storage which is a diagnosis of the co-occurrence of a minimum of three of the following medical conditions: abdominal obesity, high triglyceride levels, low HDL cholesterol levels, high fasting blood sugar, and high blood pressure. The molecular mechanisms of MetS are not fully understood. Most patients are older, sedentary, obese, and have a certain amount of insulin resistance. Important factors that are associated with MetS can include aging, diet, sedentary behavior, genetics, excessive alcohol use, or low physical activity (Poulsen et al. 2001; Pollex and Hegele. 2006; Malik et al. 2010; Edwardson et al. 2012). MetS appears to have three conceivable etiological groupings: obesity and disorders of adipose tissue; insulin resistance; and a collection of independent factors (ex: molecules of hepatic, vascular, and immunologic origin).

Inflammatory factors produced during obesity are a major pathway for developing metabolic perturbation which can lead to MetS. Experimental studies have demonstrated ID3 to be a key regulator of Monocyte chemoattractant protein-1 (MCP-1) (Kapla et al. 2015). MCP-1 is a well-known chemokine impacted by MetS (Panee. 2012). ID3 has also been reported to regulate the production

of interleukins IL-5, IL-6, IL-8, and IL-10 (Jin et al. 2011; Kuo et al. 2011; Perry et al. 2012). The induction of these chemokines have been observed in population studies of obesity and/or MetS. ID3 is also an oxidative stress regulated gene which may provide a positive feedback pathway in response to metabolic perturbations (Mueller et al. 2002; Felty and Porther. 2008). Taken together, these lines of evidence provide the basis for how ID3 can participate in metabolic perturbations via controlling the expression of inflammatory factors involved in obesity and/or MetS. A growing number of reports implicate endocrine-disrupting chemicals (EDCs) as an environmental factor that contributes to the occurrence of MetS. We performed a comprehensive search in the Comparative Toxicogenomic Database (CTD) to identify known ID3 and EDC interactions with results shown in Table 1 (Mattingly et al. 2004).

Table 1. ID3 and Endocrine Disrupting Chemicals (EDCs)

Chemical name	Chemical ID	CAS RN	Interaction count	Organism count	Interaction	PubMed ID	Authors	Title	Year	Citation	Organism
Tetrachlorodibenzodioxin	D013749	1746-01-6	18	3	Tetrachlorodibenzodioxin affects the expression of ID3 mRNA	23238561	Nault et al.	Comparison of TCDD-elicited genome-wide hepatic gene expression in Sprague-Dawley rats and C57BL/6 mice.	2013	Toxicol Appl Pharmacol. 2013 Mar 1; 267(2): 184-91.	<i>Rattus norvegicus</i>
					Tetrachlorodibenzodioxin cotreated with TIPARP gene mutant form results in decreased expression of ID3 mRNA	21496263	Dere et al.	Differences in TCDD-elicited gene expression profiles in human HepG2, mouse Hepa1c1c7, and rat H4IIE hepatoma cells.	2011	BMC Genomics. 2011; 12: 193.	<i>Rattus norvegicus</i>
					Tetrachlorodibenzodioxin results in increased expression of ID3 mRNA	19684285	Kim et al.	Comparative analysis of AhR-mediated TCDD-elicited gene expression in human liver adult stem cells.	2009	Toxicol Sci. 2009 Nov; 112(1): 229-44.	<i>Homo sapiens</i>
						17942748	Boverhof et al.	Inhibition of estrogen-mediated uterine gene expression responses by dioxin.	2008	Mol Pharmacol. 2008 Jan; 73(1): 82-93.	<i>Rattus norvegicus</i>
						15591033	Watanabe et al.	Comparative uterine gene expression analysis after dioxin and estradiol administration.	2004	J Mol Endocrinol. 2004 Dec; 33(3): 763-71.	<i>Rattus norvegicus</i>

TABLE 1: Continued.

Chemical name	Chemical ID	CAS RN	Interaction count	Organism count	Interaction	PubMed ID	Authors	Title	Year	Citation	Organism
Bisphenol A	C006780	80-05-7	5	3	Bisphenol A results in increased expression of ID3 mRNA	26982218	Porreca et al.	"Stockpile" of slight transcriptomic changes determines the indirect genotoxicity of low-dose BPA in thyroid cells. Exposure to low-dose bisphenol A impairs meiosis in the rat seminiferous tubule culture model: a physiotoxicogenomic approach.	2016	PLoS One. 2016; 11(3): e0151618.	<i>Rattus norvegicus</i>
						25181051	Ali et al.	A novel toxicogenomics-based approach to categorize (non)genotoxic carcinogens. Convergent transcriptional profiles induced by endogenous estrogen and distinct xenoestrogens in breast cancer cells. Genome-wide gene expression profiling of low-dose, long-term exposure of human osteosarcoma cells to bisphenol A and its analogs bisphenols AF and S.	2014	PLoS One. 2014; 9(9): e106245.	<i>Rattus norvegicus</i>
						25270620	Schaap et al.		2014	Arch Toxicol. 2014 Oct 2.	<i>Mus musculus</i>
						16474171	Buterin et al.		2006	Carcinogenesis. 2006 Aug; 27(8): 1567-78.	<i>Homo sapiens</i>
						25912373	Fic et al.		2015	Toxicol In Vitro. 2015 Aug; 29(5): 1060-9.	<i>Homo sapiens</i>

TABLE 1: Continued.

Chemical name	Chemical ID	CAS RN	Interaction count	Organism count	Interaction	PubMed ID	Authors	Title	Year	Citation	Organism
Ethinylestradiol	D004997	57-63-6	5	2	Ethinylestradiol inhibits the reaction [IL1B protein results in increased expression of ID3 mRNA]	12072388	Evans et al.	Estrogen receptor alpha inhibits IL-1beta induction of gene expression in the mouse liver.	2002	Endocrinology. 2002 Jul; 143(7): 2559-70.	<i>Mus musculus</i>
Benzo(a)pyrene	D001564	50-32-8	4	2	Benzo(a)pyrene results in increased expression of ID3 mRNA	22228805	Kerley-Hamilton et al.	Inherent and benzo(a)pyrene-induced differential aryl hydrocarbon receptor signaling greatly affects life span, atherosclerosis, cardiac gene expression, and body and heart growth in mice. Transcriptional signature of human macrophages exposed to the environmental contaminant benzo(a)pyrene.	2012	Toxicol Sci. 2012 Apr; 126(2): 391-404.	<i>Mus musculus</i>
						20064835	Sparfel et al.		2010	Toxicol Sci. 2010 Apr; 114(2): 247-59.	<i>Homo sapiens</i>
Coumestrol	D003375	479-13-0	3	1	Coumestrol cotreated with 2,3-bis(3'-hydroxybenzyl)butyrolactone results in decreased expression of ID3 mRNA						
					Coumestrol cotreated with resveratrol results in decreased expression of ID3 mRNA	19167446	Dip et al.	Pleiotropic combinatorial transcriptomes of human breast cancer cells exposed to mixtures of dietary phytoestrogens.	2009	Food Chem Toxicol. 2009 Apr; 47(4): 787-95.	<i>Homo sapiens</i>

TABLE 1: Continued.

Chemical name	Chemical ID	CAS RN	Interaction count	Organism count	Interaction	PubMed ID	Authors	Title	Year	Citation	Organism
Methylmercuric chloride	C004925	115-09-3	1	1	Methylmercuric chloride results in increased expression of ID3 mRNA	20864626	Robinson et al.	Embryotoxicant-specific transcriptomic responses in rat postimplantation whole-embryo culture.	2010	Toxicol Sci. 2010 Dec; 118(2): 675-85.	<i>Rattus norvegicus</i>
Methylmercury compounds	D008767		1	1	Methylmercury compounds result in increased expression of ID3 mRNA	23397585	Song et al.	Monitoring of deiodinase deficiency based on transcriptomic responses in SH-SY5Y cells.	2013	Arch Toxicol. 2013 Jun; 87(6): 1103-13.	<i>Homo sapiens</i>
Methyl methanesulfonate	D008741	66-27-3	1	1	Methyl methanesulfonate results in decreased expression of ID3 mRNA	24211769	Sakai et al.	Utilization of CDKN1A/p21 gene for class discrimination of DNA damage-induced clastogenicity.	2014	Toxicology. 2014 Jan 6; 315: 8-16.	<i>Homo sapiens</i>
Methylnitrosourea	D008770	684-93-5	1	1	Methylnitrosourea results in decreased expression of ID3 mRNA	25270620	Schaap et al.	A novel toxicogenomics-based approach to categorize (non)genotoxic carcinogens.	2014	Arch Toxicol. 2014 Oct 2.	<i>Mus musculus</i>
Monobutyl phthalate	C028577	131-70-4	1	1	Monobutyl phthalate results in increased expression of ID3 mRNA	20864626	Robinson et al.	Embryotoxicant-specific transcriptomic responses in rat postimplantation whole-embryo culture.	2010	Toxicol Sci. 2010 Dec; 118(2): 675-85.	<i>Rattus norvegicus</i>
Mustard gas	D009151	505-60-2	1	1	Mustard gas results in increased expression of ID3 mRNA	15674844	Sabourin et al.	Time- and dose-dependent analysis of gene expression using microarrays in sulfur mustard-exposed mice.	2004	J Biochem Mol Toxicol. 2004; 18(6): 300-12.	<i>Mus musculus</i>

TABLE 1: Continued.

Chemical name	Chemical ID	CAS RN	Interaction count	Organism count	Interaction	PubMed ID	Authors	Title	Year	Citation	Organism
n-Butoxyethanol	C017096	111-76-2	1	1	n-Butoxyethanol results in decreased expression of ID3 mRNA	19812364	Laifenfeld et al.	The role of hypoxia in 2-butoxyethanol-induced hemangiosarcoma.	2010	Toxicol Sci. 2010 Jan; 113(1): 254-66.	<i>Mus musculus</i>
Nickel sulfate	C029938	7786-81-4	1	1	Nickel sulfate results in increased expression of ID3 mRNA	22714537	Clancy et al.	Gene expression changes in human lung cells exposed to arsenic, chromium, nickel, or vanadium indicate the first steps in cancer.	2012	Metallicomics. 2012 Aug; 4(8): 784-93.	<i>Homo sapiens</i>
Nitrosobenzylmethylamine	C014707	937-40-6	1	1	Nitrosobenzylmethylamine results in increased expression of ID3 mRNA	17616710	Reen et al.	Effects of phenylethyl isothiocyanate on early molecular events in N-nitrosomethylbenzylamine-induced cytotoxicity in rat esophagus.	2007	Cancer Res. 2007 Jul 1; 67(13): 6484-92.	<i>Rattus norvegicus</i>
N-Nitrosomorpholine	C002741	59-89-2	1	1	N-Nitrosomorpholine results in increased expression of ID3 mRNA	19716841	Oberemm et al.	Toxicogenomic analysis of N-nitrosomorpholine induced changes in rat liver: comparison of genomic and proteomic responses and anchoring to histopathological parameters.	2009	Toxicol Appl Pharmacol. 2009 Dec 1; 241(2): 230-45.	<i>Rattus norvegicus</i>
Octylmethoxycinnamate	C118580		1	1	Octylmethoxycinnamate results in decreased expression of ID3 mRNA	23397585	Song et al.	Monitoring of deiodinase deficiency based on transcriptomic responses in SH-SY5Y cells.	2013	Arch Toxicol. 2013 Jun; 87(6): 1103-13.	<i>Homo sapiens</i>

TABLE 1: Continued.

Chemical name	Chemical ID	CAS RN	Interaction count	Organism count	Interaction	PubMed ID	Authors	Title	Year	Citation	Organism
Oxophenylarsine	C029341	637-03-6	1	1	Oxophenylarsine binds to ID3 protein	23523789	Kurooka et al.	The metalloloid arsenite induces nuclear export of Id3 possibly via binding to the N-terminal cysteine residues.	2013	Biochem Biophys Res Commun. 2013 Apr 19; 433(4): 579-85.	<i>Mus musculus</i>
Perfluorooctanoic acid	C023036	335-67-1	1	1	Perfluorooctanoic acid results in decreased expression of ID3 mRNA	19162173	Ren et al.	Evidence for the involvement of xenobiotic-responsive nuclear receptors in transcriptional effects upon perfluoroalkyl acid exposure in diverse species.	2009	Reprod Toxicol. 2009 Jun; 27(3-4): 266-77.	<i>Rattus norvegicus</i>
Phenol	D019800	108-95-2	1	1	Phenol results in increased expression of ID3 mRNA	17547211	Kawata et al.	Classification of heavy-metal toxicity by human DNA microarray analysis.	2007	Environ Sci Technol. 2007 May 15; 41(10): 3769-74.	<i>Homo sapiens</i>
Polychlorinated biphenyls	D011078		1	1	Polychlorinated biphenyls analog results in decreased expression of ID3 mRNA	16474171	Buterin et al.	Convergent transcriptional profiles induced by endogenous estrogen and distinct xenoestrogens in breast cancer cells.	2006	Carcinogenesis. 2006 Aug; 27(8): 1567-78.	<i>Homo sapiens</i>
Propiconazole	C045950	60207-90-1	1	1	Propiconazole results in decreased expression of ID3 mRNA	21278054	Nesnow et al.	Propiconazole induces alterations in the hepatic metabolome of mice: relevance to propiconazole-induced hepatocarcinogenesis.	2011	Toxicol Sci. 2011 Apr; 120(2): 297-309.	<i>Mus musculus</i>
Vanadyl sulfate	C034028	27774-13-6	1	1	Vanadyl sulfate results in decreased expression of ID3 mRNA	16330358	Li et al.	Discrimination of vanadium from zinc using gene profiling in human bronchial epithelial cells.	2005	Environ Health Perspect. 2005 Dec; 113(12): 1747-54.	<i>Homo sapiens</i>

TABLE 1: Continued.

Chemical name	Chemical ID	CAS RN	Interaction count	Organism count	Interaction	PubMed ID	Authors	Title	Year	Citation	Organism
Vinclozolin	C025643	50471-44-8	1	1	Vinclozolin results in increased expression of ID3 mRNA	23034163	Skinner et al.	Epigenetic transgenerational inheritance of somatic transcriptomes and epigenetic control regions.	2012	Genome Biol. 2012; 13(10): R91.	<i>Rattus norvegicus</i>
EthylNitrosourea	D005038	759-73-9	1	1	EthylNitrosourea results in increased expression of ID3 mRNA	15954086	Katayama et al.	Microarray analysis of genes in fetal central nervous system after ethylNitrosourea administration.	2005	Birth Defects Res B Dev Reprod Toxicol. 2005 Jun; 74(3): 255-60.	<i>Rattus norvegicus</i>
2,6-Dinitrotoluene	C023514	606-20-2	1	1	2,6-Dinitrotoluene affects the expression of ID3 mRNA	21346803	Deng et al.	Analysis of common and specific mechanisms of liver function affected by nitrotoluene compounds.	2011	PLoS One. 2011; 6(2): e14662.	<i>Rattus norvegicus</i>
3,4-Dichloroaniline	C014464	95-76-1	1	1	3,4-Dichloroaniline results in decreased expression of ID3 mRNA	24172598	Da Rocha et al.	Diuron metabolites and urothelial cytotoxicity: in vivo, in vitro, and molecular approaches.	2013	Toxicology. 2013 Dec 15; 314(2-3): 238-46.	<i>Homo sapiens</i>
4,4'-Hexafluoroisopropylidene diphenol	C583074		1	1	4,4'-Hexafluoroisopropylidene diphenol results in increased expression of ID3 mRNA	25912373	Fic et al.	Genome-wide gene expression profiling of low-dose, long-term exposure of human osteosarcoma cells to bisphenol A and its analogs bisphenols AF and S.	2015	Toxicol In Vitro. 2015 Aug; 29(5): 1060-9.	<i>Homo sapiens</i>
7,8-Dihydro-7,8-dihydroxybenzo(a)pyrene 9,10-oxide	D015123	55097-80-8	1	1	7,8-Dihydro-7,8-dihydroxybenzo(a)pyrene 9,10-oxide results in decreased expression of ID3 mRNA	19150397	Lu et al.	Early whole-genome transcriptional response induced by benzo(a)pyrene diol epoxide in a normal human cell line.	2009	Genomics. 2009 Apr; 93(4): 332-42.	<i>Homo sapiens</i>

TABLE 1: Continued.

Chemical name	Chemical ID	CAS RN	Interaction count	Organism count	Interaction	PubMed ID	Authors	Title	Year	Citation	Organism
Bis(4-hydroxyphenyl)sulfone	C543008	80-09-1	1	1	Bis(4-hydroxyphenyl)sulfone results in increased expression of ID3 mRNA	25912373	Fic et al.	Genome-wide gene expression profiling of low-dose, long-term exposure of human osteosarcoma cells to bisphenol A and its analogs bisphenols AF and S.	2015	Toxicol In Vitro. 2015 Aug; 29(5): 1060-9.	<i>Homo sapiens</i>
Bismuth tripotassium dicitrate	C002791	57644-54-9	1	1	Bismuth tripotassium dicitrate results in increased expression of ID3 mRNA	15912405	Magnusson et al.	Gene expression changes induced by bismuth in a macrophage cell line.	2005	Cell Tissue Res. 2005 Aug; 321(2): 195-210.	<i>Rattus norvegicus</i>
tert-Butylhydroperoxide	D020122	75-91-2	1	1	tert-Butylhydroperoxide results in increased expression of ID3 mRNA	15003993	Ma et al.	The effect of stress withdrawal on gene expression and certain biochemical and cell biological properties of peroxide-conditioned cell lines.	2004	FASEB J. 2004 Mar; 18(3): 480-8.	<i>Mus musculus</i>
C646 compound	C584509		1	1	C646 compound results in decreased expression of ID3 mRNA	26191083	Gaddis et al.	Altering cancer transcriptomes using epigenomic inhibitors.	2015	Epigenetics Chromatin. 2015; 8: 9.	<i>Homo sapiens</i>
Cadmium sulfate	C037123	10124-36-4	1	1	Cadmium sulfate affects the reaction [MTF1 affects the expression of ID3 mRNA]	16221973	Wimmer et al.	Two major branches of anti-cadmium defense in the mouse: MTF-1/metallothioneins and glutathione.	2005	Nucleic Acids Res. 2005; 33(18): 5715-27.	<i>Mus musculus</i>

TABLE 1: Continued.

Chemical name	Chemical ID	CAS RN	Interaction count	Organism count	Interaction	PubMed ID	Authors	Title	Year	Citation	Organism
Cobaltous chloride	C018021	7646-79-9	1	1	Cobaltous chloride results in decreased expression of ID3 mRNA	19320972	Hang et al.	Transcription and splicing regulation in human umbilical vein endothelial cells under hypoxic stress conditions by exon array.	2009	BMC Genomics. 2009; 10: 126.	<i>Homo sapiens</i>
Copper sulfate	D019327	7758-98-7	1	1	Copper sulfate results in increased expression of ID3 mRNA	19549813	Song et al.	Physiological and toxicological transcriptome changes in HepG2 cells exposed to copper.	2009	Physiol Genomics. 2009 Aug 7; 38(3): 386-401.	<i>Homo sapiens</i>
Cuprizone	D003471	370-81-0	1	1	Cuprizone affects the expression of ID3 mRNA	26577399	Abe et al.	Developmental cuprizone exposure impairs oligodendrocyte lineages differentially in cortical and white matter tissues and suppresses glutamatergic neurogenesis signals and synaptic plasticity in the hippocampal dentate gyrus of rats.	2016	Toxicol Appl Pharmacol. 2016 Jan 1; 290: 10-20.	<i>Rattus norvegicus</i>
Sodium arsenite	C017947	13768-07-5	9	2	ID3 protein inhibits the reaction [sodium arsenite results in increased expression of EGRI mRNA]	23523789	Kurooka et al.	The metalloloid arsenite induces nuclear export of ID3 possibly via binding to the N-terminal cysteine residues.	2013	Biochem Biophys Res Commun. 2013 Apr 19; 433(4): 579-85.	<i>Mus musculus</i>

TABLE 1: Continued.

Chemical name	Chemical ID	CAS RN	Interaction count	Organism count	Interaction	PubMed ID	Authors	Title	Year	Citation	Organism
Sodium arsenite					Leptomycin B inhibits the reaction [sodium arsenite affects the localization of ID3 protein]	23523789	Kurooka et al.	The metalloloid arsenite induces nuclear export of Id3 possibly via binding to the N-terminal cysteine residues.	2013	Biochem Biophys Res Commun. 2013 Apr 19; 433(4): 579-85.	<i>Mus musculus</i>
Sodium arsenite					Sodium arsenite affects the localization of ID3 protein	16966095	McNeely et al.	Exit from arsenite-induced mitotic arrest is p53 dependent.	2006	Environ Health Perspect. 2006 Sep; 114(9): 1401-6.	<i>Homo sapiens</i>
Sodium arsenite					Sodium arsenite binds to ID3 protein	12760830	Andrew et al.	Genomic and proteomic profiling of responses to toxic metals in human lung cells.	2003	Environ Health Perspect. 2003 May; 111(6): 825-35.	<i>Homo sapiens</i>
Sodium arsenite					Sodium arsenite which binds to ID3 protein promotes the reaction [XPO1 protein affects the localization of ID3 protein]	23523789	Kurooka et al.	The metalloloid arsenite induces nuclear export of Id3 possibly via binding to the N-terminal cysteine residues.	2013	Biochem Biophys Res Commun. 2013 Apr 19; 433(4): 579-85.	<i>Mus musculus</i>
Sodium arsenite					Sodium arsenite results in increased expression of ID3 mRNA	16966095	McNeely et al.	Exit from arsenite-induced mitotic arrest is p53 dependent.	2006	Environ Health Perspect. 2006 Sep; 114(9): 1401-6.	<i>Homo sapiens</i>
Cadmium chloride	D019256	10108-64-2	2	2	Cadmium chloride results in increased expression of ID3 mRNA	16221973	Wimmer et al.	Two major branches of anti-cadmium defense in the mouse: MTF-1/metalllothioneins and glutathione.	2005	Nucleic Acids Res. 2005; 33(18): 5715-27.	<i>Mus musculus</i>

TABLE 1: Continued.

Chemical name	Chemical ID	CAS RN	Interaction count	Organism count	Interaction	PubMed ID	Authors	Title	Year	Citation	Organism
Diethylhexyl phthalate	D004051	117-81-7	2	1	Diethylhexyl phthalate results in decreased expression of ID3 mRNA	19850644	Ren et al.	Characterization of peroxisome proliferator-activated receptor alpha-independent effects of PPAR-alpha activators in the rodent liver: di-(2-ethylhexyl) phthalate also activates the constitutive-activated receptor.	2010	Toxicol Sci. 2010 Jan; 113(1): 45-59.	<i>Mus musculus</i>

Table 1. ID3 and Endocrine Disrupting Chemicals (EDCs). ID3 and endocrine disrupting chemical interaction created via Comparative Toxicogenomic Database (CTD).

Adipose tissue is highly connected to steroid hormones (estrogens, androgens, and glucocorticoids) and maintains a close relationship with the immune system via adipokines. Endocrine disruption can interfere with the creation, discharge, breakdown, elimination, and imitation of natural hormones (Jin et al. 2013). EDCs can be cataloged into multiple groupings such as dioxins, organotins, plastics, and pesticides. The increased presence of EDCs in the environment may help explain the incidence of metabolic disorders and associated

complications. EDCs are found in everyday products (including food, plastic bottles, metal cans, toys, cosmetics, & pesticides) and used in the manufacture of food. Exposure to EDCs may regulate inflammatory factors via ID3. TCDD and PCB congeners have been shown to up-regulate MCP-1 expression (Kirkley and Sargis. 2014). Bisphenol A exposure has been reported to increase IL-6 (Aremma et al. 2016). Population studies furthermore have reported an association between bisphenol-A plasma levels and pro-inflammatory cytokines including IL-6 (Savastano et al. 2015). Besides inflammation, ID3 may contribute to other risk factors of MetS such as angiogenesis, adipose tissue, blood glucose levels, and insulin resistance. PCBs have been associated with MetS in epidemiological studies (Uemura et al. 2009). In the mouse model, PCB153 has been shown to produce significant metabolic changes when administered with a high fat diet that were consistent with worsened obesity and nonalcoholic fatty liver disease pathology (Wahlang et al. 2013). ID3 may contribute to MetS via visceral fat expansion that was demonstrated in mice fed a high-fat diet (Cutchins et al. 2012).

ID3 deficiency resulted in greater energy expenditure and higher metabolic rate in mice at rest. With respect to metabolic disorders involving high glucose levels; ID3 may be impacted because it was shown to be regulated by glucose in pancreatic islet β -cells (Wice and Permutt. 2001) and under chronic hyperglycemic conditions. ID family proteins were stabilized by which in turn activated metabolic genes (Gronning et al. 2006). Since a causal link of diabetes and vascular disease is chronic hyperglycemia, ID3 may also contribute to metabolic perturbations from high blood glucose levels. More importantly, however, is that mitochondrial reactive oxygen species (ROS) produced by vascular endothelial cells under hyperglycemic conditions may share a pathway similar to environmental toxicant induced oxidative stress which converges on ID3. PCBs have been reported to have estrogenic activity (Bitman and Cecil. 1970; Meek and Finch. 1999; Tavori et al. 2006; Davey et al. 2007). PCB153 is an agonist for the pregnane X receptor (PXR) and the constitutive androstane receptor (CAR) which exert their effects on energy metabolism through gene regulation (Wada et al. 2009). Hence, dysregulation of these receptors by EDCs may contribute to PCB-induced metabolic perturbations. Several epidemiological studies have reported the association of PCB exposure with the increased risk of cardiovascular disease (Hay and Tarrel. 1997; Gustavsson and Hogstedt. 1997; Tokunaga and Kataoka. 2003; Sergeev and Carpenter. 2005; Goncharov et al. 2011). Since cardiovascular disease is a chronic disease affected by metabolic perturbations, we investigated the effects of PCB153 exposure on ID3 in vascular cells. Based on known PCB blood levels from occupational exposure, we showed a significant increase in

PCB153-induced vascularization at doses of 10-100 ng/ml which was ID3 dependent (Feltz. 2011). We have shown that PCB-induced ROS mediated a highly branched neo-vascular phenotype depended on ID3 and Pyk2 (Das and Feltz. 2014). Because the level of ID3 protein is determined by the rates of protein synthesis and protein degradation, we tested if PCB153 treatment affected ID3 protein synthesis. We showed that estrogenic chemical induced ID3 did not depend on protein synthesis, instead PCB153 treatment increased ID3 protein stability in endothelial cells. The role of phosphorylation in the regulation of ID3 protein stability is not known. A search with the PhosphoMotif finder program revealed that ID3 had 38 serine kinase/phosphatase motifs and 8 tyrosine kinase/phosphatase motifs (Amanczy et al 2007). We reported that both E2 and PCB153 induced ID3 phosphorylation. E2 treatment stabilized ID3 protein during the 1-6h treatment time points and increased phosphorylated ID3 levels (Das and Feltz. 2014, Das and Feltz. 2014). It was noteworthy that we showed data of ID3 tyrosine phosphorylation by PCB153 treatment which was confirmed by MALDI-TOF MS/MS spectra data. Our findings revealed phosphorylated amino acids Tyr-11 and Tyr-48 in peptides from ID3. Interestingly, Tyr-48 is positioned in the helix-loop-helix motif that is essential for protein binding. Currently, it is not clear whether PCB153-induced phospho-ID3 leads to protein-protein interactions that may prevent its degradation; however, our findings demonstrated that ID3 is a target of post-translational modifications by the endocrine disruptor PCB153 in vascular endothelial cells. Based on these lines of evidence, estrogenic chemical-induced ID3 signaling contributes to hyperplastic vascular lesions. This vascular cell

dysfunction may be a pathway by which EDCs and ID3 contribute to cardiovascular disease (Feltz and Porter. 2008). Based on these findings, we propose that ID3 is a target of EDCs that can activate inflammatory and energy pathways susceptible to metabolic perturbation during chronic disease pathogenesis. In the next section, we intend to discuss the current mechanistic understanding of how ID3 may influence chronic diseases associated with metabolic perturbations.

ID3 & Disease Outcomes

Vascular Diseases. ID3 involvement in vascular disease has been studied together with the lipoxygenase (12/15-LO) which is known to generate pro-inflammatory changes in blood vessels that precede the development of atherosclerosis (Chakrabarti et al. 2009). 12/15-LO is an important mediator of VSMC growth and its growth-promoting effects were shown to be mediated by ID3 transcription (Taylor et al. 2005). Increased expression of 12/15-LO in the vessel wall enhanced ID3-dependent cell proliferation, fibronectin deposition, and neointimal formation. Population based studies have found a SNP (single nucleotide polymorphisms) rs11574 in the coding region of the human ID3 gene associated with subclinical atherosclerosis in the Diabetes Heart study (Doran et al 2010). ID3 SNP rs11574 showed a significant association of coronary artery disease for caucasians and to a lesser extent in African Americans and Hispanics (Manichaikul et al. 2014).

Ectopic expression of ID3 in VSMC (vascular smooth muscle cells) regulates the cell-cycle (Yang et al. 2013). ID3 has also been shown to play a

complex role with atherosclerosis. ID3 expression is increased by hyperlipidemia and oxidized LDL (Taylor et al. 2006). ID3 regulates angiotensin II and carotid intima-media thickness. Angiotensin II promotes hyperplasia through up-regulating ID3 (Owens. 2009). The ID3 SNP could be a potential loss of function mutation if it inhibits the functioning of E proteins, thus being an atheroprotective factor. As shown in Figure 1.2, ID3 may impact vascular cell dysfunction leading to intimal lesions.

Figure 1.2 ID3 molecular mechanism & vascular diseases. Summary illustration of ID3 signaling involved in vascular disease pathogenesis. ID3 signaling can lead to neo-intimal lesions or vascular remodeling by transcriptional regulation of the target genes described above.

ID3 stimulates visceral adipose VEGFA expression, depot expansion and micro-vascular blood volume (Cutchins et al. 2012). ID3 promotes angiogenesis in HFD (high-fat diet)-induced visceral adiposity (Cutchins et al. 2012). ID3 KO shows a protective effect from HFD-induced visceral fat depot expansion. Furthermore, HFD-induced VEGFA expression in visceral adipose tissue was completely

abolished by loss of ID3. BMP9 induces both ID1 and ID3 which are necessary for induction of EphrinB2 (Kim et al. 2012). A summary in Figure 1.3 shows an ID3 signaling pathway involved in vascular malformations.

Figure 1.3 ID3 involvement in arteriovenous malformation. Summary of experimental studies showing impact of ID3 in vascular cell dysfunction.

Neurological Disorders. The ID3 gene is biologically relevant to neurological and behavior research because of its involvement in the stress response, neural plasticity, and neural circuitry. Molecular genetic studies in mice have shown that ID3 is required for embryonic neurovascular development. Genetic loss of ID1 and ID3 led to deviant neurovascular formations resulting in death (Lyden et al. 1999). ID1 and ID3 mutants showed premature differentiation of CNS radial glial cells that greatly increased neurons. Since radial glial cells, function as scaffolds for developing blood vessels in the CNS, alterations to their development in ID1/ID3

knockout mice may contribute to deviant blood vessel morphogenesis and hemorrhage. ID1 and ID3 may function beyond maturation of the CNS neurovasculature because other pathways such as Notch1 activation do not support neurovascular disorders (O'Brien et al. 2012; Solozobova et al. 2012). Psychopathologies such as anxiety and depression have been associated with ID3 methylation status. Epigenetic changes in ID3 have been associated with maltreatment of children as well as demonstrated as a predictor for depression. Montalvo-Ortiz et al reported epigenetic alterations in DNA derived from saliva in three genes predicted depression in a cohort of maltreated children: ID3, Glutamate NMDA Receptor (GRIN1), and Tubulin Polymerization Promoting Protein (TPPP) (Montalvo-Ortiz et al. 2016). Studies of the expression of these genes from medial prefrontal cortex (mPFC) tissue of mice subjected to a model of maternal neglect, which comprised of maternal separation and early weaning (MSEW). Behavioral tests were performed in MSEW and control adult male mice by the higher plus maze (EPM) and forced swimming test (FST), respectively (Montalvo-Ortiz et al. 2016). Behavioral differences in the EPM and FST tests showed that these genes ID3, GRIN1 and TPPP gene could predict anxiety and depression. Based on these studies, ID3 may contribute to the etiology of anxiety and depressive phenotypes when exposed to early life stress (Montalvo-Ortiz et al. 2016) (Figure 1.4).

Figure 1.4 Epigenetic mechanism of ID3 in neurological disorders. Summary of ID3 involvement in neurological disorders is presented.

Both environmental & genetic factors contribute to the progression of MetS and neurodegenerative disorders (Campos-Peña et al. 2017). Numerous studies have demonstrated that pre-diabetes and diabetes mellitus support cognitive decline related to Alzheimer's disease (AD) and vascular dementia (Campos-Peña et al. 2017). For example, sucrose-treated mice develop mitochondrial abnormalities with significant increase in A β levels, and slight increase in pTau levels which links metabolic perturbations from sucrose consumption with the AD-like pathology. Epigenetic changes in ID3 have been associated with maltreatment of children as well as demonstrated as a predictor for depression (Weder et al. 2014). Another epigenetic study of individuals with Autism spectrum disorders (ASD) revealed a significant association with a microRNA that targets ID3 (Sarachana et al. 2010). This is interesting as ID3 is also a neuronal target of

MeCP2 which is the causative gene for Rett syndrome in which afflicted children often exhibit autistic-like behaviors (Peddada et al. 2006). The overlap in the clinical symptoms of ASD, ADHD, and neurodegenerative disorders raises the question of whether epigenetic regulation of ID3 plays a role.

Population-based studies have demonstrated an association between toxic environmental chemical exposures and impaired neurodevelopment that may impact neurobehavioral disorders (Rossignol et al. 2014). Exposure to air pollutants from traffic and coal emissions are well-known risk factors for both attention deficit hyperactivity disorder (ADHD) and autism spectrum disorders (ASD) (Heilbrun et al. 2015). Polychlorinated biphenyls (PCBs) are endocrine-disrupting chemicals shown to adversely affect cognitive performance. Children exposed to PCBs have shown behavioral impairments as well as significant deficits in verbal and full-scale IQ (Chen et al. 1994). Urban areas are important regional sources of airborne PCBs and population-scale airborne exposure. Although PCBs have not been intentionally produced in the U.S. since the late 1970s, they continue to be detected in ambient air samples throughout the world (NIEHS, NIH). PCBs are measurable in the blood of nearly 80% of Americans over age 50 years (Shanahan et al. 2015). Hence, exposure to PCBs has been proposed to disrupt developing neuronal circuits that may cause developmental brain disorders such as learning disorders (LD), ADHD, and autism.

Endothelial cells of the blood-brain barrier (BBB) may provide clues in the study of brain health, behavior, and the environment (He et al. 2014). Inhaled air pollutants can disrupt the BBB by inducing pro-inflammatory cytokines that act on

endothelial cells (Genc et al. 2012). We have shown evidence for how PCB-induced reactive oxygen species (ROS) may contribute to cerebral vascular phenotype changes with the goal of understanding consequences of environmental exposures have on the BBB (Das and Felty. 2015). Toxic chemical exposures can change brain gene expression through regulatory epigenetic mechanisms involving alterations in DNA methylation and histone acetylation. Evidence from animal studies show that epigenetic programming by fetal exposure to toxicants has long-lasting consequences for gene expression in the brain as well as behavior (Kundakovic et al. 2015). Epigenetic changes in ID3 have been associated with maltreatment of children as well as demonstrated as a predictor for depression (Weder et al. 2014). Epigenetic biomarkers in peripheral tissues (blood, saliva, or buccal cells) may be useful to predict neurodevelopmental disorders in humans. Exposures to prenatal stress, famine, and pollution/toxins, factors known to affect brain development, have been associated with epigenetic variation in human peripheral tissues (Perera et al. 2011). Based on these evidences, we postulate that ID3 can be a useful biological marker of epigenetic perturbations caused by toxic chemical exposure in children/adolescents (Figure 1.5).

Figure 1.5 ID3 signaling pathway proposed in development of Autism Spectrum Disorder. Summary of ID3 involvement shows regulation of various genes causing changes in various processes such as modulation of synapses, circadian rhythm signaling, and collagen formation/stability.

Kidney disease. Lipoprotein abnormalities have been reportedly linked to renal dysfunction in chronic kidney disease patients. Nackiewicz et al reviewed the prominent characteristics of kidney disease previously stated in ApoE^{-/-}ID3^{-/-} double knockout mice; and show that ID3 in hyperlipidemic mice directly effects vulnerability to kidney disease. ID3 deficiency may intensify CXCL1 production by glomerular cells in response to inflammatory lipids and the resulting macrophage recruitment. Because ID3 is present in multiple cell types, it is also conceivable that other glomerular cells lacking ID3 may contribute to cytokine production in vivo (Tenger and Zhou. 2003). Therefore, the reno-protective effect of ID3 may be through regulation of local chemokine production. ID3 is known to directly interact with more than 30 different transcription factors (Lynn et al. 2010). Noticeably, a

change in the ID3 function may impact a wide range of protein-protein interactions with potentially significant consequences (Nackiewicz et al. 2014). In dissimilarity to the findings of Nachkiewicz et al, in ApoE^{-/-}ID3^{-/-} mice with glomerulonephritis, the hyperlipidemic ID3^{-/-} mice did not express meaningful increase in glomerular immune complex deposition associated to hyperlipidemic WT mice. Apolipoprotein E deficiency is known to cause enlarged immune responsiveness (Zhao et al. 2016) and these results add significance to the study in dissecting the effects of ID3 alone on kidney disease.

Clinical studies deliver indication for the relationship between lipids and chronic kidney disease. Nevertheless, they fail to elucidate why certain individuals (in the absence of diabetes or MetS) are probable to develop chronic kidney disease (Liu et al. 2012). Increased susceptibility to atherosclerosis has been reported to be associated with an ID3 single nucleotide polymorphism (SNP) (Doran et al. 2010). The overall preliminary findings in humans suggest a significant association between the same ID3 SNP and proteinuria, specifically influenced by small low density lipoproteins ($p=0.0024$) (Tenger and Zhou. 2003). C57BL/6 male mice on high fat diets (60% calorie from fat) develop MetS connected with obesity, elevated plasma glucose, proteinuria and glomerulonephritis (GN) and this may be due to decrease in renal AMP activated protein kinase, a cellular energy sensor (Declèves et al. 2011). However, C57BL/6 female mice on high fat diets develop GN and proteinuria only in the absence of ID3 suggesting distinct pathogenic mechanisms between females and males. Examining molecular mechanisms in mice has recognized ID3 as a unique

transcription factor that may contribute to kidney disease and provide mechanistic links between atherosclerosis, hyperlipidemia, and kidney disease in humans. A summary of ID3 pathway contributing to chronic kidney disease is shown in Figure 1.6.

Figure 1.6 ID3 involvement in kidney disease. Summary illustration shows a link to hyperlipidemia and atherosclerosis.

Cancer. Deregulation of ID genes are reported in human cancers such as non-small cell lung cancer (NSCLC) and colon cancer. ID3 contributes in generation of hematopoietic stem and progenitor cells (HSPC) associated with myeloproliferative disease (MPD) (Keller, NCI Division of Basic Sciences). ID1 and ID3 associated with the tumor promotion and metastasis (Castanon. 2013). Poor

response to chemo-radiotherapy have been reported in NSCLC patients with elevated ID1 and ID3 protein expression (Castanon et al. 2013). Regulation of p21 by ID1 and ID3 has been seen as the vital mechanism inhibiting the accumulation of additional DNA damage and subsequent functional low energy of colon-initiating cells (CC-ICs). Genetic silencing of ID1 and ID3 increases chemotherapy sensitivity in CC-ICs suggesting that these molecules allow cancer cells to be drug resistant (O'Brien et al. 2012). Summary illustration of ID3 signaling involved in various cancer pathways is shown in Figure 1.7.

Figure 1.7 ID3 involvement in various types of cancer. Summary of ID3 signaling pathways involved in Colon and Lung Cancer (Left panel). ID3 contribution to tumor angiogenesis in brain cancer (Right panel).

Glioblastoma multiforme (GBM) tumors contain glioma stem cells or GSCs which are implicated for glioma resistance to treatment (Bao et al. 2006; Nakano and Kornblum. 2006; Chalmers. 2007; Esposito et al. 2012; Phi et al. 2013). ID3 is also shown to be connected with medulloblastoma in children (Phi et al. 2013). Inhibition of ID3 reduced proliferation, migration, and increased apoptosis of medulloblastoma cells. Potential molecular mechanisms of ID3 in brain cancer are shown in Figure 1.8.

Figure 1.8 ID3 and brain cancer mechanisms. Signaling pathways of ID3 involved in brain cancer are summarized in the illustration.

Bone Disease. Osteogenesis imperfecta (OI) is a condition of fragile or brittle bones that break easily. OI affects 1 in 15,000 live births resulting in recurrent fractures and reduced mobility, with significant influence on quality of life (Balasubramanian et al. 2016). BMPs (bone morphogenetic proteins) are

morphogenetic signaling molecules vital for embryonic modelling. To find molecular understanding into the effect of BMPs on morphogenesis, Hollnagel et al examined novel genes directly activated by BMP signaling. CDM (chemically distinct growth medium)-cultured ES cells reacted very stringently to stimulation by numerous activin A, mesoderm inducers (BMP2/4), as well as fibroblast growth factor (Hollnagel et al. 1999). Using cDNA cloning, six BMP target genes were recognized. These include ID3, which exhibited convincing mRNA initiation, and the relatively stimulated Cyr61, DEK, and eIF4All genes, as well as a gene translating a GC-binding protein. Alongside ID1, ID2, and ID3 genes were initiated by BMP4 in both ES cells and arrangement of various cell lines. ID genes encode negative regulators of basic helix-loop-helix transcription factors. In vivo, ectopic expression was observed of ID3 and Msx-2 mRNAs in Ft/1 embryos at intersecting regions of ectopic Bmp4 misexpression. As a result, Hollnagel et al proposed that the target genes of BMP4 signaling demonstrated here are part of BMP-stimulated initial processes of mammalian development. The expression arrangements of Msx-1, Msx-2, c-jun, ID1, ID2, and ID3 in normal mice versus those lacking in BMP2, BMP4, and BMP2/4-type I receptor will be of distinctive interest to compare. The promoters of the genes recognized in the analysis will aid as valuable tools to illustrate the molecular governing circuits that are overseen by BMP signaling (Hollnagel et al. 1999).

ID proteins, including ID1, ID2, and ID3, are involved with essential binding factor α -1 (Cbfa1) to trigger debilitated transcription of the osteocalcin (OCL) and alkaline phosphatase (ALP) gene, commanding to weakened ALP

action and osteocalcin (OCL) production. ID acts by hindering the specific-sequence binding of Cbfa1 to DNA and diminishing the expression of Cbfa1 in cells experiencing osteogenic differentiation (Luan et al. 2008). Summary illustration of ID3 signaling in OI or brittle bone disease shown in Figure 6. p204, an interferon-inducible protein that acts with both Cbfa1 and ID2, debilitated the ID2-mediated inhibition of Cbfa1-induced ALP action and OCL production. Luan et al establish that p204 interrupted the binding of ID2 to Cbfa1 and facilitated Cbfa1 to bind to the promoters of its target genes. Furthermore p204 stimulated the translocation from nucleus to the cytoplasm and enhanced the degradation of ID2 by ubiquitin–proteasome pathway during osteogenesis. Nucleus export signal (NES) of p204 is necessary for the p204-enhanced cytoplasmic translocation and degradation of ID2, since a p204 mutant-requiring NES lost these activities. Taken together, ID

proteins help to shape a regulatory circuit and take part to control osteoblast differentiation (Luan et al. 2008).

Figure 1.9 ID3 involvement in brittle bone disease pathogenesis. ID3 regulates various genes leading to osteoblast differentiation, which can contribute to osteogenesis imperfecta as described in the summary illustration.

ID1 and ID3 function to regulate bone metabolism in vivo (Maeda et al. 2004). ID1/ID3 heterozygous knock out mice, showed that the thickness of calvarial junctions was attenuated by more than 50% (Maeda et al. 2004). Suppression of proliferation and mineralization in osteoblasts resultant from ID1/ID3 heterozygous knock out mice was proposed as a mechanism. Moreover, ID1/ID3 heterozygous knock out mice inhibited BMP-stimulated bone development in-vivo. Hence, ID1 and ID3 are critical regulators that support bone formation in vivo (Maeda et al. 2004).

The connection between mechanisms of MetS and bone mineral density (BMD) is controversial (Muhlen et al. 2007). Muhlen et al examined the association of MetS with osteoporosis, osteoporotic fractures, and BMD. MetS was associated with decreased, not increased BMD. Frequency of osteoporotic non-vertebral breakages was greater in members with MetS. MetS may be an additional risk factor for osteoporotic fractures (Muhlen et al. 2007). Occurrence of MetS at reference was 23.5% in men and 18.2% in women. Age-adjusted analyses demonstrated in both men and women with MetS had increased BMD at total hip when compared to those without MetS ($p<0.001$ and $p=0.01$, respectively). Men not women with MetS furthermore had greater BMD at femoral neck ($p=0.05$). Subsequently modifying for BMI, these connections were inverted, such that MetS was linked with decreased and not increased BMD. Occurrence of osteoporotic non-vertebral breakages was increased in participants with MetS. The connection of MetS with increased BMD was explained by the increased BMI in those with MetS (Muhlen et al. 2007).

Autoimmune Diseases. MetS has been involved in autoimmune diseases. One particular autoimmune disease is Primary Sjögren's syndrome (pSS), which is primarily categorized by inflammatory association of the exocrine glands important to dry eye and mouth. Numerous organ systems can be disturbed which can cause a wide variety of extra-glandular indicators, such as small airway disorders, multiple sclerosis-like disease, peripheral neuropathy glomerulonephritis, and lymphoma (Maeda et al. 2004). pSS predominately affects females (9:1), with a

frequency in the overall population from 0.1 to 0.6 %. Studies evaluating patients with rheumatoid arthritis (RA) (Sakurai et al. 2004) and systemic lupus erythematosus (SLE) (Sinicato et al. 2013) have shown that inflammation plays a role in the progression of hypertension, diabetes mellitus, and MetS (Pereira et al. 2009).

The ID3 gene is involved in the growth and function of B and T cells. Deficient ID3 mice develop autoimmune disease comparable to human Sjögren's syndrome (pSS). Together B and T lymphocytes have been involved to contribute to the disease phenotype in this model (Guo et al. 2011). The up-regulation of ID1 and ID3 genes has been reported in patients with rheumatoid arthritis (RA) (Sakurai et al. 2001). Elevated expression of ID1 and ID3 in endothelial cells has been proposed to contribute to severe angiogenesis found in RA.

Conclusion

We have comprehensively reviewed the existing evidence to illustrate the association between ID3 and metabolic perturbations. Furthermore, we extended this understanding of how ID3 and metabolic perturbations by environmental factors such as EDCs can modify chronic disease risk and health outcomes. ID3 has been seen to interact with multiple diseases such as cancer, vascular, neurological, autoimmune, & bone. Epidemiological and animal model studies have shown connections between ID3 and metabolic perturbations in chronic disease. Research is warranted to better define the influence of EDCs to ID3-induced metabolic perturbations. This may lead to novel pathways for how the

interaction of ID3, EDCs, and metabolic disorders exacerbate complex chronic disease; and can help public health professionals control these metabolic disorders.

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CHAPTER III

MANUSCRIPT 2

Association of PCB exposure in Peripheral Arterial Disease (PAD) Among the U.S. Population: NHANES 1999-2004

Background. Peripheral arterial disease (PAD) is a subclinical hallmark of coronary artery disease and identifies asymptomatic individuals at high risk for cardiovascular disease events. Current reports have shown association with a number of chemicals suspected to act as endocrine disruptors (EDs) by emulating synthetic or natural estrogen resultant in an increase in disease risk such as cancer, cardiovascular & vascular, and neuro-degenerative disorders. Polychlorinated biphenyls (PCBs) have been recognized as an endocrine disruptor due to their capability to affect & interfere with reproductive function and development in animals and humans by increasing estrogen activity or blocking estrogen action. Recent animal studies have suggested that PCB exposure may have a role in several mechanisms involved in the development of CVD including oxidative stress, vascular & endothelial dysfunction, weight gain, and insulin resistance. We conducted an Environmental-Wide Association Study (EWAS), in which epidemiological data are systematically and compressively interpreted in a method corresponding to a Genome Wide Association Study (GWAS).

Objectives. The purpose of this study is to examine association between exposure to PCBs & peripheral arterial disease (PAD) in a nationally represented sample of the U.S. population.

Methods. We utilized participants in the National Health and Nutritional Examination Survey (NHANES) 1999–2004. We estimated associations between exposure to polychlorinated biphenyls (PCBs) and PAD (ankle–brachial index < 0.9) using complex survey and logistic regression models adjusted for potential confounders.

Results. Analysis demonstrated higher geometric levels of all 6 PCB congeners in both PAD diagnosed participants and defined AS participants when compared to the rest of the population. Furthermore PCB153 was shown to have the highest geometric mean amongst all PCB congeners in both participants diagnosed with PAD (53.38 ng/g) and AS defined participants (38.66 ng/g). Furthermore adjusted models for PAD confounders (diabetes, smoking, & alcohol use), with gender, age, and race showed significant association with higher exposure of PCBs and increased risk of PAD and AS.

Conclusion. Our results suggest an association between environmental exposure to polychlorinated biphenyls (PCBs) & increased risk of peripheral arterial disease (PAD) and atherosclerosis (AS) among the U.S. population. Although the use of PCBs have been discontinued, exposure via the environment still remains to pose threats on the general population's health.

Key words: ankle–brachial index, endocrine disrupting chemicals, NHANES, peripheral arterial disease, polychlorinated biphenyls, vascular disease

Introduction

Humans are exposed to a variety of environmental exposures and these exposures may interact with one another to modify health risk outcomes. The cardiovascular system is a significant target of these environmental exposures via genomic and non-genomic pathways (Chambliss et al., 2002). The estrogen receptor (ER α/β) has been shown in endothelial cells, human coronary arteries, and cardiomyocytes (Kim-Schulze et al., 1996; Yang et al., 2004). Peripheral arterial disease (PAD) can lead to lasting disability but general diagnosis of the disease frequently appears late in the process. The first nationally representative community survey done in the U.S. sponsored by the PAD coalition established 75% of respondents were unaware PAD existed and the remaining 25% knew minimal correct information (Hirsch et al., 2007). Previous studies reported an association between Bisphenol A (BPA), an endocrine disrupting chemical produced in very large volume (>2 million metric tons manufactured globally in 2003) and used in the creation of polycarbonate plastics and containers used for food and beverages (Vandenberg et al., 2009). Nevertheless, the association of other endocrine disrupting chemicals with cardiovascular disease risk has never been without debate.

One particular compound that has been known to show estrogenic-like attributes are polychlorinated biphenyls (PCBs). We previously demonstrated that planar and co-planar PCBs induce the expression of different genes compared to estradiol. Furthermore PCBs can promote vascular lesion formation by activating

gene networks involved in endothelial cell adhesion, cell growth, and pro-inflammatory molecules which were dissimilar from natural estrogen. (Felty et al., 2010). Peripheral arterial disease (PAD) is a subclinical measure of atherosclerotic vascular disease (Newman et al. 1993) that is an independent predictor of following CVD incidence (Murabito et al. 2003; Newman et al. 1993). Criqui et al. (1992) reported that individuals among originally free of CVD, an ankle–brachial index (ABI) of < 0.9 was associated with a hazard ratio of 6.6 for future risk of death from coronary heart disease even after adjusting for cardiovascular risk factors including age, sex, body mass index (BMI), smoking, and high cholesterol levels. Guidelines (American Diabetes Association 2003; Hirsch et al. 2006) exist for defining PAD based on the ABI, which can be measured even in large population-based studies (Selvin and Erlinger 2004).

In this study, we examined the independent association between 6 individual PCBs, the sum of non-dioxin-like PCBs, and the sum of dioxin-like-PCBs with diagnosis of peripheral arterial disease and participants defined with atherosclerosis in the NHANES (National Health and Nutrition Examination Survey) in three year cycles: 1999 – 2000, 2001 – 2002, and 2003 – 2004. The aim of this study is to assess: 1) the mean PCB levels in PAD defined participants and atherosclerosis compared to non-diagnosed participants; and 2) the association between higher PCB exposure and increased risk of PAD/AS.

Methods

Study design and population. The current study is based on data from NHANES 1999-2004. NHANES survey includes a stratified multistage probability sample representative of the civilian non-institutionalized U.S. population. Selection was based on counties, blocks, households, and individuals within households and included the oversampling of non-Hispanic blacks and Mexican Americans in order to provide stable estimates of these groups. Participants provided written informed consent before their participation. Furthermore approval was obtained from the Human Subjects Committee in the U.S. Department of Health and Human Services. Measurements of ABI, a subclinical measure of atherosclerosis (Criqui et al. 1992; Newman et al. 1993), were obtained for the subsample of participants ≥ 40 years of age. The current study sample consisted of participants ≥ 40 years of age among whom polychlorinated biphenyls was available. This resulted in participants (461), of whom had PAD and participants (390) whom were indicated as having atherosclerosis. Subjects who were excluded because of missing covariates were in general similar to the NHANES cohort in terms of age, sex, race/ethnicity, and education (data not shown). Secondary analysis of the nationally collected data was done using variables of interest, which were only measured concurrently during the three NHANES cycles from 1999-2000, 2001-2002, and 2003-2004. NHANES uses a multifaceted sampling design (multistage, probability, cluster and stratified) for continuous collection and release of 2-year cross-sectional data sets. Approval for these questionnaires, age, appropriate examinations and laboratory testing were given to NHANES by the CDC/ATSDR

institutional review board of the CDC. The current study was considered exempt from review by the local institutional review board.

Peripheral Arterial Disease (PAD) identification. The NHANES ABI is routinely calculated by their computer system and verified by the National Center for Health Statistics before data release. Systolic pressure is measured on the right arm (brachial artery) and both ankles (posterior tibial arteries). If the participant has a rash or open wound on the right arm, dialysis shunt, right-sided radical mastectomy or any additional condition that would interfere with accurate measurement or cause discomfort to the participant, the left arm is used for the brachial pressure measurement. Systolic blood pressure is measured twice at each site for participants aged 40-59 years and once at each site for participants aged 60 years and older. If a health technician is unable to acquire a reading at a site they may attempt an additional reading at the same site after a short-term resting period. The right ABPI was obtained by dividing the mean systolic blood pressure in the right ankle by the mean blood pressure in the arm. The left ABPI was obtained by dividing the mean systolic blood pressure in the left ankle by the mean blood pressure in the arm. The mean blood pressure value for the arm and ankles are computed based on the first and second reading at each site. Since the second reading for all participants 60+ years of age is missing the mean values are in fact the first recorded blood pressure reading at a site. This may also be true for 40-59 year old participants who have a missing value for the first or second blood pressure reading. Individuals are excluded from the exam if they have bilateral amputations or weigh over 400 pounds (due to equipment limitations). In addition

to these exclusion criteria, some participants who were eligible for the exam (40 years of age and older) might not have received the exam due to various reasons: 1) casts, ulcers, dressings, or other conditions of the participant interfered with testing, 2) participant could not understand the test instructions, 3) participant became ill and the test could not be performed, 4) there was an equipment failure, 5) participant refused, 6) participant came late or left early from the MEC and the LED exam could not be performed, or 7) some other reason. As a result, these eligible individuals will have missing data for the ABPI variables. PAD was defined as an ABI < 0.7 . Values $0.9 < \text{ABI} < 1.3$ were considered normal. Measurements $0.7 < \text{ABI} < 0.9$ were defined as indication of atherosclerosis as seen in Table 2.1.

ABI Value	Reading Interpretation
ABI < 0.7	Peripheral Arterial Disease (PAD)
$0.7 < \text{ABI} < 0.9$	Atherosclerosis (AS)
$0.9 < \text{ABI} < 1.3$	Normal

Table 2.1 ABI (Ankle-Brachial Index)

Polychlorinated Biphenyl (PCBs) measurements. Individual PCB congeners were measured through blood serum concentration in a demonstrative, random one-third subsample of people 12 years of age older in three survey cycles: 1999 - 2000, 2001 – 2002, and 2003 – 2004. Each survey cycle sampled for various PCB congeners. NHANES sampled for 22 PCB congeners in the 1999 – 2000 survey, 34 PCBs congeners in the 2001-2002 cycle, and 38 PCB congeners in the 2003-2004 cycle. PCB congeners were measured in blood serum by high-resolution gas chromatography/isotope-dilution high-resolution mass spectrometry

(HRGS/ID-HRMS). Both wet-weight and lipid adjusted values for each sample, corrected for sample weight and analyte recovery was provided by NHANES. However we used lipid adjusted values in our analysis. Results for PCBs are reported on a whole weight (ng/g or ppb) and a lipid-adjusted basis (ng/g or ppb). Laboratory methods were consistent between survey cycles and are fully available online (CDC 2013b). A detection limit variable (LOD) is specified for all PCB congeners in the dataset. The variable LOD has two numerical values: “1” designates that the results was below the limit of detection and “2” designates that the results was equal to or above the limit of detection. To account for bias with PCBs below the LOD, we selected 6 individual PCB congeners that were represented & available from 1999 – 2004 and had concentrations above the LOD in at least 60% of the study subjects. The overall analysis explored the association between peripheral arterial disease variables in the U.S. population using the following 6 PCB congeners: 2,2',3,4,4,5,5'-heptachlorobiphenyl (PCB180), 2,2',4,4',5,5'-hexachlorobiphenyl (PCB153), 2,2',3,4,4',5-hexachlorobiphenyl (PCB138), 2,3',4,4',5- pentachlorobiphenyl (PCB118), 2,2',4,4',5- pentachlorobiphenyl (PCB99), and 2,4,4',5 tetrachlorobiphenyl (PCB74). We also conducted analysis on the sum of dioxin-like PCBs (074 and 118) and non-dioxin-like PCBs (099, 138,153,180).

Covariates. We considered a variety of potential confounders based on well-established risk factors for peripheral arterial disease and review of the literature. The resulting potential confounders were either taken as a laboratory measurement or self-reported in the questionnaire interviews. The demographic

variables of gender (male/female), race (Mexican American, Other Hispanic, Non-Hispanic White, Non-Hispanic Black, and other race) and age (≥ 40 -59, 60-74, and ≥ 75) were obtained during the NHANES home interview (CDC, 2012). Others obtained include: smoking, alcohol use (yes/no), BMI (18.5 to < 25 , 25 to < 30 , ≥ 30), diabetes (yes/no), estradiol levels, sex hormone binding globulin, testosterone, education, and annual income.

Statistical Analysis. All statistical analysis were performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). These analyses used 5% significance level ($p \leq 0.05$). We used logistic regression models to calculate odds ratios (ORs) and their 95% CI (confidence intervals) to investigate the risk between PCBs and peripheral arterial disease. According to the National Center for Health Statistics guides a six year subsample weight was calculated to adjust for oversampling of minority groups in the merged 1999-2004 NHANES data cycles (CDC 2013b). Non-missing values for serum concentrations below the LOD were assigned a serum level of the LOD divided by the square root of two. In our analysis, all lipid adjusted serum PCB concentrations were log transformed to satisfy normality assumptions. Participants were categorized as the following methods depending on data analysis: $< \text{LOD}$ vs. $\geq \text{LOD}$; $< \text{LOD}$ to 50th percentile vs. ≥ 50 th percentile; and $< \text{LOD}$ to 50th percentile vs. 50th percentile to 75th percentile vs. ≥ 75 th percentile. We also conducted separate analyses on all participants with serum PCB levels $> \text{LOD}$ where PAD & AS cases were compared with normal cases. Significance was set at $p < 0.05$ for all analyses.

We utilized survey means analysis (PROC SURVEYMEANS) to account for the complex sampling design of NHANES and to obtain weighted means, 95% confidence intervals, and standard errors of individual PCBs, the sum of dioxin-like PCBs, and the sum of non-dioxin-like PCBs. A two-sided student t-test was calculated using PROC SURVEYREG to test if the mean PCB levels between participants who were identified with PAD, AS, and normal participants were significantly different. Geometric mean (GM) PCB levels and geometric standard errors (GSE) were reported for peripheral arterial disease (PAD) status, atherosclerosis (AS) status, age at interview, and race/ethnicity for all participants with individual serum PCB levels. Computational mean serum levels of dioxin-like and non-dioxin-like PCBs and 95% CIs were reported for PAD status and select confounding variables. Age was divided into three groups (≥ 40 -59 years, 60-74 years, and ≥ 75 years) that were selected based on the age distribution of PAD cases, AS cases, and normal cases in the population. Race/ethnicity was divided into five groups (Mexican American, other Hispanic, Non-Hispanic White, Non-Hispanic Black, and other race). We furthermore calculated both crude and age standardized GMs for PAD cases and non-cases in the population with PCB levels above the LOD. We used the direct technique for age adjustment provided by the CDC (CDC 2014).

Results

Descriptive Statistics. The study population includes 6,624 participants ≥ 40 years of age. Among the 6,624 participants, 461 (6.96%) were defined with PAD, 390 (5.89%) for atherosclerosis (AS), & 5773 (87.15%) were defined as normal

(Table 2.2). Participants were equally distributed among gender: males – 3,489 (49.77%) to females – 3,135 (50.23%). The majority of the participants in each of the categories were predominantly Non-Hispanic White: 268 (3.72%), 236 (4.90%), and 3125 (69.64%) respectively. Participants in age group 60-74 years old were over-represented with defined PAD (199) and $\geq$ 40-59 years old with atherosclerosis (180). When asked if PAD identified participants ever smoked 72 (1.84%) said “yes,” 10 (0.24%) said “sometimes,” and 150 (3.68%) said “no.” Various hormone levels were also assessed. Estradiol levels between 5 -95 pg/mL were overrepresented 21 (3.18%) for participants with PAD; 31 (9.20%) for participants defined with AS; and 323 (85.44%) for normal participants. For sex hormone binding globulin (nmol/L) ranges from 0.350 – 90 nmol/L were most represented by the population: 20 (2.88%) for PAD identified participants; 31 (9.31%) for participants with AS; and 306 (82.69%) normal participants. Lastly testosterone levels (ng/mL) were also assessed for the population. Measurement range from 0.020 – 9 ng/mL were overrepresented. Participants with PAD represented 22 (3.24%) participants; AS participants with 32 (9.36%); and 318 (84.49%) for normal participants.

Table 2.2 Descriptive statistics for PAD & selected covariates among participants ≥ 40 years of age, NHANES 1999 – 2004.

Variable	ABI < 0.7* n (%)	0.7 < ABI < 0.9* n (%)	0.9 < ABI < 1.3* n (%)
Total Population n (%)	461 (6.96%)	390 (5.888%)	5773 (87.152%)
Gender			
Male	228 (1.947%)	275 (3.9918%)	2986 (43.8267%)
Female	233 (2.7315%)	115 (1.7774%)	2787 (45.7256%)
Race/Ethnicity			
Mexican American	72 (0.1567%)	103 (0.3131%)	1231 (4.1428%)
Other Hispanic	12 (0.1152%)	11 (0.2016%)	221 (4.0714%)
Non-Hispanic White	268 (3.7214%)	236 (4.894%)	3125 (69.6389%)
Non-Hispanic Black	100 (0.5863%)	31 (0.1975%)	1013 (7.8865%)
Other Race	9 (0.0989%)	9 (0.1622%)	183 (3.8127%)
Age at interview (years)			
≥ 40 -59	67 (1.1721%)	180 (3.7787%)	3078 (61.729%)
60-74	199 (2.0057%)	155 (1.5675%)	1924 (20.8257%)
≥ 75	195 (1.5007%)	55 (0.423%)	771 (6.9976%)
Ever Smoked			
Yes	72 (1.8416%)	31 (1.134%)	764 (30.244%)
Sometimes	10 (0.2441%)	10 (0.2571%)	117 (3.9327%)
No	150 (3.6785%)	140 (5.0162%)	1493 (53.6517%)
Alcohol Use			
Yes	0 (0.00%)	2 (0.0411%)	24 (0.5721%)
No	333 (4.5564%)	324 (6.2234%)	4415 (88.6069%)
BMI			
Normal Weight (18.5 to < 25)	105 (1.3733%)	86 (1.7261%)	1205 (26.2679%)
Overweight (25 to <30)	129 (1.6091%)	120 (2.3676%)	1755 (34.4198%)
Obese (≥ 30)	88 (1.3574%)	119 (2.1868%)	1450 (28.692%)
Diabetes			
Yes	81 (0.9209%)	46 (0.5002%)	520 (7.4332%)
No	251 (3.527%)	283 (5.6672%)	3903 (80.3218%)
Boarderline	9 (0.1072%)	3 (0.0905%)	84 (1.3882%)
Don't know	0 (0.00%)	0 (0.00%)	2 (0.0437%)
Estradiol (pg/mL)			
<5	1 (0.0545%)	1 (0.1399%)	4 (1.1599%)
May-95	21 (3.1751%)	31 (9.1967%)	323 (85.4413%)
95-185	1 (0.0867%)	0 (0.00%)	2 (0.6376%)
> 185	0 (0.00%)	0 (0.00%)	1 (0.1084%)
Sex Hormone Binding Globulin (nmol/L)			
0.350-90	20 (2.8754%)	31 (9.3131%)	306 (82.6883%)
90-180	2 (0.3553%)	1 (0.0267%)	21 (4.3095%)
>180	1 (0.0867%)	0 (0.00%)	2 (0.3449%)

Testosterone (ng/mL)			
0.020 - 9	22 (3.2402%)	32 (9.3673%)	318 (84.4869%)
12-Sep	0 (0.00%)	0 (0.00%)	8 (2.5649%)
> 12	1 (0.087%)	0 (0.00%)	2 (0.2538%)
Education			
< 9th Grade	120 (0.6988%)	74 (0.3937%)	976 (6.1832%)
9-11th Grade (Includes 12th grade with no diploma)	76 (0.7538%)	45 (0.5289%)	860 (10.5315%)
High School Grad/GED or Equivalent	117 (1.4821%)	65 (1.1016%)	1382 (23.692%)
Some College or AA degree	91 (1.0934%)	103 (1.7124%)	1403 (25.4172%)
College Graduate or above	54 (0.6319%)	102 (2.0203%)	1142 (23.6305%)
Refused	0 (0.00%)	0 (0.00%)	3 (0.0219%)
Don't Know	3 (0.0186%)	1 (0.0124%)	6 (0.0757%)
Income			
\$ 0 to \$ 4,999	14 (0.0897%)	12 (0.1544%)	161 (1.7935%)
\$ 5,000 to \$ 9,999	56 (0.4762%)	22 (0.1865%)	378 (4.1621%)
\$10,000 to \$14,999	77 (0.6941%)	33 (0.2147%)	580 (6.1557%)
\$15,000 to \$19,999	55 (0.4726%)	22 (0.1933%)	459 (5.1039%)
\$20,000 to \$24,999	40 (0.3538%)	29 (0.3374%)	473 (5.5343%)
\$25,000 to \$34,999	67 (0.7037%)	35 (0.5199%)	670 (9.4058%)
\$35,000 to \$44,999	30 (0.3397%)	37 (0.462%)	524 (8.463%)
\$45,000 to \$54,999	27 (0.3059%)	32 (0.4788%)	458 (8.3173%)
\$55,000 to \$64,999	15 (0.276%)	21 (0.379%)	322 (6.6019%)
\$65,000 to \$74,999	10 (0.1592%)	22 (0.3727%)	253 (5.1088 %)
\$75,000 and over	25 (0.3829%)	93 (2.0818%)	995 (22.9582%)
Over \$20,000	19 (0.3009%)	14 (0.2414%)	194 (3.2409%)
Under \$20,000	9 (0.0873%)	7 (0.0566%)	99 (1.1958%)
Refused	6 (0.0394%)	5 (0.0464%)	90 (1.0123%)
Don't know	6 (0.0238%)	5 (0.0711%)	51 (0.4455%)

Estimated percent distribution after applying NHANES sampling weights

*ABI < 0.7 (PAD). 0.7 < ABI < 0.9 (AS), & 0.9 < ABI < 1.3 (Normal)

Table 2.3 presents GMs and GSE of PCB concentrations (lipid-adjusted) by PAD status, AS status, and normal status. GM PCB levels were demonstrated as higher for all 6 PCB congeners for both participants defined with PAD as well as participants identified with AS when compared to the normal population (Table 2.3). Overall GM levels of PCBs ranged from 13.47 ng/g (PCB074) being the lowest and 53.38 ng/g (PCB153) being the highest in participants with PAD.

Furthermore it was demonstrated that 6.19 ng/g (PCB099) and 38.66 ng/g (PCB153) were demonstrated as the lowest and highest GM levels in AS participants.

Table 2.3 Geometric Mean PCB levels (ng/g) above the LOD by PAD status among participants ≥ 40 years of age, NHANES 1999-2004.

Analyte ₁	Cases/Non-Cases			Geometric Mean ₂ (ng/g) (GSE)		
	ABI < 0.7*	0.7 < ABI < 0.9*	0.9 < ABI < 1.3*	ABI < 0.7	0.7 < ABI < 0.9	0.9 < ABI < 1.3
PCB074	65	49	2871	13.47 (8.80, 20.61)	8.82 (7.15, 10.88)	7.82 (7.54, 8.11)
PCB099	57	76	3767	9.95 (7.078, 14.00)	6.19 (5.35, 7.16)	5.70 (5.53, 5.87)
PCB118	40	75	2866	15.02 (8.94, 25.24)	10.53 (8.20, 13.52)	8.90 (8.56, 9.26)
PCB138	58	76	3794	37.05 (28.04, 48.97)	25.18 (20.48, 30.96)	19.79 (19.11, 20.49)
PCB153	69	66	3803	53.38 (40.68, 70.04)	38.66 (30.64, 48.78)	27.68 (26.70, 28.70)
PCB180	62	72	3800	42.13 (33.00, 53.78)	31.11 (23.84, 40.60)	20.50 (19.71, 21.31)

1 Lipid Adjusted and log transformed polychlorinated biphenyls (ng/g)

2 Geometric Means calculated after applying NHANES sampling weights

*ABI < 0.7 (PAD), 0.7 < ABI < 0.9 (AS), & 0.9 < ABI < 1.3 (Normal)

Figure 2.1 Geometric mean PCB levels (ng/g) by PAD status for participants ≥ 40 years of age, NHANES 1999-2004.

Geometric mean PCB levels were also examined by age group of participants (Table 2.4). The overall GM levels of PCBs increased significantly in

PAD participants as age group progressed except PCB099 which demonstrated a non-consistent trend (10.04 ng/g vs. 8.82 ng/g vs. 18.99 ng/g). In the first age group ($\geq 40 - 59$) GM levels of PCBs are demonstrated to be higher in PAD defined participants when compared to normal participant levels. Additionally the same trend is shown when comparing AS participants to the normal group. In the second age group (60 – 74 years old), participants had higher GM PCB levels of congeners with the exception of PCB congener 180 that demonstrated lower GM PCB levels in normal participants when compared to PAD participants (Table 2.4). The population who were AS defined when compared to the normal population were shown as being higher. In the third age group (≥ 75 years old) all PCB congeners were shown to be higher in PAD identified participants when compared to normal participants. Furthermore higher PCB levels of participants whom were at defined AS participants were demonstrated with PCB congeners 74 and 118. Interestingly PCB 099, 138, 153, and 180 were shown to have higher PCB GM in normal participants when compared to AS participants.

Table 2.4 Geometric Mean PCB levels (ng/g) above the LOD by peripheral arterial disease status by age groups, NHANES 1999-2004

Analyte,	Geometric Mean ₂ (ng/g) (GSE, n)								
	Age: $\geq 40-59$			Age: 60-74			Age: ≥ 75		
	ABI < 0.7*	0.7 < ABI < 0.9*	0.9 < ABI < 1.3*	ABI < 0.7	0.7 < ABI < 0.9	0.9 < ABI < 1.3	ABI < 0.7	0.7 < ABI < 0.9	0.9 < ABI < 1.3
PCB074	9.41 (2.59)	7.10 (0.73)	6.58 (0.14)	14.78 (0.50)	19.87 (2.53)	12.12 (4.02)	40.06 (5.46)	25.53 (5.54)	22.32 (1.05)
PCB099	10.04 (2.52)	5.54 (0.47)	5.14 (0.10)	8.82 (0.30)	8.69 (1.11)	7.61 (2.53)	18.99 (3.13)	10.27 (2.97)	11.65 (0.52)
PCB118	9.01 (2.13)	8.48 (1.15)	7.56 (0.18)	17.42 (0.76)	22.96 (3.32)	13.18 (5.67)	54.77 (10.36)	50.02 (13.18)	24.40 (1.32)
PCB138	36.90 (9.13)	22.75 (2.54)	18.20 (0.39)	39.46 (1.20)	40.84 (4.63)	36.78 (7.43)	64.60 (8.86)	46.38 (11.75)	51.76 (2.06)
PCB153	52.80 (10.55)	35.51 (4.38)	25.61 (0.57)	58.18 (1.59)	64.95 (8.62)	55.36 (10.15)	97.05 (12.95)	63.22 (10.94)	75.76 (2.71)
PCB180	38.98 (8.45)	28.80 (4.69)	18.91 (0.46)	48.13 (1.19)	50.25 (6.84)	49.84 (7.19)	65.00 (9.02)	44.38 (10.29)	60.77 (2.08)

¹ Lipid Adjusted and log transformed polychlorinated biphenyls (ng/g);

² Geometric Means calculated after applying NHANES sampling weights

*ABI < 0.7 (PAD), 0.7 < ABI < 0.9 (AS), & 0.9 < ABI < 1.3 (Normal)

Figure 2.2.1 Geometric mean PCB levels (ng/g) by PAD status for participants ≥ 40 -59 years of age, NHANES 1999-2004.

Figure 2.2.2 Geometric mean PCB levels (ng/g) by PAD status for participants 60 - 74 years of age, NHANES 1999-2004.

Figure 2.2.3 Geometric mean PCB levels (ng/g) by PAD status for participants ≥ 75 years of age, NHANES 1999-2004.

Table 2.5 presents age standardized GMs and GSEs of PCBs by race/ethnicity in participants with individual concentrations of PCBs above the LOD. Ethnic backgrounds that were demonstrated to have higher PCB concentrations when having PAD compared to normal participants include: “Mexican American,” “Non-Hispanic White,” “Other Race,” and “Non-Hispanic Black.” “Other Hispanic” demonstrated lower levels of PCB concentrations in PAD participants when compared to normal participants. Furthermore participants with AS in ethnic groups include: “Mexican American,” “Non-Hispanic White,” “Non-Hispanic Black,” and “Other Race” demonstrated higher levels of PCBs when compared to normal participants. “Other Hispanic” AS participants showed lower PCB concentrations when compared to normal participants.

Table 2.5 Age standardized geometric mean PCB levels (ng/g) above the LOD by race/ethnicity among participants ≥ 40 years old PAD by age groups, NHANES 1999-2004.

Geometric Mean ₂ (ng/g) (GSE, n)								
Mexican American			Other Hispanic					
ABI < 0.7*		0.7 < ABI < 0.9*	0.9 < ABI < 1.3*	ABI < 0.7		0.7 < ABI < 0.9	0.9 < ABI < 1.3	
Analyte ₁				Analyte				
PCB074	10.46 (6.56-16.69)	7.68 (5.50-10.72)	4.60 (4.29-4.93)	PCB074	3.90 (3.87-4.01)	5.98 (2.20-16.22)	7.03 (5.95-8.31)	
PCB099	7.53 (4.97-11.43)	5.50 (4.28-7.08)	3.55 (3.35-3.77)	PCB099	4.52 (4.10-4.35)	4.82 (2.65-8.77)	5.63 (5.00-6.35)	
PCB118	10.83 (6.03-19.46)	10.05 (6.96-14.52)	5.71 (5.34-6.12)	PCB118	3.54 (3.42-3.98)	6.85 (1.96-23.96)	9.81 (8.28-11.62)	
PCB138	28.70 (17.25-47.72)	23.30 (17.39-31.21)	9.72 (9.02-10.47)	PCB138	6.59 (6.43-8.42)	7.40 (1.84-29.80)	19.56 (16.64-22.98)	
PCB153	36.97 (23.34-58.55)	37.56 (28.53-49.44)	12.57 (11.61-13.61)	PCB153	7.97 (3.42-8.15)	8.33 (1.66-41.87)	26.36 (22.21-31.29)	
PCB180	24.46 (14.99-39.90)	27.73 (19.04-40.41)	9.02 (8.25-9.87)	PCB180	3.42 (3.14-3.88)	6.66 (2.01-22.04)	18.39 (15.48-21.85)	
Non-Hispanic White			Non-Hispanic Black					
ABI < 0.7		0.7 < ABI < 0.9	0.9 < ABI < 1.3	ABI < 0.7		0.7 < ABI < 0.9	0.9 < ABI < 1.3	
Analyte				Analyte				
PCB074	13.17 (7.67-22.61)	9.05 (7.14-11.48)	8.45 (8.08-8.84)	PCB074	18.56 (10.53-32.70)	8.91 (6.61-12.00)	7.69 (7.07-8.36)	
PCB099	9.53 (6.28-14.48)	6.27 (5.32-7.38)	5.75 (5.54-5.96)	PCB099	14.15 (7.58-26.40)	7.53 (5.99-9.47)	7.40 (6.88-7.95)	
PCB118	14.54 (7.42-28.53)	10.78 (8.13-14.30)	9.13 (8.70-9.59)	PCB118	21.73 (12.04-39.20)	12.12 (7.94-18.51)	9.80 (8.87-10.82)	
PCB138	35.72 (25.63-49.80)	26.78 (21.74-32.99)	20.65 (19.80-21.55)	PCB138	54.59 (32.07-92.93)	31.61 (14.00-71.36)	23.93 (22.01-26.02)	
PCB153	52.22 (37.92-71.90)	41.52 (33.01-52.22)	29.32 (28.07-30.62)	PCB153	78.34 (45.95-133.56)	39.37 (15.94-97.22)	32.53 (29.82-35.49)	
PCB180	42.66 (32.36-56.24)	33.69 (25.47-44.56)	22.27 (21.24-23.35)	PCB180	54.35 (30.65-96.39)	38.61 (15.76-94.60)	21.91 (20.04-23.95)	
Other Race								
ABI < 0.7		0.7 < ABI < 0.9	0.9 < ABI < 1.3					
Analyte								
PCB074	10.43 (10.22-11.23)	11.73 (11.70-11.88)	5.86 (5.02-6.85)					
PCB099	5.77 (4.42-5.96)	8.02 (8.00-8.10)	5.64 (4.94-6.43)					
PCB118	10.21 (10.15-10.45)	11.01 (11.00-11.02)	8.28 (6.89-9.94)					
PCB138	21.21 (18.32-23.32)	21.70 (21.74-21.89)	19.01 (16.21-22.29)					
PCB153	30.43 (29.23-31.75)	40.00 (40.0-40.2)	26.25 (22.15-31.11)					
PCB180	24.42 (22.32-25.32)	26.20 (26.19-26.24)	18.28 (15.08-22.17)					

1 Lipid Adjusted and log transformed polychlorinated biphenyls (ng/g)
2 Geometric Means calculated after applying NHANES sampling weight
*ABI < 0.7 (PAD), 0.7 < ABI < 0.9 (AS), & 0.9 < ABI < 1.3 (Normal)

Table 2.6 demonstrates the computational means and 95% CIs of dioxin-like serum levels of PCBs for PAD, atherosclerosis, normal, and selected covariates in the study population. Computational means of dioxin-like PCBs were higher in participants diagnosed with PAD (1.57 ng/g) compared to participants without PAD (1.35 ng/g). Furthermore PCBs were lower in participants with AS (1.33 ng/g) compared to normal participants. Mean dioxin-like PCBs increased with age in all three groups: PAD, AS, & normal participants. Other Hispanic was demonstrated to be the highest race diagnosed with PAD (1.68 ng/g) and Non-Hispanic Black with AS (1.51 ng/g). Additionally all three age groups demonstrated a higher mean of PCB levels in both PAD participants & AS participants when compared to normal participants.

Table 2.6 Serum levels of dioxin-like PCBs (ng/g) in the study population, participants ≥ 40 years of age, NHANES 1999-2004.

Variable	Cases/Non - Cases			Mean ¹ (ng/g) (95% CI)		
	ABI < 0.7* (n)	0.7 < ABI < 0.9* (n)	0.9 < ABI < 1.3* (n)	ABI < 0.7 n(%)	0.7 < ABI < 0.9 n(%)	0.9 < ABI < 1.3 n(%)
Dioxin-like PCBs (074 + 118)	107	106	1324	1.57 (1.46-1.67)	1.37 (1.25-1.40)	1.35 (1.33-1.37)
Race/Ethnicity						
Mexican American	13	35	271	1.31 (1.12-1.49)	1.29 (1.14-1.44)	1.20 (1.16-1.24)
Other Hispanic	2	3	53	1.68 (1.59-1.76)	1.33 (0.84-1.47)	1.37 (1.26-1.47)
Non-Hispanic White	64	63	742	1.57 (1.44-1.70)	1.39 (1.25-1.42)	1.35 (1.32-1.37)
Non-Hispanic Black	27	4	208	1.58 (1.44-1.73)	1.51 (1.22-1.8)	1.50 (1.44-1.56)
Other Race	1	1	50	1.45 (1.45-1.45)	1.36 (1.35-1.36)	1.30 (1.21-1.39)
Age at interview (years)						
> 40-59	19	53	697	1.28 (1.12-1.44)	1.22 (1.14-1.30)	1.25 (1.23-1.28)
60-74	45	39	450	1.58 (1.43-1.73)	1.51 (1.43-1.61)	1.52 (1.48-1.55)
≥ 75	43	14	177	1.82 (1.72-1.91)	1.77 (1.62-1.92)	1.65 (1.60-1.70)
Ever Smoked						
Yes	20	10	218	1.38 (1.13-1.63)	1.25 (0.89-1.45)	1.21 (1.17-1.26)
Sometimes	1	4	35	0.99 (0.96-1.00)	1.08 (1.01-1.15)	1.26 (1.16-1.36)
No	55	44	454	1.54 (1.43-1.67)	1.27 (1.17-1.37)	1.39 (1.35-1.43)
Alcohol Use						
Yes	0	0	7	N/A	N/A	1.20 (1.00-1.40)
No	46	67	2868	1.46 (1.26-1.67)	1.29 (1.19-1.40)	1.23 (1.21-1.25)
BMI						
Normal Weight (18.5 to < 25)	25	24	380	1.44 (1.31-1.58)	1.28 (1.11-1.45)	1.34 (1.30-1.38)
Overweight (25 to <30)	41	39	514	1.57 (1.35-1.78)	1.30 (1.20-1.39)	1.33 (1.29-1.37)
Obese (≥ 30)	33	40	405	1.59 (1.48-1.70)	1.40 (1.29-1.51)	1.38 (1.35-1.42)
Diabetes						
Yes	24	13	160	1.65 (1.46-1.85)	1.55 (1.29-1.65)	1.52 (1.46-1.58)
No	81	92	1146	1.55 (1.43-1.67)	1.32 (1.24-1.40)	1.33 (1.31-1.35)
Borderline	2	1	17	1.62 (1.52-1.71)	1.27 (1.27-1.28)	1.46 (1.33-1.59)
Don't know	0	0	1	N/A	N/A	2.23 (2.22-2.23)
Estradiol (pg/mL)						
<5	0	19	0	N/A	N/A	1.26 (1.02-1.50)
5.1-95	6	491	19	1.30 (0.95-1.66)	1.22 (1.08-1.37)	1.20 (1.16-1.23)
95.1-185	1	1	0	2.67 (2.67-2.67)	N/A	0.92 (0.92-0.93)
> 185	0	1	0	N/A	N/A	2.22 (2.21-2.22)
Education						
< 9th Grade	24	21	221	1.67 (1.43-1.91)	1.43 (1.25-1.60)	1.36 (1.29-1.44)
9-11th Grade (includes 12th grade with no	24	10	206	1.37 (1.13-1.61)	1.36 (1.12-1.60)	1.42 (1.35-1.48)
High School Grad/GED or Equivalent	30	11	291	1.63 (1.44-1.82)	1.14 (0.92-1.37)	1.36 (1.31-1.41)
Some College or AA degree	15	27	347	1.50 (1.39-1.61)	1.36 (1.19-1.53)	1.33 (1.29-1.37)
College Graduate or above	13	37	257	1.70 (1.54-1.85)	1.35 (1.26-1.44)	1.33 (1.29-1.37)
Refused	0	0	0	N/A	N/A	N/A
Don't know	1	0	2	2.15 (2.15-2.16)	N/A	0.88 (0.78-0.98)
Income						
\$ 0 to \$ 4,999	1	3	120	1.55 (1.55-1.56)	1.14 (0.88-1.41)	1.11 (1.04-1.18)
\$ 5,000 to \$ 9,999	8	4	182	1.89 (1.57-2.21)	1.12 (0.94-1.31)	1.31 (1.23-1.40)
\$10,000 to \$14,999	7	9	284	1.65 (1.20-2.10)	1.64 (1.56-1.72)	1.33 (1.27-1.39)
\$15,000 to \$19,999	3	1	255	1.34(0.75-1.94)	1.36 (1.36-1.37)	1.22 (1.16-1.27)
\$20,000 to \$24,999	3	4	262	0.93 (0.407-1.45)	1.04 (0.73-1.36)	1.26 (1.20-1.32)
\$25,000 to \$34,999	5	7	346	1.44 (0.81-2.07)	1.46 (1.32-1.59)	1.23(1.18-1.28)
\$35,000 to \$44,999	2	3	252	1.59 (1.18-2.00)	1.87 (1.69-2.04)	1.25 (1.20-1.31)
\$45,000 to \$54,999	5	7	236	1.13 (0.86-1.40)	1.10 (0.87-1.33)	1.19 (1.14-1.23)
\$55,000 to \$64,999	1	3	174	1.76 (1.76-1.77)	1.00 (0.81-1.18)	1.19 (1.12-1.25)
\$65,000 to \$74,999	0	5	111	N/A	1.51 (1.05-1.97)	1.14 (1.07-1.20)
\$75,000 and over	5	19	426	1.25 (0.94-1.56)	1.35 (1.20-1.50)	1.21 (1.17-1.24)
Over \$20,000	4	2	88	1.52 (1.09-1.95)	1.41 (1.18-1.65)	1.38(1.29-1.47)
Under \$20,000	0	1	51	N/A	1.03 (1.03-1.04)	1.28 (1.13-1.42)
Refused	0	0	42	N/A	N/A	1.36 (1.25-1.47)
Don't know	0	1	19	N/A	0.97 (0.96-0.97)	1.19 (0.92-1.45)

¹ Means calculated after applying NHANES sampling weights; lipid-adjusted and log transformed PCBs

*ABI < 0.7 (PAD). 0.7 < ABI < 0.9 (AS), & 0.9 < ABI < 1.3 (Normal)

Table 2.7 demonstrates the computational means and 95% CIs of non-dioxin-like PCBs for diagnosis of PAD, AS, and normal population. Computational means was seen higher in diagnosed PAD participants exposed to non-dioxin-like PCBs compared to the normal population (2.18 ng/g vs.2.00 ng/g). Interestingly,

means was seen lower in AS participants when compare to the normal population (1.95 ng/g vs. 2.00 ng/g). Additionally all individual races/ethnicities demonstrated higher non-dioxin-like PCB concentrations in PAD diagnosed participants when compared to the normal population. Individual races/ethnicities including: “Mexican American” and “Non-Hispanic Black” showed higher non-dioxin-like PCB exposure in participants with AS when compared to the normal population.

Table 2.7 Serum levels of non-dioxin like PCBs (ng/g) in the study population, participants ≥ 40 years of age, NHANES 1999-2004

Variable	Cases/Non - Cases			Mean ¹ (ng/g) (95% CI)		
	ABI < 0.7 (n)	0.7 < ABI < 0.9 (n)	0.9 < ABI < 1.3 (n)	ABI < 0.7 n (%)	0.7 < ABI < 0.9 n (%)	0.9 < ABI < 1.3 n (%)
Non-dioxin-like PCBs (099+138+153+180)	138	123	1663	2.18 (2.11-2.24)	1.95 (1.92-2.04)	2.00 (1.98-2.01)
Race/Ethnicity						
Mexican American	17	36	327	1.96 (1.84-2.07)	1.93 (1.83-2.03)	1.80 (1.77-1.84)
Other Hispanic	2	3	63	2.47 (2.29-2.65)	1.75 (1.43-2.07)	1.98 (1.91-2.05)
Non-Hispanic White	84	79	947	2.16 (2.09-2.24)	1.99 (1.92-2.06)	1.99 (1.97-2.00)
Non-Hispanic Black	34	4	263	2.29 (2.16-2.42)	2.30 (2.13-2.47)	2.17 (2.13-2.22)
Other Race	1	1	63	2.20 (2.20-2.21)	1.93 (1.93-1.94)	1.98 (1.91-2.05)
Age at interview (years)						
> 40-59	23	59	878	1.98 (1.86-2.10)	1.88 (1.80-1.96)	1.92 (1.90-1.94)
60-74	57	45	563	2.22 (2.14-2.30)	2.14 (2.08-2.21)	2.12 (2.10-2.14)
≥ 75	58	19	222	2.31 (2.24-2.38)	2.31 (2.22-2.40)	2.22 (2.18-2.26)
Ever Smoked						
Yes	20	10	218	2.20 (1.96-2.44)	1.89 (1.61-2.16)	2.00 (1.96-2.05)
Sometimes	1	4	35	1.49 (1.49-1.51)	1.85 (1.75-1.94)	1.96 (1.86-2.07)
No	55	44	454	2.19 (2.11-2.27)	2.01 (1.90-2.11)	2.06 (2.03-2.09)
Alcohol Use						
Yes	0	0	6	N/A	N/A	2.12 (1.99-2.24)
No	107	103	1317	2.19 (2.12-2.2)	2.00 (1.92-2.07)	2.02 (2.00-2.04)
BMI						
Normal Weight (18.5 to < 25)	25	24	380	2.17 (2.01-2.32)	1.93 (1.79-2.08)	2.04 (2.00-2.09)
Overweight (25 to < 30)	41	39	514	2.20 (2.06-2.34)	2.04 (1.85-2.05)	2.02 (1.99-2.05)
Obese (≥ 30)	33	40	406	2.15 (2.05-2.26)	2.11 (2.00-2.20)	1.20 (1.97-2.03)
Diabetes						
Yes	24	13	160	2.29 (2.09-2.48)	2.12 (1.97-2.27)	2.10 (2.09-2.20)
No	81	92	1147	2.19 (2.10-2.27)	1.95 (1.91-2.07)	2.01 (1.99-2.03)
Borderline	2	1	17	2.16 (2.14-2.17)	1.93 (1.93-1.94)	2.12 (1.99-2.24)
Don't know	0	0	1	N/A	N/A	2.64 (2.64-2.64)
Estradiol (pg/mL)						
<5	0	0	19	N/A	N/A	1.75 (1.45-2.09)
5.1-95	6	19	491	2.18 (1.79-2.57)	1.96 (1.79-2.13)	1.89 (1.85-1.92)
95.1-185	1	0	1	2.99 (2.98-3.00)	N/A	1.23 (1.23-1.24)
> 185	0	0	1	N/A	N/A	2.77 (2.76-2.77)

Education						
< 9th Grade	35	22	269	2.27 (2.12-2.42)	2.14 (2.03-2.25)	1.98 (1.92-2.03)
9-11th Grade (Includes 12th grade with no diploma)	27	11	248	2.14 (1.95-2.33)	1.96 (1.751-2.16)	2.06 (2.01-2.11)
High School Grad/GED or Equivalent	42	15	381	2.19 (2.09-2.30)	1.80 (1.61-2.00)	2.00 (1.97-2.03)
Some College or AA degree	18	34	422	2.10 (2.01-2.18)	1.95 (1.84-2.06)	1.99 (1.96-2.01)
College Graduate or above	14	41	341	2.22 (2.05-2.39)	2.03 (1.96-2.12)	1.97 (1.93-2.00)
Refused	0	0	0	N/A	N/A	N/A
Don't Know	2	0	2	2.37 (2.17-2.57)	0 (0.00 %)	1.62 (1.53-1.71)
Income						
\$ 0 to \$ 4,999	1	3	171	2.25 (2.24-2.25)	1.72 (1.28-2.15)	1.62 (1.54-1.69)
\$ 5,000 to \$ 9,999	9	4	235	2.32 (2.01-2.63)	1.88 (1.69-2.07)	1.88 (1.81-1.95)
\$10,000 to \$14,999	11	11	386	2.06 (1.79 -2.32)	2.04 (1.83-2.26)	1.84 (1.79-1.90)
\$15,000 to \$19,999	5	3	331	1.99 (1.73-2.25)	1.90 (1.81-1.99)	1.80 (1.75-1.85)
\$20,000 to \$24,999	4	4	356	2.2 (1.95-2.48)	1.45 (0.96-1.94)	1.81 (1.75-1.86)
\$25,000 to \$34,999	5	7	449	2.19 (1.77-2.60)	2.07 (1.98-2.174)	1.80 (1.76-1.85)
\$35,000 to \$44,999	2	4	350	2.43 (1.86-3.00)	1.96 (1.72-2.20)	1.80 (1.75-1.85)
\$45,000 to \$54,999	7	8	306	1.89 (1.59-2.19)	1.97 (1.64-2.308)	1.82 (1.77-1.86)
\$55,000 to \$64,999	2	3	212	2.39 (2.34-2.44)	1.726 (1.472-1.97)	1.82 (1.77-1.88)
\$65,000 to \$74,999	2	5	147	1.88 (1.84-1.92)	2.04 (1.78-2.31)	1.77 (1.70-1.83)
\$75,000 and over	5	20	595	1.97 (1.89-2.05)	2.01 (1.89-2.13)	1.77 (1.74-1.80)
Over \$20,000	4	2	103	2.13 (1.94-2.31)	2.21 (1.95-2.46)	2.00 (1.91-2.08)
Under \$20,000	0	1	57	N/A	1.78 (1.78-1.79)	1.88 (1.73-2.02)
Refused	0	0	44	N/A	N/A	1.98 (1.87-2.10)
Don't know	0	1	25	N/A	1.76 (1.76-1.77)	1.79 (1.54-2.05)

¹ Means calculated after applying NHANES sampling weights; lipid-adjusted and log transformed PCBs
*ABI < 0.7 (PAD). 0.7 < ABI < 0.9 (AS), & 0.9 < ABI < 1.3 (Normal)

Geometric Means and 95% CI's of PCB levels (lipid-adjusted) are reported in Table 2.8 for participants with PCB levels < LOD and $\geq$ LOD. All participants who reported concentrations of $\geq$ LOD who were diagnosed with PAD had higher GM levels of PCB congeners. Similarly, participants who reported concentrations of $\geq$ LOD who were AS participants had higher PCB congener levels (074, 099, 158, and 180). PCB congener 074 had higher GM in normal participants vs. ones who were AS participants (14.07 ng/g vs. 13.19 ng/g) and congener 118 demonstrated comparable GM levels (11.51 ng/g vs. 11.32 ng/g) (Table 2.8)

2.8 Geometric Mean PCB levels (ng/g) by PAD status among participants $\geq$ 40 years of age, NHANES 1999-2004.

Analyte,	Cases/Non-cases			Geometric Mean ₂ (ng/g) (95% CI)		
	ABI < 0.7*	0.7 < ABI < 0.9*	0.9 < ABI < 1.3*	ABI < 0.7	0.7 < ABI < 0.9	0.9 < ABI < 1.3
PCB074						
< LOD	16	22	1467	4.27 (3.83-4.75)	4.12 (3.87-4.40)	3.71 (3.63-3.79)
≥ LOD	29	47	1382	25.70 (19.36-34.12)	13.186 (11.47-15.15)	14.07 (13.66-14.49)
PCB099						
< LOD	11	13	1267	2.85 (2.44-3.34)	3.35 (3.16-3.54)	2.63(2.57-2.70)
≥ LOD	28	35	1322	21.21 (15.96-28.18)	10.06 (8.72-11.61)	12.29 (11.90-12.70)
PCB118						
< LOD	3	3	574	2.39 (1.55-3.69)	3.63 (3.50-3.76)	2.94 (2.85-3.033)
≥ LOD	41	66	2250	18.28 (10.88-30.71)	11.51 (8.97-14.76)	11.32 (10.89-11.77)
PCB138						
< LOD	0	0	78	N/A	N/A	1.98 (1.89-2.07)
≥ LOD	58	76	3708	37.05 (28.03-48.97)	25.18 (20.48-30.96)	20.40 (19.72-21.10)
PCB153						
< LOD	6	5	1080	4.07 (3.65-4.54)	4.99 (3.18-7.84)	5.58 (5.39-5.78)
≥ LOD	52	71	2530	62.71 (49.79-78.98)	44.28 (36.43-53.80)	40.82 (39.59-42.09)
PCB180						
< LOD	0	0	217	N/A	N/A	1.53 (1.39-1.69)
≥ LOD	58	76	3571	42.13 (33.00-53.78)	31.11 (23.84-40.60)	22.10 (21.28-22.93)

1 Lipid adjusted and log transformed polychlorinated biphenyls (ng/g)

2 Geometric means calculated after applying NHANES sampling weights

*ABI < 0.7 (PAD), 0.7 < ABI < 0.9 (AS), & 0.9 < ABI < 1.3 (Normal)

We also adjusted the PCB levels into the following two groups < LOD to 50th percentile and ≥ 50th percentile (Table 2.9). Participants in the first group (< LOD to 50th percentile) with PAD showed higher GM PCB levels compared to participants who were defined as normal participants: PCB congener 074 (3.65 ng/g vs. 3.10 ng/g); 099 (2.85 ng/g vs. 2.61 ng/g); 118 (3.82 ng/g vs. 3.60 ng/g). PCB congeners 138 (7.06 ng/g vs. 7.66 ng/g); 153 (6.51 ng/g vs. 9.59 ng/g); and 180 (5.78 ng/g vs. 6.99 ng/g) showed higher levels of PCB GMs in normal participants then in participants defined with PAD. Furthermore a significant increase was seen in the second group (≥ 50th percentile) for PCB congener 074 (17.68 vs. 11.20); 099 (13.06 vs. 8.03); 118 (28.26 vs. 14.09); 138 (45.30 vs. 35.53); 153 (64.87 vs. 50.47) 180 (51.69 vs. 41.63). GM PCB concentrations between AS participants and normal participants were demonstrated to have little to no change between the two groups with the exception of PCB 153 (12.94 vs.

9.59 for < LOD to 50%; 54.29 vs. 50.47 for ≥ 50%) and PCB 180 (44.85 vs. 41.63 for ≥ 50%) (Table 2.9).

Table 2.9 Geometric mean PCB levels (ng/g) by PAD status among participants ≥ 40 years of age, NHANES 1999-2004

Analyte,	Cases/Non-cases			Geometric Mean, (ng/g) (95% CI)		
	ABI < 0.7*	0.7 < ABI < 0.9*	0.9 < ABI < 1.3*	ABI < 0.7	0.7 < ABI < 0.9	0.9 < ABI < 1.3
PCB074						
< LOD to 50%	8	11	962	3.65 (3.15-4.23)	3.70 (3.48-3.94)	3.10 (3.02-3.18)
≥ 50%	37	58	1904	17.68 (11.43-27.35)	11.04 (9.10-13.40)	11.20 (10.82-11.59)
PCB099						
< LOD to 50%	11	13	1215	2.85 (2.44-3.33)	3.35 (3.17-3.54)	2.61 (2.55-2.68)
≥ 50%	46	63	2549	13.06 (9.41-18.11)	7.14 (6.11-8.34)	8.03 (7.80-8.26)
PCB118						
< LOD to 50%	16	14	1181	3.82 (3.06-4.78)	4.14 (3.85-4.45)	3.60 (3.52-3.68)
≥ 50%	30	55	1686	28.26 (16.81-47.52)	15.26 (12.45-18.70)	14.09 (13.54-14.66)
PCB138						
< LOD to 50%	11	16	1776	7.06 (4.42-11.29)	10.48 (7.94-13.83)	7.66 (7.41-7.92)
≥ 50%	47	60	2018	45.30 (34.93-58.75)	34.77 (29.88-40.46)	35.53 (34.49-36.60)
PCB153						
< LOD to 50%	10	12	1751	6.51 (3.86-10.96)	12.94 (8.76-19.12)	9.59 (9.23-9.96)
≥ 50%	49	64	2050	64.87 (51.40-81.85)	54.29 (47.20-62.44)	50.47 (49.05-51.93)
PCB180						
< LOD to 50%	11	15	1840	5.78 (4.02-8.32)	7.67 (5.71-10.30)	6.99 (6.69-7.29)
≥ 50%	47	60	1953	51.69 (42.09-63.47)	44.85 (37.38-53.81)	41.63 (40.51-42.78)

¹Lipid adjusted and log transformed polychlorinated biphenyls (ng/g)

²Geometric means calculated after applying NHANES sampling weights

*ABI < 0.7 (PAD), 0.7 < ABI < 0.9 (AS), & 0.9 < ABI < 1.3 (Normal)

We additionally divided lipid adjusted PCB GMs to three groups: < LOD to 50%, 50% to 75%, and ≥ 75% (Table 2.10). Participants in groups <LOD to 50% demonstrated higher PCB concentrations in PAD defined participants when compared to normal participants (PCB congeners 074, 099, 118). PCB congeners 138, 153, and 180 showed the reverse results and demonstrated higher PCB concentration levels in normal participants when compared to the participants defined with PAD. When comparing AS participants to normal, all PCB congeners were higher than PCB GM levels in normal participants for < LOD to 50% (074, 099, 118, 138, 153, 180). GM PCB concentrations did not significantly differ in group 50% to 75% for both PAD diagnosed & participants with AS when compared to the normal group. In the final group ≥ 75%, a significant change was

demonstrated through all the participants with PAD when compared to the normal group. However PCB congener 180 was only shown at being higher in AS participants compared to normal ones (Table 2.10).

Table 2.10 Serum levels of PCBs (ng/g) in the study population, participants ≥ 40 years of age, NHANES 1999 – 2004.

Analyte,	Cases/Non-cases			Geometric Mean _z (ng/g) (95% CI)		
	ABI < 0.7*	0.7 < ABI < 0.9*	0.9 < ABI < 1.3*	ABI < 0.7	0.7 < ABI < 0.9	0.9 < ABI < 1.3
PCB074						
< LOD to 50%	8	11	962	3.65 (3.15-4.23)	3.70 (3.48-3.94)	3.10 (3.02-3.18)
50% to 75%	13	19	803	5.63 (4.91-6.44)	6.15 (5.302-7.14)	6.05 (5.94-6.17)
$\geq 75\%$	24	39	1101	31.81 (24.21-41.79)	15.95 (13.57-18.74)	16.83 (16.25-17.43)
PCB099						
< LOD to 50%	11	13	1215	2.85 (2.44-3.33)	3.35 (3.17-3.54)	2.61 (2.55-2.68)
50% to 75%	19	41	1621	4.43 (4.22-4.65)	5.28 (4.78-5.83)	5.41 (5.33-5.50)
$\geq 75\%$	27	22	928	21.30 (16.02-28.33)	12.38 (10.50-14.582)	15.72 (15.20-16.26)
PCB118						
< LOD to 50%	16	14	1181	3.82 (3.06-4.78)	4.14 (3.85-4.45)	3.60 (3.52-3.68)
50% to 75%	4	15	614	5.87 (4.78-7.22)	7.04 (6.04-8.21)	7.24 (7.09-7.40)
$\geq 75\%$	26	40	1072	34.52 (22.10-53.93)	19.09 (15.52-23.48)	21.64 (20.78-22.53)
PCB138						
< LOD to 50%	11	16	1776	7.06 (4.42-11.29)	10.48 (7.93-13.83)	7.66 (7.41-7.92)
50% to 75%	19	25	817	21.63 (18.62-25.13)	20.40 (18.03-23.09)	21.70 (21.31-22.09)
$\geq 75\%$	28	35	1198	75.44 (59.77-95.22)	47.29 (42.38-52.77)	54.04 (52.47-55.66)
PCB153						
< LOD to 50%	9	11	1268	6.51 (3.86-10.96)	12.94 (8.76-19.12)	9.59 (9.23-9.96)
50% to 75%	11	19	588	30.98 (26.72-35.93)	32.36 (27.87-7.58)	30.73 (30.17-31.31)
$\geq 75\%$	24	39	997	92.40 (73.10-116.79)	68.28 (61.11-76.30)	74.12 (72.12-76.17)
PCB180						
< LOD to 50%	11	15	1840	5.78 (4.02-8.32)	7.67 (5.71-10.30)	6.99 (6.69-7.29)
50% to 75%	10	20	765	23.44 (20.60-26.68)	26.79 (23.64-30.35)	26.49 (26.03-26.96)
$\geq 75\%$	36	40	1188	67.27 (56.16-80.57)	61.44 (51.93-72.715)	60.60 (59.00-62.25)

1 Lipid adjusted and log transformed polychlorinated biphenyls (ng/g)

2 Geometric means calculated after applying NHANES sampling weight

*ABI < 0.7 (PAD), 0.7 < ABI < 0.9 (AS), & 0.9 < ABI < 1.3 (Normal)

Table 2.11 summarized adjusted odds ratios (ORs) and 95% confidence interval (CI) models for individual PCB congeners (074, 099, 118, 138, 153, and 180) in two groups: < LOD to 50% & $\geq 50\%$. Adjusted models for < LOD to 50% showed no association between PCB exposure and PAD. However adjusted models for $\geq 50\%$ showed significant associations for PCB congeners (074, 099, 118, 138, 153, and 180) and PAD. Table 2.12 demonstrated adjusted ORs and 95% CI models for individual PCB congeners (074, 099, 118, 138, 153, and 180)

in three groups: < LOD to 50%, 50 % to 75%, and $\geq$ 75%. Adjusted models for PAD confounders; gender and PAD confounders; age, race, and PAD confounders; and gender, age, race, and PAD confounders showed a significant association between exposure and increased risk of PAD in groups 50% to 75% and $\geq$ 75%. Group < LOD to 50% showed no association. Table 2.13 shows adjusted ORs and 95% CI models for individual PCB congeners in four groups: < LOD to 50%, $\geq$ 50%, < LOD to 75%, and $\geq$ 75%. Adjusted models in groups $\geq$ 50% & $\geq$ 75% showed association between higher exposure of PCBs and increased risk of PAD.

In the adjusted model for PAD confounders (diabetes, smoking, & alcohol use) at $\geq$ 50% & $\geq$ 75%, dioxin-like PCBs were significantly associated with PAD [(OR: 2.04; 95% CI: 1.81 – 3.57), (OR:2.46; 95% CI:2.01 -11.87)] as well as non-dioxin-like PCBs [(OR: 1.07; 95% CI: 1.02 – 32.59), (OR: 2.64; 95% CI: 1.62 – 10.67)] . After adjusting the model for gender and PAD confounders, dioxin-like PCBs showed association with PAD [(OR: 1.35; 95% CI: 1.05 – 1.36), (OR: 3.35; 95% CI: 1.35 – 10.36)] as well as non-dioxin-like PCBs [(OR: 2.38; 95% CI: 2.35 – 29.40), OR: 2.59; 95% CI: 2.57 – 10.62)]. Adjusting the model for age, race, & PAD confounders the model demonstrated dioxin-like PCBs association with PAD [(OR: 1.95; 95% CI: 0.94 – 2.36), (OR: 2.95; 95% CI: 0.94 – 6.00)] together with non-dioxin-like PCBs [(OR: 2.07; 95% CI: 0.96 – 12.97), (OR: 2.37; 95% CI: 0.96 – 6.97)]. After adjusting the model for gender, age, race, and PAD confounders; dioxin-like PCBs remained significantly associated with PAD [(OR: 1.68; 95% CI:

1.38 – 2.58), (OR: 3.88; 95% CI: 1.01 – 5.08)] as well as non-dioxin-like PCBs [(OR: 2.09; 95% CI: 2.09– 14.10), OR: 3.01; 95% CI: 1.10 – 8.13)].

Table 2.11 Estimated ORs & 95% CI of PAD status by concentration of PCB levels among participants ≥ 40 years of age, NHANES 1999 - 2004

Analyte ¹	ABI < 0.7*	0.7 < ABI < 0.9*	0.9 < ABI < 1.3*	Adjusted OR (95% CI) ²	Adjusted OR (95% CI) ³	Adjusted OR (95% CI) ⁴	Adjusted OR (95% CI) ⁵
PCB074							
< LOD to 50%	8	11	962	1	1	1	1
$\geq 50\%$	37	58	1904	1.61 (0.64 - 8.65)	2.72 (1.72 - 4.73)	2.63 (1.63 - 11.64)	2.76 (1.75 - 11.77)
PCB099							
< LOD to 50%	11	13	1215	1	1	1	1
$\geq 50\%$	46	63	2549	2.92 (1.91 - 5.92)	2.95 (1.94 - 5.95)	1.91 (1.60 - 3.92)	1.95 (1.94 - 2.96)
PCB118							
< LOD to 50%	16	14	1181	1	1	1	1
$\geq 50\%$	30	55	1686	2.26 (0.75 - 11.26)	3.31 (1.31 - 10.32)	4.08 (3.08 - 9.09)	4.17 (1.16 - 9.17)
PCB138							
< LOD to 50%	11	16	1776	1	1	1	1
$\geq 50\%$	47	60	2018	1.38 (2.37 - 17.39)	3.39 (2.38 - 12.40)	3.71 (1.71 - 7.72)	3.73 (1.72 - 6.70)
PCB153							
< LOD to 50%	10	12	1751	1	1	1	1
$\geq 50\%$	49	64	2050	3.45 (1.47 - 25.66)	4.64 (2.63 - 42.65)	2.16 (1.95 - 31.97)	3.33 (1.92 - 29.94)
PCB180							
< LOD to 50%	11	15	1840	1	1	1	1
$\geq 50\%$	47	60	1953	2.33 (1.52 - 19.55)	3.49 (3.47 - 30.50)	2.00 (1.59 - 17.02)	2.89 (2.87 - 17.90)

¹ Lipid adjusted and log transformed polychlorinated biphenyls (ng/g)

² Adjusted for PAD confounders (Diabetes, Smoking, and Alcohol Use)

³ Adjusted for Gender, & PAD confounders

⁴ Adjusted for Age, Race, & PAD confounders

⁵ Adjusted for Gender, Age, Race, & PAD confounders

*ABI < 0.7 (PAD), 0.7 < ABI < 0.9 (AS), & 0.9 < ABI < 1.3 (Normal)

Table 2.12 Estimated ORs & 95% CI of PAD status by concentration of PCB levels among participants ≥ 40 years of age, NHANES 1999 - 2004

Analyte ¹	ABI < 0.7*	0.7 < ABI < 0.9*	0.9 < ABI < 1.3*	Adjusted OR (95% CI) ²	Adjusted OR (95% CI) ³	Adjusted OR (95% CI) ⁴	Adjusted OR (95% CI) ⁵
PCB074							
< LOD to 50%	8	11	962	1	1	1	1
50% to 75%	13	19	803	1.78 (1.12 - 10.13)	2.11 (1.09 - 4.21)	1.35 (0.54 - 6.95)	2.45 (1.04 - 4.05)
$\geq 75\%$	24	39	1101	2.02 (1.71 - 19.73)	3.26 (1.45 - 6.87)	2.59 (0.58 - 7.60)	3.80 (1.39 - 6.99)
PCB099							
< LOD to 50%	11	13	1215	1	1	1	1
50% to 75%	19	41	1621	2.58 (1.38 - 4.59)	2.63 (1.62 - 3.63)	1.37 (0.13 - 2.85)	2.24 (0.44 - 3.45)
$\geq 75\%$	27	22	928	3.50 (2.22 - 6.51)	2.58 (1.57 - 3.59)	1.32 (0.31 - 2.34)	2.46 (0.35 - 2.78)
PCB118							
< LOD to 50%	16	14	1181	1	1	1	1
50% to 75%	4	15	614	2.21 (1.66 - 6.68)	2.80 (1.80 - 5.81)	1.76 (0.49 - 3.51)	3.00 (0.68 - 4.70)
$\geq 75\%$	26	40	1072	2.50 (2.73 - 10.75)	2.89 (2.88 - 6.91)	2.59 (2.58 - 5.60)	3.79 (2.77 - 5.80)
PCB138							
< LOD to 50%	11	16	1776	1	1	1	1
50% to 75%	19	25	817	1.75 (1.38 - 10.39)	2.39 (1.39 - 9.40)	1.76 (1.26 - 7.47)	2.48 (0.47 - 5.48)
$\geq 75\%$	28	35	1198	2.03 (1.32 - 12.74)	3.75 (2.33 - 19.76)	2.28 (1.19 - 23.09)	3.10 (2.07 - 26.11)
PCB153							
< LOD to 50%	9	11	1268	1	1	1	1
50% to 75%	11	19	588	3.69 (1.03 - 9.74)	5.64 (2.23 - 10.74)	3.07 (1.06 - 10.08)	3.04 (1.03 - 9.05)
$\geq 75\%$	24	39	997	4.81 (2.24 - 13.17)	6.84 (3.23 - 14.45)	4.81 (1.70 - 13.73)	4.85 (2.64 - 10.66)
PCB180							
< LOD to 50%	11	15	1840	1	1	1	1
50% to 75%	10	20	765	2.67 (1.23 - 8.68)	2.96 (1.65 - 30.66)	3.25 (1.66 - 25.68)	3.62 (1.61 - 19.62)
$\geq 75\%$	36	40	1188	2.95 (1.87 - 10.33)	3.04 (2.23 - 14.26)	3.92 (0.86 - 16.90)	3.98 (0.66 - 21.71)

¹ Lipid adjusted and log transformed polychlorinated biphenyls (ng/g)

² Adjusted for PAD confounders (Diabetes, Smoking, and Alcohol Use)

³ Adjusted for Gender, & PAD confounders

⁴ Adjusted for Age, Race, & PAD confounders

⁵ Adjusted for Gender, Age, Race, & PAD confounders

*ABI < 0.7 (PAD), 0.7 < ABI < 0.9 (AS), & 0.9 < ABI < 1.3 (Normal)

Table 2.13 Estimated ORs (95% CIs) of PAD status by concentration of dioxin-like & non-dioxin like PCB levels among participants ≥ 40 years of age, NHANES 1999 – 2004

Analyte ¹	ABI < 0.7*	0.7 < ABI < 0.9*	0.9 < ABI < 1.3*	Adjusted OR (95% CI) ⁴	Adjusted OR (95% CI) ⁵	Adjusted OR (95% CI) ⁶	Adjusted OR (95% CI) ⁷
Dioxin-like PCBs_50²							
< LOD to 50%	4	6	71	1	1	1	1
$\geq 50\%$	45	69	1342	2.04 (1.81 - 3.57)	1.35 (1.05 - 1.36)	1.95 (0.94 - 2.36)	1.68 (1.38 - 2.58)
Dioxin-like PCBs_75²							
< LOD to 75%	10	15	90	1	1	1	1
$\geq 75\%$	58	76	1288	2.46 (2.01 - 11.87)	3.35 (1.35 - 10.36)	2.95 (0.94 - 6.00)	3.88 (1.01 - 5.08)
Non-dioxin-like PCBs_50³							
< LOD to 50%	15	18	65	1	1	1	1
$\geq 50\%$	61	55	1710	1.07 (1.02 - 32.59)	2.38 (2.35 - 29.40)	2.07 (0.96 - 12.97)	2.09 (2.09 - 14.10)
Non-dioxin-like PCBs_75⁴							
< LOD to 75%	6	4	951	1	1	1	1
$\geq 75\%$	59	76	828	2.64 (1.62 - 10.67)	2.59 (2.57 - 10.62)	2.37 (0.96 - 6.97)	3.01 (1.10 - 8.13)

¹ Lipid adjusted and log transformed polychlorinated biphenyls (ng/g)

² Adjusted for PAD confounders (Diabetes, Smoking, and Alcohol Use)

³ Adjusted for Gender & PAD confounders

⁴ Adjusted for Age, Race, & PAD confounders

⁵ Adjusted for Gender, Age, Race, & PAD confounders

*ABI < 0.7 (PAD), 0.7 < ABI < 0.9 (AS), & 0.9 < ABI < 1.3 (Normal)

Discussion

This cross sectional study representing a sample of the United States demonstrated that higher levels of PCB concentration were associated with participants defined with PAD and atherosclerosis in the general population. We assessed 6 individual PCB congeners, the sum of dioxin-like PCBs, and the sum of non-dioxin like PCBs. Overall analysis of geometric mean levels of PCBs represented by participants identified with PAD, atherosclerosis, age, race, and various covariates was done to assess PCB concentrations in subpopulations. All PCB congeners showed a higher concentration level in both PAD participants and at AS when comparing to the normal population. Although to our knowledge this is the first study investigating PCB association with PAD and AS, our results adds developing evidence to the environmental exposure of PCBs similar to the effects of BPA, another type of endocrine disrupting chemical in cardiovascular disease (Lang et al., 2008; Melzer et al., 2010). Furthermore because PAD is a subclinical

marker for atherosclerosis, our findings may elucidate that possible effects of PCBs on atherosclerosis may be an essential mechanism previously reporting an association between PCB exposure and the development of obesity and obesity-associated atherosclerosis (Arsenescu et al., 2008).

Polychlorinated Biphenyls are a group of man-made organic chemicals that consist of chlorine, hydrogen, and carbon atoms. Today PCBs can still be released in the environment from illegal dumping of PCB wastes, burning wastes in industrial and municipal incinerators, leaks from electrical transformers containing PCBs, and improper or poorly maintained hazardous sites containing PCBs (EPA). While there has not been any previously studies done in NHANES linking PCB exposure to PAD and AS recent studies have linked exposure to BPA to peripheral arterial disease (Shankar et al., 2012) and heart disease (Melzer et al., 2010).

Numerous lines of evidence suggest that a link between levels of PCB concentrations and PAD and/or AS may be biologically conceivable. Significant associations between PCBs, lipids, age, BMI, and CVD in a Native American population demonstrate a consistency with a model in which age is considered both a biological driving mechanism and exogenous explanatory variable (Goncharov et al., 2008). Dioxin-like PCB 126 effects CVD risk factors on female experimental rodents such as blood pressure, heart weight, and serum cholesterol (Lind et al., 2004). Furthermore higher levels of PCB concentration in HDL demonstrated links to CVD (Ljunggren et al., 2014). Previously we have reported planar and coplanar PCBs induce expression of various genes compared to

estradiol that have biological relevance to signaling in pulmonary vascular lesions (Feltz et al., 2010). Additionally, ID3 a molecule that has been demonstrated to be involved in vascular diseases has provided evidence linking its vital role in regulating both vascular endothelial cell survival and microvascular lesion development by endocrine disrupting chemicals such as PCB153 (Das & Feltz., 2014).

Our study includes a nationally representative sample of the United States population and provides a rigorous methodology of collecting, analyzing, and having multiple confounding data point's accessible to the public. (NCHS 2010a, 2010b). Further strengths include availability of biological measurements of endocrine disrupting chemicals and oversampling of minority populations making it possible to have a complete representation of the US population. However a limitation that we have on our study is since it is a cross-sectional study, it makes it difficult to validate the exposure ensued the overall outcome. Additionally observed associations could be potentially confounded by lack of data on geographical location of family history and/or geographic location of some variables.

Conclusion

In a nationally representative sample of the U.S. population higher levels of PCB exposure were associated with increased risk of PAD as well as atherosclerosis subsequently adjusting for covariate factors. While our results must be established in other studies, they provide preliminary evidence that

environmental exposure to PCBs may contribute to increased risk of PAD and/or AS.

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CHAPTER IV

MANUSCRIPT 3

Association of endocrine disrupting chemical exposure in Peripheral Arterial Disease (PAD) Among the U.S. Population: NHANES 1999 – 2004

Abstract

Background. Recent reports have continuously shown that the human population get exposed to a wide range of endocrine disrupting chemicals. In particular phthalates & bisphenol A (BPA) have been detected in more than 90% of urine and blood samples collected (Silva et al 2004; Woodruff et al 2011; Calafat et al 2008). Although previous reports from various data have demonstrated association between phthalates & BPA with various diseases the bridge between phthalates & BPA with peripheral arterial disease is limited.

Objectives. The purpose of this study is to examine association between exposure to endocrine disrupting chemicals (EDCs) & peripheral arterial disease (PAD) in a nationally represented sample of the U.S. population.

Methods. We utilized participants in the National Health and Nutritional Examination Survey (NHANES) in 1999 - 2004. We estimated associations between exposure to EDCs (phthalates in 1999 - 2004 & bisphenol A in 2003 – 2004) respectively and PAD using complex survey and logistic regression models adjusted for potential confounders.

Results. Individual analyses showed phthalate metabolites: MnBP and MBzP alongside BPA to be higher among participants with PAD and AS when compared to the rest of the study population. Adjusted models for PAD confounders showed association between phthalate metabolites $\geq$ LOD and participants with PAD and AS: MnBP (OR: 1.33; 95%: 1.00 – 2.34); MBzP (OR: 1.16; 95% CI: 1.08 – 2.01) and BPA (OR: 1.22; 95% CI: 1.02 – 5.07). Furthermore after adjusting the model for gender and PAD confounders; age, race, and PAD confounders; and gender, age, race, and PAD confounders we demonstrated association between phthalate metabolites & BPA with PAD participants and AS.

Conclusion. Our findings suggest a relationship between higher environmental exposure of certain phthalate metabolites & BPA and increased risk of peripheral arterial disease (PAD) and atherosclerosis (AS).

Key words: ankle – brachial index, bisphenol A, endocrine disrupting chemicals, NHANES, peripheral arterial disease, phthalates

Introduction

The US EPA (United States Environmental Protection Agency) outlines endocrine disrupting chemicals (EDCs) as “agents that interferes with the synthesis, transport, elimination, binding, or secretion of natural hormones in the body that are responsible for the maintenance of reproduction, homeostasis, behavior, and/or development.” (Kavlock et al., 1996). While numerous chemicals have EDC activity the most studied chemicals today include phthalates and bisphenol A (BPA). Phthalates consist of a wide variety of products that include medical tubing, cosmetics, plastics, and coatings. (Gore et al., 2015). Bisphenol A is used in products such as toys, food packaging, manufacturing, and other applications. Due to the fact that essentially everyone uses these day-to-day products, the overall population is continually exposed. (Meeker and Ferguson. 2011). While earlier research has concentrated on the role of phthalates and bisphenol A (BPA) in cancer, reproductive, and developmental health problems; existing research has established associations between diabetes, obesity, and cardiovascular disease (Anway et al. 2005; Lang et al. 2008; Melzer et al. 2010; Morgan et al., 2016). Although these establishments have been seen throughout the literature it is warranted to extend that investigation to peripheral arterial disease in order to further bridge the gap in knowledge

In this study, we examined the independent association between individual phthalates and bisphenol A (BPA) with diagnosis of peripheral arterial disease and participants with atherosclerosis with participants in the NHANES (National Health

and Nutrition Examination Survey) 3-year cycle for phthalates: 1999 – 2004 and 1-year cycle 2003-2004 for bisphenol A (BPA). The aim of this study is to assess: 1) the mean phthalate levels in PAD defined participants and defined with atherosclerosis compared to non-diagnosed participants; and 2) the mean bisphenol A (BPA) levels in PAD defined participants and atherosclerosis compared to non-diagnosed participants, and 3) the association between higher EDC exposure and increased risk of PAD/AS respectively.

Methods

Study design and population. NHANES survey encompasses stratified multistage probability sample representative of the civilian non-institutionalized U.S. population. The study is recognized based on data from NHANES 1999 - 2004. Selection was based on individuals within households, households, blocks, counties and included the oversampling of non-Hispanic blacks and Mexican Americans in order to deliver consistent evaluations of these groups. Participants provided written informed consent before their participation. Additionally approval was acquired from the Human Subjects Committee in the U.S. Department of Health and Human Services. ABI measurements which is a subclinical measure of atherosclerosis (Criqui et al. 1992; Newman et al. 1993), were acquired for the subsample of participants ≥ 40 years of age. The current study sample consisted of participants ≥ 40 years of age among whom endocrine disrupting chemicals was available. This resulted in participants (461), of whom had PAD and participants (390) whom were atherosclerosis defined in NHANES 1999 – 2004 and 178 PAD

defined participants; 94 atherosclerosis participants; and 1850 normal participants respectively. Subjects who were omitted because of missing covariates were in general comparable to the NHANES group in terms of sex, age, race/ethnicity, and education (data not shown). Secondary examination of the nationally collected data was done using variables of interest, which were only measured concurrently during the three NHANES cycles from 1999 - 2000, 2001 - 2002, and 2003 - 2004. NHANES uses a multifaceted sampling design (multistage, probability, cluster and stratified) for continuous collection and release of 2-year cross-sectional data sets. Approval for these suitable examinations and laboratory testing were given to NHANES by the CDC/ATSDR institutional review board of the CDC. The current study was considered exempt from review by the local institutional review board.

Peripheral Arterial Disease (PAD) diagnosis. The NHANES ABI is regularly calculated, verified, and released by the National Center for Health Statistics. Systolic pressure is measured on the right arm (brachial artery) and both ankles (posterior tibial arteries). If the participant has one of the following: 1) a rash; 2) open wound on the right arm; 3) dialysis shunt; 4) right-sided radical mastectomy; or any extra condition that would obstruct with correct measurement or cause discomfort to the participant, the left arm is utilized for the brachial pressure measurement. Systolic blood pressure is measured twice at each site for participants 40-59 years old and once at each site for participants ≥ 60 years old and older. If a health technician cannot obtain a reading at a site they may attempt an additional reading at the same site after a short-term resting period. The right ABPI was obtained by dividing the mean systolic blood pressure in the right ankle

by the mean blood pressure in the arm. The left ABPI was obtained by dividing the mean systolic blood pressure in the left ankle by the mean blood pressure in the arm. The mean blood pressure value for the arm and ankles are computed based on the first and second reading at each site. As the second reading for all participants ≥ 60 years old is missing the mean values are in fact the first recorded blood pressure reading at a site. This may furthermore be correct for participants 40-59 years old who have a missing value for the first or second blood pressure reading. Individuals are omitted from the exam if they weigh over 400 pounds (weight limitations of equipment) or have bilateral amputations. Additionally to some participants who were eligible for the exam (≥ 40 years old) might not have received the exam due to various reasons including: casts, ulcers, dressings, or other conditions of the participant interfered with testing; participant could not understand the test instructions or became ill & the test could not be performed; there was an equipment failure; participant refused; participant came late or left early from the MEC & the LED exam could not be performed; and some other reason. As a result, these eligible individuals will have missing data for the ABPI variables. Peripheral arterial disease diagnosis was defined as having an ankle brachial index (ABI) < 0.7 . Values $0.9 < \text{ABI} < 1.3$ were considered normal. Individuals whom demonstrated $0.7 < \text{ABI} < 0.9$ was defined as atherosclerosis.

Measurements of phthalates & bisphenol A. Both phthalates and bisphenol A were calculated in urine by the SPE (solid phase extraction) coupled to reverse phase HPLC-MS/MS (high performance liquid chromatography-tandem mass spectrometry). Laboratory methods and quality control were reliable across survey

years and in which documentation is available online (CDC, 2012). Overall we investigated 7 phthalate metabolites measured in NHANES years 1999 - 2004: mono-n-butyl phthalate (MnBP), mono-cyclohexyl phthalate (MCHP), mono-ethyl phthalate (MEP), mono-2-ethylhexyl phthalate (MEHP), mono-isobutyl phthalate (MiBP), mono-n-octyl phthalate (MOP), and mono-benzyl phthalate (MBzP). BPA (bisphenol A) was the only phenol included in this study. In order to adjust for urine dilution, we divided all phthalate metabolites and BPA by urinary creatinine concentration and used the resultant corrected creatinine concentrations (mg/g) for all of our analyses. Urine samples with creatinine that represented levels > 300 mg/dL or < 30 mg/dL were excluded in the analysis because the sample(s) were over-concentrated for an accurate examination (Sata et al., 1995). Furthermore we provided a limit of detection (LOD) variable for each phthalate metabolite and BPA in the datasets. The variable is defined with two values: “1” represents the result was below the limit of detection and “2” represents the result was equal or above the limit of detection.

Covariates. We considered a range of confounders based on well-established risk factors for peripheral arterial disease and review of the literature. The resultant confounders were self-reported in the questionnaire interviews or taken as laboratory measurements. The demographic variables of gender (men/women), race (Mexican American, Other Hispanic, Non-Hispanic White, Non-Hispanic Black, and other race) and age (≥ 40 -59, 60-74, and ≥ 75) were obtained during the NHANES home interview (CDC, 2012). Others obtained include: smoking, BMI

(18.5 to < 25, 25 to <30, & $\geq$ 30), diabetes (yes/no), estradiol levels, sex hormone binding globulin, testosterone, and education.

Statistical Analysis. Analysis were performed using SAS 9.4 (SAS Institute Inc.; Cary, NC, USA). Appropriate subsampling weights were calculated according to the National Center for Health Statistics guidelines to adjust for oversampling of minority groups (CDC, 2013b). All urinary chemicals and metabolites were log transformed and creatinine adjusted. PROC SURVEYMEANS was used to account for the complex sampling design of NHANES and furthermore obtain means (weighted), 95% confidence intervals, and standard errors of individual phthalate metabolites and BPA. Age was divided into three groups ($\geq$ 40-59, 60 – 74, and $\geq$ 75 years old). A two-sided student t-test was measured using PROC SURVEYREG to test if the mean EDC (phthalates/BPA) levels between participants who were defined with PAD, AS, and normal participants were significantly different. Geometric mean (GM) levels and geometric standard errors (GSE) were reported for peripheral arterial disease status, AS status, age at interview, and race/ethnicity for all participants with individual phthalate metabolites & BPA levels. Age was divided into three groups ($\geq$ 40-59 years, 60-74 years, and $\geq$ 75 years) that were selected based on the age distribution of PAD cases, cases of AS, and normal cases in the population. Race/ethnicity was divided into five groups (Mexican American, other Hispanic, Non-Hispanic White, Non-Hispanic Black, and other race).

Results

Phthalate and Bisphenol A Descriptive Statistics. The study population for phthalates includes 3,489 men and 3,125 women from NHANES cycles 1999 – 2004 with available data and who are either identified with peripheral arterial disease ($n = 461$), atherosclerosis defined ($n = 390$), or as a normal participant ($n = 5773$) as shown in Table 3.1. Bisphenol A population from NHANES 2003 – 2004 includes 868 men and 946 women from NHANES cycle 2003 – 2004 with available data (Table 3.2). Overall 178 participants are diagnosed with PAD; 94 whom are at identified with atherosclerosis; and 1850 whom are considered normal participants. The majority of the participants in the phthalate population was Non-Hispanic White: 268 (3.72%) for PAD participants; 236 (4.90%) for participants with AS; and 3,125 (69.64%) normal diagnosed participants. Table 3.3 displays Cases/Non-Cases, geometric means (GM), and geometric standard error (GSE). GM of particular phthalate metabolites were significantly higher in participants diagnosed with PAD and AS when compared to normal diagnosed participants. Those phthalate metabolites include: MnBP (27.12 & 24.99 vs. 24.16) and MBzP (13.63 & 12.21 vs. 11.69). Furthermore, BPA also showed significant higher GM in diagnosis of PAD and AS when compared to normal participants (4.65 & 7.67 vs. 2.73). Table 3.4 illustrates estimated odds ratios (ORs) and 95 % confidence intervals (CIs) for phthalate metabolites and bisphenol A adjusted for 1) PAD confounders (diabetes, smoking, and alcohol use); 2) gender and PAD confounders 3) age, race, BMI, & PAD confounders; and 4) gender, age, race, BMI and PAD confounders. In the PAD confounders adjusted model phthalate

metabolites: MnBP (OR: 1.33; 95% CI: 1.00 – 2.34) and MBzP (OR: 1.16; 95% CI: 1.08 – 2.01) showed significant association with PAD and AS when exposure is $\geq$ LOD. Furthermore bisphenol A also showed significant association with PAD and AS when exposure is $\geq$ LOD (OR: 1.22; 95% CI: 1.02 – 5.07). In the gender and PAD confounders adjusted model phthalate metabolites: MnBP (OR: 1.83; 95% CI: 1.00 – 2.34); MCHP (OR: 1.54; 95% CI: 1.00 – 2.10), MEP (OR: 1.01; 95% CI: 0.44 – 1.09) and MBzP (OR: 1.76; 95% CI: 1.58 – 2.91) showed significant association with PAD and AS when exposure is $\geq$ LOD. BPA showed significant association with PAD and AS when exposure is $\geq$ LOD (OR: 2.89 95% CI: 1.50 – 10.19). The age, race, BMI, and PAD confounders model demonstrated that phthalate metabolites: MnBP (OR: 1.33; 0.89 – 2.95); MCHP (OR: 1.39; 95% CI: 1.00 – 1.65); and MiBP (OR: 1.48; 95% CI: 1.12 – 1.87) showed significant association with PAD and AS when exposure is $\geq$ LOD. In the final model adjusted for gender, age, race, BMI, and the PAD confounders phthalate metabolites MCHP (OR: 1.34; 95% CI: 1.22 – 1.59) and MiBP (OR: 1.41; 95% CI: 0.99 – 1.83) showed significant association with PAD and AS when exposure is $\geq$ LOD. BPA also showed significant association (OR: 3.02; 95% CI: 0.14 – 12.15) with PAD and AS when exposure is $\geq$ LOD.

Table 3.1 Descriptive statistics for PAD & selected covariates among participants ≥ 40 years of age, NHANES 1999 – 2004.

Variable	ABI < 0.7* n (%)	0.7 < ABI < 0.9* n (%)	0.9 < ABI < 1.3* n (%)
Total Population n (%)	461 (6.96%)	390 (5.888%)	5773 (87.152%)
Gender			
Male	228 (1.947%)	275 (3.9918%)	2986 (43.8267%)
Female	233 (2.7315%)	115 (1.7774%)	2787 (45.7256%)
Race/Ethnicity			
Mexican American	72 (0.1567%)	103 (0.3131%)	1231 (4.1428%)
Other Hispanic	12 (0.1152%)	11 (0.2016%)	221 (4.0714%)
Non-Hispanic White	268 (3.7214%)	236 (4.894%)	3125 (69.6389%)
Non-Hispanic Black	100 (0.5863%)	31 (0.1975%)	1013 (7.8865%)
Other Race	9 (0.0989%)	9 (0.1622%)	183 (3.8127%)
Age at interview (years)			
≥ 40 -59	67 (1.1721%)	180 (3.7787%)	3078 (61.729%)
60-74	199 (2.0057%)	155 (1.5675%)	1924 (20.8257%)
≥ 75	195 (1.5007%)	55 (0.423%)	771 (6.9976%)
Ever Smoked			
Yes	72 (1.8416%)	31 (1.134%)	764 (30.244%)
Sometimes	10 (0.2441%)	10 (0.2571%)	117 (3.9327%)
No	150 (3.6785%)	140 (5.0162%)	1493 (53.6517%)
Alcohol Use			
Yes	0 (0.00%)	2 (0.0411%)	24 (0.5721%)
No	333 (4.5564%)	324 (6.2234%)	4415 (88.6069%)
BMI			
Normal Weight (18.5 to < 25)	105 (1.3733%)	86 (1.7261%)	1205 (26.2679%)
Overweight (25 to <30)	129 (1.6091%)	120 (2.3676%)	1755 (34.4198%)
Obese (≥ 30)	88 (1.3574%)	119 (2.1868%)	1450 (28.692%)
Diabetes			
Yes	81 (0.9209%)	46 (0.5002%)	520 (7.4332%)
No	251 (3.527%)	283 (5.6672%)	3903 (80.3218%)
Boarderline	9 (0.1072%)	3 (0.0905%)	84 (1.3882%)
Don't know	0 (0.00%)	0 (0.00%)	2 (0.0437%)
Estradiol (pg/mL)			
<5	1 (0.0545%)	1 (0.1399%)	4 (1.1599%)
May-95	21 (3.1751%)	31 (9.1967%)	323 (85.4413%)
95-185	1 (0.0867%)	0 (0.00%)	2 (0.6376%)
> 185	0 (0.00%)	0 (0.00%)	1 (0.1084%)
Sex Hormone Binding Globulin (nmol/L)			
0.350-90	20 (2.8754%)	31 (9.3131%)	306 (82.6883%)
90-180	2 (0.3553%)	1 (0.0267%)	21 (4.3095%)
>180	1 (0.0867%)	0 (0.00%)	2 (0.3449%)

Testosterone (ng/mL)			
0.020 - 9	22 (3.2402%)	32 (9.3673%)	318 (84.4869%)
12-Sep	0 (0.00%)	0 (0.00%)	8 (2.5649%)
> 12	1 (0.087%)	0 (0.00%)	2 (0.2538%)
Education			
< 9th Grade	120 (0.6988%)	74 (0.3937%)	976 (6.1832%)
9-11th Grade (Includes 12th grade with no diploma)	76 (0.7538%)	45 (0.5289%)	860 (10.5315%)
High School Grad/GED or Equivalent	117 (1.4821%)	65 (1.1016%)	1382 (23.692%)
Some College or AA degree	91 (1.0934%)	103 (1.7124%)	1403 (25.4172%)
College Graduate or above	54 (0.6319%)	102 (2.0203%)	1142 (23.6305%)
Refused	0 (0.00%)	0 (0.00%)	3 (0.0219%)
Don't Know	3 (0.0186%)	1 (0.0124%)	6 (0.0757%)
Income			
\$ 0 to \$ 4,999	14 (0.0897%)	12 (0.1544%)	161 (1.7935%)
\$ 5,000 to \$ 9,999	56 (0.4762%)	22 (0.1865%)	378 (4.1621%)
\$10,000 to \$14,999	77 (0.6941%)	33 (0.2147%)	580 (6.1557%)
\$15,000 to \$19,999	55 (0.4726%)	22 (0.1933%)	459 (5.1039%)
\$20,000 to \$24,999	40 (0.3538%)	29 (0.3374%)	473 (5.5343%)
\$25,000 to \$34,999	67 (0.7037%)	35 (0.5199%)	670 (9.4058%)
\$35,000 to \$44,999	30 (0.3397%)	37 (0.462%)	524 (8.463%)
\$45,000 to \$54,999	27 (0.3059%)	32 (0.4788%)	458 (8.3173%)
\$55,000 to \$64,999	15 (0.276%)	21 (0.379%)	322 (6.6019%)
\$65,000 to \$74,999	10 (0.1592%)	22 (0.3727%)	253 (5.1088 %)
\$75,000 and over	25 (0.3829%)	93 (2.0818%)	995 (22.9582%)
Over \$20,000	19 (0.3009%)	14 (0.2414%)	194 (3.2409%)
Under \$20,000	9 (0.0873%)	7 (0.0566%)	99 (1.1958%)
Refused	6 (0.0394%)	5 (0.0464%)	90 (1.0123%)
Don't know	6 (0.0238%)	5 (0.0711%)	51 (0.4455%)

Estimated percent distribution after applying NHANES sampling weights

*ABI < 0.7 (PAD). 0.7 < ABI < 0.9 (AS), & 0.9 < ABI < 1.3 (Normal)

Table 3.2 Descriptive statistics for PAD & selected covariates among participants ≥ 40 years of age with bisphenol A (BPA) measurements NHANES 2003 - 2004.

Variable	ABI < 0.7* n (%)	0.7 < ABI < 0.9* n (%)	0.9 < ABI < 1.3* n (%)
Total Population n (%)	178 (8.40%)	94 (4.40%)	1850 (87.20%)
Gender			
Male	81 (4.01%)	39 (2.26%)	748 (41.03%)
Female	76 (4.62%)	42 (2.00%)	828 (46.11%)
Race/Ethnicity			
Mexican American	49 (0.84%)	24 (0.45%)	467 (2.03%)
Other Hispanic	12 (0.77%)	0 (0.00%)	60 (3.08%)
Non-Hispanic White	62 (5.32%)	45 (4.01%)	695 (58.81%)
Non-Hispanic Black	37 (0.81%)	20 (0.36%)	471 (11.05%)
Other Race	10 (0.78%)	4 (0.18%)	77 (5.03%)
Age at Interview (years)			
$\geq 40-59$	23 (5.65%)	8 (1.91%)	224 (53.36%)
60-74	23 (4.50%)	12 (1.68%)	197 (22.11%)
≥ 75	10 (0.79%)	7 (0.66%)	129 (11.34%)
Smoking			
Yes	35 (3.80%)	20 (2.09%)	300 (32.52%)
Sometimes	2 (0.27%)	1 (0.027%)	65 (7.16%)
No	45 (4.95%)	13 (1.47%)	435 (47.72%)
BMI			
Normal Weight (18.5 to < 25)	47 (4.12%)	25 (1.89%)	451 (33.44%)
Overweight (25 to <30)	27 (2.10%)	12 (0.80%)	310 (23.68%)
Obese (≥ 30)	35 (3.09%)	55 (4.77%)	1133 (85.97%)
Diabetes			
Yes	6 (0.40%)	3 (0.26%)	91 (4.51%)
No	150 (8.15%)	78 (3.97%)	1470 (81.80%)
Borderline	1 (0.08%)	0 (0.00%)	15 (0.83%)
Estradiol (pg/mL)			
<5	0 (0.00%)	1 (0.04%)	10 (4.40%)
5 to 95	30 (8.94%)	23 (8.47%)	253 (72.56%)
95-185	2 (0.57%)	0 (0.00%)	14 (5.03%)
> 185	0 (0.00%)	0 (0.00%)	0 (0.00%)

Sex Hormone Binding Globulin (nmol/L)			
0.350-90	30 (9.37%)	21 (7.85%)	267 (78.87%)
90-180	2 (0.11%)	3 (0.62%)	11 (3.18%)
> 180	0 (0.00%)	0 (0.00%)	0 (0.00%)
Testosterone (ng/mL)			
0.020 - 9	26 (8.75%)	23 (8.49%)	247 (77.44%)
9 to 12	3 (0.67%)	1 (0.53%)	15 (2.72%)
> 12	1 (0.29%)	0 (0.00%)	2 (1.11%)
Education			
< 9th Grade	11 (0.49%)	5 (0.14%)	129 (0.62%)
9-11th Grade (Includes 12th grade with no diploma)	11 (0.47%)	10 (0.46%)	133 (10.76%)
High School Grad/GED or Equivalent	25 (3.14%)	6 (0.74%)	200 (21.43%)
Some College or above	11 (1.92%)	9 (1.55%)	159 (20.09%)
Refused	0 (0.00%)	0 (0.00%)	1 (0.05%)
Don't Know	0 (0.00%)	0 (0.00%)	1 (0.03%)
Income			
\$ 0 to \$ 4,999	2 (0.02%)	6 (0.08%)	44 (1.62%)
\$ 5,000 to \$9,999	9 (0.29%)	3 (0.03%)	102 (3.53%)
\$10,000 to \$14,999	12 (0.44%)	12 (0.37%)	142 (4.77%)
\$15,000 to \$19,999	20 (0.68%)	9 (0.37%)	147 (5.23%)
\$20,000 to \$24,999	17 (0.71%)	7 (0.26%)	159 (6.56%)
\$25,000 to \$34,999	28 (1.24%)	9 (0.57%)	249 (11.70%)
\$35,000 to \$44,999	16 (0.73%)	6 (0.36%)	182 (10.68%)
\$45,000 to \$54,000	14 (0.90%)	4 (0.21%)	144 (8.16%)
\$55,000 to \$64,999	5 (0.30%)	6 (0.49%)	88 (5.79%)
\$65,000 to \$74,999	11 (0.99%)	2 (0.12%)	71 (5.61%)
\$75,000 and Over	27 (2.41%)	22 (2.06%)	310 (21.76%)
Over \$20,000	0 (0.00%)	0 (0.00%)	6 (0.60%)
Under \$20,000	1 (0.03%)	1 (0.02%)	10 (0.23%)
Don't Know	0 (0.00%)	0 (0.00%)	7 (0.08%)

Estimated percent distribution after applying NHANES sampling weights

*ABI < 0.7 (PAD), 0.7 < ABI < 0.9 (AS), & 0.9 < ABI < 1.3 (Normal)

Table 3.3. Geometric mean of urinary phthalate metabolites and BPA levels above the LOD by PAD status among participants ≥ 40 years of age, NHANES 1999 - 2004

	Cases/Non-Cases			Geometric Mean ³ (ng/g) (GSE)		
	ABI < 0.7*	0.7 < ABI < 0.9*	0.9 < ABI < 1.3*	ABI < 0.7	0.7 < ABI < 0.9	0.9 < ABI < 1.3
Phthalate Metabolites¹						
MnBP	384	320	5198	27.12 (22.21, 33.10)	24.99 (20.58, 31.12)	24.16 (23.24, 25.12)
MCHP	456	250	5196	0.55 (0.50, 0.60)	0.62 (0.55, 0.70)	0.58 (0.56, 0.60)
MEP	175	223	5504	127.33 (102.40, 158.34)	120.80 (98.68, 147.86)	136.31 (129.59, 143.38)
MEHP	210	520	5172	3.58 (2.87, 4.45)	2.97 (2.48, 3.55)	3.44 (3.28, 3.62)
MIBP	180	869	4853	1.05 (0.99, 1.11)	1.03 (0.97, 1.09)	1.04 (1.01, 1.07)
MOP	384	320	5198	1.22 (1.18, 1.27)	1.21 (1.18, 1.25)	1.23 (1.21, 1.24)
MBzP	400	380	5122	13.63 (11.26, 16.50)	12.21 (10.13, 14.71)	11.69 (11.13, 12.28)
Bisphenol A²						
BPA	142	77	1427	4.65 (3.27 - 6.04)	7.67 (5.75 - 9.59)	2.73 (2.64 - 5.82)

¹ Log transformed and creatinine corrected phthalate metabolites (ng/mg); NHANES 1999 - 2004

² Log transformed and creatinine corrected BPA (ng/mg); NHANES 2003 - 2004

³ Geometric means calculated after applying NHANES sampling weights

*ABI < 0.7 (PAD), 0.7 < ABI < 0.9 (AS), & 0.9 < ABI < 1.3 (Normal)

Table 3.4 Estimated ORs (95% CIs) of PAD status by concentration of creatinine adjusted urinary phthalate metabolites & BPA among participants ≥ 40 years of age, NHANES 1999 – 2004 & 2003 – 2004.

	ABI < 0.7*	0.7 < ABI < 0.9*	0.9 < ABI < 1.3*	Adjusted OR (95% CI) ²	Adjusted OR (95% CI) ⁴	Adjusted OR (95% CI) ⁵	Adjusted OR (95% CI) ⁶
Phthalate Metabolites¹							
MnBP							
< LOD	294	239	4032	1	1	1	1
$\geq$ LOD	90	81	1166	1.33 (1.00 - 2.34)	1.83 (1.00 - 2.34)	1.33 (0.89 - 2.95)	0.96 (0.85 - 1.99)
MCHP							
< LOD	400	341	5400	1	1	1	1
$\geq$ LOD	10	3	86	0.54 (0.25 - 2.10)	1.54 (1.00 - 2.10)	1.39 (1.00 - 1.65)	1.34 (1.22 - 1.59)
MEP							
< LOD	49	44	603	1	1	1	1
$\geq$ LOD	360	297	4876	0.99 (0.44 - 1.05)	1.01 (0.44 - 1.09)	0.85 (0.55 - 1.09)	0.98 (0.51 - 1.28)
MEHP							
< LOD	407	339	5429	1	1	1	1
$\geq$ LOD	3	1	48	0.72 (0.40 - 0.93)	0.92 (0.40 - 0.93)	0.94 (0.92 - 0.95)	0.92 (0.93 - 0.96)
MIBP							
< LOD	410	341	5457	1	1	1	1
$\geq$ LOD	7	13	21	0.79 (0.18 - 0.80)	0.59 (0.18 - 0.80)	1.48 (1.12 - 1.87)	1.41 (0.99 - 1.83)
MOP							
< LOD	410	341	5489	1	1	1	1
$\geq$ LOD	10	9	34	0.95 (0.95 - 1.60)	0.97 (0.95 - 1.60)	0.92 (0.70 - 1.49)	0.98 (0.47 - 1.57)
MBzP							
< LOD	131	103	1916	1	1	1	1
$\geq$ LOD	277	236	3551	1.16 (1.08 - 2.01)	1.76 (1.58 - 2.91)	0.91 (0.84 - 0.97)	1.73 (0.92 - 3.18)
Bisphenol A²							
BPA							
< LOD	8	8	118	1	1	1	1
$\geq$ LOD	141	71	1315	1.22 (1.02 - 5.07)	2.89 (1.50 - 10.19)	2.17 (1.65 - 7.17)	3.02 (0.14 - 12.15)

¹ Log transformed and creatinine corrected phthalate metabolites (ng/mg); NHANES 1999 - 2004

² Log transformed and creatinine corrected BPA (ng/mg); NHANES 2003 - 2004

³ Adjusted for PAD confounders (Diabetes, Smoking, and Alcohol Use)

⁴ Adjusted for Gender & PAD confounders

⁵ Adjusted for Age, Race, & PAD confounders

⁶ Adjusted for Gender, Age, Race, & PAD confounders

*ABI < 0.7 (PAD), 0.7 < ABI < 0.9 (AS), & 0.9 < ABI < 1.3 (Normal)

Discussion. In this cross-sectional study of participants aged ≥ 40 years old, we separately investigated 7 individual phthalate metabolites and BPA in association with peripheral arterial disease (PAD) and atherosclerosis (AS). In participants with detectable levels of phthalates and BPA, higher exposures were found in participants diagnosed with PAD and AS when compared to normal participants and phthalate metabolites: MnBP and MBzP. Furthermore when ORs and 95% CI models were adjusted for various models higher exposure phthalate metabolites & BPA ($\geq$ LOD) showed significant association with participants diagnosed with PAD and AS [1) PAD confounders model: MnBP and MBzP; 2) gender and PAD confounders model: MnBP, MCHP, MEP, and MBzP 3) age, race, BMI, and PAD

confounders model: MnBP, MCHP, and MiBP and 4) gender, age, race, BMI, & PAD confounders model: MCHP, MiBP, and MBzP].

Previous epidemiological studies showed that phthalate metabolite MMP was associated with atherosclerotic plaques in an inverted U-shaped manner (low levels of MMP have an effect) independently of cardiovascular risk factors. Some phthalates and BPA were also correlated to the echogenicity of the plaques, suggesting a role for plaque-associated chemicals in atherosclerosis (Lind and Lind. 2011). Furthermore irregular associations between phthalates and hyperlipidemia were shown with self-reported diabetes and CVD in Shanghai adults (Dong et al. 2017). BPA levels were demonstrated to be significantly associated with PAD before and after adjusting for confounders (Shankar et al. 2012). There is additional evidence that individuals with higher urinary BPA levels are more inclined to suffer from general or abdominal obesity, hypertension, and diabetes when compared with individuals having lower urinary concentrations (Rancière et al. 2015). Common carotid artery intima-media thickness (CIMT) measured by ultrasonography is a recognized indicator of subclinical atherosclerosis and independent predictor of myocardial infarction, coronary events, and stroke (Bots et al. 1997; Hodis et al. 1998; Su et al. 2006; Chien et al. 2008; Su et al. 2012). It was reported that in a cross-sectional investigation of a representative population sample of subjects aged 60 - 80 years old showed a significant association between small changes in IMT and both symptomatic and asymptomatic peripheral arterial disease (Allan et al. 1997). Lin et al further determined the relationship between levels of BPA and carotid-media thickness

(CIMT) and discovered higher serum concentrations of BPA were associated with increased CIMT in a cross-sectional study of adolescents and young adults (Lin et al. 2015). Although previous studies and our current study show significant associations between EDCs (phthalates and BPA) with peripheral arterial disease further research is warranted to elucidate mechanisms of these associations.

The main strengths of our study include oversampling of minority populations that make it highly representative and generalizable to the United States population and availability of biological measurements of environmental pollutants. Additionally this study is nationally representative and uses stringent methods to collect available data on confounders. One limitation of this study is since it is a cross – sectional design with self - reported data, it does not permit for causal inferences to be concluded. Self – reported data increases the likelihood of misclassification bias of cases and controls contributing to the possibility of incorrectly or undiagnosed reported PAD and/or AS cases. Another limitation is that BPA measurements are more likely to result in misclassification due to variability.

Conclusion

Based on our analysis of phthalate metabolites from 1999 – 2004 and BPA from 2003 – 2004 higher exposure of particular phthalate metabolites and BPA did demonstrate association with participants who are diagnosed with PAD alongside AS. Given earlier and existing research demonstrates exposure to other EDCs such as polychlorinated biphenyls are associated with cardiovascular disease, it is

biologically plausible that exposure to phthalates and BPA may contribute to higher risk of the disease. Our research findings coupled with previous epidemiological evidence concerning EDCs and peripheral arterial disease warrants the necessity for prospective future studies.

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CHAPTER V

MANUSCRIPT 4

Genome-wide gene expression profiling of Inhibitor of DNA
binding/Differentiation-3 (ID3) candidate targets in vascular-intimal lesions

Abstract

Obliterative and proliferative vascular intimal lesions are categorized by perturbations of cellular constituents & their normal patterning of vessels. The pathophysiologic pathways that transition to these end-stages are infrequently distinct. Because they may suggest the normal assembly of vessels during development, developmental biology, which combines embryologic analyses of cellular origins, lineage, and cell–cell interactions with genetic studies, holds the prospect of revealing the role of individual genes in the pathogenesis of vascular disease. The Inhibitor of DNA binding/Differentiation-3 (ID3), a member of the ID family of transcriptional regulators, has been shown to play a role in lipoxigenase (12/15-LO) which is known to produce pro-inflammatory alterations in blood vessels that lead to the development of atherosclerosis. Furthermore studies have shown overexpression of ID3 increasing OCT4 and SOX2 expression in microvascular endothelial cells. Therefore we determined the functional role of ID3 and ID3 candidate target genes in obliterative and proliferative vascular-intimal lesions. We performed a meta-analysis on published whole genome microarray datasets comparing transcriptional patterns of blood cells & tissue samples to controls. Bioinformatics network analysis identified statistically the most overrepresented processes and signaling pathways. Deciphering the pattern & role of genes involved with ID3 & ID3 targets in obliterative and proliferative vascular diseases may provide a new model by which ID3 may contribute to the growth regulation of these vascular intimal-lesions. **Keywords:** bioinformatics, ID3, vascular diseases, vascular-intimal lesions

Introduction

Although poorly understood, obliterative & proliferative vascular-intimal lesions are a significant health problem that may lead to blocked blood vessels and can cause threatening end organ damage such as ischemic stroke, blindness, right-sided heart failure, or myocardial infarction. Obliterative & proliferative vascular-intimal lesions manifest in numerous vascular diseases such as arteriosclerosis, idiopathic pulmonary arterial hypertension, peripheral arterial disease, and lymphangioleiomyomatosis. The endothelium plays an essential role in classifications of obliterative and proliferative diseases. A vital foundation of vascular biology is that the lining of the endothelial is dynamically a changeable interface. This is locally responsive to multiple types of stimuli that can originate from the emigrating & circulating cells and/or neighboring tissues and thus can actively participate in the physiologic variation or vascular dysfunction of a certain section of the vasculature. The endothelium appears to be best suited to function in this capacity, given its distinctive anatomic location between tissue & blood and ability to generate various biological effectors such as cytokines, growth stimulators & inhibitors, vasoactive peptides, pro- & anti-coagulants, and nitric oxide. Disruption in the production or interactions of these different mediators appear to effect various vital functions of the endothelium, specifically its role as a non-thrombogenic vessel for antithrombotic, growth inhibitory, and anti-inflammatory activities; and blood that aids to preserve the integrity of the vascular wall, when facing injury-related stimuli and various disease risk factors (Fishman et al. 1998).

Conceivably the most well-known studied model of activation of vascular endothelium are the effects of pro-inflammatory cytokines such as tumor necrosis factor (TNF) and interleukin-1 (IL-1). These have provided a widely used experimental model, which have been studied at the tissue level (various diseased human tissues & animal models) and cellular level (receptors, genetic regulatory events, & signaling pathways) (Fishman et al.1998; Cuthins et al. 2012; Sakurai et al. 2004). Additionally, the effects of the biochemical dynamisms produced by pulsatile blood flow including pressures, wall shear stress, & cyclic strains on endothelial structure and function as been seen as another model of vascular cell activation. The capability of laminar shear stresses to up-regulate atheroprotective endothelial genes and significantly influence the functional phenotype of endothelial cells indicates that this model of activation may be playing a significant pathophysiologic role. Although the evidence implicates tissue and cellular levels as an influence on obliterative & proliferative vascular intimal-lesions, the pathogenesis is far from clarified to date.

At the molecular level, Inhibitor of DNA binding/Differentiation-3 is a member of the ID family (ID1-ID4) of helix-loop-helix proteins encoded by an immediate-early gene responsive to mitogenic signals and oxidative stress. Besides the regulation of cell differentiation, ID proteins are also positive regulators of cell growth and play a major role in neovascularization. An increasing body of evidence suggests that ID3 may be involved in disruptions characterized by abdominal obesity, insulin resistance, hyperglycemia, dyslipidemia, and hypertension. Interactions across multiple organ systems that contribute to

disruption presents a challenge to ongoing research attempting to elucidate biological mechanisms of chronic disease such as vascular. For example, insulin resistance and systemic low-grade inflammation result from complex interactions between the vasculature, metabolic tissue, and immune cells. In the vasculature, ID3 is essential to embryonic vasculogenesis and endothelial cell activation (Sakurai et al., 2004; Felty & Portner, 2008; Elen et al., 2015). ID3 involvement in vascular disease has been studied together with the lipoxygenase (12/15-LO), which is a vital mediator of VSMC growth and its growth-promoting effects were shown to be mediated by ID3 transcription (Taylor et al., 2015). Increased expression of 12/15-LO in the vessel wall enhanced ID3-dependent cell proliferation, fibronectin deposition, and neo-intimal formation (Taylor et al., 2015). Population established studies have found an SNP (single nucleotide polymorphisms) rs11574 in the coding region of the human ID3 gene associated with subclinical atherosclerosis in the Diabetes Heart study (Doran et al., 2010). Furthermore two cohorts determined the link between the SNP directly with coronary artery pathology. ID3 has also been shown as a key mechanism of high fat diet stimulated through visceral adipose VEGFA expression, depot expansion and micro-vascular blood volume (Cuthins et al., 2012). ID3 is important to obesity because it has been suggested to stimulate angiogenesis, a major factor of HFD (high-fat diet)-induced visceral adiposity (Cuthins et al., 2012).

Our research has recently shown that ectopic overexpression of ID3 increased OCT4 and SOX2 expression in endothelial cells as well as resulted in a population of cells that were positive for the molecular stemness signature CD133+

VEGFR3+ CD34+ (Das et al., 2015). These endothelial stem cells were morphologically distinguished into smooth muscle cells and neuron cells. Based on these findings, ID3 maintains cells in a non-committed state by inhibiting the repression of pluripotency factors by TCF3. Furthermore we also recently reported that ID3 overexpression increased endothelial cell neovascularization, resistance to apoptosis, cell migration, and increased size of endothelial spheroids. In vivo, we reported that ID3 expression is higher in pulmonary vascular lesions. Our studies have also shown ID3 protein and oxidative damage highly expressed in benign and malignant cardiovascular tissues in patients (Das & Felty, 2014). In this study, we have elucidated the role of ID3 and ID3 candidate target genes in vascular-intimal lesions with the specific focus on how ID3 may play a critical role in regulating various genes and signaling pathways that are involved in cell adhesion, extracellular matrix proteolysis, & cell growth which predisposes the vessel to vascular dysfunction. A better understanding of how vascular-intimal lesions depend on ID3 regulation may open up novel approaches for both prevention & treatment of diseases these intimal-lesions manifest from.

Materials & Methods

Identification of microarray datasets & analysis. Our study was based on multiple studies carried out and deposited in NCBI Gene Express Omnibus (GEO), a public functional genomics data repository supporting MIAME-compliant data submissions in which both sequence-based and array data are accepted. Datasets were compiled on vascular diseases and respectively divided into blood & tissue studies. The following tables represents information on data collected & accessible

demographics of individual datasets. Note not all datasets are represented in the tables. Figure 3.1 represents a flow chart of our current study.

Vascular Diseases (Blood Studies)	GEO Accession Number	Platform	Affymetrix Type	Number of genes in GeneChip	Cases	Controls	Citation (Authors & Year)
Arteriovenous Malformations	GSE40960	GPL570	[HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array	21049	3	3	Young et al., 2012
Arteriovenous Malformations	GSE18014	GPL6104	Illumina humanRef-8v2.0 expression beadchip	18196	2	2	Wustehube et al., 2010
Atherosclerosis	GSE13139	GPL570	[HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array	21025	30	24	Martaliano et al., 2009
Atherosclerosis	GSE6054	GPL570	[HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array	21049	10	13	Mosig et al., 2008
Atherosclerosis	GSE36487	GPL96	[HG-U133A] Affymetrix Human Genome U133A Array	13211	2	1	Minta et al., 2012
Atherosclerosis	GSE20129	GPL6104/GPL10558	Illumina humanRef-8v2.0 expression beadchip	18196	39	6	Huang et al., 2011
Atherosclerosis	GSE12261	GPL570	[HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array	21049	6	6	Zaug et al., 2015
Atherosclerosis	GSE20484	GPL570	[HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array	21049	2	2	Geissner et al., 2010
Atherosclerosis	GSE9820	GPL6255	Illumina humanRef-8v2.0 expression beadchip	18189	18	13	Schirmer et al., 2009
Idiopathic pulmonary arterial hypertension	GSE33463	GPL6947	Illumina HumanHT-12 V3.0 expression beadchip	37805	30	41	Cheadle et al., 2012
Idiopathic pulmonary arterial hypertension	GSE22356	GPL570	[HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array	21025	8	10	Risbano et al., 2012
Infantile hemangioma	GSE43742	GPL10558	Illumina HumanHT-12 V4.0 expression beadchip	31333	2	2	Siles et al., 2013
Infantile hemangioma	GSE34989	GPL13607	Agilent-028004 SurePrint G3 Human GE 8x60K Microarray	34809	29	5	Harbi et al., 2016
Lymphangioleiomyomatosis	GSE5579	GPL570	[HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array	21025	3	1	Ingoyen et al., 2007
Peripheral Arterial Disease	GSE27034	GPL570	[HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array	21025	19	18	Masud et al., 2012
Vascular Diseases (Tissue Studies)	GEO Accession Number	Platform	Affymetrix Type	Number of genes in GeneChip	Cases	Controls	Citation (Authors & Year)
Infantile hemangioma	GSE78811	GPL2015	Agilent-067406 Human CBC lncRNA + mRNA microarray V4.0	22595	3	3	Liu et al., 2016
Lymphangioleiomyomatosis	GSE12027	GPL96	[HG-U133A] Affymetrix Human Genome U133A Array	13211	14	11	Zhang et al., 2009
Arteriovenous Malformations	GSE3515	GPL18096	Agilent-028004 SurePrint G3 Human GE 8x60K Microarray	22229	40	40	Torrington et al., 2014
Idiopathic pulmonary arterial hypertension	GSE48149	GPL16221	Illumina HumanRef-8v3.0 expression beadchip	18630	36	9	Hsu et al., 2011
Proliferative Diabetic Retinopathy	GSE50436	GPL6884	Illumina HumanWG-6v3.0 expression beadchip	25428	6	3	Ishikawa et al., 2015

Table 4.1 NCBI GEO datasets represented in study. Table is divided into two cohorts: blood and tissue samples.

Blood studies

Characteristics	Samples	
	Cases (n = 19)	Controls (n = 18)
Age, years	69.57 ± 9.18	66.88 ± 9.44
Men, %	13 (68%)	13 (72%)
Ever smoker, %	17 (89%)	12 (66%)
BMI, kg · m ⁻²	28.53 ± 5.25	30.6 ± 5.99
Systolic BP, mm Hg	139.2 ± 23.27	132.4 ± 20.1
Diastolic BP, mm Hg	74.2 ± 11.66	80.1 ± 12.3
HDL cholesterol, mg/dl	44.12 ± 8.59	49.7 ± 15
Hypertension, %	17 (89.4%)	13 (72%)
Diabetes, %	5 (26%)	4 (22%)
Lipid-lowering medication, %	11 (57%)	12(66%)
Ankle-brachial index	0.48 ± 0.20	1.12 ± 0.07
WBC count, µL	8.03 ± 2.34	7.26 ± 1.10
Values are expressed as either 'mean ± standard deviation' or as 'n (%)'		

Table 4.2.1 Characteristics of peripheral arterial disease dataset.

PatientID	Lesions	AgeAtTimeOfResection	Location&Size	Comments
1	hemEC-1	8months	1.8x0.8cm, Right eyelid	Normal karyotype*
4	hemEC-4	3months	3x3, Right flank	
5	hemEC-5	4months	5cm, Lower abdomen	Multiple hemangiomas
8	heomaEC-8B	2years	8x ankle	Normal karyotype*, Multiple
	heomaEC-8C		8x knee	hemangoendothelioma lesions of variable size, new lesions still appearing at 2 years
	heomaEC-8F		8x right foot	
	heomaEC-8G		6x inner thigh	
10	hemEC-10	2years	4cm, forehead	
12	hemEC-12	4months	1.5cm, left cheek	
13	hemEC-13	9months	3cm, left upper eyelid	Other hemangiomas on neck and labia
17	hemEC-17A	10months	A. 3cm, scalp	
	hemEC-17B		B. 3.5x3.5cm, forehead	
20	8months	8months	2.5cm, neck	Multiple hemangiomas
21	9months	9months	6.5x5.5cm, scalp	Other hemangiomas on right ear and forehead

*Endothelial cells from these patients showed a normal (46,XX) karyotype

Table 4.2.2 Characteristics of infantile hemangioma dataset.

	Homozygous FH	Heterozygous FH	Controls
Gender	Female (n=6)	Female (n=5)	Female (n=16)
	Male (n=20)	Male (n=3)	Male (n=10)
Age (years)	30.2 ± 11.1	28.8 ± 7.5	33.8 ± 11.2
LDL (mg/dl)	413.82 ± 92.33 (***)	220.64 ± 27.81 (***)	107.03 ± 17.78
HDL (mg/dl)	60.33 ± 8.09	54.17 ± 3.71	61.17 ± 6.60
Triglycerides (mg/dl)	141.31 ± 39.89	233.61 ± 132.80	107.63 ± 33.04
Lp(a) (mg/dl)	676.67 ± 347.06	442.73 ± 420.11	187.53 ± 153.59
Leukocytes (Gpt/l)	7.64 ± 0.95	5.45 ± 0.57	6.38 ± 0.8
Thrombocytes (Gpt/l)	253.12 ± 22.2	277.39 ± 37.51	258.12 ± 21.97

Values are means ± SD. FH, familial hypercholesterolemia; Gpt, Giga particles; Tpt, Terra particles.

Table 4.2.3 Characteristics of atherosclerosis dataset.

Variable	African American	White	African American	White
CAC (coronary artery calcification)	CAC < 10	CAC < 10	CAC >100	CAC > 100
n (number of patients)	17	41	13	26
Gender (male or female)	female	female	female	female
Age (years)	64.1 ± 7.7	62.9 ± 6.8	68.3 ± 8.5	69.0 ± 6.5
Body mass index (kg/m2*)	32.0 ± 6.4	25.5 ± 4.2	30.9 ± 7.1	27.4 ± 4.6
Systolic BP (mmHg)	119.1 ± 17.3	114.4 ± 18.7	130.6 ± 18.2	130.0 ± 22.6
Diastolic BP (mmHg)	68.6 ± 7.8	65.9 ± 11.1	71.4 ± 9.5	67.1 ± 8.7
Total cholesterol (mg/dl)	191.8 ± 32.4	197.5 ± 31.0	199.7 29.7	204.0 37.0
LDL cholesterol (mg/dl)	113.5 ± 27.7	110.2 ± 27.5	122.6 30.4	119.0 39.1
HDL cholesterol (mg/dl)	61.6 13.1	65.0 15.6	61.3 13.5	59.1 12.6
Triglycerides (mg/dl*§)	83.7 32.8	111.3 67.5	78.6 29.8	129.2 72.3
Fasting glucose (mg/dl)	91.5 8.0	89.7 8.5	90.6 7.5	91.0 8.7
HOMA insulin resistance	0.92 0.45	0.74 0.39	1.04 0.55	1.06 0.70
Insulin (uIU/ml)	4.4 2.0	3.6 1.8	4.8 2.4	4.9 3.1
Current smoker (%)	23.5	37.5	53.8	48
Lipid-lowering medication use (%)	5.8	15.3	38.4	52
Antihypertension medication use (%)	47	25.6	53.8	48

*P<0.05 comparing African American with white among women with coronary artery calcification (CAC) < 10
§P <0.05 comparing African American with white among women with CAC > 100

Table 4.2.4 Characteristics of atherosclerosis dataset.

Baseline characteristics	Atherosclerotic patients (n=18)	Control patients (n=13)
Age (years)	55.8 ± 7.9	52.2 ± 7.2
Male sex-no (%)	8 (44.4)	5 (38.5)
Body mass index (BMI)	25.2 ± 3.5	27.4 ± 3.5
Body surface area (BSA)	1.90 ± 0.21	1.97 ± 0.25
Family history of CAD – no. (%)	14 (77.8)	6 (46.2)
Current smoker – no. (%)	4 (22.2)	4 (30.8)
Ex smoker – no. (%)	7 (38.9)	4 (30.8)
Beta-blockers – no. (%)	15 (83.3)	10 (76.9)
Statins – no. (%)	18 (100)	13 (100)
Aspirin – no. (%)	18 (100)	13 (100)
Calcium antagonists – no. (%)	6 (33.3)	2 (16.4)
Nitrates – no. (%)	8 (44.4)	4 (30.8)
ACE-inhibitors/ARBs – no. (%)	8 (44.4)	7 (53.8)
Diuretics – no. (%)	4 (22.2)	4 (30.8)
C-reactive protein. – mg/dl	1.0 [1.0; 1.4]	1.1 [1.0; 1.7]
Troponin T – ng/ml	<0.03	<0.03
Glucose – mmol/l	5.5 ± 1.7	5.7 ± 0.6
Triglycerides – mmol/l	1.17 ± 0.90	0.90 ± 0.63
Lipoprotein (a) – mg/dl	343 [63, 569]	90 [49; 163]
LDL-cholesterol – mmol/l	2.20 ± 0.79	1.87 ± 0.53
HDL-cholesterol – mmol/l	1.39 ± 0.46	0.90 ± 0.63
1) Patients with triple-vessel coronary artery disease and control patients did not show differences in clinical characteristics except for lipoprotein		
2) CAD = coronary artery disease		
ARB = Angiotensin receptor blockers		
ACE = angiotensin converting enzyme		

* Data expressed as median [1. quartile, 3. quartile]

Table 4.2.5 Characteristics of atherosclerosis dataset.

Tissue Studies

Patient ID	Age	Gender	Position	Previous treatment	Pathology
1	2 months 15 days	Female	Left axilla	NO	IH
2	2 months 13 days	Female	Right posterior neck	Oral propranolol	IH
3	1 year	Female	Abdomen	Sclerotherapy	IH
4	8 months	Female	Right thoracic wall	Sclerotherapy	IH
5	3 months	Male	Thoracic wall	NO	IH

Table 4.2.6 Characteristics of infantile hemangioma dataset.

Family No.	Age	Sex	Phenotype
9	49	M	E, T, P, G, F
9	53	F	E, T, F
13	54	F	E, T, P, G, F
13	30	F	E, T, P, F
15	47	F	E, T, G, F
15	38	M	E, T, P, F
15	40	F	E, T, F
24	40	F	E, T, F
24	46	F	E, T, P, G, F
37	51	M	E, T, P, F
38	37	F	E, T, F
49	47	F	E, T, F
61	49	F	E, T, F
65	35	F	T, P, F
87	38	M	T, P, F
92	44	M	E, T, P
93	37	M	E, T, F
94	30	M	E, T, F
96	46	M	E, T, P, F
8	55	M	E, T, F
8	28	M	E, T, F
18	43	M	E, T, F
18	44	F	E, T, P, F
18	41	M	E, T, F
20	47	M	E, T
42	32	F	E, T, F
43	38	F	E, T, F
43	36	F	T, F
46	39	M	E, T, F
47	40	F	E, T, F
56	44	F	E, T, F
57	34	M	E, T, F
57	32	F	T, F
67	24	F	E, T, F
67	31	M	E, T, F
69	39	M	E, T, F
71	44	F	E, T, F
82	48	M	E, T, F
88	56	F	E, T, G, F
88	50	M	E, T, F

Abbreviations: AVM, arteriovenous malformation; E, epistaxis; T, Telangiectasia; P, pulmonary AVM; C, cerebral AVM; G, gastrointestinal telangiectasia/gastrointestinal bleeding; H, Hepatic AVM; F, family history. M, male; F, female.

Table 4.2.7 Characteristics of arteriovenous malformation dataset.

Patient ID	Gender (Age)	Smoking (pack years)	Skin disease (type)	Autoantibody	FVC (%)	FEV-1 (%)	DLCO (%)	FVC% / DLCO %	Mean PAP (mmHg)
1	M 56.8	0	limited	Th/To	40	48	34	1.18	18
3	F 57.8	10	limited	centromere	88	85	38	2.32	51
4	F 51.3	10	diffuse	n/a	34	43	22	1.55	30
5	F 39.3	0	diffuse	Scl-70	24	31	31	1.55	22
6	M 59.0	40	n/a	centromere	80	80	24	3.33	47
7	M 46.3	0	limited	centromere	76	80	40	1.9	48
8	F 26.5	0	sine	U11/U12 RNP	28	33	39	0.71	13
9	F 41.8	0	limited	centromere	61	69	34	1.79	62
10	F 56.4	60	limited	Th/To	64	34	29	2.2	42
11	M 45.3	0	diffuse	Scl-70	30	34	23	1.3	23
13	M 42.5	8	diffuse	U1 RNP	36	45	31	1.16	50
14	F 34.6	0	diffuse	U11/U12 RNP	28	33	32	0.88	n/a
15	F 47.7	1	diffuse	Scl-70	18	17	n/a	n/a	14
16	F 36.8	7	limited	Th/To	85	91	83	1.02	60
17	F 56.7	0	limited	centromere	74	74	30	2.47	52
18	F 42.3	15	limited	Scl-70	46	56	14	3.28	24
19	F 37.0	0	diffuse	n/a	18	21	n/a	n/a	26
21	F 56.2	55	n/a	n/a	41	29	25	1.64	43
22	M 60.0	0	diffuse	Scl-70	60	70	36	1.67	48
23	F 51.7	39	limited	n/a	36	44	20	1.8	22
24	M 45.1	0	n/a	n/a	26	28	30	0.87	23
25	F 51.8	12	diffuse	n/a	23	25	n/a	n/a	20
26	M 57.5	0	diffuse	n/a	33	46	26	1.27	24
27	F 41.6	5	diffuse	U11/U12 RNP	42	44	31	1.35	15
28	M 58.2	30	diffuse	RNA polymerase	63	77	16	3.93	38
29	F 60.1	0	limited	Scl-70	61	70	20	3.05	23
30	F 58.8	0	n/a	n/a	39	50	14	2.79	19
32	F 63.8	0	limited	centromere	59	70	n/a	n/a	44
33	F 63.5	0	limited	RNA polymerase	82	95	25	3.28	31
35	M 54.2	0	n/a	n/a	40	47	n/a	n/a	38
36	M 49.0	30	diffuse	U11/U12 RNP	76	67	n/a	n/a	43
37	M 47.2	0	diffuse	n/a	52	58	20	2.6	35
38	F 49.0	30	limited	n/a	26	32	19	1.37	21

Table 4.2.8 Characteristics of idiopathic pulmonary arterial hypertension dataset.

Figure 4.1 Flow chart summarizing the steps in this study.

Computational identification of ID3 and ID3 candidate target genes.

Respectively each dataset has a different number of genes signifying the genome (~13,000 – 35,000). To compare the data, the identification of commonly expressed genes across all studies in both blood and tissue was first established. A total number of 3665 genes were commonly represented in each study. In order to remove systematic variation in the microarray experiments which affects the measured gene expression levels, raw data was discretized using the function: (IF (Sample>Average+0.5*Standard Deviation, 2, IF (Sample>Average-0.5*Standard*, 1, 0))). Expression levels were signified as 0 (under/low-expression), 1 (normal expression), & 2 (overexpression). To classify ID3 candidate target genes, a pearson correlation test was conducted. This is a measure of linear correlation between two variables: X & Y and is represented by a value between -1 and 1, where -1 is a total negative correlation, 0 is no correlation, & 1 is a total positive correlation. Additionally the interquartile range (IQR), which is a measure of variability, based on dividing a set of data in quartiles (Q1 - 25th percentile, Q2- 50th percentile, Q3 – 75th percentile, and Q4 – 100th percentile) was calculated. Genes that were measured between Q3 (75th percentile) & Q4 (100th percentile) were identified as best correlated with ID3. Additionally an independent t-test, which compares the means of two independent groups in order to determine whether there is statistical evidence that the associated population means are significantly different was used to test statistical differences between means of two groups (disease and control). Our common gene list (3665) was used during our t-test and significance value was considered

at $p \leq 0.05$ and IQR was determined. Genes measured in the Q1 (25th percentile) was represented as significant. In order to generate the conclusive list of ID3 candidate targets, genes that were mutually expressed in Q3 and Q4 of the Pearson correlation analysis and Q1 of the t-test were included as candidate targets. This method was done separately for whole blood and tissue studies. Furthermore the top 10 most significant ID3 targets in both blood and tissue samples were also identified.

Bayesian analysis & network. Due to the development of biomedical data in biology & medicine with vast number of variables, Bayesian networks have been successfully used in developing effective algorithms that scale up to datasets connecting numbers of variables in learning high quality predictive models from genomic data (Yoo et al. 2002; Yoo and Cooper, 2004). A Bayesian network is a directed acyclic graph in which each node represents a variable and each arc represents probabilistic influence. In Bayesian Networks, each arc is interpreted as a direct influence between a parent node (variable) and a child node, relative to the other nodes in the network. Structure of Bayesian network with respect to a decomposable score such as BIC, BDe, or AIC becomes infeasible as the number of variables increases. A much simpler algorithm for finding the globally optimal Bayesian network structure without any structural limitations is used with $n < 33$ which is also known as Bene. The approach is broken down into five logical steps: Step 1. Compute the local scores for all $n^2n - 1$ different (variable, variable set) – pairs; Step 2. Using the local scores, find the best parents for all $n^2n - 1$ (variable, parent candidate set)-pairs; Step 3. Find the best sink for all $2n$ variable sets; and

Step 4. Using the results from Step 3, find a best ordering of the variables. Step 5. Find a best network using results computed in Steps 2 and 4 (Silander & Myllymak).

Genie. Genie Modular is a graphical user interface to SMILE (Structural Modeling, Interface, & Learning Engine) is a fully platform independent library of C++ classes applying decision-theoretic models & graphical probabilistic, such as Bayesian networks, influence diagrams, and structural equation models. A graph demonstrates much about the structure of a probabilistic domain but not much about its numerical properties. These are encoded in conditional probability distribution matrices (equivalent to the factors in the factorized form), called conditional probability tables (CPTs) that are associated with the nodes. There will always be nodes in the network with no predecessors. These nodes are categorized by their prior marginal probability distribution. Any probability in the joint probability distribution can be determined from these explicitly represented prior and conditional probabilities.

Gene Ontology & Pathway Analysis. To understand the functions of the gene list we applied two gene ontology tools: Princeton University Lewis-Sigler Institute for Integrative Genomics Gene Ontology (GO) Tools (<http://go.princeton.edu/>), & STRING database version 10 (<http://string-db.org/>). All tools provide a knowledgebase of functional annotation tools for investigators to understand functional meaning (biological, molecular) behind various sizes of gene lists.

Comparative Toxicogenomic Database (CTD) Analysis. CTD is a publicly available database that aims to advance understanding about how environmental exposures affect human health (Davis et al. 2016). The database provides curated information about chemical–gene/protein interactions, chemical–disease and gene–disease relationships. The data are integrated with functional and pathway data to support in development of hypotheses about the mechanisms essential environmentally predisposed diseases. To understand the association between endocrine disrupting chemicals (PCBs and BPA) & ID3/ID3 target genes, CTD was used to investigate the association between PCB/BPA associated genes & ID3/ID3 target genes identified in both blood and tissue respectively. Furthermore overlapping blood-tissue ID3 target genes and their association with both PCBs and BPA was also examined. Results were summarized in venn diagrams and final lists was provided for each.

Results

Gene expression of ID3 & ID3 candidate targets in blood & tissue studies.

We identified ID3 & ID3 candidate target genes in all blood studies and tissue studies. Table 4.3 and 4.5 shows a listing of ID3 and ID3 candidate target genes. In blood, 217-candidate target genes were identified and in tissue 134-candidate target genes were identified. Top 10 most significant ID3 target genes were identified in blood & tissue studies summarized in tables 4.4 & 4.6. Top 10 most significant ID3 targets in blood includes: COL1A2, COL6A1, CYP2C18, DIRAS3, DLG2, DZIP1, GCLM, GDF11, GRP, and KNG1. The top 10 most significant ID3

targets in tissue includes: ACLY, ACP1, ARPC4, ATP5D, CAMK1, CDKN2B, COPE, CYC1, EIF4EBP2, and MCRS1.

Gene Symbol	Entrez Gene Title
ABCA4	ATP binding cassette subfamily A member 4 (ABCA4)
ABCB6	ATP binding cassette subfamily B member 6 (Langereis blood group)(ABCB6)
ACIN1	apoptotic chromatin condensation inducer 1 (ACIN1)
ACP1	acid phosphatase 1, soluble (ACP1)
ACTA2	actin, alpha 2, smooth muscle, aorta (ACTA2)
ACTN1	actinin alpha 1 (ACTN1)
ADAMTS3	ADAM metalloproteinase with thrombospondin type 1 motif 3(ADAMTS3)
AEBP1	AE binding protein 1 (AEBP1)
AGTR2	angiotensin II receptor type 2 (AGTR2)
ANKRD26	ankyrin repeat domain 26 (ANKRD26)
ANPEP	alanyl aminopeptidase, membrane (ANPEP)
APAF1	apoptotic peptidase activating factor 1 (APAF1)
APOF	apolipoprotein F (APOF)
APOH	apolipoprotein H(APOH)
ARID5B	AT-rich interaction domain 5B (ARID5B)
ARSA	arylsulfatase A(ARSA)
ASB4	ankyrin repeat and SOCS box containing 4 (ASB4)
ASNS	asparagine synthetase (glutamine-hydrolyzing) (ASNS)
ATP7B	ATPase copper transporting beta(ATP7B)
COL1A2	collagen type I alpha 2 chain (COL1A2)
COL3A1	collagen type III alpha 1 chain (COL3A1)
COL6A1	collagen type VI alpha 1 chain (COL6A1)
COPB2	coatamer protein complex subunit beta 2 (COPB2)
CREBBP	CREB binding protein (CREBBP)
CRYAA	crystallin alpha A (CRYAA)
CTGF	connective tissue growth factor (CTGF)
CTH	cystathionine gamma-lyase (CTH)
CTNNB1	catenin beta 1 (CTNNB1)
CTNNB1	beta-catenin (ctnnb1)
CTSF	cathepsin F (CTSF)
CYP26A1	cytochrome P450 family 26 subfamily A member 1 (CYP26A1)
CYP2C18	cytochrome P450 family 2 subfamily C member 18 (CYP2C18)
CYP4F11	cytochrome P450 family 4 subfamily F member 11 (CYP4F11)
CYR61	cysteine rich angiogenic inducer 61(CYR61)
DAAM1	dishevelled associated activator of morphogenesis 1 (DAAM1)
DAB2	DAB2, clathrin adaptor protein(DAB2)
DCBLD2	discoidin, CUB and LCCL domain containing 2 (DCBLD2)
DCX	Doublecortin (DCX)
DHRS3	dehydrogenase/reductase 3 (DHRS3)
DHRS7	dehydrogenase/reductase 7 (DHRS7)
DIRAS3	DIRAS family GTPase 3 (DIRAS3)
DLG2	discs large MAGUK scaffold protein 2 (DLG2)
DNAJC7	DnaJ heat shock protein family (Hsp40) member C7 (DNAJC7)
DNMT1	DNA methyltransferase 1 (DNMT1)
DOCK9	dedicator of cytokinesis 9(DOCK9)

AVPR2	arginine vasopressin receptor 2 (AVPR2)
BCAM	basal cell adhesion molecule (Lutheran blood group)(BCAM)
BCL2L2	BCL2 like 2 (BCL2L2)
BYSL	bystin like (BYSL)
C10ORF10	chromosome 10 open reading frame 10 (C10orf10)
CACNB4	calcium voltage-gated channel auxiliary subunit beta 4 (CACNB4)
CACNG3	calcium voltage-gated channel auxiliary subunit gamma 3 (CACNG3)
CAD	carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase (CAD)
CALR	calreticulin (CALR)
CCL7	C-C motif chemokine ligand 7(CCL7)
CCT8	chaperonin containing TCP1 subunit 8(CCT8)
CDH12	cadherin 12 (CDH12)
CDH15	cadherin 15 (CDH15)
CDH16	cadherin 16 (CDH16)
CDH17	cadherin 17 (CDH17)
CDH2	cadherin 2 (CDH2)
CDH6	cadherin 6 (CDH6)
CDKL2	cyclin dependent kinase like 2 (CDKL2)
CFDP1	craniofacial development protein 1 (CFDP1)
CFTR	cystic fibrosis transmembrane conductance regulator (CFTR)
CH25H	cholesterol 25-hydroxylase (CH25H)
CHEK1	checkpoint kinase 1 (CHEK1)
CKM	creatine kinase, M-type (CKM)
CNKSR2	connector enhancer of kinase suppressor of Ras 2 (CNKSR2)
COL17A1	collagen type XVII alpha 1 chain (COL17A1)

DRD1	dopamine receptor D1 (DRD1)
DSCR4	Down syndrome critical region 4(DSCR4)
DTYMK	deoxythymidylate kinase (DTYMK)
DUSP14	dual specificity phosphatase 14 (DUSP14)
DYRK3	dual specificity tyrosine phosphorylation regulated kinase 3 (DYRK3)
DZIP1	DAZ interacting zinc finger protein 1 (DZIP1)
EEF1E1	eukaryotic translation elongation factor 1 epsilon 1 (EEF1E1)
ETS1	ETS proto-oncogene 1, transcription factor (ETS1)
FBLN2	fibulin 2 (FBLN2)
FBLN5	fibulin 5 (FBLN5)
FBP2	fructose-bisphosphatase 2 (FBP2)
FKBP4	FK506 binding protein 4 (FKBP4)
FNBP1	formin binding protein 1 (FNBP1)
FOXC2	forkhead box C2 (FOXC2)
FUT9	fucosyltransferase 9 (FUT9)
GABRA2	gamma-aminobutyric acid type A receptor alpha2 subunit (GABRA2)
GAL	galanin and GMAP prepropeptide (GAL)
GAS8	growth arrest specific 8 (GAS8)
GCLM	glutamate-cysteine ligase modifier subunit (GCLM)
GDF11	growth differentiation factor 11 (GDF11)
GFRA1	GDNF family receptor alpha 1 (GFRA1)
GLRA1	glycine receptor alpha 1 (GLRA1)
GPR135	G protein-coupled receptor 135 (GPR135)
GPR37L1	G protein-coupled receptor 37 like 1 (GPR37L1)
GRIA2	glutamate ionotropic receptor AMPA type subunit 2 (GRIA2)
GRIK5	glutamate ionotropic receptor kainate type subunit 5 (GRIK5)

GRIN2B	glutamate ionotropic receptor NMDA type subunit 2B (GRIN2B)
GRP	gastrin releasing peptide(GRP)
HDAC4	histone deacetylase 4 (HDAC4)
HDDC2	HD domain containing 2 (HDDC2)
HIST1H4C	histone cluster 1 H4 family member c (HIST1H4C)
HMGB3	high mobility group box 3 (HMGB3)
HMGCS2	3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2)
HNF4G	hepatocyte nuclear factor 4 gamma (HNF4G)
HOXD3	homeobox D3 (HOXD3)
HS3ST1	heparan sulfate-glucosamine 3-sulfotransferase 1 (HS3ST1)
HSPB2	heat shock protein family B (small) member 2 (HSPB2)
HTR4	5-hydroxytryptamine receptor 4 (HTR4)
IBSP	integrin binding sialoprotein (IBSP)
ID3	inhibitor of DNA binding 3, HLH protein (ID3)
IFIH1	interferon induced with helicase C domain 1 (IFIH1)
IFNA2	interferon alpha 2 (IFNA2)
IGFBP2	insulin like growth factor binding protein 2 (IGFBP2)
IL13RA2	interleukin 13 receptor subunit alpha 2 (IL13RA2)
IL6	interleukin 6 (IL6)
INHBB	inhibin beta B subunit (INHBB)
ITGA8	integrin subunit alpha 8 (ITGA8)
KCNH2	potassium voltage-gated channel subfamily H member 2 (KCNH2)
KCNJ4	potassium voltage-gated channel subfamily J member 4 (KCNJ4)
KCNK1	potassium two pore domain channel subfamily K member 1 (KCNK1)
KIF1A	kinesin family member 1A (KIF1A)
KNG1	kininogen 1 (KNG1)

LGR5	leucine rich repeat containing G protein-coupled receptor 5 (LGR5)
LIFR	leukemia inhibitory factor receptor alpha (LIFR)
LMO3	LIM domain only 3 (LMO3)
MAN1A2	mannosidase alpha class 1A member 2 (MAN1A2)
MCM4	minichromosome maintenance complex component 4 (MCM4)
MLLT10	myeloid/lymphoid or mixed-lineage leukemia; translocated 10 (MLLT10)
MMP11	matrix metalloproteinase 11 (MMP11)
MPP3	membrane palmitoylated protein 3 (MPP3)
MPP6	membrane palmitoylated protein 6 (MPP6)
MTTP	microsomal triglyceride transfer protein (MTTP)
MYL2	myosin light chain 2 (MYL2)
NDP	NDP, norrin cystine knot growth factor (NDP)
NDUFA7	NADH: ubiquinone oxidoreductase subunit A7 (NDUFA7)

Table 4.3 ID3 and ID3 candidate target genes identified in blood studies.

Gene Symbol	Entrez Gene Title
COL1A2	collagen type I alpha 2 chain (COL1A2)
COL6A1	collagen type VI alpha 1 chain (COL6A1)
CYP2C18	cytochrome P450 family 2 subfamily C member 18 (CYP2C18)
DIRAS3	DIRAS family GTPase 3 (DIRAS3)
DLG2	discs large MAGUK scaffold protein 2 (DLG2)
DZIP1	DAZ interacting zinc finger protein 1 (DZIP1)
GCLM	glutamate-cysteine ligase modifier subunit (GCLM)
GDF11	growth differentiation factor 11 (GDF11)
GRP	gastrin releasing peptide (GRP)
KNG1	kininogen 1 (KNG1)

Table 4.4 Top 10 most significant ID3 candidate target genes in blood.

Gene Symbol	Entrez Gene Title
ABCA1	ATP binding cassette subfamily A member 1 (ABCA1)
ABCB6	ATP binding cassette subfamily B member 6 (Langereis blood group)(ABCB6)
ACLY	ATP citrate lyase (ACLY)
ACOX1	acyl-CoA oxidase 1(ACOX1)
ACP1	acid phosphatase 1, soluble(ACP1)
ACTR1A	ARP1 actin-related protein 1 homolog A, centractin alpha(ACTR1A)
ACTR2	ARP2 actin related protein 2 homolog(ACTR2)
ADRM1	adhesion regulating molecule 1 (ADRM1)
AGGF1	angiogenic factor with G-patch and FHA domains 1(AGGF1)
AHCTF1	AT-hook containing transcription factor 1 (AHCTF1)
AK2	adenylate kinase 2(AK2)
ALDH3B1	aldehyde dehydrogenase 3 family member B1 (ALDH3B1)
ALDOC	aldolase, fructose-bisphosphate C(ALDOC)
ALPL	alkaline phosphatase, liver/bone/kidney(ALPL)
ANAPC10	anaphase promoting complex subunit 10(ANAPC10)
AOX1	aldehyde oxidase 1 (AOX1)
AP2S1	adaptor related protein complex 2 sigma 1 subunit(AP2S1)
API5	apoptosis inhibitor 5(API5)
APOD	apolipoprotein D(APOD)
APRT	adenine phosphoribosyltransferase (APRT)
ARCN1	archain 1 (ARCN1)
ARF4	ADP ribosylation factor 4(ARF4)
ARF5	ADP ribosylation factor 5(ARF5)
ARFGEF2	ADP ribosylation factor guanine nucleotide exchange factor 2 (ARFGEF2)
ARID3A	AT-rich interaction domain 3A (ARID3A)
ARL1	ADP ribosylation factor like GTPase 1(ARL1)
ARPC1A	actin related protein 2/3 complex subunit 1A (ARPC1A)
ARPC4	actin related protein 2/3 complex subunit 4 (ARPC4)

ASGR2	asialoglycoprotein receptor 2 (ASGR2)
ASPHD1	aspartate beta-hydroxylase domain containing 1(ASPHD1)
ATF6	activating transcription factor 6 (ATF6)
ATM	ATM serine/threonine kinase (ATM)
ATOX1	antioxidant 1 copper chaperone (ATOX1)
ATP5D	ATP synthase, H+ transporting, mitochondrial F1 complex, delta subunit (ATP5D)
ATP5H	ATP synthase, H+ transporting, mitochondrial Fo complex subunit D (ATP5H)
ATP6V0B	ATPase H+ transporting V0 subunit b (ATP6V0B)
ATP6V0D1	ATPase H+ transporting V0 subunit d1 (ATP6V0D1)
ATP6V1B2	ATPase H+ transporting V1 subunit B2 (ATP6V1B2)
AXIN1	axin 1 (AXIN1)
BAX	BCL2 associated X, apoptosis regulator (BAX)
BCAP31	B-cell receptor-associated protein 31 (BCAP31)
BCAR3	breast cancer anti-estrogen resistance 3 (BCAR3)
BCAT2	branched chain amino acid transaminase 2 (BCAT2)
BCL7B	BCL tumor suppressor 7B (BCL7B)
BNIP3	BCL2 interacting protein 3 (BNIP3)
BOP1	block of proliferation 1 (BOP1)
BYSL	bystin like (BYSL)
C18orf25	chromosome 18 open reading frame 25 (C18orf25)
C4BPB	complement component 4 binding protein beta (C4BPB)
CAD	carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase (CAD)
CAMK1	calcium/calmodulin dependent protein kinase I (CAMK1)
CAPNS1	calpain small subunit 1 (CAPNS1)
CASK	calcium/calmodulin dependent serine protein kinase (CASK)
CCDC6	coiled-coil domain containing 6 (CCDC6)
CCL23	C-C motif chemokine ligand 23 (CCL23)
CCNI	cyclin I (CCNI)
CD2BP2	CD2 cytoplasmic tail binding protein 2 (CD2BP2)
CD99	CD99 molecule (CD99)

CDH15	cadherin 15(CDH15)
CDK2AP2	cyclin dependent kinase 2 associated protein 2 (CDK2AP2)
CDK5	cyclin dependent kinase 5 (CDK5)
CDKN2B	cyclin dependent kinase inhibitor 2B (CDKN2B)
CEBPG	CCAAT/enhancer binding protein gamma (CEBPG)
CHMP2A	charged multivesicular body protein 2A (CHMP2A)
CLN3	CLN3, battenin (CLN3)
CLPP	caseinolytic mitochondrial matrix peptidase proteolytic subunit (CLPP)
CLTB	clathrin light chain B (CLTB)
COG7	component of oligomeric golgi complex 7 (COG7)
COMMD4	COMM domain containing 4 (COMMD4)
COPE	coatamer protein complex subunit epsilon(COPE)
COPS3	COP9 signalosome subunit 3 (COPS3)
COPS7A	COP9 signalosome subunit 7A (COPS7A)
COX5A	cytochrome c oxidase subunit 5A (COX5A)
COX7C	cytochrome c oxidase subunit 7C (COX7C)
COX8A	cytochrome c oxidase subunit 8A (COX8A)
CSF3R	colony stimulating factor 3 receptor (CSF3R)
CSPG5	chondroitin sulfate proteoglycan 5 (CSPG5)
CTNNBIP1	catenin beta interacting protein 1 (CTNNBIP1)
CTSZ	cathepsin Z (CTSZ)
CYB561D2	cytochrome b561 family member D2 (CYB561D2)
CYC1	cytochrome c1 (CYC1)
CYHR1	cysteine and histidine rich 1(CYHR1)
DAP	death associated protein(DAP)
DCBLD2	discoidin, CUB and LCCL domain containing 2 (DCBLD2)
DCTN2	dynactin subunit 2 (DCTN2)
DCTN5	dynactin subunit 5 (DCTN5)
DDB1	damage specific DNA binding protein 1 (DDB1)
DDN	Dendrin (DDN)
DDO	D-aspartate oxidase (DDO)
DEGS1	delta 4-desaturase, sphingolipid 1 (DEGS1)

DHCR7	7-dehydrocholesterol reductase (DHCR7)
DHRS3	dehydrogenase/reductase 3 (DHRS3)
DHX16	DEAH-box helicase 16 (DHX16)
DHX8	DEAH-box helicase 8 (DHX8)
DNAJB6	DnaJ heat shock protein family (Hsp40) member B6 (DNAJB6)
DNAJC8	DnaJ heat shock protein family (Hsp40) member C8 (DNAJC8)
DNASE2	deoxyribonuclease 2, lysosomal (DNASE2)
DNMT1	DNA methyltransferase 1 (DNMT1)
DPM2	dolichyl-phosphate mannosyltransferase subunit 2, regulatory (DPM2)
DYRK4	dual specificity tyrosine phosphorylation regulated kinase 4 (DYRK4)
E2F5	E2F transcription factor 5 (E2F5)
EEF2	eukaryotic translation elongation factor 2 (EEF2)
EIF2B1	eukaryotic translation initiation factor 2B subunit alpha (EIF2B1)
EIF4EBP2	eukaryotic translation initiation factor 4E binding protein 2 (EIF4EBP2)
EIF4G1	eukaryotic translation initiation factor 4 gamma 1(EIF4G1)
EIF5A	eukaryotic translation initiation factor 5A (EIF5A)
ELAC2	elaC ribonuclease Z 2 (ELAC2)
ELF4	E74 like ETS transcription factor 4(ELF4)
ENO1	enolase 1 (ENO1)
EPHA1	EPH receptor A1 (EPHA1)
EPRS	glutamyl-prolyl-tRNA synthetase (EPRS)
ERAL1	Era like 12S mitochondrial rRNA chaperone 1 (ERAL1)
ERCC2	ERCC excision repair 2, TFIIH core complex helicase subunit (ERCC2)
FAM20B	FAM20B, glycosaminoglycan xylosylkinase (FAM20B)
FAM50A	family with sequence similarity 50 member A (FAM50A)
FLAD1	flavin adenine dinucleotide synthetase 1 (FLAD1)
FLOT2	flotillin 2(FLOT2)
FPR1	formyl peptide receptor 1 (FPR1)
FRAT2	frequently rearranged in advanced T-cell lymphomas 2 (FRAT2)

FXR2	FMR1 autosomal homolog 2 (FXR2)
FZD2	frizzled class receptor 2 (FZD2)
G3BP2	G3BP stress granule assembly factor 2 (G3BP2)
GABRD	gamma-aminobutyric acid type A receptor delta subunit (GABRD)
GALK2	galactokinase 2 (GALK2)
GATA2	GATA binding protein 2 (GATA2)
GBF1	golgi brefeldin A resistant guanine nucleotide exchange factor 1(GBF1)
GLB1	galactosidase beta 1 (GLB1)
GOLGA3	golgin A3 (GOLGA3)
GOSR2	golgi SNAP receptor complex member 2 (GOSR2)
GOT1	glutamic-oxaloacetic transaminase 1(GOT1)
GPAA1	glycosylphosphatidylinositol anchor attachment 1 (GPAA1)
GPC5	glypican 5 (GPC5)
GPD1L	glycerol-3-phosphate dehydrogenase 1-like (GPD1L)
GPI	glucose-6-phosphate isomerase (GPI)
GPR39	G protein-coupled receptor 39 (GPR39)
GPR4	G protein-coupled receptor 4 (GPR4)
GPX1	glutathione peroxidase 1 (GPX1)
GRIN2D	glutamate ionotropic receptor NMDA type subunit 2D (GRIN2D)
GTF2F2	general transcription factor IIF subunit 2 (GTF2F2)
H1F0	H1 histone family member 0 (H1F0)
HAGH	hydroxyacylglutathione hydrolase (HAGH)
HCLS1	hematopoietic cell-specific Lyn substrate 1 (HCLS1)
HERC3	HECT and RLD domain containing E3 ubiquitin protein ligase 3 (HERC3)
HERC4	HECT and RLD domain containing E3 ubiquitin protein ligase 4 (HERC4)
HESX1	HESX homeobox 1 (HESX1)
HIBCH	3-hydroxyisobutyryl-CoA hydrolase (HIBCH)
HIST1H1B	histone cluster 1 H1 family member b (HIST1H1B)
HIST1H1D	histone cluster 1 H1 family member d (HIST1H1D)
HMGCR	3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR)

HSP90AB1	heat shock protein 90 alpha family class B member 1 (HSP90AB1)
HTATIP2	HIV-1 Tat interactive protein 2 (HTATIP2)
HTR2A	5-hydroxytryptamine receptor 2A (HTR2A)
ICA1	islet cell autoantigen 1 (ICA1)
ICAM3	intercellular adhesion molecule 3 (ICAM3)
ID3	inhibitor of DNA binding 3, HLH protein (ID3)
IDH2	isocitrate dehydrogenase (NADP(+)) 2, mitochondrial (IDH2)
IDH3B	isocitrate dehydrogenase 3 (NAD(+)) beta (IDH3B)
IDH3G	isocitrate dehydrogenase 3 (NAD(+)) gamma (IDH3G)
IFNGR2	interferon gamma receptor 2 (interferon gamma transducer 1) (IFNGR2)
IGFBP5	insulin like growth factor binding protein 5 (IGFBP5)
IL2RA	interleukin 2 receptor subunit alpha (IL2RA)
ILF2	interleukin enhancer binding factor 2 (ILF2)
IMP4	IMP4 homolog, U3 small nucleolar ribonucleoprotein (IMP4)
ITGAM	integrin subunit alpha M (ITGAM)
ITGB5	integrin subunit beta 5 (ITGB5)
IVD	isovaleryl-CoA dehydrogenase (IVD)
KIAA0196	KIAA0196 (KIAA0196)
KIFAP3	kinesin associated protein 3 (KIFAP3)
KLK2	kallikrein related peptidase 2 (KLK2)
KPNA4	karyopherin subunit alpha 4 (KPNA4)
KRT19	keratin 19 (KRT19)
LASP1	LIM and SH3 protein 1 (LASP1)
LGALS1	galectin 1 (LGALS1)
LGALS3BP	galectin 3 binding protein (LGALS3BP)
LMAN1	lectin, mannose binding 1 (LMAN1)
LMNA	lamin A/C (LMNA)
LRRC42	leucine rich repeat containing 42(LRRC42)
LSM3	LSM3 homolog, U6 small nuclear RNA and mRNA degradation associated(LSM3)
LY96	lymphocyte antigen 96(LY96)

M6PR	mannose-6-phosphate receptor, cation dependent (M6PR)
MAPK1	mitogen-activated protein kinase 1(MAPK1)
MAPK13	mitogen-activated protein kinase 13(MAPK13)
MAPKAPK3	mitogen-activated protein kinase-activated protein kinase 3 (MAPKAPK3)
MAPRE1	microtubule associated protein RP/EB family member 1(MAPRE1)
MCM3	minichromosome maintenance complex component 3 (MCM3)
MCM4	minichromosome maintenance complex component 4 (MCM4)
MCRS1	microspherule protein 1 (MCRS1)
MFGE8	milk fat globule-EGF factor 8 protein (MFGE8)
MFSD5	major facilitator superfamily domain containing 5(MFSD5)
MGAT1	Mannosyl (alpha-1,3-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase(MGAT1)
MGST3	microsomal glutathione S-transferase 3 (MGST3)
MIF	macrophage migration inhibitory factor (glycosylation-inhibiting factor)(MIF)
MPDU1	mannose-P-dolichol utilization defect 1 (MPDU1)
MPI	mannose phosphate isomerase(MPI)
MPPE1	metallophosphoesterase 1 (MPPE1)
MPV17	MPV17, mitochondrial inner membrane protein (MPV17)
MR1	major histocompatibility complex, class I-related(MR1)
MRPL12	mitochondrial ribosomal protein L12 (MRPL12)
MRPS11	mitochondrial ribosomal protein S11 (MRPS11)
MRPS12	mitochondrial ribosomal protein S12 (MRPS12)
MSN	Moesin (MSN)
MTHFS	5,10-methylenetetrahydrofolate-synthetase(5-formyltetrahydrofolate-cyclo-ligase) (MTHFS)
MTM1	myotubularin 1 (MTM1)
MVK	mevalonate kinase (MVK)
MYBPC2	myosin binding protein C, fast type (MYBPC2)
MYO7A	myosin VIIA(MYO7A)
NAGLU	N-acetyl-alpha-glucosaminidase (NAGLU)

NAGPA	N-acetylglucosamine-1-phosphodiester-alpha-N-acetylglucosaminidase (NAGPA)
NCK1	NCK adaptor protein 1 (NCK1)
NCSTN	Nicastrin (NCSTN)
NDST1	N-deacetylase and N-sulfotransferase 1 (NDST1)
NDUFA1	NADH: ubiquinone oxidoreductase subunit A1(NDUFA1)
NDUFA7	NADH: ubiquinone oxidoreductase subunit A7 (NDUFA7)
NDUFS4	NADH: ubiquinone oxidoreductase subunit S4 (NDUFS4)
NDUFV1	NADH: ubiquinone oxidoreductase core subunit V1 (NDUFV1)
NDUFV2	NADH: ubiquinone oxidoreductase core subunit V2 (NDUFV2)
NEDD8	neural precursor cell expressed, developmentally down-regulated 8 (NEDD8)

Table 4.5 ID3 and ID3 candidate target genes in tissue studies.

Gene Symbol	Entrez Gene Title
ACLY	ATP citrate lyase (ACLY)
ACP1	acid phosphatase 1, soluble (ACP1)
ARPC4	actin related protein 2/3 complex subunit 4 (ARPC4)
ATP5D	ATP synthase, H ⁺ transporting, mitochondrial F1 complex, delta subunit (ATP5D)
CAMK1	calcium/calmodulin dependent protein kinase I (CAMK1)
CDKN2B	cyclin dependent kinase inhibitor 2B (CDKN2B)
COPE	coatamer protein complex subunit epsilon (COPE)
CYC1	cytochrome c1 (CYC1)
EIF4EBP2	eukaryotic translation initiation factor 4E binding protein 2 (EIF4EBP2)
MCRS1	microspherule protein 1 (MCRS1)

Table 4.6 Top 10 most significant ID3 candidate target genes in tissue.

Gene expression in blood versus tissue studies. Overlapping ID3 & ID3 candidate target genes were identified between blood & tissue samples. Genes include: ABCB6, ACP1, BYSL, CAD, CDH15, DCBLD2, DHRS3, DNMT1, ID3, MCM4, and NDUFA7 as shown in Table 4.7 & Figure 4.3 Furthermore we demonstrated the results in both Bene and Genie models (Figure 4.4 & 4.5.1 – 4.5.4) illustrating a fully connected structure and probability distribution of gene expression.

Gene Symbol	Entrez Gene Title
ABCB6	ATP binding cassette subfamily B member 6 (Langereis blood group) (ABCB6)
ACP1	acid phosphatase 1, soluble (ACP1)
BYSL	bystin like (BYSL)
CAD	carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase (CAD)
CDH15	cadherin 15 (CDH15)
DCBLD2	discoidin, CUB and LCCL domain containing 2 (DCBLD2)
DHRS3	dehydrogenase/reductase 3 (DHRS3)
DNMT1	DNA methyltransferase 1 (DNMT1)
ID3	inhibitor of DNA binding 3, HLH protein (ID3)
MCM4	minichromosome maintenance complex component 4 (MCM4)
NDUFA7	NADH: ubiquinone oxidoreductase subunit A7 (NDUFA7)

Table 4.7 ID3 and common candidate target genes between blood and tissue studies.

Figure 4.2 Venn diagram illustration summarizes ID3 & ID3 candidate target genes. Left circle represent genes in blood studies (134), right circle represent genes in tissue studies (217), & the middle intersection of the two circles represent the shared genes between both blood & tissue studies (11).

Figure 4.3 Representation of ID3 and overlapping ID3-candidate target genes in both blood and tissue samples. Bene, a method for finding the globally optimal Bayesian network produced a fully connected structure.

Figure 4.4.1 Best fit model based on Genie Modeler with overlapping ID3 & ID3-candidate target genes.

Figure 4.4.2 Representation of ID3 & ID3-candidate targets in vascular state. Left panel represents model in disease state and right panel represents model in non-disease state.

Figure 4.4.3 Representation of ID3 & ID3-candidate targets in location of vascular disease. Left panel represents model in blood & right panel represents model in tissue.

Figure 4.4.4 Representation of models based on expression of ID3. Left panel summarizes ID3 low-expression, middle panel represents ID3 normal-expression, & the right panel summarizes ID3-high expression.

Gene expression of ID3 & ID3 candidate target genes in specific vascular diseases in blood & tissue. Our investigation indicated the expression of ID3 & ID3 candidate target genes within each vascular disease. Figure 4.6.1 illustrates the diverse number of candidate targets and overlapping genes between each disease in blood studies. Number of candidate targets genes (n) include: atherosclerosis (357); arteriovenous malformation (36); peripheral arterial disease (190); proliferative diabetic retinopathy (403); infantile hemangioma (84); idiopathic pulmonary arterial hypertension (75); & Lymphangioleiomyomatosis (363). The analysis also identified ID3 & ID3 candidate target genes within each vascular disease in tissue studies. Figure 4.6.2 illustrates the diverse number of candidate targets and overlapping genes between each disease. Number of candidate targets genes (n) include: arteriovenous malformation (277); proliferative diabetic

retinopathy (403); infantile hemangioma (98); idiopathic pulmonary arterial hypertension (973); & Lymphangioleiomyomatosis (231).

Figure 4.5.1 Venn diagram illustration summarizes ID3 & ID3 candidate target genes in vascular diseases in blood studies. Each shape represents a different disease that includes blood samples. Diseases include: IH (Infantile Hemangioma), PAD (peripheral arterial disease), AVM (arteriovenous malformation), LAM (Lymphangioleiomyomatosis), IPAH (idiopathic pulmonary arterial hypertension), & AS (atherosclerosis).

Figure 4.5.2 Venn diagram summarizes ID3 & ID3 candidate target genes in specific diseases in tissue studies. Each ovular shape represents a vascular disease. Diseases in tissue studies include: IH (Infantile Hemangioma), AVM (arteriovenous malformation), LAM (Lymphangioleiomyomatosis), PDR (Proliferative Diabetic Retinopathy), & IPAH (Idiopathic pulmonary arterial hypertension).

ID3 & ID3 gene-associated biological processes

ID3 and ID3 candidate target genes in blood. In order to understand the roles of these genes in functional processes a gene ontology approach was used via the Princeton University Lewis-Sigler Institute for Integrative Genomics Gene Ontology (GO) Tool to demonstrate the processes in which ID3 & ID3 candidate target genes in blood were represented. The genes were demonstrated in 122 biological processes and are represented in Table 4.8.

Gene Ontology ID	Biological Process	Corrected p-value	Number List Annotation	FDR Rate	Expected False Positive	Annotated Genes
GO:0048856	anatomical structure development	9.30E-12	34	0.00%	0	IFNA2, NDP, COL3A1, LGR5, DRD1, CTGF, AGTR2, ACTA2, ARID5B, CTH, DLG2, APOH, LIFR, DZIP1, ID3, COL17A1, GAL, IBSP, COL6A1, FUT9, CCL7, FOXC2, GPR37L1, CDH15, CFDP1, DAB2, BYSL, GFRA1, HDAC4, HIST1H4C, HMGCS2, INHBB, ASB4, ITGA8
GO:0009653	anatomical structure morphogenesis	9.64E-07	19	0.00%	0	NDP, CCL7, FOXC2, COL3A1, CFDP1, LGR5, CTGF, DAB2, BYSL, AGTR2, ACTA2, ARID5B, APOH, LIFR, ID3, ASB4, ITGA8, GAL, COL6A1
GO:0048513	animal organ development	2.35E-08	23	0.00%	0	IFNA2, NDP, GPR37L1, FOXC2, COL3A1, LGR5, DRD1, CTGF, DAB2, BYSL, AGTR2, ACTA2, ARID5B, APOH, LIFR, HDAC4, HIST1H4C, HMGCS2, ID3, INHBB, ITGA8, GAL, IBSP
GO:0006915	apoptotic process	1.73E-05	16	0.00%	0	IFNA2, GCLM, FOXC2, CFDP1, CTGF, KNG1, DAB2, AGTR2, CTH, APOH, HDAC4, LIFR, GRIK5, INHBB, ID3, GAL
GO:0022610	biological adhesion	0.001153274	13	0.00%	0	IFNA2, CDH12, FOXC2, COL3A1, CFDP1, CDH15, COL17A1, CTGF, ITGA8, KNG1, BYSL, IBSP, COL6A1
GO:0065007	biological regulation blood	3.15E-05	47	0.00%	0	IFNA2, DCBLD2, NDP, GCLM, MPP3, GPR135, COL3A1, LGR5, DRD1, CTGF, HSPB2, KNG1, AGTR2, CACNG3, DLG2, ACTA2, ARID5B, CTH, APOH, LIFR, IFIH1, DZIP1, GRIN2B, ID3, COL17A1, GAL, IL13RA2, CCL7, KCNJ4, FOXC2, GPR37L1, CDH15, CFDP1, ADAMTS3, DAB2, HNF4G, DIRAS3, GFRA1, GRP, HDAC4, HIST1H4C, INHBB, GRIK5, CNKSR2, ITGA8, ASB4, GLRA1
GO:0008015	circulation	2.50E-06	10	0.00%	0	ACTA2, KCNJ4, HDAC4, GCLM, FOXC2, DRD1, CTGF, KNG1, AGTR2, CACNG3

GO:0072358	cardiovascular system development	0.00440161	8	0.02%	0.02	CTH, ACTA2, NDP, APOH, FOXC2, COL3A1, CTGF, ASB4
GO:0007155	cell adhesion	0.001106077	13	0.00%	0	IFNA2, CDH12, FOXC2, COL3A1, CFDP1, CDH15, COL17A1, CTGF, ITGA8, KNG1, BYSL, IBSP, COL6A1
GO:0007154	cell communication	1.50E-08	37	0.00%	0	IFNA2, DCBLD2, NDP, GCLM, MPP3, GPR135, COL3A1, LGR5, DRD1, CTGF, AGTR2, CACNG3, ARID5B, CTH, DLG2, LIFR, IFIH1, DZIP1, GRIN2B, GAL, IL13RA2, CCL7, FOXC2, GPR37L1, ADAMTS3, DAB2, HNF4G, DIRAS3, GFRA1, GRP, HDAC4, GRIK5, INHBB, CNKSR2, ITGA8, ASB4, GLRA1
GO:0008219	cell death	6.23E-06	17	0.00%	0	IFNA2, GCLM, GPR37L1, FOXC2, CFDP1, CTGF, KNG1, DAB2, AGTR2, CTH, APOH, HDAC4, LIFR, ID3, GRIK5, INHBB, GAL
GO:0048468	cell development	0.000651109	14	0.00%	0	ARID5B, ACTA2, LIFR, HDAC4, GPR37L1, FOXC2, DZIP1, COL3A1, LGR5, INHBB, DRD1, ITGA8, GAL, AGTR2
GO:0030154	cell differentiation	1.88E-08	25	0.00%	0	IFNA2, GPR37L1, FOXC2, COL3A1, CDH15, LGR5, DRD1, CTGF, DAB2, BYSL, AGTR2, ACTA2, ARID5B, CTH, LIFR, HIST1H4C, HDAC4, DZIP1, ID3, INHBB, ITGA8, ASB4, GAL, IBSP, COL6A1
GO:0008283	cell proliferation	0.000725251	14	0.00%	0	IFNA2, NDP, APOH, LIFR, HDAC4, GPR37L1, FOXC2, CFDP1, LGR5, CTGF, DAB2, BYSL, GAL, AGTR2
GO:0007166	cell surface receptor signaling pathway	1.09E-07	23	0.00%	0	IFNA2, NDP, CCL7, GCLM, GPR37L1, FOXC2, COL3A1, LGR5, ADAMTS3, CTGF, DAB2, AGTR2, CACNG3, ARID5B, GFRA1, LIFR, DZIP1, GRIN2B, INHBB, GRIK5, ITGA8, IL13RA2, GLRA1
GO:0007267	cell-cell signaling	0.000107771	14	0.00%	0	DLG2, IFNA2, NDP, CCL7, GRIN2B, LGR5, INHBB, DRD1, GRIK5, CTGF, DAB2, GAL, AGTR2, GLRA1
GO:0016043	cellular component organization	0.000335738	28	0.00%	0	DCBLD2, NDP, MPP6, CCL7, GCLM, FOXC2, COL3A1, CFDP1, CDH15, ADAMTS3, DRD1, CTGF, DAB2, AGTR2, ARID5B, CTH, CDH12, LIFR, HIST1H4C, HDAC4, DZIP1, GRIK5, COL17A1, ITGA8, GAL, IBSP, COL6A1, GLRA1
GO:0071840	cellular component organization or biogenesis	0.000170434	29	0.00%	0	DCBLD2, NDP, MPP6, CCL7, GCLM, FOXC2, COL3A1, CFDP1, CDH15, ADAMTS3, DRD1, CTGF, DAB2, BYSL, AGTR2, ARID5B, CTH, CDH12, LIFR, HIST1H4C, HDAC4, DZIP1, GRIK5, COL17A1, ITGA8, GAL, IBSP, COL6A1, GLRA1
GO:0048869	cellular developmental process	1.14E-09	27	0.00%	0	IFNA2, CCL7, GPR37L1, FOXC2, COL3A1, CFDP1, CDH15, LGR5, DRD1, CTGF, DAB2, BYSL, AGTR2, ACTA2, ARID5B, CTH, LIFR, HIST1H4C, HDAC4, DZIP1, ID3, INHBB, ITGA8, ASB4, GAL, IBSP, COL6A1
GO:0009987	cellular process	0.000288023	57	0.00%	0	IFNA2, DCBLD2, NDP, MPP6, GCLM, MPP3, GPR135, COL3A1, LGR5, DRD1, CTGF, KNG1, AGTR2, CACNG3, CYP2C18, DLG2, CTH, ACTA2, ARID5B, APOH, LIFR, IFIH1, DZIP1, GRIN2B, ID3, COL17A1, GAL, IL13RA2, IBSP, COL6A1, FUT9, CCL7, KCNJ4, GPR37L1, FOXC2, CDH15, CFDP1, ADAMTS3, CH25H, DAB2, HNF4G, BYSL, DIRAS3, GFRA1, CDH12, GRP, HMGS2, HIST1H4C, HDAC4, INHBB, GRIK5, CNKSR2, ITGA8, ASB4, CKM, FBP2, GLRA1
GO:0071229	cellular response to acid chemical	0.00625897	5	0.02%	0.02	ID3, HMGS2, COL6A1, GLRA1, COL3A1
GO:0071230	cellular response to amino acid stimulus	0.001480522	4	0.00%	0	HMGS2, COL6A1, GLRA1, COL3A1

GO:0070887	cellular response to chemical stimulus	8.68E-11	25	0.00%	0	IFNA2, CCL7, GPR37L1, FOXC2, COL3A1, ADAMTS3, DRD1, CTGF, DAB2, HNF4G, BYSL, AGTR2, CYP2C18, CTH, LIFR, HDAC4, HMGC2, ID3, INHBB, GRIK5, ITGA8, IL13RA2, IBSP, COL6A1, GLRA1
GO:0071495	cellular response to endogenous stimulus	3.24E-05	13	0.00%	0	HMGC2, HDAC4, FOXC2, COL3A1, INHBB, DRD1, CTGF, ITGA8, DAB2, HNF4G, AGTR2, COL6A1, GLRA1
GO:0071363	cellular response to growth factor stimulus	0.005538798	8	0.02%	0.02	ADAMTS3, CTGF, ITGA8, DAB2, BYSL, IBSP, AGTR2, COL3A1
GO:0032870	cellular response to hormone stimulus	0.005482186	8	0.02%	0.02	HMGC2, HDAC4, FOXC2, DRD1, INHBB, DAB2, HNF4G, AGTR2
GO:1901699	cellular response to nitrogen compound	0.0018159	8	0.00%	0	HMGC2, FOXC2, COL3A1, DRD1, INHBB, AGTR2, COL6A1, GLRA1
GO:0071310	cellular response to organic substance	1.35E-10	23	0.00%	0	IFNA2, CCL7, FOXC2, COL3A1, ADAMTS3, DRD1, CTGF, DAB2, HNF4G, BYSL, AGTR2, CTH, LIFR, HDAC4, HMGC2, ID3, INHBB, GRIK5, ITGA8, IL13RA2, IBSP, COL6A1, GLRA1
GO:0071417	cellular response to organ nitrogen compound	0.000506385	8	0.00%	0	HMGC2, FOXC2, COL3A1, DRD1, INHBB, AGTR2, COL6A1, GLRA1
GO:1901701	cellular response to oxygen-containing compound	6.01E-07	13	0.00%	0	CCL7, HMGC2, GPR37L1, FOXC2, COL3A1, ID3, INHBB, GRIK5, DRD1, BYSL, AGTR2, COL6A1, GLRA1
GO:0051716	cellular response to stimulus	1.72E-12	44	0.00%	0	IFNA2, DCBLD2, NDP, GCLM, MPP3, GPR135, COL3A1, LGR5, DRD1, CTGF, AGTR2, CACNG3, CYP2C18, ARID5B, CTH, LIFR, IFIH1, DZIP1, GRIN2B, ID3, GAL, IL13RA2, IBSP, COL6A1, CCL7, KCNJ4, FOXC2, GPR37L1, ADAMTS3, DAB2, BYSL, HNF4G, DIRAS3, GFRA1, GRP, HDAC4, HIST1H4C, HMGC2, INHBB, GRIK5, CNKSR2, ITGA8, ASB4, GLRA1
GO:0072359	circulatory system development	0.001387394	10	0.00%	0	CTH, ACTA2, NDP, APOH, FOXC2, COL3A1, ID3, CTGF, ASB4, AGTR2
GO:0003013	circulatory system process	2.68E-06	10	0.00%	0	ACTA2, KCNJ4, HDAC4, GCLM, FOXC2, DRD1, CTGF, KNG1, AGTR2, CACNG3
GO:0032502	developmental process	9.02E-11	34	0.00%	0	IFNA2, NDP, COL3A1, LGR5, DRD1, CTGF, AGTR2, ACTA2, ARID5B, CTH, DLG2, APOH, LIFR, DZIP1, ID3, COL17A1, GAL, IBSP, COL6A1, FUT9, CCL7, FOXC2, GPR37L1, CDH15, CFDP1, DAB2, BYSL, GFRA1, HDAC4, HIST1H4C, HMGC2, INHBB, ASB4, ITGA8
GO:0007167	enzyme linked receptor signaling pathway	4.56E-05	12	0.00%	0	GFRA1, ARID5B, LIFR, FOXC2, GRIN2B, COL3A1, ADAMTS3, INHBB, CTGF, ITGA8, DAB2, AGTR2
GO:0030198	extracellular matrix organization	0.00036749	8	0.00%	0	ADAMTS3, CTGF, ITGA8, FOXC2, IBSP, ID3, COL6A1, COL3A1
GO:0043062	extracellular structure organization	0.000374985	7	0.00%	0	ADAMTS3, CTGF, ITGA8, FOXC2, IBSP, COL6A1, COL3A1
GO:0060322	head development	0.008012499	8	0.02%	0.02	DRD1, ARID5B, ITGA8, HMGC2, GPR37L1, BYSL, AGTR2, COL3A1
GO:0001822	kidney development	9.56E-05	7	0.00%	0	ID3, ARID5B, ACTA2, ITGA8, HMGC2, FOXC2, AGTR2
GO:0001656	metanephros development	0.006881579	4	0.02%	0.02	ID3, ITGA8, FOXC2, AGTR2
GO:0007275	multicellular organism development	2.56E-10	31	0.00%	0	FUT9, IFNA2, NDP, GPR37L1, FOXC2, COL3A1, CFDP1, LGR5, DRD1, CTGF, DAB2, BYSL, AGTR2, GFRA1, ACTA2,

						ARID5B, CTH, DLG2, APOH, LIFR, HMGC2, HIST1H4C, HDAC4, DZIP1, ID3, INHBB, ITGA8, ASB4, GAL, IBSP, COL6A1
GO:0032501	multicellular organismal process	2.22E-12	39	0.00%	0	IFNA2, NDP, GCLM, COL3A1, LGR5, DRD1, CTGF, KNG1, AGTR2, CACNG3, ACTA2, ARID5B, CTH, DLG2, APOH, LIFR, IFIH1, DZIP1, GRIN2B, ID3, GAL, IBSP, COL6A1, FUT9, KCNJ4, FOXC2, GPR37L1, CFDP1, ADAMTS3, DAB2, BYSL, GFRA1, HDAC4, HIST1H4C, HMGC2, INHBB, ASB4, ITGA8, GLRA1
GO:0061061	muscle structure development	0.000174968	9	0.00%	0	CTH, ARID5B, HDAC4, FOXC2, COL3A1, CDH15, ID3, ITGA8, AGTR2
GO:0003012	muscle system process	0.003487104	7	0.02%	0.02	DRD1, ACTA2, CTGF, KNG1, HDAC4, AGTR2, GLRA1
GO:0048519	negative regulation of biological process	1.28E-05	25	0.00%	0	IFNA2, DCBLD2, GCLM, GPR37L1, FOXC2, COL3A1, CFDP1, DRD1, CTGF, KNG1, DAB2, AGTR2, ARID5B, CTH, DLG2, APOH, LIFR, HIST1H4C, HDAC4, IFIH1, ID3, INHBB, GAL, IL13RA2, GLRA1
GO:0060548	negative regulation of cell death	0.006253691	9	0.02%	0.02	CTH, APOH, LIFR, GCLM, GPR37L1, FOXC2, CFDP1, CTGF, DAB2
GO:0048523	negative regulation of cellular process	8.67E-06	24	0.00%	0	IFNA2, DCBLD2, GCLM, GPR37L1, FOXC2, COL3A1, CFDP1, DRD1, CTGF, KNG1, DAB2, AGTR2, ARID5B, CTH, DLG2, APOH, LIFR, HIST1H4C, HDAC4, ID3, INHBB, GAL, IL13RA2, GLRA1
GO:0051093	negative regulation of developmental process	0.002394213	9	0.02%	0.02	ID3, IFNA2, APOH, HIST1H4C, HDAC4, GPR37L1, GAL, FOXC2, COL3A1
GO:0051241	negative regulation of multicellular organismal process	6.69E-09	15	0.00%	0	IFNA2, APOH, HIST1H4C, HDAC4, IFIH1, GPR37L1, FOXC2, COL3A1, ID3, INHBB, DRD1, KNG1, GAL, AGTR2, GLRA1
GO:0048585	negative regulation of response to stimulus	0.005272508	11	0.02%	0.02	CTH, IFNA2, APOH, GCLM, GPR37L1, COL3A1, DRD1, KNG1, DAB2, AGTR2, IL13RA2
GO:0007399	nervous system development	0.000672503	15	0.00%	0	GFRA1, FUT9, DLG2, NDP, LIFR, HMGC2, HDAC4, GPR37L1, COL3A1, ID3, DRD1, ITGA8, BYSL, GAL, AGTR2
GO:0043065	positive regulation of apoptotic process	0.002443163	8	0.02%	0.02	HDAC4, GRIK5, INHBB, ID3, CTGF, KNG1, GAL, AGTR2
GO:0048518	positive regulation of biological process	2.77E-08	30	0.00%	0	IFNA2, NDP, CCL7, GPR37L1, FOXC2, COL3A1, CDH15, LGR5, ADAMTS3, DRD1, CTGF, KNG1, DAB2, AGTR2, ACTA2, ARID5B, CTH, APOH, LIFR, HIST1H4C, HDAC4, IFIH1, ID3, INHBB, GRIK5, ITGA8, ASB4, GAL, IL13RA2, GLRA1
GO:0010647	positive regulation of cell communication	2.99E-05	14	0.00%	0	CTH, IFNA2, CCL7, GPR37L1, COL3A1, LGR5, ADAMTS3, INHBB, DRD1, CTGF, ITGA8, DAB2, GAL, AGTR2
GO:0010942	positive regulation of cell death	0.003745574	8	0.02%	0.02	HDAC4, GRIK5, INHBB, ID3, CTGF, KNG1, GAL, AGTR2
GO:0048522	positive regulation of cellular process	4.39E-05	24	0.00%	0	IFNA2, NDP, CCL7, GPR37L1, FOXC2, COL3A1, CDH15, LGR5, ADAMTS3, DRD1, CTGF, KNG1, DAB2, AGTR2, CTH, APOH, LIFR, HDAC4, ID3, GRIK5, INHBB, ITGA8, ASB4, GAL
GO:0009893	positive regulation of metabolic process	0.006424653	16	0.02%	0.02	IFNA2, NDP, CCL7, GPR37L1, FOXC2, DRD1, CTGF, DAB2, AGTR2, ACTA2, APOH, HDAC4, HIST1H4C, INHBB, ITGA8, GAL
GO:0044093	positive regulation of molecular function	0.002933859	14	0.02%	0.02	GFRA1, CTH, ARID5B, NDP, CCL7, APOH, HDAC4, GCLM, GRIN2B, DRD1, CTGF, HSPB2, GAL, AGTR2

GO:0051240	positive regulation of multicellular organismal process	0.00065788	12	0.00%	0	CTH, APOH, IFIH1, GPR37L1, FOXC2, INHBB, CTGF, ASB4, KNG1, DAB2, GAL, AGTR2
GO:0043068	positive regulation of programmed cell death	0.002588551	8	0.02%	0.02	HDAC4, GRIK5, INHBB, ID3, CTGF, KNG1, GAL, AGTR2
GO:0048584	positive regulation of response to stimulus	0.000260258	15	0.00%	0	CTH, IFNA2, CCL7, APOH, IFIH1, GPR37L1, FOXC2, COL3A1, LGR5, ADAMTS3, INHBB, CTGF, ITGA8, DAB2, AGTR2
GO:0009967	positive regulation of signal transduction	0.000653226	12	0.00%	0	CTH, IFNA2, CCL7, GPR37L1, COL3A1, LGR5, ADAMTS3, INHBB, CTGF, ITGA8, DAB2, GAL, AGTR2
GO:0023056	positive regulation of signaling	3.10E-05	14	0.00%	0	CTH, IFNA2, CCL7, GPR37L1, COL3A1, LGR5, ADAMTS3, INHBB, DRD1, CTGF, ITGA8, DAB2, GAL, AGTR2
GO:0012501	programmed cell death	2.03E-05	16	0.00%	0	IFNA2, GCLM, FOXC2, CFDP1, CTGF, KNG1, DAB2, AGTR2, CTH, APOH, HDAC4, LIFR, GRIK5, INHBB, ID3, GAL
GO:0042981	regulation of apoptotic process	3.06E-06	15	0.00%	0	CTH, APOH, LIFR, HDAC4, GCLM, FOXC2, CFDP1, ID3, INHBB, GRIK5, CTGF, KNG1, DAB2, GAL, AGTR2
GO:0050789	regulation of biological process	1.39E-05	46	0.00%	0	IFNA2, DCBLD2, NDP, GCLM, MPP3, GPR135, COL3A1, LGR5, DRD1, CTGF, KNG1, AGTR2, CACNG3, DLG2, ACTA2, ARID5B, CTH, APOH, LIFR, IFIH1, DZIP1, GRIN2B, ID3, COL17A1, GAL, IL13RA2, CCL7, KCNJ4, FOXC2, GPR37L1, CDH15, CFDP1, ADAMTS3, DAB2, HNF4G, DIRAS3, GFRA1, GRP, HDAC4, HIST1H4C, INHBB, GRIK5, CNKSR2, ITGA8, ASB4, GLRA1
GO:0065008	regulation of biological quality	0.00567202	18	0.02%	0.02	IFNA2, CCL7, GCLM, FOXC2, COL3A1, CFDP1, DRD1, CTGF, KNG1, AGTR2, ACTA2, APOH, HIST1H4C, DZIP1, GRIK5, INHBB, GAL, GLRA1
GO:1903522	regulation of blood circulation	0.003207853	6	0.02%	0.02	DRD1, CTGF, KCNJ4, HDAC4, AGTR2, CACNG3
GO:0050880	regulation of blood vessel size	9.19E-05	6	0.00%	0	DRD1, ACTA2, KNG1, GCLM, FOXC2, AGTR2
GO:0010646	regulation of cell communication	9.89E-05	19	0.00%	0	IFNA2, CCL7, GCLM, GPR37L1, COL3A1, LGR5, ADAMTS3, DRD1, CTGF, DAB2, AGTR2, CACNG3, CTH, GRIK5, INHBB, CNKSR2, ITGA8, GAL, GLRA1
GO:0010941	regulation of cell death	8.10E-07	16	0.00%	0	GCLM, GPR37L1, FOXC2, CFDP1, CTGF, KNG1, DAB2, AGTR2, CTH, APOH, HDAC4, LIFR, GRIK5, INHBB, ID3, GAL
GO:0045595	regulation of cell differentiation	0.001437131	12	0.00%	0	CTH, IFNA2, HIST1H4C, HDAC4, GPR37L1, COL3A1, CDH15, ID3, CTGF, ASB4, DAB2, GAL
GO:0030334	regulation of cell migration	0.006514346	8	0.02%	0.02	CCL7, APOH, HDAC4, FOXC2, COL3A1, DRD1, DAB2, AGTR2
GO:0050794	regulation of cellular process	0.000172246	43	0.00%	0	IFNA2, DCBLD2, NDP, GCLM, MPP3, GPR135, COL3A1, LGR5, DRD1, CTGF, KNG1, AGTR2, CACNG3, DLG2, ARID5B, CTH, APOH, LIFR, IFIH1, DZIP1, GRIN2B, ID3, GAL, IL13RA2, CCL7, FOXC2, GPR37L1, CDH15, CFDP1, ADAMTS3, DAB2, HNF4G, DIRAS3, GFRA1, GRP, HDAC4, HIST1H4C, INHBB, GRIK5, CNKSR2, ITGA8, ASB4, GLRA1
GO:0050793	regulation of developmental process	1.36E-05	17	0.00%	0	CTH, IFNA2, CCL7, APOH, HIST1H4C, HDAC4, GPR37L1, FOXC2, COL3A1, CFDP1, CDH15, ID3, CTGF, ASB4, DAB2, GAL, AGTR2
GO:0032879	regulation of localization	0.000479144	16	0.00%	0	IFNA2, CCL7, KCNJ4, FOXC2, COL3A1, DRD1, KNG1, DAB2, AGTR2, CACNG3, APOH, HDAC4, GRIK5, INHBB, GAL, IL13RA2

GO:0065009	regulation of molecular function	5.94E-05	20	0.00%	0	NDP, CCL7, GCLM, DRD1, CTGF, HSPB2, KNG1, DAB2, AGTR2, DIRAS3, CACNG3, ARID5B, CTH, DLG2, GFRA1, APOH, HDAC4, GRIN2B, ID3, GAL
GO:2000026	regulation of multicellular organismal development	0.000776707	13	0.00%	0	CTH, IFNA2, APOH, HIST1H4C, HDAC4, GPR37L1, FOXC2, COL3A1, CTGF, ASB4, DAB2, GAL, AGTR2
GO:0051239	regulation of multicellular organismal process	5.78E-08	21	0.00%	0	IFNA2, KCNJ4, GPR37L1, FOXC2, COL3A1, DRD1, CTGF, KNG1, DAB2, AGTR2, CACNG3, CTH, APOH, HDAC4, HIST1H4C, IFIH1, ID3, INHBB, ASB4, GAL, GLRA1
GO:0043067	regulation of programmed cell death	3.41E-06	15	0.00%	0	CTH, APOH, LIFR, HDAC4, GCLM, FOXC2, CFDP1, ID3, INHBB, GRIK5, CTGF, KNG1, DAB2, GAL, AGTR2
GO:0048583	regulation of response to stimulus	5.75E-06	23	0.00%	0	IFNA2, CCL7, GCLM, GPR37L1, FOXC2, COL3A1, LGR5, ADAMTS3, DRD1, CTGF, KNG1, DAB2, AGTR2, CACNG3, CTH, APOH, HDAC4, IFIH1, INHBB, COL17A1, CNKSR2, ITGA8, IL13RA2
GO:0023051	regulation of signaling	0.000137005	19	0.00%	0	IFNA2, CCL7, GCLM, GPR37L1, COL3A1, LGR5, ADAMTS3, DRD1, CTGF, DAB2, AGTR2, CACNG3, CTH, GRIK5, INHBB, CNKSR2, ITGA8, GAL, GLRA1
GO:0044057	regulation of system process	3.15E-06	10	0.00%	0	KCNJ4, HDAC4, DRD1, INHBB, CTGF, KNG1, GAL, AGTR2, CACNG3, GLRA1
GO:0035150	regulation of tube size	9.53E-05	6	0.00%	0	DRD1, ACTA2, KNG1, GCLM, FOXC2, AGTR2
GO:0072001	renal system development	5.98E-06	8	0.00%	0	ARID5B, ACTA2, HMGC2, FOXC2, LGR5, ID3, ITGA8, AGTR2
GO:0001101	response to acid chemical	0.00039036	7	0.00%	0	ID3, DRD1, CTGF, HMGC2, COL6A1, GLRA1, COL3A1
GO:0097305	response to alcohol	0.00908155	5	0.02%	0.02	DRD1, CCL7, HMGC2, GRIN2B, GLRA1
GO:0043200	response to amino acid	8.76E-06	6	0.00%	0	DRD1, CTGF, HMGC2, COL6A1, GLRA1, COL3A1
GO:0042221	response to chemical	7.76E-11	30	0.00%	0	IFNA2, CCL7, GCLM, GPR37L1, FOXC2, COL3A1, ADAMTS3, DRD1, CTGF, HSPB2, DAB2, HNF4G, BYSL, AGTR2, CYP2C18, CTH, APOH, LIFR, HMGC2, HDAC4, GRIN2B, ID3, INHBB, GRIK5, ITGA8, GAL, IL13RA2, IBSP, COL6A1, GLRA1
GO:0009719	response to endogenous stimulus	2.42E-05	14	0.00%	0	HMGC2, HDAC4, FOXC2, COL3A1, INHBB, DRD1, CTGF, ITGA8, DAB2, HNF4G, GAL, AGTR2, COL6A1, GLRA1
GO:0045471	response to ethanol	0.001478928	5	0.00%	0	DRD1, CCL7, HMGC2, GRIN2B, GLRA1
GO:0009605	response to external stimulus	0.000577938	15	0.00%	0	ACTA2, IFNA2, CCL7, APOH, KCNJ4, HMGC2, HDAC4, IFIH1, COL3A1, INHBB, DRD1, KNG1, BYSL, IL13RA2, GLRA1
GO:0070848	response to growth factor	0.007266335	8	0.02%	0.02	ADAMTS3, CTGF, ITGA8, DAB2, BYSL, IBSP, AGTR2, COL3A1
GO:0009725	response to hormone	0.000668949	10	0.00%	0	HMGC2, HDAC4, FOXC2, INHBB, DRD1, CTGF, DAB2, HNF4G, GAL, AGTR2
GO:0033993	response to lipid	0.008318598	9	0.02%	0.02	APOH, HMGC2, ID3, DRD1, CTGF, DAB2, BYSL, HNF4G, AGTR2
GO:1901698	response to nitrogen compound	0.000228868	11	0.00%	0	IFNA2, HMGC2, FOXC2, COL3A1, INHBB, DRD1, CTGF, GAL, AGTR2, COL6A1, GLRA1
GO:0014070	response to organic cyclic compound	0.001729965	10	0.00%	0	IFNA2, LIFR, HMGC2, ID3, DRD1, CTGF, DAB2, BYSL, HNF4G, AGTR2
GO:0010033	response to organic substance	3.57E-12	27	0.00%	0	IFNA2, CCL7, FOXC2, COL3A1, ADAMTS3, DRD1, CTGF, HSPB2, DAB2, HNF4G, BYSL, AGTR2, CTH, APOH, LIFR, HDAC4, HMGC2, GRIN2B, ID3, INHBB, GRIK5, ITGA8, GAL, IL13RA2, IBSP, COL6A1, GLRA1
GO:0010243	response to organ nitrogen compound	0.000644137	10	0.00%	0	HMGC2, FOXC2, COL3A1, INHBB, DRD1, CTGF, GAL, AGTR2, COL6A1, GLRA1
GO:1901700	response to oxygen-	2.29E-08	17	0.00%	0	CCL7, GPR37L1, FOXC2, COL3A1, DRD1, CTGF, BYSL, AGTR2, APOH,

	containing compound response to peptide response to peptide hormone					HMGCS2, GRIN2B, ID3, GRIK5, INHBB, GAL, COL6A1, GLRA1 INHBB, DRD1, CTGF, HMGCS2, GAL, FOXC2, AGTR2
GO:1901652		0.003249007	7	0.02%	0.02	
GO:0043434		0.00187346	7	0.00%	0	INHBB, DRD1, CTGF, HMGCS2, GAL, FOXC2, AGTR2
						IFNA2, DCBLD2, NDP, GCLM, MPP3, GPR135, COL3A1, LGR5, DRD1, CTGF, HSPB2, KNG1, AGTR2, CACNG3, CYP2C18, ACTA2, ARID5B, CTH, APOH, LIFR, IFIH1, DZIP1, GRIN2B, ID3, COL17A1, GAL, IL13RA2, IBSP, COL6A1, CCL7, KCNJ4, FOXC2, GPR37L1, ADAMTS3, DAB2, HNF4G, BYSL, DIRAS3, GFRA1, GRP, HMGCS2, HDAC4, HIST1H4C, INHBB, GRIK5, CNKSR2, ITGA8, ASB4, GLRA1
GO:0050896	response to stimulus	6.27E-14	49	0.00%	0	
GO:0006950	response to stress response to	0.000366184	21	0.00%	0	IFNA2, DCBLD2, CCL7, GCLM, GPR37L1, FOXC2, COL3A1, DRD1, CTGF, HSPB2, KNG1, AGTR2, CTH, APOH, HDAC4, HIST1H4C, HMGCS2, IFIH1, ID3, INHBB, GAL
GO:0009611	wounding	0.004128371	8	0.02%	0.02	ID3, IFNA2, DCBLD2, CTGF, APOH, KNG1, FOXC2, COL3A1
						IFNA2, DCBLD2, NDP, GCLM, MPP3, GPR135, COL3A1, LGR5, DRD1, CTGF, AGTR2, CACNG3, ARID5B, CTH, LIFR, IFIH1, DZIP1, GRIN2B, GAL, IL13RA2, CCL7, FOXC2, GPR37L1, ADAMTS3, DAB2, HNF4G, DIRAS3, GFRA1, GRP, HDAC4, GRIK5, INHBB, CNKSR2, ASB4, ITGA8, GLRA1
GO:0007165	signal transduction	1.06E-08	36	0.00%	0	
						IFNA2, DCBLD2, NDP, GCLM, MPP3, GPR135, COL3A1, LGR5, DRD1, CTGF, AGTR2, CACNG3, ARID5B, CTH, DLG2, LIFR, IFIH1, DZIP1, GRIN2B, GAL, IL13RA2, CCL7, KCNJ4, FOXC2, GPR37L1, ADAMTS3, DAB2, HNF4G,
GO:0023052	signaling	2.47E-09	38	0.00%	0	
						DIRAS3, GFRA1, GRP, HDAC4, GRIK5, INHBB, CNKSR2, ITGA8, ASB4, GLRA1
GO:0044700	single organism signaling	2.21E-09	38	0.00%	0	IFNA2, DCBLD2, NDP, GCLM, MPP3, GPR135, COL3A1, LGR5, DRD1, CTGF, AGTR2, CACNG3, ARID5B, CTH, DLG2, LIFR, IFIH1, DZIP1, GRIN2B, GAL, IL13RA2, CCL7, KCNJ4, FOXC2, GPR37L1, ADAMTS3, DAB2, HNF4G, DIRAS3, GFRA1, GRP, HDAC4, GRIK5, INHBB, CNKSR2, ITGA8, ASB4, GLRA1
GO:0044707	single-multicellular organism process	3.98E-14	38	0.00%	0	IFNA2, NDP, COL3A1, LGR5, DRD1, CTGF, KNG1, AGTR2, CACNG3, ACTA2, ARID5B, CTH, DLG2, APOH, LIFR, IFIH1, DZIP1, GRIN2B, ID3, GAL, IBSP, COL6A1, FUT9, KCNJ4, FOXC2, GPR37L1, CFDP1, ADAMTS3, DAB2, BYSL, GFRA1, HDAC4, HIST1H4C, HMGCS2, INHBB, ASB4, ITGA8, GLRA1
GO:0044763	single-organism cellular process	7.06E-11	49	0.00%	0	IFNA2, DCBLD2, NDP, GCLM, MPP3, COL3A1, LGR5, DRD1, CTGF, KNG1, AGTR2, CYP2C18, DLG2, CTH, ACTA2, ARID5B, APOH, LIFR, DZIP1, GRIN2B, ID3, COL17A1, GAL, IL13RA2, IBSP, COL6A1, FUT9, CCL7, KCNJ4, FOXC2, GPR37L1, CDH15, CFDP1, ADAMTS3, CH25H, DAB2, BYSL, DIRAS3, GFRA1, CDH12, HMGCS2, HIST1H4C, HDAC4, INHBB, GRIK5, ITGA8, ASB4, CKM, GLRA1
GO:0044767	single-organism developmental process	4.15E-10	33	0.00%	0	IFNA2, NDP, COL3A1, LGR5, DRD1, CTGF, AGTR2, ACTA2, ARID5B, CTH, DLG2, APOH, LIFR, DZIP1, ID3, GAL, IBSP, COL6A1, FUT9, CCL7, FOXC2, GPR37L1, CDH15, CFDP1, DAB2, BYSL, GFRA1, HDAC4, HIST1H4C, HMGCS2, INHBB, ASB4, ITGA8
GO:0044710	single-organism metabolic process	0.001255126	25	0.00%	0	FUT9, CCL7, GCLM, APOF, MPP3, GPR37L1, COL3A1, ADAMTS3, DRD1, CTGF, CH25H, CYP2C18, GFRA1, ARID5B, CTH, DLG2, APOH, HMGCS2,

						HDAC4, GRIN2B, INHBB, CKM, GAL, FBP2, COL6A1
GO:0044699	single-organism process	2.61E-10	57	0.00%	0	IFNA2, DCBLD2, NDP, GCLM, MPP3, GPR135, COL3A1, LGR5, DRD1, CTGF, KNG1, AGTR2, CACNG3, CYP2C18, DLG2, CTH, ACTA2, ARID5B, APOH, LIFR, IFIH1, DZIP1, GRIN2B, ID3, COL17A1, GAL, IL13RA2, IBSP, COL6A1, FUT9, CCL7, KCNJ4, APOF, GPR37L1, FOXC2, CDH15, CFDP1, ADAMTS3, CH25H, DAB2, HNF4G, BYSL, DIRAS3, GFRA1, CDH12, GRP, HMGCS2, HIST1H4C, HDAC4, INHBB, GRIK5, CNKSR2, ITGA8, ASB4, CKM, FBP2, GLRA1
GO:0048731	system development	2.82E-09	28	0.00%	0	FUT9, IFNA2, NDP, GPR37L1, FOXC2, COL3A1, LGR5, DRD1, CTGF, DAB2, BYSL, AGTR2, ACTA2, ARID5B, CTH, DLG2, GFRA1, APOH, LIFR, HMGCS2, HIST1H4C, HDAC4, ID3, INHBB, ITGA8, ASB4, GAL, IBSP
GO:0003008	system process	7.67E-06	17	0.00%	0	DLG2, ACTA2, NDP, KCNJ4, HDAC4, GCLM, FOXC2, GRIN2B, INHBB, DRD1, CTGF, ITGA8, KNG1, GAL, AGTR2, GLRA1, CACNG3
GO:0009888	tissue development	2.21E-06	16	0.00%	0	FOXC2, COL3A1, LGR5, CTGF, DAB2, AGTR2, ACTA2, ARID5B, HDAC4, HMGCS2, COL17A1, ID3, ITGA8, GAL, COL6A1, IBSP
GO:0001655	urogenital system development	1.57E-05	8	0.00%	0	ARID5B, ACTA2, HMGCS2, FOXC2, LGR5, ID3, ITGA8, AGTR2
GO:0003018	vascular process in circulatory system	0.000196727	6	0.00%	0	DRD1, ACTA2, KNG1, GCLM, FOXC2, AGTR2
GO:0001944	vasculature development	0.004084262	8	0.02%	0.02	CTH, ACTA2, NDP, APOH, FOXC2, COL3A1, CTGF, ASB4
GO:0042311	vasodilation	0.007959111	3	0.02%	0.02	DRD1, KNG1, AGTR2

Table 4.8 Demonstration of biological processes that ID3 & ID3 target genes are involved with in blood.

ID3 and ID3 candidate target genes in tissue. In order to understand the roles of these genes in functional processes gene ontology methodology was used to demonstrate the processes in which ID3 & ID3 candidate target genes in tissue were overrepresented. Candidate target genes in tissue were overrepresented in 89 biological processes.

Gene Ontology ID	Biological Process	Corrected p-value	Number List Annotation	FDR Rate	Expected False Positive	Annotated Genes
GO:0048856	anatomical structure development	8.28E-05	26	0.00%	0	MTM1, FLOT2, NAGLU, CDKN2B, NEDD8, MFGE8, FRAT2, GPR4, CDH15, AGGF1, CTSZ, MAPK1, BYSL, EE2, LGALS1, AXIN1, CEBPG, CDK5, ID3, FZD2, DCTN5, HSP90AB1, BCAR3, CTNNBIP1, ATF6, EPHA1
GO:0009653	anatomical structure morphogenesis	0.0006562	16	0.00%	0	MTM1, NAGLU, NEDD8, MFGE8, GPR4, AGGF1, CTSZ, MAPK1, BYSL, AXIN1, CDK5, FZD2, ID3, BCAR3, CTNNBIP1, EPHA1
GO:0048513	animal organ development beta-catenin destruction complex	0.000127866	19	0.00%	0	MTM1, NAGLU, CDKN2B, GPR4, CTSZ, MAPK1, BYSL, EE2, LGALS1, AXIN1, CEBPG, CDK5, ID3, DCTN5, FZD2, BCAR3, HSP90AB1, CTNNBIP1, ATF6
GO:1904886	disassembly	0.004245634	3	0.00%	0	FZD2, AXIN1, FRAT2
GO:0065007	biological regulation	4.61E-07	52	0.00%	0	DCBLD2, NEDD8, LY96, GPR4, COX8A, CCL23, BCL7B, C4BPB, COMMD4, EE2, MIF, HIST1H1D, GABRD, CDK5, DDN, ID3, CD2BP2, BCAR3, LMAN1, CTNNBIP1, AHCTF1, GBF1, MTM1, FLOT2, CDKN2B, MFGE8, FRAT2, CDH15, AGGF1, MR1, KIFAP3, HIST1H1B, ARFGEF2, CTSZ, MAPK1, GRIN2D, GOT1, KPNA4, DAP, LGALS1, GTF2F2, AXIN1, LSM3, CEBPG, FZD2, HSP90AB1, FXR2, MAPRE1, ATF6, EPHA1, FAM20B, EIF4EBP2
GO:0009056	catabolic process	0.003827336	16	0.00%	0	MTM1, GOT1, NAGLU, DAP, HERC4, NEDD8, AXIN1, LSM3, C18orf25, CDK5, HSP90AB1, COX8A, FXR2, CTSZ, C4BPB, H1F0
GO:0007154	cell communication	0.000898247	31	0.00%	0	GBF1, MTM1, FLOT2, DCBLD2, CDKN2B, NEDD8, LY96, FRAT2, GPR4, MR1, KIFAP3, ARFGEF2, BCL7B, CCL23,

GO:0030154	cell differentiation	0.002005838	19	0.00%	0	MAPK1, GRIN2D, GOT1, DAP, LGALS1, MIF, GTF2F2, AXIN1, CDK5, GABRD, FZD2, HSP90AB1, BCAR3, CTNNBIP1, ATF6, EPHA1, EIF4EBP2
GO:0008283	cell proliferation	0.001483763	14	0.00%	0	FLOT2, NAGLU, HERC4, CDKN2B, GPR4, CDH15, AGGF1, BCL7B, MAPK1, BYSL, EEF2, LGALS1, AXIN1, CEBPG, CDK5, ID3, FZD2, CTNNBIP1, EPHA1
GO:0007166	cell surface receptor signaling pathway	0.001988295	18	0.00%	0	EEF2, CDKN2B, MIF, MFGE8, FRAT2, CDK5, AGGF1, KIFAP3, CCL23, MAPRE1, MAPK1, BYSL, CTNNBIP1, EPHA1
GO:0007267	cell-cell signaling	0.008900922	12	0.02%	0.02	CDKN2B, NEDD8, LY96, FRAT2, BCL7B, CCL23, MAPK1, GRIN2D, GOT1, MIF, AXIN1, GTF2F2, CDK5, FZD2
GO:0044248	cellular catabolic process	0.008928515	14	0.02%	0.02	HSP90AB1, CTNNBIP1, EPHA1, EIF4EBP2
GO:0022607	cellular component assembly	9.75E-05	19	0.00%	0	FLOT2, AXIN1, FRAT2, GABRD, CDK5, FZD2, BCL7B, CCL23, MAPK1, CTNNBIP1, GRIN2D, EIF4EBP2
GO:0044085	cellular component biogenesis	1.55E-05	21	0.00%	0	MTM1, GOT1, DAP, HERC4, NEDD8, AXIN1, LSM3, C18orf25, CDK5, HSP90AB1, COX8A, FXR2, CTSZ, H1F0
GO:0016043	cellular component organization	4.30E-08	35	0.00%	0	MTM1, FLOT2, NDUFA1, KIFAP3, HIST1H1B, CTSZ, MIF, AXIN1, GTF2F2, LSM3, CDK5, HIST1H1D, CD2BP2, LMAN1, CTNNBIP1, AHCTF1, MAPRE1, EPHA1, H1F0
						DCBLD2, COX8A, C4BPB, MIF, MRPS12, HIST1H1D, CDK5, CD2BP2, LMAN1, CTNNBIP1, AHCTF1, H1F0, GBF1, MTM1, FLOT2, NAGLU, CDKN2B, NDUFA1, FRAT2, MFGE8, CDH15, KIFAP3, HIST1H1B, ARFGEF2, CTSZ, MAPK1, LGALS1, AXIN1, GTF2F2, LSM3, FZD2, HSP90AB1, MAPRE1, FAM20B, EPHA1

GO:0071840	cellular component organization or biogenesis	3.68E-09	37	0.00%	0	DCBLD2, COX8A, C4BPB, MIF, MRPS12, HIST1H1D, CDK5, CD2BP2, LMAN1, AHCTF1, CTNNBIP1, H1F0, GBF1, MTM1, FLOT2, NAGLU, CDKN2B, NDUFA1, IMP4, FRAT2, MFGE8, CDH15, KIFAP3, HIST1H1B, ARFGEF2, CTSZ, BYSL, MAPK1, LGALS1, AXIN1, GTF2F2, LSM3, FZD2, HSP90AB1, MAPRE1, FAM20B, EPHA1
GO:0048869	cellular developmental process	0.00022203	21	0.00%	0	MTM1, FLOT2, NAGLU, HERC4, CDKN2B, GPR4, CDH15, AGGF1, BCL7B, MAPK1, BYSL, EEF2, LGALS1, MIF, AXIN1, CEBPG, CDK5, ID3, FZD2, CTNNBIP1, EPHA1
GO:0044260	cellular macromolecule metabolic process	0.000350622	42	0.00%	0	NEDD8, C18orf25, CCL23, C4BPB, COMMD4, EEF2, MIF, MRPS12, HIST1H1D, CDK5, DDN, ID3, CD2BP2, BCAR3, LMAN1, AHCTF1, CTNNBIP1, H1F0, MTM1, CDKN2B, HERC4, IMP4, DNAJC8, MFGE8, AGGF1, HIST1H1B, ARFGEF2, CTSZ, MAPK1, BYSL, GRIN2D, DAP, AXIN1, GTF2F2, LSM3, CEBPG, FZD2, HSP90AB1, ATF6, EPHA1, EIF4EBP2
GO:0044237	cellular metabolic process	0.000178547	48	0.00%	0	NEDD8, C18orf25, COX8A, CCL23, C4BPB, CYC1, COMMD4, EEF2, MIF, MRPS12, HIST1H1D, CDK5, DDN, ID3, CD2BP2, BCAR3, LMAN1, CTNNBIP1, AHCTF1, H1F0, GBF1, MTM1, HERC4, CDKN2B, NDUFA1, IMP4, DNAJC8, MFGE8, AGGF1, HIST1H1B, ARFGEF2, CTSZ, MAPK1, BYSL, GRIN2D, GOT1, DAP, AXIN1, GTF2F2, LSM3, CEBPG, FZD2, HSP90AB1, FXR2, ATF6, EPHA1, FAM20B, EIF4EBP2
GO:0009987	cellular process	1.22E-06	63	0.00%	0	DCBLD2, NEDD8, LY96, C18orf25, GPR4, COX8A, CCL23, BCL7B, C4BPB, CYC1, COMMD4, EEF2, MIF, MRPS12, HIST1H1D, GABRD, CDK5, DDN, ID3, CD2BP2, BCAR3, LMAN1, CTNNBIP1,

						AHCTF1, H1F0, GBF1, MYBPC2, MTM1, FLOT2, NAGLU, HERC4, CDKN2B, NDUFA1, IMP4, DNAJC8, MFGE8, FRAT2, CDH15, AGGF1, MR1, KIFAP3, HIST1H1B, ARFGEF2, CTSZ, MAPK1, BYSL, GRIN2D, GOT1, KPNA4, DAP, LGALS1, GTF2F2, AXIN1, LSM3, CEBPG, FZD2, HSP90AB1, FXR2, MAPRE1, ATF6, EPHA1, FAM20B, EIF4EBP2
GO:0070887	cellular response to chemical stimulus	0.001346639	17	0.00%	0	GBF1, CDKN2B, NEDD8, LY96, CCL23, MAPK1, BYSL, GOT1, EEF2, LGALS1, AXIN1, GTF2F2, MIF, ID3, HSP90AB1, ATF6, EIF4EBP2
GO:0071310	cellular response to organic substance	0.000551398	16	0.00%	0	GOT1, EEF2, LGALS1, CDKN2B, NEDD8, LY96, MIF, GTF2F2, AXIN1, ID3, HSP90AB1, CCL23, MAPK1, BYSL, ATF6, EIF4EBP2
GO:0051716	cellular response to stimulus	9.15E-05	35	0.00%	0	DCBLD2, NEDD8, LY96, GPR4, COX8A, BCL7B, CCL23, EEF2, MIF, GABRD, CDK5, ID3, BCAR3, CTNNBIP1, GBF1, MTM1, CDKN2B, FRAT2, KIFAP3, MR1, ARFGEF2, BYSL, MAPK1, GRIN2D, GOT1, DAP, LGALS1, AXIN1, GTF2F2, CEBPG, FZD2, HSP90AB1, EPHA1, ATF6, EIF4EBP2
GO:0032502	developmental process	4.49E-06	29	0.00%	0	MTM1, FLOT2, NAGLU, HERC4, CDKN2B, NEDD8, MFGE8, FRAT2, GPR4, CDH15, AGGF1, CTSZ, BCL7B, MAPK1, BYSL, EEF2, LGALS1, MIF, AXIN1, CEBPG, CDK5, ID3, FZD2, DCTN5, HSP90AB1, BCAR3, CTNNBIP1, ATF6, EPHA1
GO:0010467	gene expression	0.000684211	31	0.00%	0	FLOT2, CDKN2B, NEDD8, IMP4, DNAJC8, AGGF1, HIST1H1B, CTSZ, MAPK1, BYSL, C4BPB, COMMD4, EEF2, DAP, MIF, GTF2F2, AXIN1, LSM3, CEBPG, MRPS12, HIST1H1D, CDK5, DDN, ID3, FZD2, CD2BP2, FXR2, AHCTF1, CTNNBIP1, ATF6, EIF4EBP2
GO:0002376	immune system process	0.000525349	18	0.00%	0	GBF1, FLOT2, CDKN2B, LY96, MR1, KIFAP3, COX8A, CCL23, MAPK1, C4BPB, EEF2, LGALS1, MIF, CEBPG, DCTN5, FXR2, HSP90AB1, CTNNBIP1
GO:0065003	macromolecular complex assembly	0.000231204	15	0.00%	0	NDUFA1, MIF, GTF2F2, AXIN1, LSM3, HIST1H1D, CD2BP2, KIFAP3, LMAN1, HIST1H1B, CTSZ, MAPRE1, AHCTF1, CTNNBIP1, H1F0
GO:0043933	macromolecular complex subunit organization	5.09E-06	18	0.00%	0	NDUFA1, FRAT2, KIFAP3, HIST1H1B, CTSZ, AXIN1, GTF2F2, MIF, LSM3, MRPS12, HIST1H1D, FZD2, CD2BP2, LMAN1, CTNNBIP1, AHCTF1, MAPRE1, H1F0
GO:0009057	macromolecule catabolic process	0.000993401	12	0.00%	0	MTM1, NAGLU, HERC4, NEDD8, AXIN1, LSM3, C18orf25, CDK5, HSP90AB1, CTSZ, C4BPB, H1F0
GO:0043170	macromolecule metabolic process	0.000978265	44	0.00%	0	NEDD8, C18orf25, CCL23, C4BPB, COMMD4, EEF2, MIF, MRPS12, HIST1H1D, CDK5, DDN, ID3, CD2BP2, BCAR3, LMAN1, AHCTF1, CTNNBIP1, H1F0, MTM1, FLOT2, NAGLU, CDKN2B, HERC4, IMP4, DNAJC8, MFGE8, AGGF1, HIST1H1B, ARFGEF2, CTSZ, MAPK1, BYSL, GRIN2D, DAP, AXIN1, GTF2F2, LSM3, CEBPG, FZD2, HSP90AB1, FXR2, ATF6, EPHA1, EIF4EBP2
GO:0008152	metabolic process	0.001714225	51	0.00%	0	NEDD8, C18orf25, COX8A, CCL23, C4BPB, CYC1, COMMD4, EEF2, MIF, MRPS12, HIST1H1D, CDK5, DDN, ID3, CD2BP2, BCAR3, LMAN1, CTNNBIP1, AHCTF1, H1F0, GBF1, MTM1, FLOT2, NAGLU, HERC4, CDKN2B, NDUFA1, IMP4, DNAJC8, MFGE8, AGGF1, HIST1H1B, ARFGEF2, CTSZ, MAPK1, BYSL, GRIN2D, GOT1, DAP, GTF2F2, AXIN1, LSM3, CEBPG, FZD2, HSP90AB1, FXR2, ATF6, EPHA1, FAM20B, CYB561D2, EIF4EBP2

GO:0007275	multicellular organism development	0.000988858	23	0.00%	0	MTM1, NAGLU, CDKN2B, MFGE8, FRAT2, GPR4, AGGF1, CTSZ, MAPK1, BYSL, EEF2, LGALS1, AXIN1, CEBPG, CDK5, ID3, DCTN5, FZD2, HSP90AB1, BCAR3, CTNNBIP1, ATF6, EPHA1, LY96, GPR4, C4BPB, EEF2, MIF, CDK5, ID3, BCAR3, LMAN1, CTNNBIP1, MTM1, MYBPC2, NAGLU, CDKN2B, HERC4, FRAT2, MFGE8, AGGF1, MR1, ARFGEF2, CTSZ, BYSL, MAPK1, GRIN2D, LGALS1, AXIN1, CEBPG, DCTN5, FZD2, HSP90AB1, EPHA1, ATF6, EIF4EBP2, GBF1, KPNA4, LGALS1, HERC4, LY96, MIF, GTF2F2, MFGE8, MR1, COX8A, FXR2, HSP90AB1, MAPK1, BYSL, EIF4EBP2
GO:0032501	multicellular organismal process	1.21E-06	33	0.00%	0	MTM1, FLOT2, EEF2, LGALS1, AXIN1, CDK5, CDH15, ID3, FZD2, MTM1, FLOT2, DCBLD2, CDKN2B, LY96, GPR4, KIFAP3, HIST1H1B, CCL23, MAPK1, C4BPB, DAP, LGALS1, MIF, AXIN1, LSM3, CEBPG, CDK5, HIST1H1D, ID3, CD2BP2, HSP90AB1, FXR2, MAPRE1, CTNNBIP1, EPHA1, EIF4EBP2
GO:0051704	multi-organism process	0.003807121	15	0.00%	0	MTM1, CDKN2B, HIST1H1B, C4BPB, DAP, AXIN1, MIF, CDK5, HIST1H1D, CD2BP2, ID3, FXR2, HSP90AB1, CTNNBIP1, EPHA1, EIF4EBP2
GO:0061061	muscle structure development	0.00028054	9	0.00%	0	MTM1, DCBLD2, CDKN2B, LY96, KIFAP3, HIST1H1B, CCL23, MAPK1, C4BPB, DAP, LGALS1, MIF, AXIN1, LSM3, CEBPG, CDK5, HIST1H1D, ID3, CD2BP2, FXR2, HSP90AB1, MAPRE1, CTNNBIP1, EPHA1, EIF4EBP2
GO:0048519	negative regulation of biological process	2.06E-06	27	0.00%	0	MTM1, CDKN2B, HIST1H1B, C4BPB, DAP, AXIN1, MIF, CDK5, HIST1H1D, CD2BP2, ID3, FXR2, HSP90AB1, CTNNBIP1, EPHA1, EIF4EBP2
GO:0031324	negative regulation of cellular metabolic process	0.00086878	16	0.00%	0	MTM1, CDKN2B, HIST1H1B, C4BPB, DAP, AXIN1, MIF, CDK5, HIST1H1D, CD2BP2, ID3, FXR2, HSP90AB1, CTNNBIP1, EPHA1, EIF4EBP2
GO:0048523	negative regulation of cellular process	0.000152704	23	0.00%	0	MTM1, CDKN2B, HIST1H1B, C4BPB, DAP, AXIN1, MIF, CDK5, HIST1H1D, CD2BP2, ID3, FXR2, HSP90AB1, CTNNBIP1, EPHA1, EIF4EBP2
GO:0010629	negative regulation of gene expression	0.001748952	13	0.00%	0	FLOT2, DAP, MIF, AXIN1, LSM3, HIST1H1D, CDK5, ID3, FXR2, HIST1H1B, C4BPB, CTNNBIP1, EIF4EBP2
GO:0010605	negative regulation of macromolecule metabolic process	0.000279927	17	0.00%	0	MTM1, FLOT2, CDKN2B, HIST1H1B, C4BPB, DAP, AXIN1, MIF, LSM3, CDK5, HIST1H1D, ID3, FXR2, HSP90AB1, CTNNBIP1, EPHA1, EIF4EBP2
GO:0009892	negative regulation of metabolic process	0.000168458	18	0.00%	0	MTM1, FLOT2, CDKN2B, HIST1H1B, C4BPB, DAP, AXIN1, MIF, LSM3, CDK5, HIST1H1D, ID3, CD2BP2, FXR2, HSP90AB1, CTNNBIP1, EPHA1, EIF4EBP2
GO:0051172	negative regulation of nitrogen compound metabolic process	0.000411432	16	0.00%	0	MTM1, FLOT2, DAP, CDKN2B, MIF, AXIN1, HIST1H1D, CDK5, ID3, HSP90AB1, FXR2, HIST1H1B, C4BPB, CTNNBIP1, EPHA1, EIF4EBP2
GO:0006807	nitrogen compound metabolic process	0.000315375	47	0.00%	0	NEDD8, C18orf25, COX8A, CCL23, C4BPB, CYC1, COMMD4, EEF2, MIF, MRPS12, HIST1H1D, CDK5, DDN, ID3, CD2BP2, BCAR3, LMAN1, AHCTF1, CTNNBIP1, H1F0, MTM1, FLOT2, NAGLU, CDKN2B, HERC4, NDUFA1, IMP4, DNAJC8, MFGE8, AGGF1, HIST1H1B, CTSZ, MAPK1, BYSL, GRIN2D, GOT1, DAP, AXIN1, GTF2F2, LSM3, CEBPG, FZD2, HSP90AB1, FXR2, ATF6, EPHA1, EIF4EBP2
GO:0071704	organic substance metabolic process	0.002140448	48	0.00%	0	NEDD8, C18orf25, COX8A, CCL23, C4BPB, CYC1, COMMD4, EEF2, MIF, MRPS12, HIST1H1D, CDK5, DDN, ID3, CD2BP2, BCAR3, LMAN1, CTNNBIP1, AHCTF1, H1F0, MTM1, FLOT2, NAGLU, HERC4, CDKN2B, NDUFA1, IMP4, DNAJC8, MFGE8, AGGF1, HIST1H1B, ARFGEF2, CTSZ, MAPK1, BYSL, GRIN2D, GOT1, DAP, AXIN1, GTF2F2, LSM3, CEBPG, FZD2, HSP90AB1, FXR2, ATF6, EPHA1, EIF4EBP2
GO:1901564	organonitrogen compound	0.007895034	33	0.02%	0.02	NEDD8, C18orf25, COX8A, CCL23, C4BPB, CYC1, EEF2, MIF, MRPS12,

	metabolic process					HIST1H1D, CDK5, BCAR3, LMAN1, FLOT2, MTM1, NAGLU, CDKN2B, HERC4, NDUFA1, MFGE8, HIST1H1B, CTSZ, MAPK1, GRIN2D, GOT1, DAP, AXIN1, CEBPG, FZD2, FXR2, HSP90AB1, EPHA1, EIF4EBP2
GO:0048518	positive regulation of biological process	7.85E-10	33	0.00%	0	LY96, COX8A, CCL23, C4BPB, EEF2, MIF, CDK5, DDN, ID3, BCAR3, LMAN1, CTNNBIP1, FLOT2, MTM1, CDKN2B, MFGE8, CDH15, AGGF1, KIFAP3, HIST1H1B, ARFGEF2, MAPK1, DAP, LGALS1, AXIN1, GTF2F2, CEBPG, FZD2, HSP90AB1, MAPRE1, ATF6, EPHA1, FAM20B
GO:0031325	positive regulation of cellular metabolic process	0.001413453	17	0.00%	0	CDKN2B, COX8A, HIST1H1B, CCL23, MAPK1, DAP, EEF2, AXIN1, GTF2F2, MIF, CEBPG, CDK5, DDN, FZD2, BCAR3, HSP90AB1, ATF6
GO:0048522	positive regulation of cellular process	4.27E-08	29	0.00%	0	FLOT2, CDKN2B, LY96, MFGE8, CDH15, AGGF1, COX8A, HIST1H1B, CCL23, MAPK1, DAP, EEF2, LGALS1, MIF, GTF2F2, AXIN1, CEBPG, CDK5, DDN, ID3, FZD2, HSP90AB1, BCAR3, LMAN1, MAPRE1, CTNNBIP1, ATF6, EPHA1, FAM20B
GO:0010604	positive regulation of macromolecule metabolic process	0.000664684	17	0.00%	0	CDKN2B, HIST1H1B, CCL23, MAPK1, C4BPB, DAP, EEF2, MIF, AXIN1, GTF2F2, CEBPG, CDK5, DDN, FZD2, BCAR3, HSP90AB1, ATF6
GO:0009893	positive regulation of metabolic process	0.000650123	18	0.00%	0	CDKN2B, COX8A, HIST1H1B, CCL23, MAPK1, C4BPB, DAP, EEF2, MIF, AXIN1, GTF2F2, CEBPG, CDK5, DDN, FZD2, BCAR3, HSP90AB1, ATF6
GO:0044093	positive regulation of molecular function	0.001093635	15	0.00%	0	GBF1, DAP, MIF, AXIN1, CEBPG, CDK5, FZD2, HSP90AB1, BCAR3, ARFGEF2, CCL23, MAPRE1, MAPK1, EPHA1, GRIN2D
GO:0051173	positive regulation of nitrogen compound metabolic process	0.000544576	17	0.00%	0	CDKN2B, HIST1H1B, CCL23, MAPK1, C4BPB, DAP, EEF2, MIF, AXIN1, GTF2F2, CEBPG, CDK5, DDN, FZD2, BCAR3, HSP90AB1, ATF6
GO:0010638	positive regulation of organelle organization	0.008993792	9	0.02%	0.02	CDKN2B, CDK5, COX8A, HIST1H1B, LMAN1, MAPK1, MAPRE1, EPHA1, FAM20B
GO:0051247	positive regulation of protein metabolic process	0.002261232	12	0.00%	0	EEF2, DAP, MIF, AXIN1, CEBPG, CDK5, HSP90AB1, BCAR3, HIST1H1B, CCL23, MAPK1, C4BPB
GO:0044238	primary metabolic process	0.006764705	46	0.02%	0.02	NEDD8, C18orf25, COX8A, CCL23, C4BPB, CYC1, COMMD4, EEF2, MIF, MRPS12, HIST1H1D, CDK5, DDN, ID3, CD2BP2, BCAR3, LMAN1, AHCTF1, CTNNBIP1, H1FO, MTM1, FLOT2, CDKN2B, HERC4, NDUFA1, IMP4, DNAJC8, MFGE8, AGGF1, HIST1H1B, CTSZ, MAPK1, BYSL, GRIN2D, GOT1, DAP, AXIN1, GTF2F2, LSM3, CEBPG, FZD2, HSP90AB1, FXR2, ATF6, EPHA1, EIF4EBP2
GO:0071822	protein complex subunit organization	0.002568001	13	0.00%	0	NDUFA1, MIF, GTF2F2, AXIN1, FRAT2, MRPS12, FZD2, KIFAP3, LMAN1, CTSZ, MAPRE1, AHCTF1, CTNNBIP1
GO:0008104	protein localization	0.008115101	16	0.02%	0.02	GBF1, MTM1, FLOT2, KPNA4, NEDD8, MIF, AXIN1, CDK5, KIFAP3, HSP90AB1, HIST1H1B, ARFGEF2, MAPRE1, MAPK1, AHCTF1, FAM20B
GO:0051098	regulation of binding	0.003677534	6	0.00%	0	HSP90AB1, CCL23, MAPRE1, CTNNBIP1, CEBPG, CDK5
GO:0050789	regulation of biological process	3.13E-08	52	0.00%	0	DCBLD2, NEDD8, LY96, GPR4, COX8A, CCL23, BCL7B, C4BPB, COMMD4, EEF2, MIF, HIST1H1D, GABRD, CDK5, DDN, ID3, CD2BP2, BCAR3, LMAN1, CTNNBIP1, AHCTF1, GBF1, MTM1,

						FLOT2, CDKN2B, MFGE8, FRAT2, CDH15, AGGF1, MR1, KIFAP3, HIST1H1B, ARFGEF2, CTSZ, MAPK1, GRIN2D, GOT1, KPNA4, DAP, LGALS1, GTF2F2, AXIN1, LSM3, CEBPG, FZD2, HSP90AB1, FXR2, MAPRE1, ATF6, EPHA1, FAM20B, EIF4EBP2
GO:0051128	regulation of cellular component organization	0.00156244	16	0.00%	0	MTM1, DCBLD2, LGALS1, CDKN2B, GTF2F2, MFGE8, CDK5, COX8A, LMAN1, HIST1H1B, MAPRE1, MAPK1, C4BPB, CTNNBIP1, EPHA1, FAM20B
GO:0031323	regulation of cellular metabolic process	0.005750123	29	0.00%	0	MTM1, CDKN2B, NEDD8, COX8A, HIST1H1B, CCL23, MAPK1, C4BPB, COMMD4, DAP, EE2, MIF, GTF2F2, AXIN1, CEBPG, HIST1H1D, CDK5, DDN, ID3, FZD2, CD2BP2, HSP90AB1, FXR2, BCAR3, AHCTF1, CTNNBIP1, EPHA1, ATF6, EIF4EBP2
GO:0050794	regulation of cellular process	1.08E-07	50	0.00%	0	DCBLD2, NEDD8, LY96, GPR4, COX8A, CCL23, BCL7B, C4BPB, COMMD4, EE2, MIF, HIST1H1D, GABRD, CDK5, DDN, ID3, CD2BP2, BCAR3, LMAN1, CTNNBIP1, AHCTF1, GBF1, MTM1, FLOT2, CDKN2B, MFGE8, FRAT2, CDH15, AGGF1, MR1, KIFAP3, HIST1H1B, ARFGEF2, MAPK1, GRIN2D, GOT1, KPNA4, DAP, LGALS1, GTF2F2, AXIN1, CEBPG, FZD2, HSP90AB1, FXR2, MAPRE1, ATF6, EPHA1, FAM20B, EIF4EBP2
GO:0032268	regulation of cellular protein metabolic process	0.001072431	16	0.00%	0	MTM1, EE2, DAP, CDKN2B, MIF, AXIN1, CDK5, HSP90AB1, FXR2, BCAR3, HIST1H1B, CCL23, MAPK1, C4BPB, EPHA1, EIF4EBP2
GO:0050793	regulation of developmental process	0.006558277	14	0.02%	0.02	MTM1, FLOT2, LGALS1, MIF, AXIN1, GPR4, CDK5, CDH15, AGGF1, ID3, FZD2, MAPK1, CTNNBIP1, EPHA1
GO:0060255	regulation of macromolecule	0.001199016	30	0.00%	0	MTM1, FLOT2, CDKN2B, NEDD8, HIST1H1B, CTSZ, CCL23, MAPK1, C4BPB, COMMD4, DAP, EE2, MIF,
	metabolic process					GTF2F2, AXIN1, LSM3, CEBPG, HIST1H1D, CDK5, DDN, ID3, FZD2, HSP90AB1, FXR2, BCAR3, AHCTF1, CTNNBIP1, ATF6, EPHA1, EIF4EBP2
GO:0019222	regulation of metabolic process	0.000428052	32	0.00%	0	NEDD8, COX8A, CCL23, C4BPB, COMMD4, EE2, MIF, HIST1H1D, CDK5, DDN, ID3, CD2BP2, BCAR3, AHCTF1, CTNNBIP1, FLOT2, MTM1, CDKN2B, HIST1H1B, CTSZ, MAPK1, DAP, AXIN1, GTF2F2, CEBPG, LSM3, FZD2, FXR2, HSP90AB1, ATF6, EPHA1, EIF4EBP2
GO:0065009	regulation of molecular function	0.000832051	19	0.00%	0	GBF1, CDKN2B, ARFGEF2, CCL23, MAPK1, GRIN2D, DAP, MIF, AXIN1, CEBPG, CDK5, ID3, CD2BP2, FZD2, BCAR3, HSP90AB1, MAPRE1, CTNNBIP1, EPHA1
GO:0051171	regulation of nitrogen compound metabolic process	0.002189663	29	0.00%	0	MTM1, FLOT2, CDKN2B, NEDD8, HIST1H1B, CTSZ, CCL23, MAPK1, C4BPB, COMMD4, DAP, EE2, MIF, GTF2F2, AXIN1, CEBPG, HIST1H1D, CDK5, DDN, ID3, FZD2, HSP90AB1, FXR2, BCAR3, AHCTF1, CTNNBIP1, ATF6, EPHA1, EIF4EBP2
GO:0080090	regulation of primary metabolic process	0.003080236	29	0.00%	0	MTM1, FLOT2, CDKN2B, NEDD8, HIST1H1B, CTSZ, CCL23, MAPK1, C4BPB, COMMD4, DAP, EE2, MIF, GTF2F2, AXIN1, CEBPG, HIST1H1D, CDK5, DDN, ID3, FZD2, HSP90AB1, FXR2, BCAR3, AHCTF1, CTNNBIP1, ATF6, EPHA1, EIF4EBP2
GO:0043393	regulation of protein binding	0.004726962	5	0.00%	0	HSP90AB1, CCL23, MAPRE1, CTNNBIP1, CDK5
GO:0051246	regulation of protein metabolic process	1.54E-05	19	0.00%	0	MTM1, FLOT2, CDKN2B, HIST1H1B, CCL23, CTSZ, MAPK1, C4BPB, DAP, EE2, MIF, AXIN1, CEBPG, CDK5, BCAR3, FXR2, HSP90AB1, EPHA1, EIF4EBP2
GO:0042221	response to chemical	0.002166211	21	0.00%	0	GBF1, CDKN2B, NEDD8, LY96, CCL23, MAPK1, BYSL, CYC1, GOT1, EE2, KPNA4, LGALS1, MIF, AXIN1, GTF2F2,

						CDK5, ID3, BCAR3, HSP90AB1, ATF6, EIF4EBP2
GO:0009605	response to external stimulus	0.001245943	15	0.00%	0	GBF1, EEF2, DAP, CDKN2B, LY96, MIF, CDK5, MR1, FXR2, COX8A, CCL23, MAPK1, BYSL, C4BPB, GRIN2D
GO:0014070	response to organic cyclic compound	0.002874616	10	0.00%	0	GOT1, EEF2, LGALS1, NEDD8, AXIN1, CDK5, ID3, HSP90AB1, BYSL, MAPK1, CDKN2B, NEDD8, LY96, CCL23, MAPK1, BYSL, CYC1, GOT1, EEF2, LGALS1, AXIN1, GTF2F2, MIF, CDK5, ID3, HSP90AB1, ATF6, EIF4EBP2
GO:0010033	response to organic substance	0.000605808	18	0.00%	0	
GO:1901700	response to oxygen-containing compound	0.002276073	12	0.00%	0	GOT1, KPNA4, EEF2, LGALS1, LY96, MIF, CDK5, ID3, BYSL, MAPK1, CYC1, EIF4EBP2
GO:0050896	response to stimulus	3.43E-06	41	0.00%	0	DCBLD2, NEDD8, LY96, GPR4, COX8A, BCL7B, CCL23, C4BPB, CYC1, EEF2, MIF, GABRD, CDK5, ID3, BCAR3, LMAN1, CTNNBIP1, GBF1, MTM1, CDKN2B, MFGE8, FRAT2, MR1, KIFAP3, ARFGEF2, BYSL, MAPK1, GRIN2D, GOT1, DAP, KPNA4, LGALS1, AXIN1, GTF2F2, CEBPG, FZD2, FXR2, HSP90AB1, ATF6, EPHA1, EIF4EBP2
GO:0006950	response to stress	0.000241022	22	0.00%	0	DCBLD2, LY96, GPR4, MR1, COX8A, CCL23, MAPK1, C4BPB, DAP, EEF2, KPNA4, LGALS1, MIF, AXIN1, CEBPG, CDK5, ID3, FXR2, HSP90AB1, LMAN1, CTNNBIP1, ATF6
GO:0009611	response to wounding	0.006154923	8	0.03%	0.02	ID3, DCBLD2, LMAN1, LGALS1, MAPK1, C4BPB, GPR4, CDK5
GO:0007165	signal transduction	0.000690034	30	0.00%	0	GBF1, MTM1, DCBLD2, CDKN2B, NEDD8, LY96, FRAT2, GPR4, MR1, KIFAP3, ARFGEF2, BCL7B, CCL23, MAPK1, GRIN2D, GOT1, DAP, LGALS1, MIF, GTF2F2, AXIN1, CDK5, GABRD, FZD2, HSP90AB1, BCAR3, CTNNBIP1, ATF6, EPHA1, EIF4EBP2
GO:0023052	signaling	0.000880782	31	0.00%	0	GBF1, MTM1, FLOT2, DCBLD2, CDKN2B, NEDD8, LY96, FRAT2, GPR4, MR1, KIFAP3, ARFGEF2, BCL7B, CCL23, MAPK1, GRIN2D, GOT1, DAP, LGALS1, MIF, GTF2F2, AXIN1, CDK5, GABRD, FZD2, HSP90AB1, BCAR3, CTNNBIP1, ATF6, EPHA1, EIF4EBP2
GO:0044700	single organism signaling	0.000814049	31	0.00%	0	GBF1, MTM1, FLOT2, DCBLD2, CDKN2B, NEDD8, LY96, FRAT2, GPR4, MR1, KIFAP3, ARFGEF2, BCL7B, CCL23, MAPK1, GRIN2D, GOT1, DAP, LGALS1, MIF, GTF2F2, AXIN1, CDK5, GABRD, FZD2, HSP90AB1, BCAR3, CTNNBIP1, ATF6, EPHA1, EIF4EBP2
GO:0044707	single-multicellular organism process	2.90E-07	31	0.00%	0	MTM1, NAGLU, CDKN2B, LY96, MFGE8, FRAT2, GPR4, AGGF1, MR1, CTSZ, ARFGEF2, MAPK1, BYSL, C4BPB, GRIN2D, EEF2, LGALS1, MIF, AXIN1, CEBPG, CDK5, ID3, FZD2, DCTN5, HSP90AB1, BCAR3, LMAN1, CTNNBIP1, ATF6, EPHA1, EIF4EBP2
GO:0044763	single-organism cellular process	4.72E-07	46	0.00%	0	DCBLD2, LY96, GPR4, COX8A, BCL7B, CCL23, CYC1, EEF2, MIF, MRPS12, GABRD, CDK5, ID3, LMAN1, AHCTF1, CTNNBIP1, H1FO, GBF1, MYBPC2, MTM1, FLOT2, NAGLU, CDKN2B, HERC4, NDUFA1, MFGE8, FRAT2, CDH15, AGGF1, KIFAP3, HIST1H1B, ARFGEF2, CTSZ, BYSL, MAPK1, GRIN2D, GOT1, DAP, LGALS1, AXIN1, CEBPG, FZD2, HSP90AB1, MAPRE1, EPHA1, EIF4EBP2
GO:0044767	single-organism developmental process	1.50E-05	28	0.00%	0	MTM1, FLOT2, NAGLU, HERC4, CDKN2B, MFGE8, FRAT2, GPR4, CDH15, AGGF1, CTSZ, BCL7B, MAPK1, BYSL, EEF2, LGALS1, MIF, AXIN1, CEBPG, CDK5, ID3, FZD2, DCTN5, HSP90AB1, BCAR3, CTNNBIP1, ATF6, EPHA1
GO:0044699	single-organism process	1.81E-06	55	0.00%	0	DCBLD2, NEDD8, LY96, GPR4, COX8A, CCL23, BCL7B, C4BPB, CYC1, EEF2,

						MIF, MRPS12, GABRD, CDK5, ID3, BCAR3, LMAN1, AHCTF1, CTNNBIP1, H1FO, GBF1, MYBPC2, MTM1, FLOT2, NAGLU, HERC4, CDKN2B, NDUFA1, MFGE8, FRAT2, CDH15, AGGF1, MR1, KIFAP3, HIST1H1B, ARFGEF2, CTSZ, MAPK1, BYSL, GRIN2D, GOT1, KPNA4, DAP, LGALS1, GTF2F2, AXIN1, CEBPG, DCTN5, FZD2, HSP90AB1, MAPRE1, ATF6, EPHA1, CYB561D2, EIF4EBP2, MTM1, NAGLU, CDKN2B, MFGE8, GPR4, AGGF1, CTSZ, MAPK1, BYSL, EE2, LGALS1, AXIN1, CEBPG, CDK5, ID3, DCTN5, FZD2, BCAR3, HSP90AB1, CTNNBIP1, ATF6, EPHA1
GO:0048731	system development	0.000379327	22	0.00%	0	

Table 4.9 Biological processes that ID3 & ID3 candidate target genes in tissue.

ID3 & ID3 gene-associated signaling pathways

ID3 and ID3 candidate target genes in blood. In order to understand the potential relationships among the genes in the most over-represented processes STRING, a database of predicted & identified protein-protein interactions was used to show direct (physical) and indirect (functional) associations. ID3 & ID3 candidate target genes in blood (134) was matched to the database and generated network maps for the genes involved in over-represented processes. We show that ID3 and ID3 candidate target genes in blood are involved in 5 pathways (Arrhythmogenic right ventricular-cardiomyopathy, Neuro-active ligand-receptor interaction, focal adhesion, ECM-receptor interaction, & hypertrophic cardiomyopathy as shown in Table 4.10 Interestingly two of the pathways represented ID3 as matching proteins. Focal adhesion pathway includes proteins: ACTN1, COL1A2, COL3A1, COL6A1, CTNNB1, IBSP, ID3, ITGA8, and MYL2. ECM-receptor interaction includes proteins: COL1A2, COL3A1, COL6A1, IBSP, ID3, and ITGA8. Figure 4.7.1 and 4.7.2 demonstrate both networks. Furthermore we also represented the biological

processes these genes (134) are signified as seen in Table 4.11. Overall 119 biological processes are over-represented via ID3 & ID3 candidate genes in blood.

Pathway ID	Pathway Description	Gene Count	False Discovery Rate	Matching proteins in your network
5412	Arrhythmogenic right ventricular cardiomyopathy (ARVC)	6	0.00241	ACTN1, CACNB4, CACNG3, CDH2, CTNNB1, ITGA8
4080	Neuroactive ligand-receptor interaction	9	0.00686	AGTR2, AVPR2, DRD1, GABRA2, GLRA1, GRIA2, GRIK5, GRIN2B, HTR4
4510	Focal adhesion	9	0.00686	ACTN1, COL1A2, COL3A1, COL6A1, CTNNB1, IBSP, ID3, ITGA8, MYL2
4512	Extracellular matrix-receptor interaction	6	0.0138	COL1A2, COL3A1, COL6A1, IBSP, ID3, ITGA8
5410	Hypertrophic cardiomyopathy (HCM)	5	0.0138	CACNB4, CACNG3, IL6, ITGA8, MYL2

Table 4.10 Demonstrated pathways of ID3 and ID3 candidate targets genes in blood.

Figure 4.6.1 Network demonstrated from STRING database of ID3-involved pathway in Extra Cellular Matrix Receptor (ECM) interaction. Genes (6) highlighted in red are involved in the pathway: COL1A2, COL3A1, COL6A1, IBSP, ID3, and ITGAB

Figure 4.6.2 Network demonstrated from STRING database of ID3-involved pathway in focal adhesion. Genes (9) highlighted in red are involved in the pathway: ACTN1, COL6A1, COL6A2, COL3A1, CTNNB1, IBSP, ID3, ITGAB, and MYL2.

Pathway ID	Pathway Description	Gene Count	False Discovery Rate	Matching Proteins in the Network
GO.0034332	adherens junction organization	7	0.000433	ACTN1,CDH12,CDH15,CDH17,CDH2,CDH6,CTNNB1
GO.0048856	anatomical structure development	45	0.00335	ABCB6,ACIN1,ACTA2,ACTN1,AEBP1,AGTR2,ANPEP,APOH,ASNS,ATP7B,AVPR2,BCL2L2,BYSL,CAD,CALR,CDH15,CFTR,COL17A1,COL1A2,COL6A1,CREBBP,CTNNB1,CYR61,DAB2,DHRS3,DLG2,DRD1,DTYMK,DZIP1,ETS1,FOXC2,FUT9,GAL,GFRA1,GPR37L1,HMGCS2,HNF4G,IBSP,LD3,IFNA2,IL6,LGR5,LIFR,MAN1A2,MYL2
GO.0009653	anatomical structure morphogenesis	27	0.0147	ACTA2,ACTN1,AGTR2,ANPEP,APAF1,ARID5B,BYSL,CACNB4,CDH2,COL1A2,COL6A1,CREBBP,CTGF,CTNNB1,CYR61,DHRS3,DZIP1,ETS1,GDF11,GFRA1,GRIN2B,ID3,IL6,LGR5,LIFR,MYL2,NDP
GO.0008015	blood circulation	10	0.00335	ACTA2,AGTR2,ANPEP,COL1A2,DRD1,FOXC2,GCLM,KCNH2,KNG1,MYL2
GO.0072358	cardiovascular system development	16	0.00533	ACTA2,AGTR2,ANPEP,CAD,CALR,CDH2,COL1A2,CTGF,CTNNB1,CYR61,DHRS3,ETS1,FOXC2,ID3,MYL2,NDP
GO.0007155	cell adhesion	17	0.0326	ACTN1,BCAM,BYSL,CDH12,CDH17,CFDP1,COL17A1,COL3A1,COL6A1,CTNNB1,FBLN5,HOXD3,IBSP,ID3,IFNA2,IL6,ITGA8
GO.0007154	cell communication	53	0.00156	ABCA4,AGTR2,ARID5B,ASB4,ASNS,BCAM,BCL2L2,CACNG3,CALR,CCL7,CDKL2,CFTR,COL1A2,COL3A1,CREBBP,CTH,CTNNB1,CYR61,DAAM1,DAB2,DCBLD2,DCX,DHRS3,DIRAS3,DLG2,DOCK9,DRD1,FKBP4,GAL,GDF11,GFRA1,GPR135,GPR37L1,GRIA2,GRP,HDAC4,HIST1H4C,HNF4G,HOXD3,HTR4,IFIH1,IFNA2,IGFBP2,IL13RA2,IL6,INHBB,ITGA8,KCNJ4,KCNK1,LGR5,LIFR,MPP3,NDP
GO.0048468	cell development	22	0.0305	ACTA2,ACTN1,AGTR2,ARID5B,CDH2,CFTR,COL3A1,COL6A1,CREBBP,CTNNB1,CYR61,DRD1,DZIP1,FOXC2,GFRA1,GRIN2B,HDAC4,IL6,ITGA8,LGR5,LIFR,MYL2
GO.0030154	cell differentiation	34	0.0301	ACIN1,ACTA2,ACTN1,AGTR2,ANPEP,APAF1,ARID5B,BYSL,CALR,CDH15,CFTR,COL3A1,COL6A1,CREBBP,CTGF,CTNNB1,CYR61,DAB2,DRD1,DTYMK,DZIP1,ETS1,FKBP4,GFRA1,GPR37L1,GRIN2B,HOXD3,IBSP,ID3,IFNA2,IL6,LGR5,LIFR,MYL2
GO.0034329	cell junction assembly	8	0.00283	ACTN1,CDH12,CDH15,CDH17,CDH2,CDH6,COL17A1,CTNNB1
GO.0008283	cell proliferation	13	0.0329	BCL2L2,BYSL,CREBBP,CTGF,CTNNB1,CYR61,DAB2,DTYMK,FOXC2,IFNA2,IL6,LGR5,NDP
GO.0007267	cell-cell signaling	19	0.0015	AGTR2,CACNG3,CCL7,CTGF,CTNNB1,CYR61,DLG2,DRD1,GABRA2,GAL,GLRA1,GRIA2,HTR4,IFNA2,IL6,KCNH2,KCNJ4,KCNK1,NDP
GO.0007160	cell-matrix adhesion	10	0.000131	ACTN1,BCAM,COL17A1,COL3A1,CTGF,CTNNB1,FBLN5,HOXD3,ID3,ITGA8
GO.0016043	cellular component organization	50	0.00226	ABCA4,ACIN1,ADAMTS3,APAF1,ARID5B,CACNB4,CALR,CCL7,CCT8,CDH12,CDH15,CDH17,CDH6,CHEK1,COL17A1,COL3A1,COL6A1,COPB2,CREBBP,CRYA,CTGF,CTH
GO.0071840	cellular component organization or biogenesis	51	0.00205	CTNNB1,CYR61,DAAM1,DAB2,DLG2,DNMT1,DRD1,DZIP1,ETS1,FBLN2,FBLN5,FKBP4,FOXC2,GCLM,GFRA1,GRIK5,GRIN2B,HDAC4,HIST1H4C,HMGB3,IBSP,IL6,LIFR,MCM4,MPP6,MYL2,NDP,NDUFA7
GO.0071230	cellular response to amino acid stimulus	5	0.00398	ABCA4,ACIN1,ADAMTS3,APAF1,ARID5B,BYSL,CACNB4,CALR,CCL7,CCT8,CDH12,CDH15,CDH17,CDH6,CHEK1,COL17A1,COL3A1,COL6A1,COPB2,CREBBP,CRYAA,CTGF,CTH,CTNNB1,CYR61,DAAM1,DAB2,DLG2,DNMT1,DRD1,DZIP1,ETS1,FBLN2,FBLN5,FKBP4,FOXC2,GCLM,GFRA1,GRIK5,GRIN2B,HDAC4,HIST1H4C,HMGB3,IBSP,IL6,LIFR,MCM4,MPP6,MYL2,NDP,NDUFA7
GO.0070887	cellular response to chemical stimulus	32	0.0015	COL1A2,COL3A1,COL6A1,DNMT1,HMGCS2
GO.0071495	cellular response to endogenous stimulus	16	0.0448	AGTR2,APAF1,AVPR2,BYSL,CAD,CFTR,COL6A1,CREBBP,CTGF,CTH,CTNNB1,CYP26A1,CYP2C18,CYP4F11,DNMT1,DRD1,DTYMK,ETS1,FOXC2,GCLM,GFRA1,GRIK5,HDAC4,HMGCS2,HNF4G,IBSP,IFNA2,IGFBP2,IL13RA2,IL6,KCNH2,LIFR
GO.0071310	cellular response to organic substance	27	0.00335	AGTR2,ASNS,AVPR2,CAD,CFTR,COL6A1,CTGF,CTNNB1,DNMT1,DRD1,FOXC2,HDAC4,HMGCS2,HNF4G,IGFBP2,IL6
GO.1901701	cellular response to oxygen-containing compound	16	0.0057	AGTR2,APAF1,AVPR2,BYSL,CAD,CALR,CCL7,CFTR,COL6A1,CTGF,CTH,CTNNB1,DNMT1,DRD1,DTYMK,FOXC2,GFRA1,GRIK5,HDAC4,HMGCS2,HNF4G,IBSP,IFNA2,IGFBP2,IL13RA2,IL6,LIFR
GO.0051716	cellular response to stimulus	63	0.000131	AGTR2,BYSL,CCL7,CFTR,COL1A2,COL3A1,COL6A1,CTNNB1,DNMT1,DRD1,ETS1,FOXC2,GRIK5,HMGCS2,IL6,INHBB
GO.0072359	circulatory system development	16	0.00533	ABCA4,ACIN1,AGTR2,ARID5B,ASB4,BCAM,BCL2L2,BYSL,CACNB4,CAD,CALR,CDKL2,CFTR,CHEK1,COL6A1,CREBBP,CTH,CTNNB1,CYP26A1,CYP2C18,CYP4F11,CYR61,DAAM1,DAB2,DCBLD2,DCX,DHRS3,DIRAS3,DNAJC7,DNMT1,DOCK9,DRD1,DTYMK,ETS1,GAL,GCLM,GDF11,GFRA1,GLRA1,GPR135,GPR37L1,GRIA2,GRIN2B,GRP,HDAC4,HIST1H4C,HMGCS2,HNF4G,HOXD3,HTR4,IBSP,IFIH1,IFNA2,IGFBP2,IL13RA2,IL6,ITGA8,KCNH2,KCNJ4,LGR5,LIFR,MPP3,NDP
GO.0030574	collagen catabolic process	6	0.00226	ACTA2,AGTR2,ANPEP,CAD,CALR,CDH2,COL1A2,CTGF,CTNNB1,CYR61,DHRS3,ETS1,FOXC2,ID3,MYL2,NDP
GO.0030199	collagen fibril organization	5	0.00197	ADAMTS3,COL17A1,COL1A2,COL3A1,COL6A1,MMP11
				ADAMTS3,COL1A2,COL3A1,FOXC2,MMP11

GO.0032502	developmental process	49	0.00335	ABCB6, ACIN1, ACTA2, ACTN1, AEBP1, AGTR2, ANPEP, APOH, ASNS, ATP7B, AVPR2, BCL2L2, BYSL, CAD, CALR, CDH15, CDKL2, CFDP1, CFTR, CHEK1, COL17A1, COL1A2, COL6A1, CREBBP, CTNNB1, CYR61, DAB2, DHRS3, DLG2, DRD1, DTYMK, DZIP1, FOXC2, FUT9, GAL, GFRA1, GPR37L1, HMGB3, HMGCS2, HNF4G, IBSP, IFNA2, IGFBP2, IL6, LGR5, LIFR, MAN1A2, MMP11, MYL2
GO.0030198	extracellular matrix organization	14	0.000433	ACTN1, ADAMTS3, COL17A1, COL1A2, COL3A1, COL6A1, CTGF, CYR61, FBLN2, FBLN5, FOXC2, IBSP, ID3, ITGA8
GO.0007214	gamma-aminobutyric acid signaling pathway	3	0.0458	CACNB4, GABRA2, HTR4
GO.0048732	gland development	10	0.0312	ASNS, ATP7B, CACNB4, CAD, CTNNB1, ETS1, FKBP4, HMGCS2, HOXD3, IL6
GO.0072144	glomerular mesangial cell development	2	0.0329	ACTA2, FOXC2
GO.0072008	glomerular mesangial cell differentiation	2	0.0443	ACTA2, FOXC2
GO.0060322	head development	13	0.04	ABCB6, AGTR2, APAF1, ARID5B, AVPR2, BYSL, COL3A1, CTNNB1, DCX, DRD1, ETS1, HMGCS2, ITGA8
GO.0007507	heart development	10	0.0476	AGTR2, CAD, CALR, COL3A1, CTNNB1, CYR61, DHRS3, FOXC2, ID3, MYL2
GO.0007156	hemophilic cell adhesion via plasma membrane adhesion molecules	6	0.0388	CDH12, CDH15, CDH16, CDH17, CDH2, CDH6
GO.0035235	ionotropic glutamate receptor signaling pathway	3	0.0499	GRIA2, GRIK5, GRIN2B
GO.0001822	kidney development	8	0.0242	ACTA2, AGTR2, ARID5B, CTNNB1, FOXC2, GDF11, HMGCS2, ID3
GO.0040011	locomotion	18	0.0346	ANPEP, ARID5B, CACNB4, CCL7, CDH2, COL1A2, COL3A1, COL6A1, CTGF, CTNNB1, CYR61, DCX, DRD1, ETS1, GAS8, GFRA1, GRIN2B, IL6
GO.0001656	metanephros development	6	0.00352	AGTR2, CTNNB1, FOXC2, GDF11, ID3, ITGA8
GO.0006928	movement of cell or subcellular component	19	0.04	ARID5B, CACNB4, CCL7, CDH2, COL1A2, COL3A1, COL6A1, CTGF, CTNNB1, CYR61, DCX, DRD1, ETS1, GAS8, GFRA1, GRIN2B, IL6, KIF1A, MYL2
GO.0007275	multicellular organismal development	45	0.00328	ABCB6, ACIN1, ACTA2, ACTN1, AEBP1, AGTR2, ANPEP, APOH, ASNS, ATP7B, AVPR2, BCL2L2, BYSL, CAD, CALR, CFDP1, COL1A2, COL6A1, CREBBP, CTGF, CTNNB1, CYR61, DAB2, DHRS3, DLG2, DRD1, DZIP1, ETS1, FOXC2, FUT9, GAL, GFRA1, GPR37L1, HMGB3, HMGCS2, HNF4G, IBSP, IFNA2, IL6, INHB, LGR5, LIFR, MAN1A2, MMP11, MYL2
GO.0032501	multicellular organismal process	51	0.0428	ABCB6, ACIN1, ACTA2, ACTN1, ADAMTS3, AEBP1, AGTR2, ANPEP, APOH, ASNS, ATP7B, CAD, CALR, CFDP1, CFTR, COL17A1, COL1A2, COL6A1, CREBBP, CRYAA, CTGF, CTNNB1, CYP4F11, CYR61, DAB2, DOCK9, DRD1, DZIP1, ETS1

GO.0044703	multi-organism reproductive process	15	0.04	1, FOXC2, FUT9, GAL, GCLM, GFRA1, GPR37L1, HDAC4, HMGB3, HMGCS2, HNF4G, HTR4, IBSP, IFNA2, IGFBP2, IL6, KCNH2, KNG1, LGR5, LIFR, MAN1A2, MMP11, MYL2
GO.0061061	muscle structure development	12	0.00533	BCL2L2, BYSL, CAD, CALR, CCT8, CFTR, CTNNB1, DRD1, DZIP1, ETS1, FKBP4, GLRA1, IGFBP2, INHBB, LGR5
GO.0048519	negative regulation of biological process	45	0.00527	AEBP1, AGTR2, ARID5B, CACNB4, CALR, CDH15, COL3A1, DTYMK, FOXC2, ID3, ITGA8, MYL2
GO.0010648	negative regulation of cell communication	18	0.0326	ACIN1, ACTN1, AGTR2, ANKRD26, APOH, ARID5B, ASNS, AVPR2, BCL2L2, CALR, CFDP1, CHEK1, COL3A1, CREBBP, CRYAA, CTH, CTNNB1, CYP26A1, DAB2, DCBLD2, DHRS3, DLG2, DRD1, DUSP14, EEF1E1, FOXC2, GAS8, GCLM, GDF11, GLRA1, GRIK5, HDAC4, HIST1H4C, HMGB3, IFIH1, IFNA2, IL13RA2, IL6, INHB, KCNH2, KNG1, LIFR, LMO3, MMP11, MYL2
GO.0010721	negative regulation of cell development	8	0.0301	AGTR2, ANKRD26, BCL2L2, CALR, CDH2, CTH, CTNNB1, CYP26A1, DAB2, DHRS3, DRD1, DUSP14, GCLM, GLRA1, GPR37L1, IL6, INHB, LMO3
GO.0045596	negative regulation of cell differentiation	18	0.000155	CALR, COL3A1, CTNNB1, FKBP4, FOXC2, GAL, GDF11, GPR37L1
GO.0048523	negative regulation of cellular process	45	0.0015	ANKRD26, CALR, CDH2, COL3A1, CREBBP, CTNNB1, FKBP4, FOXC2, GAL, GDF11, GPR37L1, HDAC4, HIST1H4C, HMGB3, ID3, IFNA2, IL6, MMP11
GO.0051093	negative regulation of developmental process	19	0.000629	ACIN1, ACTN1, AGTR2, ANKRD26, APOH, ARID5B, ASNS, BCL2L2, CALR, CFDP1, CHEK1, COL3A1, CREBBP, CRYAA, CTGF, CTH, CTNNB1, CYP26A1, DAB2, DCBLD2, DHRS3, DLG2, DNMT1, DRD1, DUSP14, EEF1E1, ETS1, FOXC2, GAS8, GCLM, GDF11, GLRA1, HDAC4, HIST1H4C, HMGB3, IFNA2, IL13RA2, IL6, INHB, KCNH2, KNG1, LIFR, LMO3, MMP11, MYL2
GO.0051241	negative regulation of multicellular organismal process	22	0.000131	ANKRD26, APOH, CALR, CDH2, COL3A1, CREBBP, CTNNB1, FKBP4, FOXC2, GAL, GDF11, GPR37L1, HDAC4, HIST1H4C, HMGB3, ID3, IFIH1, IFNA2, IL6, INHB, KNG1
GO.0048585	negative regulation of response to stimulus	20	0.0172	AGTR2, ANKRD26, APOH, BCL2L2, CALR, CDH2, COL3A1, CTH, CTNNB1, CYP26A1, DAB2, DHRS3, DRD1, DUSP14, ETS1, GCLM, IFNA2, IL13RA2, KNG1, LMO3
GO.0023057	negative regulation of signaling	18	0.0305	AGTR2, ANKRD26, BCL2L2, CALR, CDH2, CTH, CTNNB1, CYP26A1, DAB2, DHRS3, DRD1, DUSP14, GCLM, GLRA1, GPR37L1, IL6, INHB, LMO3
GO.0007399	nervous system development	25	0.0333	ABCB6, AGTR2, AVPR2, BYSL, CACNB4, CALR, COL3A1, COL6A1, CTNNB1, DLG2, DRD1, ETS1, FKBP4, FUT9, GAL, GFRA1, GPR37L1, GRIN2B, HDAC4, HMGCS2, ID3, IL6, ITGA8, LIFR, NDP
GO.0007270	neuron-neuron synaptic transmission	5	0.00789	CACNB4, DRD1, GLRA1, GRIA2, GRIK5
GO.0048513	organ development	42	1.86E-05	ABCB6, ACIN1, ACTA2, ACTN1, AEBP1, AGTR2, APAF1, APOH, ASNS, ATP7B, AVPR2

				BCL2L2,BYSL,CACNB4,CAD,CALR,COL1A2,CTGF,CTNNB1,CYR61,DCX,DHRS3,DRD1,ETS1,FKBP4,FOXC2,GDF11,GRIN2B,HDAC4,HMGCS2,HNF4G,HOXD3,IBSP,ID3,IFNA2,IL6,INHBB,LGR5,LIFR,MAN1A2,MYL2,NDP
GO.0009887	organ morphogenesis	16	0.0132	ACTA2,AGTR2,ARID5B,COL1A2,COL3A1,CTGF,CTNNB1,CYR61,DHRS3,FOXC2,GDF11,HOXD3,ID3,IL6,ITGA8,MYL2
GO.0043065	positive regulation of apoptotic process	13	0.0069	ACIN1,AGTR2,CTGF,CTNNB1,CYR61,EEF1E1,GAL,GRIK5,HDAC4,ID3,IL6,INHBB,KNG1
GO.0048518	positive regulation of biological process	54	0.00065	ACIN1,ACTA2,ADAMTS3,AGTR2,APOH,ARID5B,ASB4,ASNS,AVPR2,CACNB4,CALR,CCL7,CDH15,CDKL2,CFTR,CHEK1,COL3A1,CREBBP,CTGF,CTH,CTNNB1,CYR61,DAB2,DNMT1,DOCK9,DRD1,EEF1E1,ETS1,FBLN2,FOX2,GCLM,GDF11,GLRA1,GRIK5,GRIN2B,HDAC4,HNF4G,HOXD3,HSPB2,HTR4,ID3,IFIH1,IFNA2,IGFBP2,IL13RA2,IL6,ITGA8,KCNH2,KNG1,LGR5,LIFR,LMO3,MLLT10,NDP
GO.0009891	positive regulation of biosynthetic process	23	0.0357	AGTR2,ARID5B,AVPR2,CALR,CHEK1,CREBBP,CTGF,CTH,CTNNB1,CYR61,DAB2,DRD1
GO.0010647	positive regulation of cell communication	22	0.0242	ETS1,FOXC2,GAL,HDAC4,HNF4G,HTR4,IFNA2,IL6,ITGA8,MLLT10,NDP
GO.0030335	positive regulation of cell migration	9	0.0357	ACIN1,ADAMTS3,AGTR2,APAF1,CDH2,CFTR,COL3A1,CTGF,CTH,CYR61,DAB2,DRD1,EEF1E1,GAL,GDF11,GPR37L1,GRIN2B,IFNA2,IL6,ITGA8,LGR5,LMO3
GO.0048522	positive regulation of cellular process	46	0.00464	CALR,CCL7,CYR61,DAB2,DRD1,ETS1,FOXC2,HDAC4,IL6
GO.0010628	positive regulation of gene expression	24	0.00868	ACIN1,ADAMTS3,AGTR2,ARID5B,ASB4,ASNS,AVPR2,CALR,CCL7,CDH15,CDKL2,CFTR,CHEK1,COL3A1,CREBBP,CTGF,CTH,CTNNB1,CYR61,DAB2,DNMT1,DRD1,EEF1E1,ETS1,FBLN2,FOX2,GDF11,GLRA1,GRIK5,GRIN2B,HDAC4,HNF4G,HOXD3,HTR4,ID3,IFNA2,IGFBP2,IL6,ITGA8,KCNH2,KNG1,LGR5,LIFR,LMO3,MLLT10,NDP
GO.0032846	positive regulation of homeostatic process	6	0.0436	ACTA2,AGTR2,ARID5B,AVPR2,CALR,CHEK1,CREBBP,CTGF,CTH,CTNNB1,CYR61,DAB2,DNMT1,ETS1,FOXC2,GAL,HDAC4,HNF4G,HOXD3,IFNA2,IL6,ITGA8,MLLT10,NDP
GO.0009893	positive regulation of metabolic process	36	0.0438	AGTR2,CFTR,CTNNB1,DRD1,ETS1,KNG1
GO.0045935	positive regulation of nucleobase-containing compound metabolic process	22	0.04	ACIN1,ACTA2,AGTR2,APAF1,APOH,ARID5B,AVPR2,CALR,CDH2,CHEK1,CREBBP,CTGF,CTH,CTNNB1,CYR61,DAB2,DNMT1,DOCK9,DRD1,ETS1,FOXC2,GAL,GCLM,GDF11,GPR37L1,HDAC4,HNF4G,HOXD3,HSPB2,HTR4,IFNA2,IL6,INHBB,ITGA8,MLLT10,NDP
GO.0048584	positive regulation of response to stimulus	27	0.00457	AGTR2,ARID5B,AVPR2,CALR,CHEK1,CREBBP,CTH,CTNNB1,CYR61,DAB2,DRD1,ETS1,FOXC2,GAL,HDAC4,HNF4G,HTR4,IFNA2,IL6,ITGA8,MLLT10,NDP
GO.0023056	positive regulation of signaling	21	0.0205	ACIN1,ADAMTS3,AGTR2,APAF1,APOH,CACNB4,CALR,CCL7,CDH2,CFTR,COL3A1,CREBBP,CTGF,CTH,CYR61,DAB2,DRD1,EEF1E1,FOXC2,GDF11
GO.0090100	positive regulation of transmembrane receptor protein serine/threonine kinase signaling pathway	5	0.04	GPR37L1,IFIH1,IFNA2,IL6,ITGA8,LGR5,LMO3
GO.0042981	regulation of apoptotic process	27	0.000131	ADAMTS3,AGTR2,APAF1,CDH2,CFTR,COL3A1,CTGF,CTH,CYR61,DAB2,DRD1,EEF1E1,GAL,GDF11,GPR37L1,GRIN2B,IFNA2,IL6,ITGA8,LGR5,LMO3
GO.0065008	regulation of biological quality	38	0.00145	CYR61,DAB2,GDF11,INHBB,ITGA8
GO.0050880	regulation of blood vessel size	6	0.00197	ACIN1,ACTN1,AGTR2,APOH,ASNS,BCL2L2,CALR,CFDP1,CRYAA,CTGF,CTH,CTNNB1,CYR61,DAB2,EEF1E1,ETS1,FOXC2,GAL,GCLM,GDF11,GRIK5,HDAC4,ID3,IFIH1,IL6,KNG1,LIFR
GO.0010646	regulation of cell communication	34	0.00717	ABCA4,ABCB6,ACIN1,ACTA2,ACTN1,AGTR2,ANKRD26,ANPEP,APOH,CAD,CALR,CCL7,CDH2,CFDP1,CFTR,COL1A2,COL3A1,CREBBP,CTGF,CTNNB1,CYP26A1,CYP4F11,DHRS3,DOCK9,DRD1,FOXC2,GABRA2,GAL,GCLM,GRIK5,GRIN2B,HIST1H4C,HTR4,IFNA2,IL6,KCNH2,KCNK1,KNG1
GO.0010941	regulation of cell death	26	0.000485	ACTA2,AGTR2,DRD1,FOXC2,GCLM,KNG1
GO.0045595	regulation of cell differentiation	26	0.000485	ACIN1,ADAMTS3,AGTR2,ANKRD26,APAF1,BCL2L2,CACNG3,CALR,CDH2,CFTR,CNKSR2,COL3A1,CREBBP,CTGF,CTH,CYP26A1,CYR61,DAB2,DHRS3,DRD1,DUSP14,EEF1E1,GAL,GCLM,GDF11,GLRA1,GRIK5,GRIN2B,IFNA2,IGFBP2,IL6,ITGA8,LGR5,LMO3
GO.0030334	regulation of cell migration	12	0.0399	ACIN1,ACTN1,AGTR2,APOH,ASNS,BCL2L2,CALR,CFDP1,CRYAA,CTH,CTNNB1,DAB2,EEF1E1,ETS1,FOXC2,GAL,GCLM,GDF11,GPR37L1,GRIK5,HDAC4,ID3,IFIH1,IL6,KNG1,LIFR
GO.0051270	regulation of cellular component movement	14	0.0199	ACIN1,ANKRD26,ASB4,CALR,CDH15,COL3A1,CREBBP,CTGF,CTNNB1,CYR61,DAB2,ETS1,FKBP4,FOXC2,GAL,GDF11,GPR37L1,HDAC4,HIST1H4C,HMGB3,HOXD3,ID3,IFNA2,IL6,LMO3,MMP11

GO.0050793	regulation of developmental process	29	0.00335	ACIN1,AGTR2,ANKRD26,APOH,ASB4,CALR,CCL7,CDH15,CFDP1,COL3A1,CREBBP,CTGF,CTNNB1,CYR61,DAB2,FKBP4,FOXC2,GAL,GDF11,GPR37L1,HDAC4,HIST1H4C,HMGB3,HOXD3,ID3,IFNA2,IL6,LMO3,MMP11
GO.0032879	regulation of localization	30	0.00517	ACTN1,AGTR2,APOH,AVPR2,CACNG3,CALR,CCL7,CDH2,CDKL2,CFTR,COL3A1,CRYAA,CTNNB1,CYR61,DAB2,DRD1,ETS1,FOXC2,GAL,GLRA1,GRIK5,HDAC4,IFNA2,IL13RA2,IL6,INHBB,KCNH2,KCNJ4,KCNK1,KNG1
GO.0040012	regulation of locomotion	13	0.0397	AGTR2,APOH,CALR,CCL7,COL3A1,CYR61,DAB2,DRD1,ETS1,FOXC2,HDAC4,IFNA2,IL6
GO.2000026	regulation of multicellular organismal development	22	0.0172	ACIN1,AGTR2,APOH,ASB4,CALR,CDH2,COL3A1,CTGF,CTNNB1,CYR61,DAB2,FKBP4,FOXC2,GAL,GDF11,GPR37L1,HDAC4,HIST1H4C,HMGB3,HOXD3,IFNA2,IL6
GO.0051239	regulation of multicellular organismal process	32	0.00205	ACIN1,AGTR2,ANKRD26,APOH,ASB4,CALR,CDH2,COL3A1,CREBBP,CTGF,CTNNB1,CYR61,DAB2,DHRS3,DRD1,FKBP4,FOXC2,GAL,GDF11,GPR37L1,HDAC4,HIST1H4C,HMGB3,HOXD3,ID3,IFIH1,IFNA2,IL6,INHBB,KCNH2,KNG1,MYL2
GO.0031399	regulation of protein modification process	21	0.0476	AGTR2,ANKRD26,AVPR2,CDH2,CHEK1,CTGF,CTNNB1,CYR61,DAB2,DIRAS3,DLG2,DNMT1,DRD1,DUSP14,GDF11,GPR37L1,HDAC4,IFNA2,IL6,INHBB,LMO3
GO.0035813	regulation of renal sodium excretion	3	0.0426	AGTR2,AVPR2,KNG1
GO.0048583	regulation of response to stimulus	44	0.000131	ACIN1,ADAMTS3,AGTR2,ANKRD26,APAF1,APOH,BCL2L2,CACNB4,CACNG3,CALR,CCL7,CDH2,CFTR,CHEK1,CNKS2,COL3A1,CREBBP,CTGF,CTH,CYP26A1,CYR61,DAB2,DHRS3,DNAJC7,DRD1,DUSP14,EEF1E1,ETS1,FBLN5,FKBP4,FOXC2,GCLM,GDF11,GRIN2B,HDAC4,IFIH1,IFNA2,IGFBP2,IL13RA2,IL6,ITGA8,KNG1,LGR5,LMO3
GO.0009966	regulation of signal transduction	28	0.0399	ADAMTS3,AGTR2,ANKRD26,APAF1,BCL2L2,CACNG3,CALR,CDH2,CNKS2,COL3A1,CREBBP,CTGF,CTH,CYP26A1,CYR61,DAB2,DHRS3,DUSP14,EEF1E1,GCLM,GDF11,GRIN2B,IFNA2,IGFBP2,IL6,ITGA8,LGR5,LMO3
GO.0023051	regulation of signaling regulation of system	33	0.00666	ADAMTS3,AGTR2,ANKRD26,APAF1,BCL2L2,CACNG3,CALR,CDH2,CFTR,CNKS2,COL3A1,CREBBP,CTGF,CTH,CYP26A1,CYR61,DAB2,DHRS3,DRD1,DUSP14,EEF1E1,GAL,GCLM,GDF11,GLRA1,GRIK5,GRIN2B,IFNA2,IGFBP2,IL6,ITGA8,LGR5,LMO3
GO.0044057	renal system process	10	0.0382	AGTR2,CTGF,DRD1,GAL,HDAC4,IL6,INHBB,KCNH2,KNG1,MYL2
GO.0072001	renal system development	9	0.00695	ACTA2,AGTR2,ARID5B,CTNNB1,FOXC2,GDF11,HMGCS2,ID3,LGR5

GO.0009628	response to abiotic stimulus	21	0.0015	ABCA4,APAF1,ASNS,CACNB4,CCL7,CHEK1,CREBBP,CTGF,DCX,DHRS3,DNAJC7,DRD1,ETS1,FKBP4,GRIN2B,HDAC4,HMGCS2,IGFBP2,IL6,INHBB
GO.0001101	response to acid chemical	10	0.00457	KCNJ4
GO.0097305	response to alcohol	12	0.000521	ASNS,COL1A2,COL3A1,COL6A1,CTGF,DNMT1,DRD1,HMGCS2,IGFBP2,IL6
GO.0043200	response to amino acid	9	0.000103	AGTR2,CAD,CALR,CCL7,CTGF,CTNNB1,DRD1,ETS1,GRIN2B,HMGCS2,IGFBP2,IL6
GO.0031960	response to corticosteroid	6	0.0464	ASNS,COL1A2,COL3A1,COL6A1,CTGF,DNMT1,DRD1,HMGCS2,IL6
GO.0042493	response to drug	12	0.00335	AGTR2,CAD,CTGF,HMGCS2,IGFBP2,IL6
GO.0009719	response to endogenous stimulus	20	0.0336	CAD,CALR,CTNNB1,CYP2C18,DRD1,GAL,GCLM,HDAC4,HMGCS2,IGFBP2,IL6,KCNH2
GO.0032355	response to estradiol	6	0.023	AGTR2,ASNS,AVPR2,CAD,CALR,CFTR,COL6A1,CTGF,CTNNB1,DNMT1,DRD1,DTYMK,ETS1,FOXC2,GAL,HDAC4,HMGCS2,HNF4G,IGFBP2,IL6
GO.0043627	response to estrogen	8	0.00771	CALR,CTGF,CTNNB1,DRD1,ETS1,IGFBP2
GO.0009725	response to hormone	17	0.00464	CALR,CTGF,CTNNB1,DRD1,DTYMK,ETS1,GAL,IGFBP2
GO.0070555	response to interleukin-1	5	0.0205	AGTR2,ASNS,AVPR2,CAD,CALR,CTGF,CTNNB1,DRD1,DTYMK,ETS1,FOXC2,GAL,HDAC4,HMGCS2,HNF4G,IGFBP2,IL6
GO.0033993	response to lipid	14	0.0379	BYSL,CREBBP,ETS1,HDAC4,IL6
GO.0009612	response to mechanical stimulus	10	0.000485	AGTR2,BYSL,CAD,CALR,CTGF,CTNNB1,DRD1,DTYMK,ETS1,GAL,HMGCS2,HNF4G,IGFBP2,IL6
GO.1901698	response to nitrogen compound	18	0.00199	ASNS,CHEK1,COL3A1,ETS1,GRIN2B,HDAC4,IGFBP2,IL6,INHBB,KCNJ4
GO.0014070	response to organic cyclic compound	18	0.000911	AGTR2,ASNS,AVPR2,CAD,CFTR,COL1A2,COL3A1,COL6A1,CTGF,CTNNB1,DNMT1,DRD1,FOXC2,GAL,HMGCS2,IFNA2,IL6,INHBB
GO.0010033	response to organic substance	30	0.0152	AGTR2,ASNS,BYSL,CAD,CALR,CFTR,CTGF,CTNNB1,DRD1,DTYMK,ETS1,GAL,HMGCS2,HNF4G,IFNA2,IGFBP2,IL6,LIFR
GO.0010243	response to organo-nitrogen compound	17	0.00205	AGTR2,APAF1,BYSL,CAD,CCL7,CFTR,COL6A1,CREBBP,CTGF,CTH,CTNNB1,DNMT1,DRD1,DTYMK,ETS1,FOXC2,GAL,GFRA1,GRIK5,GRIN2B,HDAC4,HMGCS2,HNF4G,HSPB2,IBSP,IFNA2,IGFBP2,IL13RA2,IL6,LIFR
GO.1901700	response to oxygen-containing compound	24	0.00065	AGTR2,ASNS,AVPR2,CAD,CFTR,COL1A2,COL3A1,COL6A1,CTGF,CTNNB1,DNMT1,DRD1,FOXC2,GAL,HMGCS2,IL6,INHBB
GO.0048545	response to steroid hormone	13	0.000834	AGTR2,ASNS,AVPR2,BYSL,CAD,CALR,CCL7,CFTR,COL1A2,COL3A1,COL6A1,CTGF,CTNNB1,DNMT1,DRD1,ETS1,FOXC2,GAL,GRIK5,GRIN2B,HMGCS2,IGFBP2,IL6,INHBB
GO.0050896	response to stimulus	60	0.0242	AGTR2,CAD,CALR,CTGF,CTNNB1,DRD1,DTYMK,ETS1,GAL,HMGCS2,HNF4G,IGFBP2,IL6

				P26A1,CYP2C18,CYP4F11,CYR61,DAAM1,DAB2,DCBLD2,DHRS3,DIRAS3,DNAJC7,DNMT1,DRD1,DYMK,GDF11,GFRA1,GPR135,GPR37L1,GRIA2,GRP,HDAC4,HIST1H4C,HMGCS2,HNF4G,HOXD3,HSPB2,HTR4,IBSP,ID3,IFIH1,IFNA2,IGFBP2,IL6,ITGA8,KCNH2,KCNJ4,KNG1,LGR5,LIFR,MPP3,NDP
GO.0009611	response to wounding	14	0.0305	ACTN1,APOH,COL1A2,COL3A1,CTGF,CYP4F11,CYR61,DCBLD2,DOCK9,ETS1,ID3,IFNA2,IL6,KNG1
GO.0051208	sequestering of calcium ion	2	0.0329	ATP7B,CALR
GO.0007165	signal transduction	54	0.000131	ABCA4,AGTR2,ARID5B,ASB4,ASNS,BCAM,BCL2L2,CACNB4,CALR,CCL7,CDKL2,CFTR,COL1A2,COL3A1,CREBBP,CTH,CTNNB1,CYR61,DAAM1,DAB2,DCBLD2,DCX,DHRS3,DIRAS3,DOCK9,DRD1,FKBP4,GAL,GDF11,GFR A1,GLRA1,GPR135,GPR37L1,GRIA2,GRIK5,GRIN2B,GRP,HDAC4,HIST1H4C,HNF4G,HOXD3,HTR4,IFIH1,IFNA2,IGFBP2,IL13RA2,IL6,INHBB,ITGA8,KCNH2,LGR5,LIFR,MPP3,NDP
GO.0044700	single organism signaling	54	0.000517	ABCA4,AGTR2,ARID5B,ASB4,ASNS,BCAM,BCL2L2,CACNB4,CALR,CCL7,CDKL2,CFTR,COL1A2,COL3A1,CREBBP,CTH,CTNNB1,CYR61,DAAM1,DAB2,DCBLD2,DCX,DHRS3,DIRAS3,DOCK9,DRD1,FKBP4,GAL,GDF11,GFRA1,GPR135,GPR37L1,GRIA2,GRP,HDAC4,HIST1H4C,HNF4G,HOXD3,HTR4,IFIH1,IFNA2,IGFBP2,IL13RA2,IL6,INHBB,ITGA8,KCNH2,KCNJ4,KCNK1,LGR5,LIFR,MPP3,NDP
GO.0044707	single-multicellular organism process	53	0.00603	ABCB6,ACIN1,ACTA2,ACTN1,ADAMTS3,AEBP1,AGTR2,ANPEP,APOH,ASNS,ATP7B,BCL2L2,BYSL,CAD,CALR,CFDP1,CFTR,COL17A1,COL1A2,COL6A1,CREBBP,CRYAA,CTGF,CTNNB1,CYP4F11,CYR61,DAB2,DOCK9,DRD1,DZIP1,ETS1,FOXC2,FUT9,GAL,GCLM,GFRA1,GPR37L1,HDAC4,HMGB3,HMGCS2,HNF4G,HTR4,IBSP,IFNA2,IL6,INHBB,KCNH2,KNG1,LGR5,LIFR,MAN1A2,MMP11,MYL2
GO.0044763	single-organism cellular process	81	0.0067	ABCB6,ACIN1,ACTA2,ADAMTS3,AGTR2,ANPEP,APOH,ARID5B,ARSA,ASB4,ASNS,BCAM,BYSL,CACNB3,CAD,CALR,CCL7,CCT8,CDH12,CDH15,CDH17,CDH6,CDKL2,CFTR,CHEK1,CKM,COL17A1,COL1A2,COL3A1,COL6A1,COPB2,CTH,CTNNB1,CYP26A1,CYP2C18,CYP4F11,CYR61,DAB2,DCBLD2,DHRS3,DIRAS3,DLG2,DNMT1,DOCK9,DRD1,DZIP1,EEF1E1,ETS1,FB LN2,FBIN5,FUT9,GAL,GAS8,GFRA1,GPR135,GRIA2,GRP,HDAC4,HIST1H4C,HNF4G,HOXD3,HTR4,IBSP,ID3,IFIH1,IFNA2,IGFBP2,IL13RA2,IL6,KCNH2,KCNJ4,KCNK1,KIF1A,KNG1,LIFR,MAN1A2,MCM4,MPP3,MYL2,NDP,NDUFA7
GO.0044767	single-organism developmental process	48	0.0048	ABCB6,ACIN1,ACTA2,ACTN1,AEBP1,AGTR2,ANPEP,APOH,ASNS,ATP7B,AVPR2,BCL2L2,BYSL,CAD,CALR,CDH15,CFDP1,CFTR,CHEK1,COL1A2,COL6A1,CREBBP,CTGF,CTNNB1,CYR61,DAB2,DHRS3,DLG2,DRD1,DYMK,DZIP1,FOXC2,FUT9,GAL,GFRA1,GPR37L1,HMGB3,HMGCS2,HNF4G,IBSP,IFNA2,IGFBP2,IL6,LGR5,LIFR,MAN1A2,MMP11,MYL2
GO.0001501	skeletal system development	12	0.00529	AEBP1,ARID5B,COL1A2,COL3A1,CTGF,CTNNB1,CYR61,DHRS3,FOXC2,GDF11,HDAC4,HOXD3
GO.0001964	startle response	3	0.0499	DRD1,GLRA1,GRIN2B
GO.0048731	system development	40	0.00529	ABCB6,ACIN1,ACTA2,ACTN1,AEBP1,AGTR2,ANPEP,APOH,ASNS,ATP7B,AVPR2,BCL2L2,BYSL,CAD,CALR,COL1A2,COL6A1,CTGF,CTNNB1,CYR61,DHRS3,DLG2,DRD1,ETS1,FOXC2,FUT9,GAL,GFRA1,GPR37L1,HMGB3,HMGCS2,HNF4G,IBSP,ID3,IFNA2,IL6,INHBB,LGR5,LIFR,MAN1A2,MYL2
GO.0001655	urogenital system development	10	0.00412	ACTA2,AGTR2,ARID5B,CTNNB1,FKBP4,FOXC2,GDF11,HMGCS2,ID3,LGR5

Table 4.11 Biological processes represented through STRING database of ID3 & ID3-candidate targets proteins in blood. Table represents 119 biological processes.

ID3 and ID3 candidate target genes in tissue. Furthermore we also used STRING to investigate the potential relationships among ID3 & ID3 candidate target genes in tissue (217). These genes demonstrate to be involved in 20 pathways as shown in Table 4.12. Additionally two of the pathways represented have a role in vascular diseases. Oxidative phosphorylation pathway included proteins: ATP5D, ATP5H, ATP6V0B, ATP6V0D1, ATP6V1B2, COX5A, COX7C, COX8A, CYC1, ID3, NDUFA1, NDUFA7, NDUFS4, NDUFV1, and NDUFV2. Cell cycle pathway includes: ANAPC10, ATM, CDKN2B, E2F5, MCM3, and MCM4.

Figures 4.8.1 and 4.8.2 represent both pathways. We also show the biological processes in which these genes (217) are over-represented in. A total of 116 processes are shown in Table 4.13.

Pathway ID	Pathway Description	Gene Count	False Discovery Rate	Matching Proteins in the Network
				ACLY, ACOX1, AK2, ALDOC, ALPL, AOX1, APRT, ATP5D, ATP5H, ATP6V0B, ATP6V0D1, ATP6V1B2, BCAT2, CAD, COX5A, COX7C, COX8A, CYC1, DEGS1, DHCR7, DHRS3, DNMT1, DPM2, EPRS, FLAD1, GALK2, GLB1, GOT1, GPA1, GPI, HIBCH, HMGCR, IDH2, IDH3B, IDH3G, IVD, MGAT1, MPI, MTM1, MVK, NAGLU, NDST1, NDUFA1, NDUFA7, NDUFS4, NDUFV1, NDUFV2
1100	Metabolic pathways	47	2.68E-13	ATF6, ATP5D, ATP5H, CDK5, COX5A, COX7C, COX8A, CYC1, GRIN2D, MAPK1, NCSTN, NDUFA1, NDUFA7, NDUFS4, NDUFV1, NDUFV2
5010	Alzheimer's disease	16	3.04E-09	ATP5D, ATP5H, ATP6V0B, ATP6V0D1, ATP6V1B2, COX5A, COX7C, COX8A, CYC1, NDUFA1, NDUFA7, NDUFS4, NDUFV1, NDUFV2
190	Oxidative phosphorylation	14	7.49E-09	AP2S1, ATP5D, ATP5H, BAX, CLTB, COX5A, COX7C, COX8A, CYC1, DCTN2, GPX1, NDUFA1, NDUFA7, NDUFS4, NDUFV1, NDUFV2
5016	Huntington's disease	16	7.49E-09	ATP5D, ATP5H, COX5A, COX7C, COX8A, CYC1, NDUFA1, NDUFA7, NDUFS4, NDUFV1, NDUFV2
5012	Parkinson's disease	11	1.55E-05	ATP6V0B, ATP6V0D1, CLN3, CLTB, CTSZ, DNASE2, GLB1, M6PR, NAGLU, NAGPA
4142	Lysosome	10	2.82E-05	BCAT2, GOT1, IDH2, IDH3B, IDH3G
1210	2-Oxocarboxylic acid metabolism	5	2.87E-05	BAX, COX5A, COX7C, COX8A, CYC1, NDUFA1, NDUFA7, NDUFS4, NDUFV1, NDUFV2
4932	Non-alcoholic fatty liver disease (NAFLD)	10	0.000135	ALDOC, GOT1, GPI, HIBCH, IDH2, IDH3B, IDH3G
1200	Carbon metabolism	7	0.00301	ALDOC, BCAT2, GOT1, IDH2, IDH3B, IDH3G
1230	Biosynthesis of amino acids	6	0.00301	ACLY, IDH2, IDH3B, IDH3G
20	Citrate cycle (TCA cycle)	4	0.00529	AP2S1, ATP6V0B, ATP6V0D1, ATP6V1B2, CLTB
4721	Synaptic vesicle cycle	5	0.0102	ATP6V0B, ATP6V0D1, BAX, CEBPG, IFNGR2, ITGAM, MAPK1, MAPK13
5152	Tuberculosis	8	0.0102	C4BPB, ITGAM, LY96, MAPK1, MAPK13
5133	Pertussis	5	0.0167	AOX1, BCAT2, HIBCH, IVD
280	Valine, leucine and isoleucine degradation	4	0.0226	ACOX1, DDO, IDH2, MPV17, MVK
4146	Peroxisome	5	0.0254	ANAPC10, ATM, CDKN2B, E2F5, MCM3, MCM4
4110	Cell cycle	6	0.0304	NCK1, ATM, AOX1, C4BPB
5014	Amyotrophic lateral sclerosis (ALS)	4	0.0304	
	Pathogenic Escherichia coli infection	4	0.0333	ARPC1A, HCLS1, LY96, NCK1
5130	Shigellosis	4	0.0442	ARPC1A, HCLS1, MAPK1, MAPK13

Table 4.12 Demonstrated pathways of ID3 & ID3 candidate target proteins in tissue.

Figure 4.7.1 Network demonstrated from STRING database of ID3 candidate targets involved in the oxidative phosphorylation pathway. Proteins involved in the pathway: ATP5D, ATP5H, ATP6V0B, ATP6V0D1, ATP6V1B2, COX5A, COX7C, COX8A, CYC1, NDUFA1, NDUFA7, NDUFS4, NDUFV1, and NDUFV2.

Figure 4.7.2 Network demonstrated from STRING database of ID3 candidate targets involved in the cell cycle pathway. Proteins include: ANAPC10, ATM, CDKN2B, E2F5, MCM3, and MCM4.

Pathway ID	Pathway Description	Gene Count	False Discovery Rate	Matching Proteins in the Network
GO.0048646	anatomical structure formation involved in morphogenesis	22	0.0351	ACTR2,AGGF1,APOD,ATM,ATP6V0D1,AXIN1,BAX,BYSL,CCDC6,CDK5,CSF3R,EPAH1,ERCC2,FZD2,GPI,GPX1,HESX1,HTATIP2,ITGB5,KRT19,MAPK1,MFGE8
GO.0009653	anatomical structure morphogenesis	38	0.0152	ACTR2,AGGF1,ALPL,AP2S1,APOD,ARPC1A,ARPC4,ATM,ATP6V0D1,BAX,BCAR3,BYSL,CSF3R,CTNNBIP1,CTSZ,DHRS3,DNAJB6,E2F5,EPAH1,ERCC2,FZD2,GATA2,GPI,GPX1,HESX1,HSP90AB1,HTATIP2,ID3,IGFBP5,ITGB5,KRT19,MFGE8,MTM1,NAGLU,NCK1,NCSTN,NDST1,NEDD8
GO.0048002	antigen processing and presentation of peptide antigen	8	0.0458	ACTR1A,AP2S1,BCAP31,DCTN2,DCTN5,ITGB5,KIFAP3,MR1
GO.0046034	ATP metabolic process	12	7.94E-05	AK2,ALDOC,ATP5D,ATP5H,ATP6V1B2,ENO1,GPI,NDUFA1,NDUFA7,NDUFS4,NDUFV1,NDUFV2
GO.1901137	carbohydrate derivative biosynthetic process	18	0.00821	AK2,APRT,ARF4,ATP5D,ATP5H,COG7,CSPG5,DPM2,GLB1,GPA1,GP5C,LMAN1,MGAT1,MPDU1,MPI,MPPE1,NAGPA,NDST1
GO.1901135	carbohydrate derivative metabolic process	32	7.17E-06	ACLY,AK2,ALDOC,APRT,ARF4,ASGR2,ATP5D,ATP5H,ATP6V1B2,BAX,CLN3,COG7,CSPG5,DPM2,ENO1,GLB1,GPA1,GP5C,GP1L,HMGCR,LMAN1,MPDU1,MPI,MPPE1,NAGLU,NAGPA,NDST1,NDUFA1,NDUFA7,NDUFS4,NDUFV1,NDUFV2
GO.0005975	carbohydrate metabolic process	23	0.000955	ACLY,ALDOC,APOD,ARF4,COG7,CSPG5,DPM2,ENO1,GLB1,GOT1,GP5C,GP1L,GPI,HAGH,IDH2,IDH3G,IGFBP5,LMAN1,MGAT1,MPDU1,MPI,NAGLU,NAGPA
GO.0007166	cell surface receptor signaling pathway	38	0.0121	ABCA1,ACTR2,AP2S1,ARF4,ARPC1A,ARPC4,ASGR2,ATP6V0B,ATP6V0D1,ATP6V1B2,AXIN1,BAX,CCDC6,CDKN2B,CLN3,CSF3R,CTNNBIP1,DDB1,E2F5,EIF4EBP2,EIF4G1,EPAH1,FRAT2,GRIN2D,HSP90AB1,ICAM3,IFNGR2,ITGAM,ITGB5,KPNA4,LY96,MAPK13,MAPKAPK3,MIF,NCK1,NCSTN,NDST1,NEDD8
GO.0016043	cellular component organization	73	0.00129	ABCA1,ACOX1,ACTR1A,ADRM1,AHCTF1,ANAPC10,AP2S1,APOD,ARCN1,ARF4,ARFGEF2,ARL1,ARPC1A,ARPC4,ATP6V0B,ATP6V0D1,ATP6V1B2,AXIN1,BAX,BCAP31,BNIP3,CAPNS1,CASK,CCDC6,CD2BP2,CDH15,CHMP2A,CLN3,CLPP,COPE,DCTN2,DDB1,DNASE2,DNMT1,E2F5,ELAC2,EPAH1,EPRS,ERAL1,FLOT2,GBF1,GOSR2,GPA1,HCLS1,HIST1H1D,HMGCR,HSP90AB1,ICAM3,ITGAM,ITGB5,KIAA0196,KIFAP3,KLK2,KPNA4,KRT19,LMNA,MAPK1,MAPRE1,MCM3,MCM4,MCRS1,MFGE8,MIF,MPV17,MRPL12,MRPS11,MRPS12,MTM1,NAGLU,NAGPA,NCSTN,NDUFA7,NDUFS4
GO.0042592	homeostatic process	27	0.0251	ABCA1,ABCB6,ACOX1,ASGR2,ATM,ATOX1,ATP6V0B,ATP6V0D1,ATP6V1B2,BCAP31,CCL23,CEBPB,CLN3,DDB1,DNASE2,ERCC2,GATA2,GOT1,GPR39,GPX1,HCLS1,IGFBP5,IL2RA,LGALS1,MPV17,MTM1,NCSTN
GO.1902600	hydrogen ion transmembrane transport	6	0.0376	ATP5D,ATP6V0B,ATP6V0D1,COX5A,COX7C,COX8A
GO.0002376	immune system process	37	0.0124	ACTR1A,ACTR2,AP2S1,ARPC1A,ARPC4,BAX,BCAP31,BNIP3,C4BPB,CCDC6,CL23,CEBPB,CSF3R,DCTN2,DCTN5,DNASE2,EEF2,ELF4,EPRS,ERCC2,GATA2,GBF1,GPI,HCLS1,HSP90AB1,ICAM3,IFNGR2,ILF2,ITGB5,KIFAP3,LGALS1,LY96,MAPKAPK3,MIF,MSN,NCK1,NCSTN
GO.0046907	intracellular transport	35	4.27E-05	ABCA1,AP2S1,ARCN1,ARFGEF2,ATOX1,ATP5D,ATP5H,AXIN1,BAX,BCAP31,BNIP3,CAMK1,CDK5,CLN3,CLTB,COG7,COPE,CSPG5,EIF5A,GATA2,GBF1,GOLGA3,GOSR2,HTATIP2,HTR2A,KIFAP3,KPNA4,LMAN1,LMNA,M6PR,MAPK1,MPPE1,MTM1,MYO7A,NAGPA
GO.0006102	isocitrate metabolic process	3	0.00292	IDH2,IDH3B,IDH3G
GO.0065003	macromolecular complex assembly	24	0.0474	ABCA1,ADRM1,AHCTF1,AP2S1,ARPC4,AXIN1,BAX,CD2BP2,CHMP2A,CLPP,EPRS,ERAL1,GPA1,H1F0,HCLS1,HIST1H1B,HIST1H1D,HMGCR,KIAA0196,KIFAP3,KPNA4,MIF,NCK1,NDUFS4
GO.0033036	macromolecule localization	37	0.0177	ABCA1,AHCTF1,APOD,ARCN1,ARF4,ARF5,ARFGEF2,ARL1,ATP6V0B,ATP6V0D1,ATP6V1B2,BAX,BCAP31,CDK5,CHMP2A,CLTB,COG7,COPE,DCTN2,DNAJB6,EIF5A,FLOT2,G3BP2,GBF1,GOSR2,GPA1,HIST1H1B,HTR2A,KIAA0196,KIFAP3,LMAN1,MAPK1,MAPRE1,MTM1,MYO7A,NAGPA,NEDD8
GO.0008152	metabolic process	113	0.0474	ABCA1,ABCB6,ACOX1,ADRM1,AGGF1,AK2,ALDOC,ALPL,ANAPC10,APOD,ARF4,ARF5,ARFGEF2,ARID3A,ARL1,ASGR2,ASPHD1,ATF6,ATM,ATP5D,ATP5H,BNIP3,BYSL,C4BPB,CAD,CAMK1,CAPNS1,CASK,CD2BP2,CDK5,CDKN2B,CLN3,CLPP,COG7,COMMD4,COPS3,COPS7A,COX5A,COX8A,CSPG5,CTSZ,CYB561D2,CYC1,DAP,DCTN2,DDB1,DHCR7,DHX8,DNAJB6,DNAJC9,DNASE2,DNMT1,DPM2,DYRK4,E2F5,EIF2B1,EIF4EBP2,EIF4G1,ELAC2,ELF4,ENO1,EPAH1,ERCC2,FAM20B,FLAD1,GBF1,GLB1,GOSR2,GPA1,GP5C,GP1L,GPX1,GTTF2F2,H1F0,HERC3,HERC4,HESX1,HIBCH,HMGCR,HSP90AB1,HTATIP2,ID3,IDH2,IMP4,IVD,KL2,LMAN1,LMNA,LSM3,LY96,MAPK1,MAPK13,MAPKAPK3,MCRS1,MGST3,MIF,MPDU1,MPI,MPPE1,MRPL12,MRPS11,MRPS12,MTHFS,MVK,NAGLU,NAGPA,NCK1,NDUFA1,NDUFA7,NDUFS4,NDUFV1,NDUFV2,NEDD8
GO.0042775	mitochondrial ATP synthesis coupled electron transport	5	0.0474	NDUFA1,NDUFA7,NDUFS4,NDUFV1,NDUFV2
GO.0006120	mitochondrial electron transport, NADH to ubiquinone	5	0.0187	NDUFA1,NDUFA7,NDUFS4,NDUFV1,NDUFV2
GO.0051704	multi-organism process	38	0.0351	ABCA1,ACOX1,ALPL,AP2S1,ATM,ATP6V0B,ATP6V0D1,ATP6V1B2,BAX,BCAP31,BNIP3,CAD,CCNI,CHMP2A,DDB1,DDO,EIF4EBP2,EIF4G1,ENO1,ERCC2,FAM50

				ATA2,GBF1,GLB1,GOSR2,GPA1,GPC5,GPD1L,GPR4,GPX1,GRIN2D,GT2F2F2,H AGH,HCLS1,HERC3,HERC4,HESX1,HIBCH,HIST1H1D,HSP90AB1,HTATIP2,ICA M3,ID3,IDH2,IFNGR2,ILF2,IMP4,ITGB5,IVD, KIAA0196,KIFAP3,KLK2,KPNA4,LASP1,LGALS3BP,LMNA,LM3,LY96, M6PR,MAPK1,MAPK13,MAPKAPK3,MAPRE1,MCRS1,MFGE8,MFSD5,MGST3,MI F,MPDU1,MPI,MPPE1,MPV17,MR1,MRPL12,MRPS11,MRPS12,MSN,MTHFS,MV K,MYBPC2,NAGLU,NAGPA,NDUFA1,NDUFA7,NDUFS4,NDUFV1,NDUFV2,NEDD 8
GO.00346 13	cellular protein localization	24	0.0349	AP2S1,ARCN1,ARF4,ARL1,BAX,BCAP31,CDK5,CLTB,COG7,DCTN2,DNAJB6, EIF5A,FLOT2,G3BP2,GBF1,GOSR2,GPA1,HIST1H1B,HTR2A,KPNA4, MAPK1,MAPRE1,MYO7A,NAGPA
GO.00442 67	cellular protein metabolic process	54	0.0121	ABCA1,ACP1,ANAPC10,ARF4,ASPHD1,ATF6,ATM,ATP6V0D1,BNIP3,CAD,CAMK 1,CASK,CLPP,COG7,COPS3,COPST7A,CTSZ,DDB1,DNAJB6,DPM2,DYRK4,EEF2, EIF2B1,EIF4EBP2,EIF4G1,EPHA1,ERCC2,GLB1,GOSR2,GPA1,GPX1,HERC3,H ERC4,HSP90AB1,IGFBP5,LMAN1,LMNA,MAPK1,MAPK13,MAPKAPK3,MCRS1,M GAT1,MPDU1,MPI,MPPE1,MRPL12,MRPS11,MRPS12,NAGPA, NCK1,NCSTN,NDST1,NDUFA7,NEDD8
GO.00453 33	cellular respiration	15	9.67E-07	ATP5D,ATP5H,BAX,COX5A,COX7C,COX8A,CYC1,IDH2,IDH3B,IDH3G,NDUFA1, NDUFA7,NDUFS4,NDUFV1,NDUFV2
GO.00708 87	cellular response to chemical stimulus	44	0.00145	ABCA1,ALPL,AP2S1,APRT,ATF6,ATP6V0D1,ATP6V1B2,AXIN1,BAX,B NIP3,BYSL,CAD,CCDC6,CCL23,CDKN2B,CSF3R,DNMT1,E2F5,EIF4EBP2,EIF4G 1,EPRS,GBF1,GOSR2,GOT1,GPR39,HCLS1,HSP90AB1,IFNGR2,IGFBP5,IL2RA,I TGAM,ITGB5,KPNA4, LGALS1,LMNA,LY96,MAPK13,MAPKAPK3,MGST3,MPV17, NCSTN,NDST1,NEDD8
GO.00713 10	cellular response to organic substance	37	0.0038	ABCA1,ALPL,AP2S1,APRT,ATF6,ATP6V0D1,ATP6V1B2,AXIN1,BAX,BY SL, CAD,CCDC6,CDKN2B,CSF3R,DNMT1,E2F5,EIF4EBP2,EIF4G1,EPRS,GOSR2,G OT1,HCLS1,HSP90AB1,IFNGR2,IGFBP5,IL2RA,ITGB5,KPNA4,LGALS1, LMNA,LY96,MAPK13,MAPKAPK3,NCSTN,NDST1,NEDD8
GO.00061 01	citrate metabolic process	4	0.039	ACLY,IDH2,IDH3B,IDH3G
GO.00067 32	coenzyme metabolic process	10	0.0214	ALDOC,ENO1,FLAD1,GPD1L,GPI,HMGCR,IDH3B,IDH3G,MTHFS,MVK
GO.00511 86	cofactor metabolic process	11	0.024	ABCB6,ALDOC,ENO1,FLAD1,GPD1L,GPI,HMGCR,IDH3B,IDH3G,MTHFS,MVK
GO.00482 05	COP1 coating of Golgi vesicle	3	0.0365	ARCN1,COPE,GBF1
GO.00359 64	COP1-coated vesicle budding	3	0.0365	ARCN1,COPE,GBF1
GO.00164 82	cytoplasmic transport	19	0.0151	ARFGEF2,AXIN1,BCAP31,CAMK1,CDK5,COPE,EIF5A,GBF1,GOSR2,HTATIP2,H TR2A,KPNA4,LMAN1,LMNA,M6PR,MAPK1,MPPE1,MTM1,NAGPA

GO.00718 40	cellular component organization or biogenesis	75	0.000868	ABCA1,ACOX1,ACTR1A,ADRM1,AHCTF1,ANAPC10,AP2S1,APOD, ARCN1,ARF4,ARFGEF2,ARL1,ARPC1A,ARPC4,ATP6V0D1,ATP6V1B2, AXIN1,BAX,BCAP31,BNIP3,BYSL,CAPNS1,CASK,CCDC6,CD2BP2,CDH15,CHMP 2A, CLN3,CLPP,COPE,DCTN2,DDB1,DNASE2,DNMT1,E2F5,ELAC2,EPHA1,EPRS, ERAL1,FLOT2,GBF1,GOSR2,GPA1,HCLS1,HIST1H1D,HMGCR,HSP90AB1, ICAM3,IMP4,ITGAM,ITGB5, KIAA0196,KIFAP3,KLK2,KPNA4,KRT19,LMNA,MAPK1, MAPRE1,MCM3,MCM4,MCRS1,MFGE8,MIF,MPV17, MRPL12,MRPS11,MRPS12,MTM1,NAGLU,NAGPA,NCSTN, NDUFA7,NDUFS4
GO.00516 41	cellular localization	40	0.00464	ABCA1,AP2S1,ARCN1,ARF4,ARFGEF2,ATOX1,ATP5D, ATP5H,AXIN1,BAX,BCAP31,BNIP3,CAMK1,CASK,CLN3, CLTB,COG7,COPE,CSPG5,DNAJB6,EIF5A,FLOT2,G3BP2, GATA2,GBF1,GOLGA3,GOSR2,GPA1,HIST1H1B,HTATIP2, KIFAP3,KPNA4,LMAN1,M6PR,MAPK1,MAPRE1,MPPE1,MTM1, MYO7A,NAGPA
GO.00442 37	cellular metabolic process	113	0.000254	ABCB6,ACOX1,ADRM1,AGGF1,AK2,ALDOC,ALPL,ANAPC10, AOX1,ARF4,ARFGEF2,ARID3A,ARL1,ASGR2,ASPHD1,ATF6, ATM,ATP5D,ATP5H,ATP6V0D1,ATP6V1B2,BAX,BNIP3,BYSL, CAD,CAMK1,CASK,CD2BP2,CDK5,CDKN2B,CLN3,CLPP,COG7, COMMD4,COPS3,COPST7A,COX5A,COX7C,COX8A,CSPG5,CTSZ, CYC1,DAP,DDB1,DEGS1,DHRS3,DHX8,DNAJB6,DNAJC8,DNASE2, DNMT1,DPM2,DYRK4,E2F5,EEF2,EIF2B1,EIF4EBP2,EIF4G1,ELAC2, ELF4,ENO1,EPHA1,ERCC2,FAM20B,FLAD1,GALK2,GBF1,GLB1,GOSR2, GPA1,GPC5,GPD1L,GPX1,GT2F2F2,H1F0,HAGH,HERC3,HERC4,HESX1, HIBCH,HSP90AB1,ID3,IDH2,IGFBP5,ILF2,IMP4,IVD,LMAN1,LMNA, LSM3,MAPK1,MAPK13,MAPKAPK3,MCM3,MCRS1,MGST3,MIF, MPDU1,MPI,MPPE1,MRPL12,MRPS11,MRPS12,MTHFS,MVK,NAGPA, NCK1,NDUFA1,NDUFA7,NDUFS4,NDUFV1,NDUFV2,NEDD8
GO.00346 41	cellular nitrogen compound metabolic process	72	0.0302	ABCB6,ACLY,ADRM1,AGGF1,AK2,ALDOC,AXO1,ARID3A,ATF6, ATP5D,ATP5H,ATP6V1B2,AXIN1,BCAT2,BYSL,CASK,CD2BP2,CDKN2B, CLN3,COMMD4,COX5A,COX7C,COX8A,CTSZ,DDB1,DHX8,DNAJC8, DNASE2,DNMT1,E2F5,EEF2,EIF2B1,EIF4EBP2,EIF4G1,ELAC2,ELF4, ENO1,ERCC2,FLAD1,GOT1,GPD1L,GPI,GPX1,GT2F2F2,H1F0,HAGH, HESX1,HIBCH,HMGCR,ID3,IDH3B,IDH3G,ILF2,IMP4,IVD,LSM3, MAPK1,MAPK13,MCM3,MCRS1,MGAT1,MPI,MRPL12,MRPS11, MRPS12,MTHFS,NCSTN,NDUFA1,NDUFA7,NDUFS4,NDUFV1,NDUFV2
GO.00099 87	cellular process	141	0.0153	ABCA1,ABCB6,ACOX1,ACTR1A,ADRM1,AGGF1,AHCTF1,AK2,ALDOC,ANAPC10, AOX1,AP2S1,APIS,APOD,ARCN1,ARF5,ARFGEF2,ARID3A,ARPC1A,ARPC4,AS PHD1,ATF6,ATM,ATP5D,AXIN1,BAX,BCAP31,BNIP3,BYSL,CAD,CAMK1,CAPNS1, CASK,CCDC6,CCL23,CD2BP2,CDH15,CDK5,CDKN2B,CHMP2A,CLN3,COG7,CO MMD4,COPE,COPS3,COPST7A,COX5A,COX8A,CSPG5,CTNNBP1,CTSZ,CYC1,D AP,DCBLD2,DEGS1,DHCR7,DHRS3,DHX8,DNAJC8,DPM2,DYRK4,E2F5,ELAC2,E LF4,ENO1,EPHA1,ERAL1,FAM20B,FLAD1,FLOT2,FPR1,FRAT2,G3BP2,GALK2,G

GO.00068 97	endocytosis	18	0.000868	ABCA1, ACTR2, AP2S1, ARPC1A, ARPC4, ASGR2, CDK5, CLN3, CLTB, GATA2, HSP90AB1, LGALS3BP, M6PR, MAPK1, MAPKAPK3, MFGE8, MYO7A, NCK1
GO.00159 80	energy derivation by oxidation of organic compounds	16	0.000119	ACLY, ATP5D, ATP5H, BAX, COX5A, COX7C, COX8A, CYC1, IDH2, IDH3B, IDH3G, NDUF1, NDUF7, NDUF54, NDUFV1, NDUFV2
GO.00071 67	enzyme linked receptor protein signaling pathway	22	0.0177	ACTR2, AP2S1, ARF4, ARPC1A, ARPC4, ATP6V0B, ATP6V0D1, ATP6V1B2, CDC6, CDKN2B, E2F5, EIF4EBP2, EIF4G1, EPHA1, ITGB5, MAPK1, MAPK13, MAPKAPK3, NCK1, NCSTN, NDST1, NEDD8
GO.00480 13	ephrin receptor signaling pathway	6	0.04	ACTR2, AP2S1, ARPC1A, ARPC4, EPHA1, NCSTN
GO.00068 88	ER to Golgi vesicle-mediated transport	5	0.0474	BCAP31, COPE, GOSR2, LMAN1, MPPE1
GO.00512 34	establishment of localization	57	0.00551	ABCA1, ABCB6, ACTR1A, AHCTF1, AP2S1, APOD, APRT, ARCN1, ARF4, ARF5, ARFGF2, ARPC1A, ARPC4, ASGR2, ATP5D, AXIN1, BAX, BCAP31, CAD, CAMK1, CLN3, CLTB, COG7, COPE, COX5A, COX7C, COX8A, CSPG5, DCTN2, EIF5A, FLOT2, G3BP2, GATA2, GBF1, GOLGA3, GOSR2, GRIN2D, HIST1H1B, HSP90AB1, HTATIP2, HTR2A, ICAT, KIAA0196, KIFAP3, LASP1, LGALS3BP, LMAN1, LMNA, MAPKAPK3, MFGE8, MFS, D5, MPDU1, MPPE1, MTM1, MYO7A, NAGPA, NCK1
GO.00516 49	establishment of localization in cell	36	0.00296	ABCA1, AP2S1, ARCN1, ARFGF2, ATOX1, ATP5D, ATP5H, AXIN1, BAX, BCAP31, BNI, P3, CAMK1, CASK, CLN3, CLTB, COG7, COPE, CSPG5, DCTN2, EIF5A, GATA2, GBF1, GOLGA3, GOSR2, HTATIP2, HTR2A, KIFAP3, KPNA4, LMAN1, LMNA, M6PR, MAPK1, MPPE1, MTM1, MYO7A, NAGPA
GO.00451 84	establishment of protein localization	28	0.0125	ABCA1, AHCTF1, ARCN1, ARF4, ARF5, ARFGF2, ATP6V0B, ATP6V0D1, ATP6V1B2, BAX, BCAP31, CDK5, CHMP2A, CLTB, COG7, COPE, EIF5A, FLOT2, GBF1, GOSR2, HIST1H1B, KIAA0196, LMAN1, LMNA, MAPK1, MTM1, MYO7A, NAGPA
GO.00380 96	Fc-gamma receptor signaling pathway involved in phagocytosis	6	0.02	ACTR2, ARPC1A, ARPC4, HSP90AB1, MAPK1, NCK1
GO.00060 91	precursor metabolites and energy	20	7.69E-06	ACLY, ACOX1, ALDOC, ATP5D, ATP5H, BAX, COX5A, COX7C, COX8A, CYC1, ENO1, GPI, IDH2, IDH3B, IDH3G, NDUF1, NDUF7, NDUF54, NDUFV1, NDUFV2
GO.00066 64	glycolipid metabolic process	6	0.0474	BAX, CLN3, DPM2, GLB1, GAA1, MPPE1
GO.00481 93	Golgi vesicle transport	12	0.00145	ARCN1, ARFGF2, ARL1, BCAP31, CHMP2A, COG7, COPE, GBF1, GOLGA3, GOSR2, LMAN1, MPPE1
GO.00193 18	hexose metabolic process	8	0.0474	ALDOC, APOD, ENO1, GALK2, GLB1, GOT1, GPI, IGF8P5
GO.00022 62	myeloid cell homeostasis	6	0.0421	A, GBF1, GPX1, GTF2F2, HERC4, HSP90AB1, HTATIP2, IFNGR2, KIAA0196, KPNA4, KRT19, LMNA, LY96, MAPK1, MAPKAPK3, MFGE8, MR1, NCK1
GO.00067 34	NADH metabolic process	6	0.00048	BAX, CEBPG, DNASE2, ERCC2, HCLS1, NCSTN
GO.00322 69	negative regulation of cellular protein metabolic process	22	0.0261	ANAPC10, ATM, BAX, C4BPB, CD2BP2, CDKN2B, CLN3, DNAJB6, DNMT1, EIF2B1, EIF4EBP2, EPHA1, EPRS, FXR2, GPD1L, GPI, GPX1, HMGCR, HSP90AB1, IGF8P5, MIF, NCK1
GO.00512 48	negative regulation of protein metabolic process	25	0.00551	ANAPC10, APOD, ATM, AXIN1, BAX, C4BPB, CD2BP2, CDKN2B, CLN3, DNAJB6, DNMT1, EIF2B1, EIF4EBP2, EPHA1, EPRS, FLOT2, FXR2, GPD1L, GPI, GPX1, HMGCR, HSP90AB1, IGF8P5, MIF, NCK1
GO.00068 07	nitrogen compound metabolic process	79	0.00641	ABCB6, ACLY, ADRM1, AGGF1, AK2, ALDOC, AOX1, ARID3A, ATF6, ATP5D, ATP5H, ATP6V1B2, AXIN1, BAX, BYSL, CAD, CASK, CD2BP2, CDKN2B, CLN3, COMMD4, COX5A, COX7C, COX8A, CSPG5, CTS2, DDB1, DEGS1, DHX8, DNAJC8, DNASE2, DNMT1, E2F5, EEF2, EIF2B1, EIF4EBP2, EIF4G1, ELAC2, ELF4, ENO1, ERCC2, FLAD1, GLB1, GPC5, GPD1L, GPI, GPX1, GTF2F2, H1FO, HAGH, HESX1, HIBCH, HMGCR, ID3, IDH3B, IDH3G, ILF2, IMP4, IVD, LSM3, MAPK1, MAPK13, MCM3, MCRS1, MGAT1, MGST3, MPI, MRPL12, MRPS11, MRPS12, MTHFS, NAGLU, NCSTN, NDST1, NDUF1, NDUF7, NDUF54, NDUFV1, NDUFV2
GO.00550 86	containing small molecule metabolic process	23	3.18E-06	ACLY, AK2, ALDOC, ATP5D, ATP5H, ATP6V1B2, CASK, ENO1, FLAD1, GPD1L, GPI, GPX1, HMGCR, IDH3B, IDH3G, MAPK1, MGAT1, MPI, NDUF1, NDUF7, NDUF54, NDUFV1, NDUFV2
GO.00091 17	nucleotide metabolic process	24	2.85E-07	ACLY, AK2, ALDOC, APRT, ATP5D, ATP5H, ATP6V1B2, CAD, CASK, ENO1, FLAD1, GPD1L, GPI, GPX1, HMGCR, IDH3B, IDH3G, MGAT1, MPI, NDUF1, NDUF7, NDUF54, NDUFV1, NDUFV2
GO.00069 96	organelle organization	52	0.000868	ABCA1, ACOX1, ACTR1A, AHCTF1, ANAPC10, AP2S1, ARCN1, ARF4, ARFGF2, ARL1, ARPC1A, ARPC4, ATP6V0B, ATP6V0D1, ATP6V1B2, AXIN1, BAX, BNIP3, CDK5, CHMP2A, CLN3, COPE, DCTN2, DDB1, DNMT1, ELAC2, ERL1, ERCC2, GBF1, GOSR2, H1FO, HCLS1, HIST1H1D, ITGB5, KIAA0196, KIFAP3, KRT19, LMNA, MAPRE1, MCM3, MCM4, MCRS1, MPV17, MRPL12, MRPS11, MRPS12, MTM1, NAGLU, NAGPA, NCK1, NDUF7, NDUF54
GO.00717 04	organic substance metabolic process	113	0.00145	ABCA1, ABCB6, ACOX1, ACP1, ADRM1, AGGF1, AK2, ALDOC, ANAPC10, AOX1, APOD, ARF4, ARFGF2, ARID3A, ASGR2, ASPHD1, ATF6, ATM, ATP5D, ATP5H, ATP6V0D1, ATP6V1B2, BNIP3, BYSL, C4BPB, CAD, CAMK1, CAPNS1, CASK, CD2BP2, CDKN2B, CLN3, CLPP, COG7, COMMD4, COPS3, COPS7A, COX5A, COX7C, COX8A, CSPG5, CTS2, DAP, DDB1, DEGS1, DHCT7, DHRS3, DHX8, DNAJB6, DNAJC8, DNASE2, DNMT1, DPM2, DYRK4, E2F5, EEF2, EIF2B1, EIF4EBP2, EIF4G1, ELAC2, ELF4, ENO1, EPHA1, ERCC2, FLAD1, GLB1, GOSR2, GAA1, GPC5, GPD1L, GPX1, GTF2F2, H1FO, HERC3, HERC4, HESX1, HIBCH, HMGCR, HSP90AB1, ID3, IDH2, ILF2, IMP4, IVD, KKL2, LMA

				N1,LMNA,LSM3,LY96,MAPK1,MAPK13,MAPKAPK3,MCM3,MCRS1,MGST3,MIF,M PDU1,MPI,MPPE1,MRPL12,MRPS11,MRPS12,MTFHS,MVK,NAGLU,NAGPA,NCK 1,NDUFA1,NDUFA7,NDUFS4,NDUFV1,NDUFV2,NEDD8
GO.19015 64	Organo-nitrogen compound metabolic process	45	2.85E-07	AK2,APRT,ATM,ATP5D,ATP5H,CAD,DPM2,FLAD1,GPAA1,GPD1L,MPI,MPPE1,M TM1,MVK
GO.00904 07	organophosphate biosynthetic process	14	0.0262	ACLY,AK2,ALDOC,APRT,ATM,ATP5D,ATP5H,ATP6V1B2,CAD,CASK,CLN3,DPM 2,ENO1,FLAD1,GPAA1,GPD1L,GPX1,HMGCR,IDH3B,IDH3G,MGAT1,MPI,MPPE1 ,MTM1,MVK,NDUFA1,NDUFA7,NDUFS4,NDUFV1,NDUFV2
GO.00196 37	organophosphate metabolic process	30	7.88E-07	ACLY,ACOX1,ALDOC,ASPHD1,ATP5D,ATP5H,BAX,COX5A,COX8A,CYB561D2,C YC1,DDO,DEGS1,DHCR7,DHRS3,ENO1,GPD1L,GPI,GPX1,HMGCR,HTATIP2,ID H2,IDH3B,IDH3G,IVD,MGST3,NDUFA1,NDUFA7,NDUFS4,NDUFV1,NDUFV2
GO.00551 14	oxidation- reduction process	31	1.56E-05	ATP5D,NDUFA1,NDUFA7,NDUFS4,NDUFV1,NDUFV2
GO.00061 19	oxidative phosphorylation oxidoreduction	6	0.0145	ATP5D,NDUFA1,NDUFA7,NDUFS4,NDUFV1,NDUFV2
GO.00067 33	coenzyme metabolic process	7	0.0115	ALDOC,ENO1,GPD1L,GPI,HMGCR,IDH3B,IDH3G
GO.00069 09	phagocytosis	10	0.00198	ABCA1,ACTR2,ARPC1A,ARPC4,GATA2,HSP90A1,MAPK1,MFGE8,MYO7A NCK1
GO.00067 96	phosphate- containing compound metabolic process	43	2.46E-05	AK2,ALDOC,ALPL,APRT,ATM,ATP5D,ATP5H,ATP6V1B2,AXIN1,CAD,CAMK1,CA SK,CDK5,CLN3,DPM2,DYRK4,ENO1,EPHA1,ERCC2,FAM20B,FLAD1,GALK2,GP AA1,GPD1L,GPX1,HMGCR,IDH3B,IDH3G,MAPK1,MAPK13,MAPKAPK3,MGAT1, MPI,MPPE1,MVK,NCK1,NDST1,NDUFA1,NDUFA7,NDUFS4,NDUFV1,NDUFV2
GO.00067 93	phosphorus metabolic process	42	7.04E-05	AK2,ALDOC,ATM,ATP5D,AXIN1,CAD,CAMK1,CASK,CDK5,DYRK4,ENO1,EPHA1, ERCC2,FAM20B,GALK2,GPI,MAPK1,MAPK13,MAPKAPK3,MVK,NDST1,NDUFA1, NDUFA7,NDUFS4,NDUFV1,NDUFV2
GO.00163 10	phosphorylation	26	0.00426	ABCA1,ACLY,ACOX1,ACTR2,ADRM1,AGGF1,ANAPC10,ARF4,ARFGEF2, ARID3A,ARL1,ARPC1A,ARPC4,ATF6,ATM,AXIN1,BAX,BCAP31,BCAR3,BNIP3 C4BPB,CAMK1,CAPNS1,CASK,CDC6,CDH15,CDKN2B,CBPB,CHMP2A, COX8A,CSPG5,CTNNBIP1,DAP,DDB1,DNAJB6,DNMT1,E2F5,EEF2,EIF2B1, EIF4G1,ELF4,EPHA1,ERCC2,FAM20B,FLAT2,FPR1,FRAT2,FZD2,GBF1, GPD1L,GPR39,HCLS1,HIST1H1B,HMGCR,ICAM3,ID3,IGFBP5,IL2RA,ILF2,ITGAM ,KIFAP3,LASP1,LMAN1,MAPK13,MAPKAPK3,MFGE8,MIF,MRPL12,MSN, NCK1,NCSTN,NDUFS4
GO.00485 18	positive regulation of biological process	72	0.0117	
GO.00485 22	positive regulation of cellular process	62	0.0394	ABCA1,ACLY,ACTR2,ADRM1,AGGF1,ANAPC10,ARF4,ARID3A,ARPC1A,ARPC4, ATF6,ATM,AXIN1,BAX,BCAP31,BCAR3,BNIP3,CAMK1,CAPNS1,CASK,CDC6,C DH15,CDKN2B,CBPB,CHMP2A,COX8A,CSPG5,CTNNBIP1,DAP,DDB1,DNMT1, E2F5,EEF2,EIF4G1,ELF4,EPHA1,ERCC2,FAM20B,FLAT2,FPR1,FRAT2,FZD2, GPD1L,GPR39,HCLS1,HIST1H1B,HMGCR,ID3,IGFBP5,IL2RA, ILF2,KIFAP3,LASP1,LMAN1,MAPKAPK3,MFGE8,MIF,MRPL12,MSN,NCK1, NCSTN,NDUFS4
GO.00442 38	primary metabolic process	111	0.00145	ABCA1,ACOX1,ACP1,ADRM1,AGGF1,AK2,ALDOC,ANAPC10,APOD,ARF4, ARID3A,ASGR2,ASPHD1,ATF6,ATM,ATP5D,ATP5H,ATP6V0D1,ATP6V1B2, BNIP3,BYSL,C4BPB,CAD,CAMK1,CAPNS1,CASK,CD2BP2,CDKN2B,CLN3, CLPP,COG7,COMMD4,COPS3,COP5A,COX5A,COX7C,COX8A,CSPG5, CTSZ,DAP,DDB1,DEGS1,DHCR7,DHRS3,DHX8,DNAJB6,DNAJC8,DNASE2, DNMT1,DPM2,DYRK4,E2F5,EEF2,EIF2B1,EIF4EBP2,EIF4G1,ELAC2,ELF4, ENO1,EPHA1,ERCC2,FLAD1,GLB1,GOSR2,GPAA1,GPC5,GPD1L,GPI, GTF2F2,H1F0,HAGH,HERC3,HERC4,HESX1,HIBCH,HMGCR,HSP90AB1, ID3,IDH2,ILF2,IMP4,IVD,KLK2,LMAN1,LMNA,LSM3,LY96,MAPK1,MAPK13, MAPKAPK3,MCM3,MCRS1,MGST3,MIF,MPDU1,MPI,MPPE1,MRPL12, MRPS11,MRPS12,MTFHS,MVK,NAGLU,NAGPA,NCK1,NDUFA1,NDUFA7, NDUFS4,NDUFV1,NDUFV2,NEDD8
GO.00064 61	protein complex assembly	21	0.0487	ADRM1,AHCTF1,AP2S1,ARPC4,AXIN1,BAX,CHMP2A,CLPP,EPRS,GPAA1,H1F0, HCLS1,HIST1H1B,HIST1H1D,HMGCR,KIAA0196,KIFAP3,KPNA4,MIF, NCK1,NDUFS4
GO.00702 71	protein complex biogenesis	21	0.0487	ADRM1,AHCTF1,AP2S1,ARPC4,AXIN1,BAX,CHMP2A,CLPP,EPRS,GPAA1,H1F0, HCLS1,HIST1H1B,HIST1H1D,HMGCR,KIAA0196,KIFAP3,KPNA4,MIF, NCK1,NDUFS4
GO.00718 22	protein complex subunit organization	29	0.0186	ACTR2,ADRM1,AHCTF1,AP2S1,ARPC1A,ARPC4,AXIN1,BAX,CHMP2A,CLPP,DC TN2,DNAJB6,EPRS,GPAA1,H1F0,HCLS1,HIST1H1B,HIST1H1D,HMGCR,ITGB5,K IAA0196,KIFAP3,KPNA4,MIF,MRPL12,MRPS11,MRPS12,MTM1, NDUFS4
GO.00081 04	protein localization	36	0.00145	ABCA1,AHCTF1,ARCN1,ARF4,ARF5,ARFGEF2,ARL1,ATP6V0B,ATP6V0D1,ATP6 V1B2,BAX,BCAP31,CDK5,CHMP2A,CLTB,COG7,COPE,DCTN2,DNAJB6,EIF5A,F LOT2,G3BP2,GBF1,GOSR2,GPAA1,HIST1H1B,HTR2A,KIAA0196, KIFAP3,LMAN1,MAPK1,MAPRE1,MTM1,MYO7A,NAGPA,NEDD8
GO.00709 73	protein localization to endoplasmic reticulum exit site	2	0.0377	BCAP31,GBF1
GO.00333 65	protein localization to organelle	16	0.0151	ARF4,ARL1,BAX,BCAP31,COG7,DCTN2,DNAJB6,GBF1,GOSR2,GPAA1, HIST1H1B,HTR2A,KPNA4,MAPK1,MAPRE1,NAGPA
GO.00195 38	protein metabolic process	60	0.0164	ACP1,ANAPC10,ARF4,ASGR2,ASPHD1,ATF6,ATM,ATP6V0D1,BNIP3,C4BPB,CA D,CAMK1,CAPNS1,CASK,CLN3,CLPP,COG7,COPS3,COPS7A,CSPG5,CTSZ,DA P,DDB1,DNAJB6,DPM2,DYRK4,EEF2,EIF2B1,EIF4EBP2,EIF4G1,EPHA1,ERCC2, GLB1,GOSR2,GPAA1,GPC5,GPX1,HERC3,HERC4,HSP90AB1,IGFBP5,KLK2,LM AN1,LMNA,LY96,MAPK1,MAPK13,MAPKAPK3,MCRS1,MGAT1,MPDU1,MPI,MPP E1,MRPL12,MRPS11,MRPS12,NAGPA,NCK1,NDUFA7,NEDD8

GO.00150				ABCA1,AHCTF1,ARCN1,ARF4,ARF5,ARFGEF2,ATP6V0B,ATP6V0D1,ATP6V1B2,BCAP31,CDK5,CHMP2A,CLTB,COG7,COPE,EIF5A,GBF1,GOSR2,KIAA0196,LMAN1,LMNA,MAPK1,MTM1,MYO7A,NAGPA
GO.00061	protein transport	25	0.04	
63	purine nucleotide metabolic process	16	8.13E-05	ACLY,AK2,ALDOC,APRT,ATP5D,ATP5H,ATP6V1B2,ENO1,GPI,GPX1,HMGCR,NDUFA1,NDUFA7,NDUFS4,NDUFV1,NDUFV2
GO.00461	ribonucleoside metabolic process	15	6.29E-05	ACLY,AK2,ALDOC,APRT,ATP5D,ATP5H,ATP6V1B2,ENO1,GPI,HMGCR,NDUFA1,NDUFA7,NDUFS4,NDUFV1,NDUFV2
GO.00091	purine monophosphate metabolic process	13	5.57E-05	AK2,ALDOC,APRT,ATP5D,ATP5H,ATP6V1B2,ENO1,GPI,NDUFA1,NDUFA7,NDUFS4,NDUFV1,NDUFV2
67	purine ribonucleoside metabolic process	15	0.00017	ACLY,AK2,ALDOC,APRT,ATP5D,ATP5H,ATP6V1B2,ENO1,GPI,HMGCR,NDUFA1,NDUFA7,NDUFS4,NDUFV1,NDUFV2
GO.00091	ribonucleotide metabolic process	15	0.00017	ACLY,AK2,ALDOC,APRT,ATP5D,ATP5H,ATP6V1B2,ENO1,GPI,HMGCR,NDUFA1,NDUFA7,NDUFS4,NDUFV1,NDUFV2
50	purine-containing compound metabolic process	15	0.00113	ACLY,AK2,ALDOC,ATP5D,ATP5H,ATP6V1B2,ENO1,GPI,GPX1,HMGCR,NDUFA1,NDUFA7,NDUFS4,NDUFV1,NDUFV2
GO.00725	pyridine-containing compound metabolic process	7	0.00671	ALDOC,AOX1,ENO1,GPD1L,GPI,IDH3B,IDH3G
24				ABCA1,ABC86,ACOX1,ACTR2,APRT,ARPC1A,ARPC4,ASGR2,ATM,ATOX1,ATP6V0B,ATP6V0D1,ATP6V1B2,BCAP31,BCAT2,BNIP3,C4BPB,CAD,CAMK1,CASK,CDC6,CCL23,CEBPB,CLN3,COG7,CTNBP1,CTSZ,DDB1,DDO,DHRS3,DNA2,DPM2,EIF4EBP2,ERCC2,FLOT2,G3BP2,GOT1,GPAA1,GPD1L,GPI,GPR39,GPX1,HCLS1,HIST1H1B,HSP90AB1,IGFBP5,IL2RA,ITGAM,KIFAP3,LGALS1,LMAN1,MPV17,MTM1,NCK1,NCSTN
GO.00650	regulation of biological quality	55	0.000335	ACTR2,ANAPC10,AP2S1,APOD,ARPC1A,ARPC4,ATM,BAX,BNIP3,C4BPB,CAMK1,CCDC6,CHMP2A,COX8A,CSPG5,CTNBP1,DCBLD2,DNAJB6,DNMT1,EIF5A,ENO1,EPHA1,FAM20B,GPX1,GTFF2F2,HCLS1,HIST1H1B,IGFBP5,LGALS1,LMAN1,LMNA,MAPK1,MAPRE1,MFGE8,MSN,MTM1,NCK1
GO.00511	regulation of cellular component organization	37	0.0376	ACTR2,AP2S1,APOD,ARPC1A,ARPC4,ATM,BAX,C4BPB,COX8A,CSF3R,CSPG5,CTNBP1,FLOT2,FXR2,GATA2,GPX1,HCLS1,HSP90AB1,ICAM3,IFNGR2,IL2RA,ITGAM,LGALS1,LY96,MAPKAPK3,MIF,MSN,NCK1
GO.00026	regulation of immune system process	28	0.0186	ACTR2,ANAPC10,ARPC1A,ARPC4,ATM,BAX,BNIP3,CDK5,CHMP2A,COX8A,CSPG5,DNMT1,EPHA1,FAM20B,GATA2,GPX1,HCLS1,HIST1H1B,LMAN1,LMNA,MAPK1,MAPRE1,MSN,MTM1,NCK1
GO.00330	regulation of organelle organization	25	0.0125	AXIN1,BAX,BCAR3,CAMK1,GPD1L,HCLS1,MIF,NCK1
GO.00331	regulation of peptidyl-serine phosphorylation	8	0.00551	
35				
GO.00512	regulation of protein metabolic process	40	0.0458	ADRM1,ANAPC10,APOD,ATM,AXIN1,BCAP31,BCAR3,C4BPB,CAMK1,CCDC6,CDC2B,CDKN2B,CEBPB,CLN3,CTSZ,DAP,DNAJB6,DNMT1,EEF2,EIF4EBP2,EIF4G1,EPHA1,EPRS,FLOT2,FPR1,FXR2,GATA2,GPD1L,GPI,GPX1,HCLS1,HIST1H1B,HMGCR,IGFBP5,LY96,MAPKAPK3,MIF,MSN,NCK1,NDUFS4
GO.00801	regulation of response to stress	28	0.0318	AP2S1,APOD,ATM,AXIN1,BAX,BCAP31,C4BPB,CASK,CEBPB,CLN3,COX8A,CSPG5,DNAJB6,FXR2,GPX1,HMGCR,HSP90AB1,ICAM3,IFNGR2,IL2RA,ITGAM,LY96,MAPK1,MAPK13,MAPKAPK3,MIF,MVK,NCK1
GO.00229	respiratory electron transport chain	11	5.92E-05	ATP5D,ATP5H,COX5A,COX7C,COX8A,CYC1,NDUFA1,NDUFA7,NDUFS4,NDUFV1,NDUFV2
GO.00422	response to chemical	58	0.0121	ABCA1,ACTR2,ALPL,AP2S1,APOD,APRT,ARPC1A,ARPC4,ATF6,ATOX1,ATP5D,ATP6V0B,ATP6V0D1,ATP6V1B2,AXIN1,BCAR3,BNIP3,BYSL,CAD,CCDC6,CCL23,CDKN2B,CSF3R,CYC1,DNMT1,E2F5,EIF2B1,EIF4EBP2,EIF4G1,EPHA1,EPRS,FPR1,GATA2,GBF1,GOSR2,GOT1,GPR39,GPX1,HCLS1,HMGCR,IFNGR2,IGFBP5,IL2RA,ITGAM,ITGB5,KRT19,LGALS1,LMNA,LY96,MAPK13,MAPKAPK3,MFGE8,MIF,MPV17,NCK1,NCSTN,NDST1,NDUFS4
GO.00097	response to endogenous stimulus	28	0.0285	ABCA1,ALPL,APRT,ATP6V0B,ATP6V0D1,ATP6V1B2,BAX,CAD,CDK5,CDKN2B,CYC1,DNMT1,E2F5,EIF2B1,EIF4EBP2,EIF4G1,GOT1,GPX1,HCLS1,HTR2A,IGFBP5,ITGB5,KRT19,MFGE8,MGST3,NDST1,NDUFS4,NEDD8
GO.00100	response to organic substance	44	0.00665	ABCA1,ALPL,AP2S1,APRT,ATF6,ATP6V0B,ATP6V0D1,ATP6V1B2,AXIN1,BYSL,CAD,CCDC6,CDK5,CSF3R,CYC1,DNMT1,E2F5,EIF2B1,EIF4EBP2,EIF4G1,EPRS,GATA2,GOSR2,GOT1,GPX1,HCLS1,HMGCR,HTR2A,IFNGR2,IGFBP5,IL2RA,ITGB5,KPNA4,KRT19,LGALS1,LMNA,LY96,MAPK13,MAPKAPK3,MFGE8,MGST3,NCSTN,NDST1,NDUFS4
GO.00102	response to organonitrogen compound	19	0.0349	APRT,ATP6V0B,ATP6V0D1,ATP6V1B2,BAX,CAD,CDK5,CYC1,DNMT1,EIF2B1,EIF4EBP2,EIF4G1,GOT1,GPX1,HTR2A,IGFBP5,MAPK1,MGST3,NDUFS4
GO.19017	response to oxygen-containing compound	30	0.00308	ABCA1,ALPL,APOD,APRT,ATOX1,ATP6V0B,ATP6V0D1,ATP6V1B2,BAX,BNIP3,BYSL,CAD,CCDC6,CDK5,CYC1,DNMT1,EIF2B1,EIF4EBP2,EIF4G1,GOT1,GPX1,HMGCR,HTR2A,IGFBP5,KPNA4,LGALS1,LY96,MAPKAPK3,MPV17,NDUFS4
GO.00068	retrograde vesicle-mediated transport, Golgi to ER	4	0.0365	ARCN1,COG7,COPE,GBF1
GO.00091	ribonucleoside metabolic process	16	4.86E-05	ACLY,AK2,ALDOC,APRT,ATP5D,ATP5H,ATP6V1B2,CAD,ENO1,GPI,HMGCR,NDUFA1,NDUFA7,NDUFS4,NDUFV1,NDUFV2
GO.00091	ribonucleoside monophosphate metabolic process	14	2.46E-05	AK2,ALDOC,APRT,ATP5D,ATP5H,ATP6V1B2,CAD,ENO1,GPI,NDUFA1,NDUFA7,NDUFS4,NDUFV1,NDUFV2
61				

GO.0009199	ribonucleoside triphosphate metabolic process	13	5.57E-05	AK2,ALDOC,ATP5D,ATP5H,ATP6V1B2,CAD,ENO1,GPI,NDUFA1,NDUFA7,NDUFS4,NDUFV1,NDUFV2
GO.0009259	ribonucleotide metabolic process	16	7.94E-05	ACLY,AK2,ALDOC,APRT,ATP5D,ATP5H,ATP6V1B2,CAD,ENO1,GPI,HMGCR,NDUFA1,NDUFA7,NDUFS4,NDUFV1,NDUFV2
GO.0007165	signal transduction	66	0.0269	ABCA1,ACTR2,AHCTF1,AP2S1,ARF4,ARF5,ARFGEF2,ARL1,ARPC1A,ARPC4,ASGR2,ATF6,ATM,ATP6V0B,ATP6V0D1,ATP6V1B2,AXIN1,BAX,BCAP31,BNIP3,CAMK1,CCL23,CDKN2B,CLN3,COPS3,CTNNBIP1,DAP,DCBLD2,DDB1,DHRS3,E2F5,EIF4G1,EPHA1,FPR1,FRAT2,G3BP2,GABRD,GOSR2,GPC5,GPR39,GPR4,GPX1,GRIN2D,HCLS1,HSP90AB1,ICAM3,IFNGR2,ITGAM,ITGB5,KIFAP3,KLK2,KNP4,LGALS1,LGALS3BP,LY96,M6PR,MAPK13,MAPKAPK3,MAPRE1,MGST3,MR1,NCK1,NCSTN,NDST1,NDUFS4,NEDD8
GO.0044708	single-organism behavior	13	0.0256	APRT,ARCN1,CDK5,CLN3,DDO,EIF4EBP2,EIF4G1,GPI,GPR39,GRIN2D,HMGCR,HTR2A,NAGLU
GO.0044711	single-organism biosynthetic process	31	0.0012	AK2,ALDOC,APRT,ATM,ATP5D,ATP5H,BCAT2,CSPG5,DDB1,DEGS1,DHCR7,DPM2,EEF2,EIF5A,ENO1,FLAD1,GPA1,GPC5,GPD1L,HMGCR,MGST3,MIF,MPDU1,MPI,MPPE1,MRPL12,MRPS11,MRPS12,MTM1,MVK,NDUFA7
GO.0044723	single-organism carbohydrate metabolic process	17	0.0153	ALDOC,APOD,ARF4,COG7,DPM2,ENO1,GALK2,GOT1,GPI,IDH2,IGFBP5,LMAN1,MGAT1,MPDU1,MPI,NAGPA,NDST1
GO.0044763	single-organism cellular process	128	0.00039	ABCA1,ABCB6,ACOX1,ACTR1A,AGGF1,AHCTF1,AK2,ALDOC,ALPL,ANAPC10,AXO1,AP2S1,APIS,APOD,ARCN1,ARF5,ARFGEF2,ARPC1A,ARPC4,ASGR2,ATF6,ATM,ATP5D,AXIN1,BAX,BCAP31,BNIP3,BYSL,CAD,CAMK1,CAPNS1,CASK,CCDC6,CCL23,CDH15,CDKN2B,CEBPG,CHMP2A,CLN3,COG7,COPE,COPS3,COX5A,COX8A,CSPG5,CTNNBIP1,CYC1,DAP,DCBLD2,DEGS1,DHCR7,DHRS3,DNMT1,DPM2,E2F5,EIF2B1,EIF5A,ELAC2,ELF4,ENO1,EPHA1,FLAD1,FLOT2,FPR1,FRAT2,G3BP2,GATA2,GBF1,GLB1,GOSR2,GPA1,GPC5,GPD1L,GPR39,GPR4,GPX1,GRIN2D,H1F0,HCLS1,HERC4,HIBCH,HSP90AB1,HTATIP2,ICAM3,ID3,IDH2,IFNGR2,ITGB5,IVD,KIAA0196,KIFAP3,KLK2,KNP4,LASP1,LGALS3BP,LMAN1,LMNA,LY96,M6PR,MAPK13,MAPKAPK3,MAPRE1,MCM3,MCM4,MCRS1,MFGE8,MFSD5,MGST3,MIF,MPI,MPPE1,MPV17,MR1,MRPL12,MRPS11,MRPS12,MSN,MTHFS,MVK,MYBPC2,NAGLU,NAGPA,NDUFA1,NDUFA7,NDUFS4,NDUFV1,NDUFV2,NEDD8
GO.1902582	single-organism intracellular transport	27	0.00394	ABCA1,ARCN1,ARFGEF2,ATOX1,ATP5D,ATP5H,BAX,BCAP31,BNIP3,CLN3,COG7,COPE,EIF5A,GATA2,GBF1,GOLGA3,GOSR2,HTR2A,KIFAP3,KNP4,LMAN1,LMNA,M6PR,MAPK1,MPPE1,MTM1,NAGPA
GO.0044710	single-organism metabolic process	68	0.000702	ABCA1,ACOX1,AK2,ALDOC,APOD,ARF4,ARL1,ASPHD1,ATM,ATP5D,ATP5H,ATP6V1B2,AXIN1,BAX,BNIP3,C4BPB,CAD,CASK,CCDC6,CLN3,COG7,COX5A,COX8A,CSPG5,CYB561D2,CYC1,DDB1,DHCR7,DPM2,EEF2,EIF5A,ELAC2,ENO1,ERCC2,FLAD1,GALK2,GPA1,GPC5,GPD1L,GPX1,HIBCH,HMGCR,HTATIP2,IDH2,IGFBP5,IVD,LMAN1,MAPK13,MAPKAPK3,MCRS1,MGST3,MIF,MPDU1,MPI,MPPE1,MRPL12,MRPS11,MRPS12,MTHFS,MTM1,MVK,NAGLU,NAGPA,NDUFA1,NDUFA7,NDUFS4,NDUFV1,NDUFV2
GO.0044281	small molecule metabolic process	46	7.94E-05	ABCA1,ACOX1,AK2,ALDOC,AXO1,ATP5D,ATP5H,ATP6V1B2,CAD,CASK,CLN3,COX5A,COX7C,COX8A,CSPG5,CYC1,DEGS1,DHCR7,DHRS3,DPM2,ENO1,ERCC2,FLAD1,GLB1,GPC5,GPD1L,GPX1,HIBCH,HMGCR,IDH2,IVD,MAPK1,MGAT1,MGST3,MIF,MPI,MTHFS,MTM1,MVK,NAGLU,NDST1,NDUFA1,NDUFA7,NDUFS4,NDUFV1,NDUFV2
GO.0044403	symbiosis, encompassing mutualism through parasitism	19	0.0376	AP2S1,ATP6V0B,ATP6V0D1,ATP6V1B2,BAX,BNIP3,CHMP2A,DDB1,EIF4G1,ERCC2,GBF1,GPX1,GT2F2,HSP90AB1,HTATIP2,KNP4,KRT19,MAPK1,MFGE8
GO.0007169	transmembrane receptor protein tyrosine kinase signaling pathway	18	0.0262	ACTR2,AP2S1,ARF4,ARPC1A,ARPC4,ATP6V0B,ATP6V0D1,ATP6V1B2,CCDC6,EIF4EBP2,EIF4G1,EPHA1,MAPK1,MAPK13,MAPKAPK3,NCK1,NCSTN,NDST1
GO.0006810	transport	55	0.00821	ABCA1,ABCB6,ACTR1A,AHCTF1,AP2S1,APOD,APRT,ARCN1,ARF4,ARF5,ARFGEF2,ARPC1A,ARPC4,ASGR2,ATP5D,AXIN1,BAX,BCAP31,CAD,CAMK1,CLN3,CLTB,COG7,COPE,COX5A,COX7C,COX8A,CSPG5,DCTN2,EIF5A,G3BP2,GATA2,GBF1,GOLGA3,GOSR2,GRIN2D,HSP90AB1,HTATIP2,HTR2A,ICA1,KIAA0196,KIFAP3,LASP1,LGALS3BP,LMAN1,LMNA,MAPKAPK3,MFGE8,MFSD5,MPDU1,MPPE1,MTM1,MYO7A,NAGPA,NCK1
GO.0007034	vacuolar transport	6	0.0238	CHMP2A,CLN3,GOSR2,M6PR,MTM1,NAGPA
GO.0016192	vesicle-mediated transport	31	0.000102	ABCA1,ACTR1A,ACTR2,AP2S1,ARCN1,ARF4,ARF5,ARFGEF2,ARPC1A,ARPC4,ASGR2,BCAP31,CHMP2A,CLN3,CLTB,COG7,COPE,GATA2,GBF1,GOLGA3,GOSR2,HSP90AB1,LGALS3BP,LMAN1,M6PR,MAPK1,MAPKAPK3,MFGE8,MPPE1,MYO7A,NCK1

Table 4.13 Biological processes of ID3 and ID3 candidate target genes in tissue. Gene ontology (GO) shown via STRING database demonstrate 116 biological processes.

ID3 & ID3 candidate target genes & EDC-associated genes. Comparative Toxicogenomic Database examination yielded various results. When comparing ID3 candidate target genes in blood (134) with PCB associated genes (648) results demonstrated 12 genes which include: AEBP1, APOH, CHEK1, DNMT1, DTYMK, FKBP4, GFRA1, HIST1H4C, ID3, IL6, LIFR, and MTTP (Figure 4.9.1 & Table 4.15.1). Furthermore, when comparing ID3 candidate target genes in tissue (217) with PCB associated genes (648) results showed 13 genes including: ACOX1, ACTR2, ALDOC, CDK2AP2, CSF3R, CTSZ, DNMT1, GPI, HSP90AB1, ID3, ILF2, ITGB5, LY96 (Figure 4.9.2 & Table 4.15.2). Additionally, we also investigated two specific PCB congeners (PCB153 and PCB180) and examined their associated genes with ID3/ID3 candidate target genes. ID3 candidate target genes in blood (134) and PCB153-associated genes (2016) resulted in 19 genes including: CCL7, CTNNB1, DCX, DLG2, DTYMK, DUSP14, FNBP1, GRIN2B, HMGB3, HNF4G, HS3ST1, HTR4, IFIH1, IGFBP2, IL6, LIFR, MCM4, MMP11, and NDUFA7 (Figure 4.9.4 & Table 4.15.4). ID3 candidate target genes in tissue (217) and PCB153-associated genes (2016) resulted in 24 genes: ABCA1, AOX1, APOD, ASGR2, ATM, BAX, COG7, EEF2, ELF4, GOT1, GPC5, GPX1, HIST1H1B, HMGCR, HTATIP2, IDH2, IL2RA, MAPK1, MCM3, MCM4, MGST3, MTM1, MVK, NDUFA7 (Figure 4.9.5 & Table 4.15.5). When comparing ID3 candidate target genes in blood (134) with PCB180-associated genes (1876) results yielded 20 genes including: ACTN1, ARID5B, CACNG3, CCL7, CHEK1, DCX, DLG2, DUSP14, FBLN5, FNBP1, GRIN2B, HDAC4, HMGB3, HNF4G, HS3ST1, HTR4, IFIH1, IL6, MCM4, and NDUFA7 (Figure 4.9.7 & Table 4.15.7). ID3 candidate target genes in

tissue (217) vs. PCB180-associated genes (1876) resulted in 18 genes: APOD, ATM, BAX, COG7, ELF4, GPC5, HIBCH, HSP90AB1, HTR2A, IL2RA, LGALS1, LGALS3BP, MAPK1, MCM3, MCM4, MGST3, MTM1, and NDUFA7 (Figure 4.9.8 & Table 4.15.8). Lastly we also demonstrated BPA-associated genes (19169) with ID3 candidate targets in both blood and tissue respectively. These results indicated 126 genes in blood and 202 genes in tissue further demonstrated in Figures 4.10.1 & 4.10.2 and Tables 4.16.1 & 4.16.2.

Figure 4.8.1 Venn diagram illustrating ID3 candidate target genes in blood with PCBs. ID3 candidate target genes (134), PCB associated genes (648), and overlapping genes (12).

Associated ID3 Target Genes in blood with PCBs	
	AEBP1
	APOH
	CHEK1
	DNMT1
	DTYMK
	FKBP4
	GFRA1
	HIST1H4C
	ID3
	IL6
	LIFR
	MTTP

Table 4.14.1 ID3 candidate target genes in blood associated with polychlorinated biphenyls (PCBs).

Figure 4.8.2 Venn diagram illustrating ID3 candidate target genes in tissue with PCBs. ID3 candidate target genes (217), PCB associated genes (648), and overlapping genes (13)

Associated ID3 Target Genes in tissue with PCBs	
	ACOX1
	ACTR2
	ALDOC
	CDK2AP2
	CSF3R
	CTS2
	DNMT1
	GPI
	HSP90AB1
	ID3
	ILF2
	ITGB5
	LY96

Table 4.14.2 ID3 candidate target genes in tissue associated with polychlorinated biphenyls (PCBs).

Figure 4.8.3 Venn diagram summarizing overlapping ID3 candidate target genes in blood-tissue with PCBs. ID3 candidate target genes (11), PCB associated genes (648), and overlapping genes (2)

Overlapping ID3 Candidate Target Genes
Genes in blood-tissue with PCBs
DNMT1
ID3

Table 4.14.3 Overlapping ID3 candidate target genes in blood-tissue associated with polychlorinated biphenyls (PCBs).

Figure 4.8.4 Venn diagram summarizing ID3 candidate target genes in blood with PCB153. ID3 candidate target genes (134), PCB153 associated genes (2016), and overlapping genes (19)

Associated ID3 Candidate Target Genes in blood with PCB153	
	CCL7
	CTNNB1
	DCX
	DLG2
	DTYMK
	DUSP14
	FNBP1
	GRIN2B
	HMGB3
	HNF4G
	HS3ST1
	HTR4
	IFIH1
	IGFBP2
	IL6
	LIFR
	MCM4
	MMP11
	NDUFA7

Figure 4.14.4 ID3 candidate target genes in blood associated with polychlorinated biphenyl 153 (PCB153).

Figure 4.8.5 Venn diagram summarizing ID3 candidate target genes in tissue with PCB153. ID3 candidate target genes (217), PCB153 associated genes (2016), and overlapping genes (24).

Associated ID3 Candidate Target Genes in tissue with PCB153	
ABCA1	
AOX1	
APOD	
ASGR2	
ATM	
BAX	
COG7	
EEF2	
ELF4	
GOT1	
GPC5	
GPX1	
HIST1H1B	
HMGCR	
HTATIP2	
IDH2	
IL2RA	
MAPK1	
MCM3	
MCM4	
MGST3	
MTM1	
MVK	
NDUFA7	

Figure 4.14.5 ID3 target genes in tissue associated with polychlorinated biphenyl 153 (PCB153).

Figure 4.8.6 Venn diagram summarizing ID3 candidate target genes in blood-tissue. ID3 candidate target genes (11), PCB153 associated genes (2016), and overlapping genes (2).

Overlapping ID3 Candidate Target Genes in blood-tissue with PCB153
MCM4
NDUFA7

Figure 4.14.6 ID3 target genes in blood-tissue associated with polychlorinated biphenyl 153 (PCB153).

Figure 4.8.7 Venn diagram summarizing ID3 target genes in blood with PCB180. ID3 candidate target genes (134), PCB180 associated genes (1876), and overlapping genes (20).

Associated ID3 Candidate Target Genes in blood with PCB180
ACTN1
ARID5B
CACNG3
CCL7
CHEK1
DCX
DLG2
DUSP14
FBLN5
FNBP1
GRIN2B
HDAC4
HMGB3
HNF4G
HS3ST1
HTR4
IFIH1
IL6
MCM4
NDUFA7

Figure 4.14.7 ID3 candidate target genes in blood associated with polychlorinated biphenyl 180 (PCB180).

Figure 4.8.8 Venn diagram summarizing ID3 candidate target genes in tissue with PCB180. ID3 candidate target genes (217), PCB180 associated genes (1876), and overlapping genes (18).

Associated ID3 Candidate Target Genes in tissue with PCB180	
	APOD
	ATM
	BAX
	COG7
	ELF4
	GPC5
	HIBCH
	HSP90AB1
	HTR2A
	IL2RA
	LGALS1
	LGALS3BP
	MAPK1
	MCM3
	MCM4
	MGST3
	MTM1
	NDUFA7

Figure 4.14.8 ID3 candidate target genes in tissue associated with polychlorinated biphenyl 180 (PCB180).

Figure 4.8.9 Venn diagram summarizing overlapping ID3 candidate target genes in blood-tissue with PCB180. ID3 candidate target genes (11), PCB180 associated genes (1876), and overlapping genes (2).

Overlapping ID3 Candidate Target
Genes in blood-tissue with PCB180
MCM4
NDUFA7

Table 4.14.9 ID3 candidate target genes in blood-tissue associated with polychlorinated biphenyl 180 (PCB180).

Figure 4.9.1. Venn diagram summarizing ID3 candidate target genes in blood with BPA. ID3 candidate target genes (134), Bisphenol A associated genes (19169), and overlapping genes (126).

ABCA4	COL1A2	GLRA1
ABCB6	COL3A1	GPR135
ACIN1	COL6A1	GPR37L1
ACP1	COPB2	GRIA2
ACTA2	CREBBP	GRIK5
ACTN1	CRYAA	GRIN2B
ADAMTS3	CTGF	GRP
AEBP1	CTH	HDAC4
AGTR2	CTNNB1	HDHC2
ANKRD26	CTSF	HMGB3
ANPEP	CYP26A1	HMGCS2
APAF1	CYP4F11	HNF4G
APOF	CYR61	HOXD3
APOH	DAAM1	HS3ST1
ARID5B	DAB2	HSPB2
ARSA	DCX	HTR4
ASB4	DHRS3	IBSP
ASNS	DHRS7	ID3
ATP7B	DIRAS3	IFIH1
AVPR2	DLG2	IGFBP2
BCAM	DNAJC7	IL13RA2
BCL2L2	DNMT1	IL6
BYSL	DOCK9	INHBB
C10ORF10	DRD1	ITGA8
CACNB4	DTYMK	KCNH2
CACNG3	DUSP14	KCNJ4
CAD	DYRK3	KCNK1
CALR	DZIP1	KIF1A
CCL7	EEF1E1	KNG1
CCT8	ETS1	LGR5
CDH15	FBLN2	LIFR
CDH16	FBLN5	LMO3
CDH17	FBP2	MAN1A2
CDH2	FKBP4	MCM4
CDH6	FBNP1	MLLT10
CFDP1	FUT9	MMP11
CFTR	GABRA2	MPP3
CH25H	GAL	MPP6
CHEK1	GAS8	MTTP
CKM	GCLM	MYL2
CNKSR2	GDF11	NDP
COL17A1	GFRA1	NDUFA7

Table 4.15.1 ID3 candidate target genes in blood associated with BPA.

Figure 4.9.2. Venn diagram summarizing ID3 candidate target genes in tissue with BPA. ID3 candidate target genes (217), Bisphenol A associated genes (19169), and overlapping genes (202)

ABCA1	BAX	DCTN2	GLB1	LASP1	NDUFV1
ABCB6	BCAP31	DCTN5	GOLGA3	LGALS1	NDUFV2
ACLY	BCAR3	DDB1	GOSR2	LGALS3BP	NEDD8
ACOX1	BCAT2	DDN	GOT1	LMAN1	
ACP1	BCL7B	DDO	GPAA1	LMNA	
ACTR1A	BNIP3	DEGS1	GPC5	LRRC42	
ACTR2	BYSL	DHCR7	GPD1L	LSM3	
ADRM1	C4BPB	DHRS3	GPI	LY96	
AGGF1	CAD	DHX16	GPR39	M6PR	
AHCTF1	CAMK1	DHX8	GPR4	MAPK1	
AK2	CASK	DNAJB6	GPX1	MAPK13	
ALDH3B1	CCDC6	DNAJC8	GRIN2D	MAPKAPK3	
ALDOC	CCNI	DNASE2	H1FO	MAPRE1	
ALPL	CD2BP2	DNMT1	HAGH	MCM3	
ANAPC10	CD99	DPM2	HCLS1	MCM4	
AOX1	CDH15	DYRK4	HERC4	MCRS1	
AP2S1	CDK2AP2	E2F5	HESX1	MFGE8	
API5	CDK5	EEF2	HIBCH	MFSD5	
APOD	CDKN2B	EIF2B1	HIST1H1B	MGAT1	
APRT	CEBPG	EIF4EBP2	HIST1H1D	MGST3	
ARCN1	CHMP2A	EIF4G1	HMGCR	MIF	
ARF4	CLN3	EIF5A	HSP90AB1	MPDU1	
ARF5	CLPP	ELAC2	HTATIP2	MPI	
ARFGEF2	CLTB	ENO1	HTR2A	MPPE1	
ARID3A	COG7	EPHA1	ICA1	MR1	
ARL1	COMMD4	EPRS	ID3	MRPL12	
ARPC1A	COPE	ERAL1	IDH2	MRPS12	
ARPC4	COPS3	ERCC2	IDH3B	MSN	
ASGR2	COPS7A	FAM20B	IDH3G	MTHFS	
ASPHD1	COX5A	FAM50A	IFNGR2	MTM1	
ATF6	COX7C	FLAD1	IGFBP5	MYBPC2	
ATM	COX8A	FLOT2	IL2RA	MYO7A	
ATOX1	CSF3R	FRAT2	ILF2	NAGLU	
ATP5D	CSPG5	FXR2	IMP4	NAGPA	
ATP5H	CTNBP1	FZD2	ITGAM	NCK1	
ATP6V0B	CTSZ	G3BP2	ITGB5	NCSTN	
ATP6V0D1	CYB561D2	GABRD	IVD	NDST1	
ATP6V1B2	CYC1	GALK2	KIFAP3	NDUFA1	
AXIN1	CYHR1	GATA2	KPNA4	NDUFA7	
BAX	DAP	GBF1	KRT19	NDUFS4	

Table 4.15.2 ID3 candidate target genes in tissue associated with BPA.

Discussion

To our knowledge this is a novelty study approach investigating ID3 and ID3 candidate target genes in vascular-intimal lesions. Our analysis intergrading both statistical & bioinformatics methods attempts to identify ID3 and ID3 candidate target genes alongside key gene networks that differentiate vascular disease subjects from non-disease subjects. We have used these approaches to elucidate a core set of genes in both blood & tissue that represent ID3 and potential targets. Interestingly 11 of these genes are mutually expressed in the two locations of

vascular diseases: blood & tissue. These genes whose involvement in various biological processes such as extracellular matrix, focal adhesion, & cell growth may help explain how ID3 signaling drives vascular dysfunction in the pathogenesis of these vascular diseases, in which vascular-intimal lesions manifest from. All mutually represented ID3 candidate target genes in both blood & tissue demonstrate known roles in vascular diseases forming a tight cluster that is largely centered on the regulation of these biological processes.

The identification of ID3 & ID3 candidate target genes helped truncate our vast quantity of mutually expressed genes (3665) across all the data. In our analysis we identified ID3 and ID3 candidate target genes in blood (134) and tissue (217) studies. In blood, 5 pathways were overrepresented by these genes. Interestingly, 2 pathways contribute to vascular perturbation in the lesion: extracellular matrix-receptor interaction & focal adhesion. The extracellular matrix (ECM); which is a dynamic composite of various proteins that form structures connecting cells, provides an architectural framework within the network. Blood vessels are inflated by blood pressure and thus require ECM mechanisms with enough tensile strength and elasticity to resist rupture (Ponticos & Smith. 2014). Furthermore focal adhesion, a type of adhesive contact between the cell and extracellular matrix is essential for proliferation, cell migrations, and embryonic development (Eleniste & Bruzzaniti. 2012). Together both regulate & control cellular function through biochemical and mechanical signaling. Cells respond to the ECM by alternating their environment which becomes dysregulated in vascular diseases such as atherosclerosis, restenosis, and hypertension (Ponticos & Smith.

2013). Both components are important for maintaining vascular integrity and prevention of unfavorable vascular remodeling (Raines. 2000). Overrepresented genes in both pathways include COL1A2, COL3A1, COL6A1, IBSP, ID3, & ITGA8. Type I collagens COL1A1 and COL1A2 are part of the fibillar α -cellular component of basement membranes and connective tissue (Yurchenco, 2011). Most recently it was shown that the expression of pro-inflammatory genes such as TNF- α and MCP-1 was significantly up-regulated as well as the expression of the pro-remodeling genes collagen type I, fibronectin and the metalloproteinase MMP-2 and TIMP-1 (Zelko et al., 2016). Interestingly ID3 snp rs11574 was demonstrated to up-regulate MMP3 & down-regulate COL6A3 leading to the stimulation of atherogenesis, which includes biological processes such as apoptosis, cell movement, cellular proliferation, immune trafficking, & adhesion (Doran et al., 2010). Integrin binding sialoprotein protein (IBSP) is a major structural protein of the bone matrix. The expression of IBSP also significantly increased on collagen type I (7 fold), vitronectin (5 fold), and laminin during a study where the role of ECM proteins in regulating the proliferation and osteogenic differentiation of bone marrow derived human mesenchymal stem cells (hMSCs) was examined (Matthews et al., 2012). In tissue, 20 pathways were overrepresented by ID3 candidate target genes. Two pathways were identified as a contributing factor to vascular dysfunction: oxidative phosphorylation & cell cycle pathway. Oxidative phosphorylation, which is the metabolic pathway in which cells use enzymes to oxidize nutrients is essential in the pathogenesis of vascular disease. Overproduction of reactive oxygen species (ROS) under various pathophysiologic

conditions can trigger certain pathways that may lead to vascular inflammation causing vessel & plaque rupture (Madamanchi et al. 2005; Gao et al. 2008). Vascular smooth muscle cell proliferation is a key component in the healing process of vascular dysfunction. This process is facilitated by multiple growth factors and cytokines which share a common pathway in inducing cell proliferation: the cell cycle (Braun-Dullaeus et al. 1998; Charron et al. 2006). Key molecular events in atherogenesis such as phospholipids, endothelial cell activation, oxidative modification of lipoproteins, and macrophage activation/infiltration are facilitated by vascular oxidative stress and inhibited by endothelial nitric oxide (Förstermann et al., 2017). Furthermore angiogenesis in the 3D collagen gel was demonstrated recently to be regulated by the JAK/STAT3 pathway which involves alterations in vessel diameter, vessel length, and sprout number. The essential mechanism was that the JAK/STAT3 signaling pathway regulated angiogenesis by perturbations in numbers of angiogenesis-associated growth factors and by direct regulation of cell cycle (Xue et al., 2016). Furthermore the top 10 most significant ID3 candidate target genes in both blood and tissue were demonstrated. In blood these genes include: COL1A2, COL6A1, CYP2C18, DIRAS3, DLG2, DZIP1, GCLM, GDF11, GRP, and KNG1. In tissue these genes include: ACLY, ACP1, ARPC4, ATP5D, CAMK1, CDKN2B, COPE, CYC1, EIF4EBP2, and MCRS1.

Additionally, genes including: ABCB6, ACP1, BYSL, CAD, CDH15, DCBLD2, DHRS3, DNMT1, ID3, MCM4, & NDUFA7 are represented both in blood & tissue data. In order to initially establish if these genes had an interaction within their network, a globally optimal Bayesian network structure without any structural

limitations (Silander & Myllymak) provided us with a fully connected structure. In order to further validate the model, the mutually common genes were implemented into a Bayesian network that allowed us to determine the probability distribution of gene expression (low, normal, & high), location of disease (tissue and blood), and vascular state (disease and no disease). Represented by our initial model it was predicted that ID3 was predominately expressed as high (high: 79%, normal: 4%, & low: 17%). Also the vascular state was in close proximity of one another (disease state: 56%, control state: 44%). We demonstrated in the model when ID3 was expressed at various levels at 100% (high, normal, & low). Interestingly when ID3 is highly expressed the genes in the model also became highly expressed predominantly occurring in blood with an evenly distributed vascular state (disease: 51%, control state: 49%). When ID3 expression is normal, the network is modeled more in tissue and the vascular state is principally geared toward vascular disease (disease: 65%, control: 35%). Low expression of ID3 represents activity principally in tissue toward vascular disease (disease: 66%, control: 34%). These changes in the model may elucidate to ID3 regulation that exists in this network. Furthermore we extended our analysis of ID3 candidate target genes in both blood & tissue and investigated which genes are additionally estrogenic/endocrine disrupting chemical associated. Results demonstrated that gene DNMT1 is associated with ID3 candidate genes in both blood & tissue and PCBs; and genes MCM4 & NDUFA7 are associated with ID3 candidate target genes in both blood & tissue and PCB153 & PCB180.

Interestingly each of these genes have been known to be involved in various vascular diseases through the literature. ABCB6 has been shown to have a new role in preventing atherosclerosis growth by inhibiting platelet production, reactivity, and chemokine (C-C motif) ligand-5 deposition in atherosclerotic lesions (Murphy et al., 2014). ACP1 is identified to be associated with atherosclerosis and autoimmune diseases in Caucasians. It was shown that rs79716074 genotype of GA polymorphism in ACP1 is associated with decreased risk of coronary arterial disease in Chinese northeast population especially in females (Li et al., 2015). CAD (carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase) has been demonstrated as an unacknowledged mechanism that regulates NOTCH/VEGF signaling during angiogenesis (Caxam et al., 2015). DCBLD2 which is also known as ESDN has been established as a potential diagnostic and therapeutic target for graft atherosclerosis, which still remains a cause of progressive, chronic failure in cardiac allograft (Sadeghi et al., 2007). MCM4, part of a family of pre-replication complexes show that MCM2, MCM4, and MCM7 mRNA levels are increased in VSMCs during G1 phase, as indicated by increased Cyclin D1 levels (Guo et al., 2013). Furthermore this family of proteins is mutually modified by ID3 influence (MCM2, MCM3 and MCM10), such that genes down-regulated in PMEL-1 RAG1^{-/-}ID3^{-/-} T cells were induced by overexpression of ID3 (Ji et al., 2011). Also, DNMT1, a differentially expressed gene that is associated with VSMC-associated diseases which is regulated by 17 miRNAs, where these miRNAs are possible targets for developing anti-R based miRNA therapy, was recently established (Chen et al., 2017). DHRS3, was

recently reported to be associated with rheumatoid arthritis (RA), a complex and prevalent complex inflammatory disease primarily affecting the joint (Wang et al., 2017). The vascular disease in RA encompasses a large spectrum ranging from rheumatoid vasculitis to atherosclerotic lesions (Tanasescua et al., 2009). NDUFA7, a subunit of the complex-I in the mitochondrial respiratory chain was also demonstrated to be associated with rheumatoid arthritis (RA). Association of NDUFA7 and RA suggest reactive oxygen species (ROS) in the pathogenesis of the disease (Mitsunaga et al., 2015). Additionally we have previously shown that ID3 is an important determinant of ROS-induced proliferation of estrogen exposed endothelial cells & the overall results suggest ID3 may serve as a beneficial target for estrogen-dependent vascular-proliferative diseases (Felty & Porther, 2008). We also demonstrated ID3 overexpressing cells which supported the formation of a 3-D microvascular lesion co-cultured with smooth muscle cells (Das JK et al., 2015) and overexpression of ID3 increased neovascularization, cell migration, and spheroid growth of human cerebral microvascular endothelial cells, hCMEC/D3 (Das & Felty, 2015). We postulate that ID3 does have an essential role in regulation of these genes that take part in perturbations of vascular-intimal lesions.

Conclusion

In summary, our analysis strongly indicates that ID3 does have a molecular mechanism that affect changes in extracellular matrix, cell adhesion, & cell growth which predisposes the blood vessel to vascular dysfunction. Common genes identified in blood and tissue samples show a strong suggestion for their several roles in vascular diseases. Therapeutic interventions which aim to target these

particular processes may indicate great effectiveness in the treatment and prevention of vascular disease.

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CHAPTER VI

OVERALL CONCLUSIONS

The main objective of the findings in this dissertation was to assess the role of Inhibitor of DNA-binding/differentiaon-3 (ID3) in vascular diseases with respect to its regulation of vascular-intimal lesion formation and explanation of how ID3 predisposes the vessel to vascular dysfunction & endocrine disrupting pollutants. Our approach to bridging gaps between ID3, endocrine disrupting chemicals & vascular disease warrants unique work.

The initial goal addressed in manuscript 1 was utilized as a literature review to highlight the current understanding of how ID3 may contribute to multifaceted chronic diseases via metabolic perturbations such as cancer, neurological disorders, vascular, autoimmune, and bone diseases. Furthermore the existing literature suggests chronic exposure to endocrine disrupting chemicals (EDCs) within the human body are connected with metabolic disorders, and how the influence of these chemicals affect ID3. Also ID3 has the ability to inhibit or stimulate gene expression resultant in cellular hyper-proliferation or stemness markers. Additionally ID3 has been shown to be involved in metabolic syndrome (MetS) and EDCs mainly focusing on the three conceivable etiological factors such as insulin resistance, obesity and adipose tissue disruption, and a group of independent factors. We also address ID3 and its involvement in molecular mechanisms & signaling pathways that lead to these various disease outcomes.

The second aim addressed in manuscript 2 explored the association of exposure to an endocrine disrupting chemical (PCBs) and participants diagnosed with peripheral arterial disease as well as participants defined with atherosclerosis. We analyzed a population 40 years and older with available PCB data from 1999 – 2004. Exposure assessment was based on lipid adjusted concentrations of 6 individual PCB congeners (074, 099, 118, 138, 153, and 180), the sum of dioxin-like PCBs (074+118), and the sum of non-dioxin-like PCBs (099+138+153+180) in conjunction with examination data obtained from NHANES. We calculated GMs (geometric means) to compare concentrations of PCBs in participants with PAD definition, atherosclerosis, and normal participants. GMs of the 6 individual PCB congeners were established to be higher among participants with PAD diagnosis as well as atherosclerosis when compared to the rest of the study population. The overall GM levels of PCBs increased significantly in PAD participants as age group progressed except PCB099, which demonstrated a non-consistent trend (10.04 ng/g vs. 8.82 ng/g vs. 18.99 ng/g). Furthermore consistent trends were shown in the first and second age group ($\geq 40 - 59$) & ($60 - 74$) years old respectively, however in the final age group ≥ 75 only 2 PCB congeners showed higher concentration levels in the diseased status compared to the normal and 4 congeners (099, 138, 153, and 180) shown to have higher PCB GMs in normal participants. We also demonstrated adjusted models for PAD confounders; gender and PAD confounders; age, race, BMI, and PAD confounders; and gender, age, race, BMI, and PAD confounders. All models showed significant association with individual PCB congeners and increased risk of PAD when $LOD \geq 50$.

Furthermore, adjusted models for both dioxin-like and non-dioxin-like PCBs also demonstrated significant association with higher exposure and increased risk of PAD.

We further extended the second aim in manuscript 3 by exploring the association between additional types of endocrine disrupting chemicals (phthalates, BPA) with PAD. GM levels demonstrated higher levels of phthalate metabolites: MnBP and MBzP in participants diagnosed with PAD and AS when compared to the rest of the population. Furthermore GM levels of BPA was shown to be higher in participants diagnosed with PAD and AS when compared to normal participants. Adjusted models for PAD confounders; gender and PAD confounders; age, race, BMI and PAD confounders; and gender, age, race, BMI, and PAD confounders demonstrated significant association with PAD and higher exposure of phthalate metabolites ($\geq$ LOD) as well as BPA ($\geq$ LOD). PAD confounder adjusted models showed significant association with phthalate metabolites: MnBP (OR: 1.33; 95% CI: 1.00 – 2.34) and MBzP (OR: 1.16; 95% CI: 1.08 – 5.07). Additionally PAD confounder adjusted model also demonstrated significant association with BPA (OR: 1.22; 95% CI: 1.02 – 5.07). Adjusted model for gender and PAD confounders showed significant association with phthalate metabolites: MnBP (OR: 1.83; 95% CI: 1.00 – 2.34), MCHP (OR: 1.54 95% CI: 1.00 – 2.10), MEP (OR: 1.01; 95% CI: 0.44 – 1.09), and MBzP (OR: 1.76; 95% CI: 1.58 -2.91) as well as BPA (OR: 2.89; 1.50 – 10.19). Age, race, BMI, and PAD confounders adjusted model demonstrated significant association with phthalate metabolites: MnBP (OR: 1.33; 95% CI: 0.89 - 2.95), MCHP (OR: 1.39; 95% CI:

1.00 -1.65), MiBP (OR: 1.48; 95% CI: 1.12 – 1.87) alongside BPA (OR: 2.17; 95% CI: 1.65 – 7.15). The final model with gender, age, race, BMI, and the PAD confounders demonstrate MCHP (OR: 1.34; 95% CI: 1.22 – 1.59), MiBP (OR: 1.41; 95% CI: 0.99 – 1.83), MBzP (OR: 1.73; 95% CI: 0.92 – 3.18) alongside BPA (OR: 3.02; 95% CI: 0.14 – 12.15) showed significant association with higher exposure.

The aim addressed in manuscript 4 investigated the role of ID3 and ID3 candidate target genes in vascular diseases in respect to elucidating the specific focus of how ID3 plays a role in vascular-intimal lesion formation. ID3 has been previously shown to be important to the embryonic vasculogenesis and endothelial cell activation. Additionally it has been shown in various vascular disease mechanisms such as neo-intimal formation, VSMC growth, visceral adipose VEGFA expression, & increased endothelial cell neovascularization. We compiled microarray expression data using NCBI GEO on vascular diseases. Using computational analysis such as pearson correlation & independent t-test we identified ID3 candidate targets in blood and tissue respectively. We identified 217-candidate target genes in blood and 134-candidate target genes in tissue. Gene ontology methodology further supported us to explore the role of the identified ID3 candidate target genes in blood and tissue samples (217 and 134 respectively). Overlapping genes in both blood and tissue were furthermore identified and include: ABCB6, ACP1, BYSL, CAD, CDH15, DCBLD2, DHRS3, DNMT1, ID3, MCM4, and NDUFA7. Utilizing Bayesian network structure without any structural limitations provided us with a fully connected structure validating an interactive model. Furthermore we implemented those same genes using Genie to allow us

to determine probability distribution of gene expression within the network. Our initial model predicted ID3 to be predominately expressed high (79%) with the vascular state of the disease being in close proximity of one another (disease state: 56%, control state: 44%). Interestingly these mutually represented genes in both blood and tissue have been demonstrated in various vascular diseases throughout the literature such as atherosclerosis and vascular pathology of rheumatoid arthritis.

In conclusion, the major findings from the research conducted in this dissertation indicate a number of results. We investigated the role of endocrine disrupting chemicals (PCBs, phthalates, and BPA) and their association with vascular disease (PAD). Our findings show higher PCB, specific phthalate metabolites, and BPA concentrations in participants diagnosed with PAD as well as those with atherosclerosis when compared to the normal population. Furthermore, adjusted models demonstrated significant association with higher exposure to these endocrine disrupting chemicals and increased risk of PAD. ID3 may play a role in regulating certain genes in vascular diseases that take part in different biological processes that are significant drivers in the growth of vascular-intimal lesions such as cell adhesion, extracellular matrix, & cell growth. These genes include: ABCB6, ACP1, BYSL, CAD, CDH15, DCBLD2, DHRS3, DNMT1, ID3, MCM4, and NDUFA7. Overall, the exposure to EDCs particularly to vascular disease may contribute to the underlying understanding of how the mechanism of ID3 regulates certain genes that are essential components of the growth of vascular-intimal lesions.

Limitations

There were a number of limitations to our study. For our NHANES study since it was a cross sectional design it does not allow for casual interpretations due to self-reported data. Self – reported data increases the likelihood of misclassification bias of cases and controls contributing to the risk of incorrectly or undiagnosed reported PAD and/or AS cases. Furthermore, missing data also limits the overall finding of our study. Additionally, observed associations could be potentially confounded due to lack of information. For our GEO study not all dataset characteristics were represented which could lead to bias with various variables.

Future Studies

Although our findings should be viewed upon with thoughtfulness, they indicate that research involving the complex combination of environmental & molecular determinants should warrant further consideration. Additional research needs to be completed in order to account for the vast intricacy of how these types of determinants can change the development of not only vascular diseases but chronic diseases such as cancer and neurological disorders. The combined effects of EDCs and their influence on the molecular mechanisms that affect disease outcomes should be evaluated at a more complete perspective. Widespread exposure to EDCs and overall concern for growth in vascular disease risk, especially with sensitive molecular & genetic mechanisms permits a strong need for further research.

VITA

VINCENT E. AVECILLA

Date of Birth: December 5, 1987

Hometown: Tappan, New York

2015 - Present	Doctor of Philosophy (PhD) Candidate Dept. of Environmental Health Sciences Robert Stempel College of Public Health & Social Work Florida International University Miami, Florida
2016 - Present	Medical Science Liaison Banco De Éxito Ventures Miami, Florida
2016	Assistant Office Manger Tutor Doctor Miami, Florida
2014 - 2017	Academic Tutor (Science, Math, Reading Comprehension) Club-Z In - Home Tutoring Services of Doral Miami, Florida
2013 - 2016	Academic Tutor (Science & Math) Tutor Doctor Miami, Florida
2014	Graduate Research Assistant Chaplin School of Hospitality & Tourism Management Florida International University Miami, Florida
2012 – 2013	Academic Tutor (Science & Math) Homework Done Right -Tutoring Services, LLC

	Miami, Florida
2011 – 2012	Graduate Research Assistant
	Dept. of Environmental & Occupational Health
	Robert Stempel College of Public Health & Social Work
	Florida International University
	Miami, Florida
2011 - 2012	Master of Public Health (MPH)
	Dept. of Environmental & Occupational Health
	Robert Stempel College of Public Health & Social Work
	Florida International University
	Miami, Florida
2006 - 2010	Bachelor of Arts (BA): Psychology
	College of Arts & Sciences
	University of Rhode Island
	Kingston, RI

Publications & Presentations:

Avecilla V, Doke M, Felty Q. Contribution of Inhibitor of DNA Binding/Differentiation-3 and Endocrine Disrupting Chemicals to Pathophysiological Aspects of Chronic Disease. *BioMed Research International*. 2017; vol. 2017, Article ID 6307109, 22 pages (Published; Subject Area: Toxicology).

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Avecilla A, Avecilla V. Developmental Effects of Endocrine Disrupting Chemicals (EDCs) and Onset of Neuropsychiatric Disorders: Translation from Environmental Health Sciences to Clinical Psychology Practice. *Frontiers in Psychology: Clinical Health & Psychology Practice*. (In-Preparation)

Doke M, Assaggaf H, Avecilla V, Das J, Felty Q. Vascular cell dysfunction from exposure to polychlorinated biphenyls contributes to lung toxicity. Society of Toxicology (SOT), San Antonio, 2018. (Accepted - Abstract/Poster Presentation)