Associations of Environmental Risk Factors with Age-Related Cataract and Glaucoma

by

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ABSTRACT

Cataract and Glaucoma are two leading causes of visual impairment and blindness, showing an increasing prevalence with age. However, in spite of this significance, the etiology of age-related cataract (ARC) or glaucoma is still unclear. Previous studies implied that although genes play a role in the development of ARC and glaucoma, knowledge regarding the influence of environmental factors is also emerging. Much evidence suggests that oxidative stress increases the risk of ARC and glaucoma, while heavy metal exposure, a well-known source of increased oxidative stress, may be linked to the risk of disease. However, for glaucoma, previous epidemiological studies on heavy metals were mainly conducted in Asian populations and were cross-sectional, raising concerns related to causal inferences and problems of reverse causality. Furthermore, lead exposure measurements were based on blood or hair lead levels, reflecting relatively recent doses, which limits inferences regarding chronic effects of cumulative exposure. For cataract, studies on the association of environmental pollutants other than heavy metals with ARC were very limited.

We thus examined the following three aims: 1) the association between bone lead levels measured via K x-ray fluorescence and incident primary open-angle glaucoma (POAG); 2) effect modification by dietary patterns and dietary vitamin intake in the association between bone lead levels and incident POAG; and 3) a two-stage environment-wide association study (EWAS) to discover potential environmental risk factors for cataract surgery. Aims 1 and 2 were conducted using data from the **Normative Aging Study**, a prospective cohort study established by the United

States Department of Veterans Affairs. Aim 3 utilized data from the **National Health and Nutrition Examination Survey (NHANES)**, a national population-based public dataset established by the United States Centers for Disease Control and Prevention.

We found that bone lead may be an important risk factor of POAG. A 10-fold increase in patella lead level was associated with more than 5-fold higher risk of POAG during 15 years of follow-up. Further analysis on effect modification by dietary pattern suggests that people who had high adherence to prudent dietary pattern, which contains plentiful legumes, vegetables, seafood, onions, tomatoes, fruits and poultry, were less susceptible to the toxicity of patella lead on the risk of POAG. For cataract, we found that urinary heavy metals (cadmium, cobalt and tungsten), and serum PCBs 44 and 49, were positively associated with cataract surgery by using the conventional EWAS approach. We further identified urinary mono-(3-carboxypropyl) phthalate and two VOCs (urinary N-acetyl-S-(3-hydroxypropyl-1-methyl)-L-cysteine and urinary N-acetyl-S- (3-hydroxypropyl)-L-cysteine) as potential risk factors for cataract via weighted EWAS approach accounting for biological half-lives of pollutants.

This dissertation revealed the effects of multiple unrecognized environmental risk factors for glaucoma and cataract. Our research can help better understand the role of environmental risk factors in the pathogenesis of ARC and POAG, providing new ideas for interventions regarding these two important ocular diseases, and ultimately decrease the global burden of visual impairment and blindness effectively.

CHAPTER I

Introduction

1. Epidemiology of Cataract and Glaucoma

Cataract and Glaucoma are two leading causes of visual impairment and blindness, and show an increasing prevalence with age (Pascolini and Mariotti 2011). Previous meta-analysis reported that approximately 13.4 million (3.3 million to 31.6 million) people would be estimated to be blind because of cataract by year 2020 (Flaxman et al. 2017). The prevalence of cataract in the United States (U.S.) in 2010 was 17% in people aged 40 years or older and more than 50% in people aged 70 years or older (Friedman et al. 2012). Surgery, which removes the patient's cataract and replaces it with an artificial lens, is currently the only effective treatment for visually significant cataract, and approximately 23.1% of cataract patients in the U.S. underwent at least one surgery in a cross-sectional study collected from year 2001 to 2011 (Kauh et al. 2016). Although cataract surgery rates are increasing worldwide, developing countries still face numerous challenges in access to surgical care, including high cost, low population awareness, lack of trained specialists, and post-surgical side-effects (Khanna et al. 2011; Rao et al. 2011; Tabin et al. 2008).

Glaucoma is the second leading cause of blindness in the world, after cataract, and the leading cause of irreversible loss of vision (Prum et al. 2016). Approximately 8% of blindness is caused by glaucoma, according to the 2010 WHO report (Pascolini and Mariotti 2012). A previous meta-analysis showed that the global prevalence of glaucoma in the population aged 40–80 years

was 3.54% (95% CI, 2.09–5.82) (Tham et al. 2014). In the U.S., the National Eye Institute reported that approximately 1.9% of the population aged 40 years and older were suffering from primary open-angled glaucoma (POAG, a major subtype of age-related glaucoma in the U.S.) in 2010, with the number of cases rising from 2.22 million to 2.72 million since the year 2000. Primary angle-closure glaucoma (PACG, the second major subtype), only accounted for approximately 0.1% of all prevalent glaucoma cases in the U.S. population (Prum et al. 2016; Glaucoma, Open-angle | National Eye Institute; Glaucoma Prevalence Rates by State). Since glaucoma is a neurodegenerative disease, the loss of visual function is irreversible once symptomatic, and there is no cure (Prum et al. 2016). Hence, the goal of clinical treatment is only to prevent further damage to the optic nerve.

2. Risk Factors for Age-related Cataract (ARC) and glaucoma

Despite their high prevalence and severe consequences, the etiologies of both age-related cataract (ARC) and glaucoma are still unclear. It is believed that the pathogenesis of ARC and glaucoma is affected by both genetic and environmental factors.

2.1. Risk Factors for Glaucoma

2.1.1. Definition and Etiology of Glaucoma

Glaucoma is commonly defined as a disease where characteristic optic neuropathy results in visual field loss (Prum et al. 2016). Glaucoma can be diagnosed by evaluating the health of the optic nerve head via ophthalmoscopy. Clinically significant features include the cup-to-disc ratio (CDR is the ratio of the optic nerve cup to the optic disc, and glaucomatous optic neuropathy

usually results in a characteristically enlarged or asymmetric CDR), the shape/color or presence of hemorrhages on the cup and disc and their rims, and the distribution of retinal vessels (Prum et al. 2016). More than half of glaucoma cases are caused by increased intraocular pressure (>21 mmHg), which may compress axonal fibers of the optic nerve, and thus induce neuropathy through decreased nerve head perfusion and/or disrupt the autoregulation of retinal veins (Prum et al. 2016). Furthermore, dysfunctions of the aqueous humor drainage system in the anterior chamber, such as due to abnormalities of cell function or anatomical obstruction of the trabecular meshwork or Schlemm's canal, can disrupt the outflow of aqueous humor from the eye, and result in the increase of intraocular pressure (Babizhayev 2012; Prum et al. 2016). Newer concepts about the translaminar pressure differential between intraocular pressure and intracranial pressure have emerged as important factors for this disease (Roy Chowdhury and P Fautsch 2015; Zhao et al. 2016). For those glaucoma patients whose intraocular pressure is within a normal range, suffering from so-called normal-tension glaucoma (NTG), their etiology is even less understood.

2.1.2. Genetic Risk factors for Glaucoma Development

POAG is a complex disease affected by both environmental and genetic factors. Genetic risk related to glaucoma is mainly polygenic and genes have incomplete penetrance (Prum et al. 2016). In addition to traditional familial linkage studies, the National Eye Institute (NEI) has established the NEI Glaucoma Human genetic collaBORation (NEIGHBOR) consortium to conduct genome-wide association studies (GWAS) to identify genetic variants related to POAG development (Wiggs et al. 2013). Current known genes associated with POAG includes Myocilin (*MYOC*), Atonal BHLH transcription Factor 7 (*ATOH7*), Transmembrane And Coiled-Coil Domains 1 (*TMCO1*), SIX Homeobox 1/SIX Homeobox 6 (*SIX1/SIX6*), Growth Arrest Specific 7 (*GAS7*), (Abu-Amero et al. 2015) etc. While multiple genes were already found to be associated

with POAG, those mutations only cover part of all POAG variance (Mabuchi et al. 2015). Previous twin study conducted in 1987 reported that the heritability of POAG was 0.13 (Teikari 1987), while recent GWAS reported that the genetic attribution on the total phenotype variation of POAG ranged from 24% to 42% (Cuellar-Partida et al. 2016; Ge et al. 2017). The remaining portion of glaucoma variants should thus be influenced by environmental risk factors (Mabuchi et al. 2015).

2.1.3. Oxidative Stress and Glaucoma Development

The commonly known non-genetic risk factors for glaucoma include increasing age, intraocular hypertension, thin central corneas, racial background, optic nerve susceptibility, and positive family history (Prum et al. 2016). Other risk factors, such as various systemic diseases (diabetes, hypertension, ischemic vascular diseases, etc.) and unhealthy behaviors (smoking, alcohol consumption), remain inconsistent among different studies (Doshi et al. 2008; Fan et al. 2004; Ko et al. 2016; Prum et al. 2016; Renard et al. 2013).

A large body of evidence implies that oxidative stress may be associated with glaucoma. For example, oxidative stress and vascular damage are two major factors that affect the function of the trabecular meshwork and aqueous humor drainage system, which is the target tissue of glaucoma in the anterior chamber (Saccà et al. 2015; Zhao et al. 2016). Excessive oxidative stress and lipid peroxidation may lead to the accumulation of free radicals and their derivatives (reactive oxygen species, ROS), overwhelming the antioxidant defense system, and thus affecting the extracellular matrix (ECM) structure and inducing ECM accumulation. This in turn can cause the loss of cell adhesion and changes in the cytoskeletal structure of trabecular meshwork cells, affecting their membrane permeability, and thus resulting in dysfunction of the trabecular meshwork and Schlemm's canal (Babizhayev 2012; Saccà et al. 2016; Zhao et al. 2016). ROS accumulation may also induce apoptosis via mitochondrial damage, cell inflammation, endothelial

dysregulation and hypoxia, and corresponding cellular changes were also observed in experiments *in vitro* (Zhao et al. 2016). Such damage to the aqueous humor drainage system can increase intraocular pressure, and cause glaucoma (Babizhayev 2012). Elevated biomarkers of oxidative stress, such as superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) levels, have been measured in the aqueous humor of POAG/PACG patients (Babizhayev 2012; Goyal et al. 2014; Majsterek et al. 2011). Significantly higher mitochondrial DNA damage and lipid peroxidation products were also observed in the trabecular meshwork of glaucoma patients (Izzotti et al. 2003; Zanon-Moreno et al. 2008; Zhao et al. 2016). Mouse models lacking the glutamate transporter genes, which are critical to the generation of major antioxidant glutathione in the retina, exhibit typical retinal ganglion cell death and optic neuropathy (Harada et al. 2007; Kimura et al. 2017). Western blot analysis using specific antibodies also detected a significant increase in 4-hydroxy-2-nonenal (HNE) adducts, which are generated by free-radical attack in the glaucomatous retina, implying that oxidative stress may also directly harm the optic nerve or retina (Tezel et al. 2010).

Consistent with these findings, antioxidants may protect against glaucoma. Higher intake of antioxidants such as vitamin A/retinol and its equivalents (carotenoids), as well as vitamin C, vitamin E and glutathione was associated with decreased likelihood of glaucoma (Giaconi et al. 2012; Ramdas et al. 2012; Veach 2004; Wang et al. 2013). Other visual-function-related nutritional factors such as dietary omega-3 fatty acids and nitrate intake were also reported to be negatively associated with intraocular pressure or POAG (Kang et al. 2016; Renard et al. 2013). A potential protective effect of vitamin D against glaucoma was also observed in multiple studies (Goncalves et al. 2015; Yoo et al. 2014).

2.1.4. Lead Exposure and Glaucoma Development

Heavy metals are an environmental source of oxidative stress which may result in glaucoma. As early as 1990, a study reported higher copper levels in the aqueous humor of glaucoma patients versus controls (Akyol et al. 1990), and a number of more recent studies have noticed a significant association between heavy metal biomarkers and glaucoma. An analysis of the Korean general population found that higher blood mercury and lower blood manganese levels were associated with higher prevalence of glaucoma (Lin et al. 2015). The authors also found that higher blood cadmium levels were associated with higher glaucoma risk, particularly in men with NTG (baseline intraocular pressure <15 mmHg) (Lee et al. 2016; Lin et al. 2015). A case-control study conducted in Japan reported that higher hair lead levels were associated with POAG in females, especially in NTG (Yuki et al. 2009). Furthermore, elevated lead levels were found to be associated with a higher risk of other ocular diseases such as ARC and age-related macular disease (AMD), and may increase the blood-retina permeability, which itself is a risk factor for retinal vascular diseases (Erie et al. 2009; Hwang et al. 2015; Mosad et al. 2010; Schaumberg et al. 2004; Shen et al. 2016). However, previous epidemiological studies on heavy metals and glaucoma were mainly conducted in Asian populations and were cross-sectional, raising concerns related to causal inferences and problems of reverse causality. Moreover, lead exposure measures were based on blood or hair lead levels, reflecting relatively recent doses, which limits inferences regarding chronic effects of cumulative exposure, since lead has a relatively short half-life of about only 1 month in the blood (Hu et al. 1995, 2007). The hair lead levels utilized in the Japanese case-control study are not a good indicator of cumulative lead exposure either, since they can be greatly affected by the frequency of hair washing and cutting.

2.2. Risk Factors for Age-related Cataract (ARC)

The detailed etiology of ARC has been better investigated compared with glaucoma. However, it is still incompletely understood.

2.2.1. Definition and Etiology of ARC

Cataract is defined as any opacification or clouding of lens tissue which affects vision (Bobrow et al. 2015; Cataracts | National Eye Institute). Most cataracts are age-related, since the accumulation of protein and lipid aggregations in the lens increases with age. Nuclear sclerotic, cortical and posterior subcapsular cataracts are three major subtypes of ARC and are believed to have distinct but overlapping etiologies. Nuclear sclerosis, which starts from the middle of the lens, is likely primarily induced by the aging process (Bobrow et al. 2015). On the other hand, systemic diseases such as diabetes may significantly increase the risk of cortical and posterior subcapsular cataracts, which are caused by the opacification of peripheral lens fibers and the posterior cortical layer (Delcourt et al. 2000; Hennis et al. 2004). In contrasts with ARC, secondary cataracts can develop following other ocular diseases, surgeries, or trauma. Congenital cataracts are inherited or attributed to an infection during pregnancy/infancy/childhood (Bobrow et al. 2015).

Oxidative stress may promote cataract formation through the modification of lens cell function. For example, the excessive generation of reactive oxygen species (ROS) can disrupt the synthesis of UV filter compounds in the lens (those compounds serve to protect the lens from photo-oxidation), overwhelm the antioxidant defense system (superoxide dismutase, catalase, lipid peroxidases), impair the DNA repair mechanisms or directly cause oxidative damage to functional DNA, enhance apoptosis of epithelial cells of the lens, and induce protein and lipid aggregation in the lens (Babizhayev 2012; Bobrow et al. 2015; Spector 1995; Truscott 2005; Tweeddale et al.

2016). Previous epidemiological studies have reported protective effects of dietary or supplemental intake of antioxidants, such as vitamins A, C and E, on ARC (Beebe et al. 2010; Chang et al. 2013; Cui et al. 2013; Thiagarajan and Manikandan 2013; Zoric et al. 2008).

2.2.2. Genetic Risk Factors for ARC Development

Genetic variants related to congenital cataract are comparatively likely to be highly penetrant Mendelian traits that can cause severe disruptions of the homeostasis of lens cells (Shiels and Hejtmancik 2013, 2007). Conversely, relatively mild mutations in the same genes can contribute to ARC, and those variants usually have low penetrance (Shiels and Hejtmancik 2013, 2007). Linkage studies and GWAS have identified multiple genes and loci that may be associated with ARC, such as *galactokinase (GALK1)* and *Eph-receptor type-A2 (EPHA2)* (Liao et al. 2014; Shiels and Hejtmancik 2013). Furthermore, the contribution of genetic factors to ARC risk varies from 35% to 74%, with cortical cataracts more heritable than nuclear types (Shiels and Hejtmancik 2013). However, a recent study suggested that genetic factors might only explain about one-third of the variation in the progression of nuclear cataracts in a longitudinal sample, indicating that the very large remaining variance may to be explained by environmental risk factors (Yonova-Doing et al. 2016).

2.2.3. Environmental and Other Non-Genetic Risk Factors for ARC Development

Commonly known risk factors for ARC other than genetic factors include increasing age, smoking, obesity, hyperglycemia, UV light/radiation exposure, and intake of specific pharmaceuticals (such as corticosteroids) (Bobrow et al. 2015). Alcohol intake and physical activities may affect the progression of ARC, but these findings were inconsistent across studies (Shiels and Hejtmancik 2013).

Although oxidative stress in the lens may be largely due to photo-oxidation caused by UV radiation, heavy metal exposure can also lead to oxidative stress through the depletion of the glutathione and thiol pools, disrupting the antioxidant defense system (Ercal et al. 2001; Jomova and Valko 2011; Valko et al. 2016). Previous studies have reported that lead exposure may be associated with ARC (Schaumberg et al. 2004). Elevated levels of lead and cadmium were also observed in cataractous lens tissues, especially among smokers (Cekic 1998; Harding 1995; Mosad et al. 2010; Rácz and Erdöhelyi 1988; Ramakrishnan et al. 1995). I conducted a cross-sectional study of cadmium and lead in relation to cataract surgery using data from NHANES, and found that urinary cadmium is positively associated with the risk of cataract surgery (Wang et al. 2016). My study also found that more than 50% of the effect of cigarette consumption on the prevalence of cataract surgery may be due to cadmium exposure (Wang et al. 2016). The remaining portion of the detrimental effect of smoking may be mediated by other toxicants present in cigarettes. Nicotine exposure may also exacerbate the development of cataracts, according to rat models (Everekliogl u et al. 2004; Tirgan et al. 2012).

Several case-control and population-based cross-sectional studies have reported that indoor smoke generated from household usage of cooking fuels can increase the risk of cataract (Mishra et al. 1999; Mohan et al. 1989; Pokhrel et al. 2005; Ravilla et al. 2016; Smith et al. 2014). Indoor cooking smoke may play a similar role in cataract development as tobacco/cigarette smoking, whereby exposure to toxicants increases the accumulation of superoxide radicals in lens epithelial cells (Pokhrel et al. 2005). Several animal studies and clinical observations have also suggested an association between cataract and exposure to polycyclic aromatic hydrocarbons (PAH) such as naphthalene and formaldehyde, which can be released in large amounts during the burning of biofuels (Hayasaka et al. 2001; Pokhrel et al. 2005; Xu et al. 1992).

In summary, few studies have evaluated environmental factors other than radiation, heavy metals, and indoor smoke from biomass fuel as risk factors for ARC.

3. Specific Aims and Hypothesis

The goal of this dissertation was thus to better understand the role of environmental factors in the pathogenesis of age-related cataract and glaucoma. We 1) examined the association between bone lead levels, as measured using K x-ray fluorescence in the bones, and incident primary open-angle glaucoma; 2) evaluated the effect modification in the association between bone lead level and incident primary open-angle glaucoma by dietary patterns and dietary vitamin intake; 3) conducted an Environment-Wide Association Study (EWAS) to identify potential environmental risk factors for ARC. Aims 1 and 2 were conducted using data from the **Normative Aging Study**, a prospective cohort study established by the United States Department of Veterans Affairs. For Aim 3, we took advantage of large sample sizes as well as a large number of biomarkers of environmental pollutants using data from the **National Health and Nutrition Examination Survey (NHANES)**.

The following three aims were examined:

Aim 1: Examine the association between bone lead levels and incident primary open-angle glaucoma, using data from the Normative Aging Study.

Hypothesis: higher bone lead is associated with higher risk of incident glaucoma, after adjustment for covariates including age, educational level, job types, BMI, systemic hypertension, ocular

hypertension, diabetes mellitus, and smoking.

Aim 2: Evaluate the potential effect modification caused by dietary patterns and dietary intake of vitamins (vitamins A, C, D and E) in the association between bone lead levels and incident glaucoma as stipulated in Aim 1.

Hypothesis: after adjustment for covariates, individuals with higher adherence to prudent dietary pattern, which is abundant for vegetables and fruits and antioxidant vitamins, may have a less pronounced association between bone lead levels and incident glaucoma.

Aim 3: Develop a two-stage EWAS to discover potential environmental pollutants for cataract surgery in U.S. adults using NHANES 1999-2008.

Hypothesis 1: higher exposure to environmental pollutants is associated with a higher prevalence of cataract surgery, after adjustment for conventional risk factors.

Hypothesis 2: Those pollutants with longer half-lives in human body are more likely to be selected by conventional EWAS.

Hypothesis 3: After corrected by weights based on half-life of the pollutants in EWAS, those chemicals with shorter half-live can be identified.

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CHAPTER II

Bone Lead Levels and Risk of Incident Primary Open-Angle Glaucoma: the VA Normative Aging Study

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1. Abstract

Background: Oxidative stress may play an important role in the etiology of primary open-angle glaucoma (POAG). The association between risk of POAG and lead exposure, which is an environmental source of oxidative stress, has not been fully investigated yet.

Objective: To determine the association between bone lead—a biomarker of cumulative lead dose (tibia lead) or an endogenous source of stored lead (patella lead)—and incident POAG.

Methods: We examined a prospective cohort of 634 POAG free males (mean baseline age=66.8 years (SD=6.7)) from the Normative Aging Study (NAS) who had tibia and patella K X-ray fluorescence lead measurements between January 1, 1991 and December 31, 1999. They also had standard ocular evaluations by NAS optometrists until December 31, 2014. POAG cases were identified by consistent reports of enlarged or asymmetric cup-to-disc ratio together with visual field defect or existence of disc hemorrhage. We used Cox proportional hazards regressions to estimate hazard ratios (HRs) of incident POAG and adjusted survival curves to examine changes in the risk of POAG during follow-up according to bone lead quartiles.

Results: We identified 44 incident POAG by the end of follow-up (incidence rate=74 per 10,000 person-years; median follow-up=10.6 years). In fully adjusted models, 10-fold increases in patella lead and tibia lead were associated with HRs of 5.06 (95% CI: 1.61, 15.88, p=0.005) and 3.07 (95% CI: 0.94, 10.0, p=0.06), respectively. The HRs comparing participants in the third and fourth quartiles with the lowest quartile were 3.41 (95% CI: 1.34, 8.66) and 3.24 (95% CI: 1.22, 8.62) for patella lead (p-for-trend=0.01), and 3.84 (95% CI: 1.54, 9.55) and 2.61 (95% CI: 0.95, 7.21) for tibia lead (p-for-trend=0.02).

Conclusions: Our study provided longitudinal evidence that bone lead may be an important risk factor of POAG in the U.S. population.

2. Introduction

Glaucoma accounts for approximately 8% of global blindness according to the 2010 World Health Organization report (Pascolini and Mariotti 2012). It is the second leading cause of blindness in the world after cataract, and the leading cause of irreversible loss of vision (Pascolini and Mariotti 2012). Despite the large patient population and severe consequences, the exact etiology of glaucoma is still unclear. Based on glaucoma clinical trials, the established risk factors for glaucoma include older age, intraocular pressure, race, myopia, optic nerve susceptibility, and positive family history (Jonas et al. 2017). Other clinical risk factors, such as various systemic diseases (diabetes, hypertension, ischemic vascular diseases, etc.) and unhealthy behaviors (smoking, alcohol consumption), remain inconsistent among different studies (Doshi et al. 2008; Fan et al. 2004; Ko et al. 2016; Prum et al. 2016; Renard et al. 2013). Although there is a large population burden and severe consequence to quality of life, there is a gap in knowledge to advance our understanding beyond the established clinical risk factors for glaucoma.

In addition to clinical risk factors, genetic risk factors for glaucoma have been established through the Mendelian studies and genome-wide association studies (GWAS) (Mabuchi et al. 2015; Prum et al. 2016; Sakurada and Mabuchi 2015; Wiggs 2015; Wiggs et al. 2013). Adult-onset glaucoma occurs mostly among individuals older than 40 years. Primary open-angle glaucoma (POAG) is the major form of adult-onset glaucoma in the United States (prevalence: 1.9%) (Prevalence of Open-Angle Glaucoma Among Adults in the United States 2004). Recent heritability estimate to quantify the proportion of genetic attribution on the total phenotype variation of POAG was about 42%, which was lower than its proportion for age-related macular degeneration (AMD) (>70%) (Cuellar-Partida et al. 2016). Although the various genetic risk

alleles for specific forms of glaucoma have been successfully identified by linkage and GWAS approaches, the environmental risk factors for glaucoma have proven difficult to identify.

Oxidative stress plays a role in glaucoma pathogenesis (Babizhayev 2012; Goyal et al. 2014; Majsterek et al. 2011; Zhao et al. 2016). The pathophysiology of glaucoma involves complex tissues in the anterior segment that regulate aqueous humor fluid dynamics and determine intraocular pressure and posterior segment end organ damage of the optic nerve, which is recently reviewed in Jonas et al (Jonas et al. 2017). The complex relationships among the delicate ganglion cells that contribute to the axonal fibers of the optic nerve, the vascular supply, the glial support tissue and connective support tissues in the optic nerve canal, and counter-pressure from the cerebral spinal fluid are active areas of research (Jonas et al. 2017). Within these tissues, markers of oxidative stress, such as superoxide dismutase, glutathione peroxidase and catalase levels, are elevated in the aqueous humor of patients with POAG (Babizhayev 2012; Goyal et al. 2014; Majsterek et al. 2011). Oxidative stress can disrupt the normal function of trabecular meshwork cells, block the outflow of aqueous humor, and increase the intraocular pressure (Babizhayev 2012; Saccà et al. 2016; Zhao et al. 2016). In the posterior segment, elevated 4-hydroxy-2-nonenal adducts generated by free-radicals have been detected in the glaucomatous retina cases, implying that oxidative stress plays a pathogenic role damaging the retina-optic nerve (Tezel et al. 2010).

As a key environmental source of oxidative stress, heavy metals may be an important risk factor for glaucoma. As early as 1990, a study reported higher copper levels in the aqueous humor of glaucoma patients (Akyol et al. 1990). Recent studies have also indicated a significant association between heavy metal and glaucoma. A cross-sectional analysis of the Korean National Health and Nutrition Examination Survey (KNHANES) found that higher blood

mercury and lower blood manganese levels were associated with higher prevalence of glaucoma (Lin et al. 2015). Another KNHANES study found that higher blood cadmium levels were associated with higher glaucoma risk, particularly in men with intraocular pressures within the normal range (Lee et al. 2016). A case-control study conducted in Japan reported that higher hair lead level was associated with POAG especially normal tension glaucoma in females (Yuki et al. 2009).

The threat of non-occupational cumulative exposure to low-dose lead has been reported since lead was banned from gasoline and paint in the United States (U.S.) in the 1990's. As there is a gap in knowledge on heavy metals as potential environmental risk factors of glaucoma, we propose an epidemiological study to test the hypothesis that cumulative lead exposure increases the risk of POAG. To the best of our knowledge, no epidemiologic study has ever tested the association between cumulative lead exposure and risk of POAG. Results of previous studies, which were mostly based on Asian populations, may not be generalizable to the U.S. population. Moreover, no previous lead-glaucoma study has ever utilized bone lead levels as a biomarker of cumulative lead dose (tibia lead) or an endogenous source of stored lead (patella lead) (Hu et al. 2007). Bone lead, which represents the majority of the body's lead burden with a half-life spanning years to decades, is known to be a better biomarker to assess chronic health effects than blood or urinary lead (Hu et al. 2007). Further, cross-sectional studies raise causal inferences and reverse causality concerns. In this study, we aim to examine the association between bone lead levels and incident POAG in a male population in the Boston area, the Normative Aging Study (NAS).

3. Method

3.1. Study population

The NAS is a longitudinal study of aging started in 1963 by the U.S. Department of Veterans Affairs (Glynn et al. 1982). The study recruited 2280 healthy male participants who were predominantly whites and free of systemic disease at the time of enrollment. Participants underwent a comprehensive physical examination, including a standard ocular evaluation, every 3-5 years (Schaumberg et al. 2004). Informed consent was provided by participants at each visit. From 1991 to 1999, 868 participants underwent bone lead measurements via K x-ray fluorescence (KXRF). We set the date of the first bone lead measurement as the baseline of the longitudinal study. Since the cohort has a long follow-up time which may accumulate survival bias, we restricted the study cohort within 15 years' follow-up. The inclusion criteria for this project were pre-cohort ophthalmology examination prior to the KXRF measurement, minimum of one ophthalmology examinations after the baseline, and no missing for covariates used for data analysis. 702 participants had both complete ophthalmology evaluations and bone lead measurement. After excluding those who were not eligible (8 for no ophthalmology examination after bone lead measurement, 30 for missing covariate data, 18 for missing information on inverse probability weighting (more details described below), 8 for pre-existing diagnosis of either open-angle glaucoma, secondary glaucoma, or narrow angle glaucoma, 1 for unacceptable uncertainty for patella lead, 3 for follow-up less than 2 years), we included a total of 634 individuals into this study (Figure II.1). The current study was reviewed and approved by the Institutional Review Boards of each participating institute, the University of Michigan School of Public Health, the Harvard School of Public Health and the Department of Veterans Affairs Boston Healthcare System.

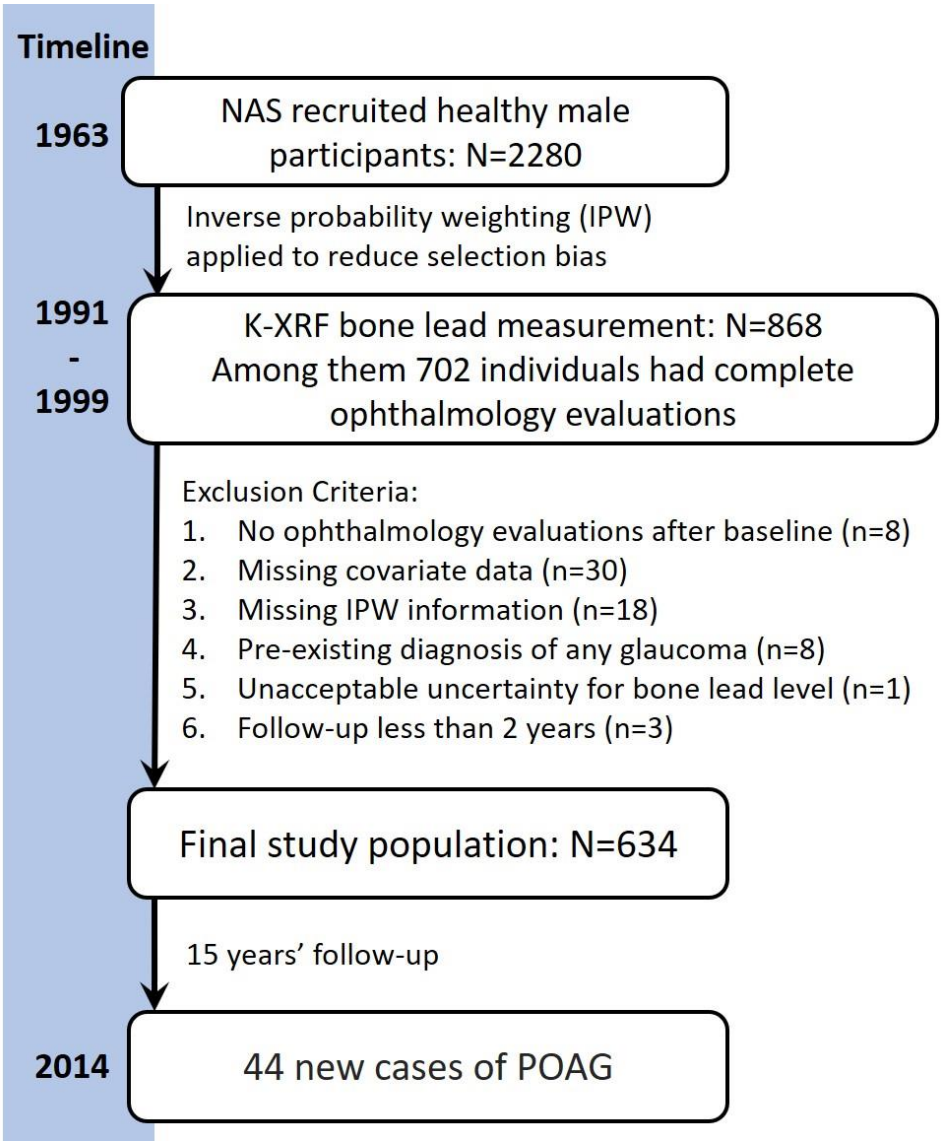

Figure II.1. Diagram illustrating the establishment cohort structure of the study population, from the NAS original recruitment in 1963 to the KXRF measurement in 1990s, which is the baseline of our study, until the end of 15 years' follow-up.

3.2. Bone lead measurements

Bone lead levels (µg/g, microgram of lead per gram of bone mineral) at the mid-tibial shaft and patella were measured for the NAS using a KXRF instrument. Tibia and patella are representative of two typical bone structures: cortical bone and trabecular bone (Hu et al. 1995). Tibia lead is a biomarker of past life-time exposure, and patella lead is a biomarker of an

endogenous source of lead body burden (Hu et al. 1995, 2007; Wilker et al. 2011). The KXRF instrument utilizes low-dose gamma radiation to provoke the release of X-rays which are specific and proportional to the lead level in bones (Hu et al. 1995). It provides a non-invasive and safe method to precisely evaluate bone lead concentrations. The physical principles, technical specifications, and validation of this instrument have been described in detail elsewhere (Burger et al. 1990).

We have multiple measurements of bone lead over time in this cohort. We used the first measurements in our study, instead of time-varying bone lead levels. One reason is that not all subjects had multiple measurements. Besides, a previous study with repeatedly measured bone lead levels in the same population showed that tibia lead decreased slightly over 11 years (1.4% annual decline) after cessation of exposure, whereas patella lead had an initial decline of 5% per year during the first 5 years but then did not change much (0.4% annual decline) after 5 years (Wilker et al. 2011). We assumed that baseline tibia lead levels did not change much during follow-up, and baseline patella lead levels can reasonably capture the average of exogenous exposures that had gradually decreased since the phase out of lead from gasoline and paint.

A subset of bone lead levels measured by KXRF had negative values (3 for tibia lead levels and 5 for patella lead levels) since the instrument provided an unbiased point estimate that may oscillate around the true value (Kim et al. 1995). In order to better present the true distribution of bone lead levels, we used original values, including negative values, rather than using a substitution method. As a quality control procedure, we adopted the measurement uncertainty for each bone lead measurement to evaluate the chance of estimated level corresponding to a true level (Hu et al. 1995). The measurement uncertainty is equal to 1 standard deviation of replicated measurements; the higher uncertainty a bone lead measurement has, the lower reliability this value

possesses. We only included those participants who had bone lead levels within an acceptable uncertainty (10 μg/g for tibia and 15 μg/g for patella).

3.3. Glaucoma identification

The NAS standard ocular evaluation includes family and personal ocular/systemic disease history, medical history, visual acuity data, biomicroscopy, tonometry and ophthalmoscopy (Schaumberg et al. 2004). A staff optometrist performed examinations at the NAS examination facility and results were reviewed/cosigned by a second qualified person. For the current study, we reviewed the de-identified medical records spanning the years 1991 to 2014. Variables were extracted for glaucoma identification, including personal and family history of glaucoma, medication, visual acuity, intraocular pressures of each eye (pre-dilated, measured in the morning), the vertical cup-to-disc ratios (CDR) of each eye and other descriptions from the fundus exam, additional testing that included visual field, and ocular diagnoses made by the NAS optometrists. Central cornea thickness was not part of the NAS standard ophthalmology examination.

The ascertainment of incident POAG cases were adopted from the glaucoma phenotype description defined from the National Eye Institute Glaucoma Human genetics collaBORation (NEIGHBOR) Consortium (Wiggs et al. 2013). POAG cases were identified in participants who showed one of the following characteristics (Table II.1): 1) either eye having CDR greater than or equal to 0.7; 2) the difference of two eyes' CDRs equal to 0.2 or larger which indicates asymmetric cup-to-disc ratio; 3) any eye's CDR equal to 0.6 or larger, with either disc hemorrhage or visual field defect; 4) vision loss due to nerve fiber layer loss. In addition, an open angle was assumed based upon biomicroscopic description of deep chamber and lack of NAS optometric description of narrow angles. Those who had glaucoma prior to the baseline

were defined as baseline glaucoma cases and were excluded from the longitudinal analysis (n=8, prevalence at baseline=1.1%). All eligible participants were followed until the end of 15 years since baseline, the last recorded visit if lost to follow-up, or the date of the first vision test identifying the onset of POAG or other types of glaucoma (Table II.1).

Table II.1. Identification of Primary Open-Angle Glaucoma Cases

Diseases	Criteria
POAG patients	
POAG[a]	1) Either eye CDR ≥ 0.7, open angle[b] 2) The difference between two eyes' CDRs ≥ 0.2, open angle 3) Either eye CDR ≥ 0.6, together with disc hemorrhage or visual field defect, open angle 4) Vison loss of either eye together with nerve fiber layer loss, open angle
Non-POAG	
PACG	Same as criteria of POAG, but angle narrowed or closed (angle≤1/4, or being diagnosed as PACG by NAS optometrist)
Secondary glaucoma	1) Pseudoexfoliation glaucoma 2) Pigment dispersion glaucoma 3) Glaucoma secondary to other diseases or accidents (trauma, stroke, surgery, etc.)
Glaucoma suspects	Being diagnosed as a glaucoma suspect by NAS optometrist, without any of the above characteristics

Abbreviations: POAG, primary open-angle glaucoma; CDR, cup-to-disc ratio; PACG, primary angle-closure glaucoma; NAS, the Normative Aging Study.
[a] Criteria of POAG were adopted from the NEI Glaucoma Human genetics collaBORation (NEIGHBOR) Consortium (Wiggs et al. 2013) and were modified to be more applicable to the NAS population.
[b] Angle was defined as the angle between cornea and iris in the anterior chamber of eye; an open angle was assumed based upon biomicroscopic description of deep chamber and lack of NAS optometric description of narrow angles.

3.4. Other variables

Established risk factors for POAG include older age, elevated intraocular pressure (IOP) defined as greater than or equal to 23 mmHg, and myopia (Jonas et al. 2017). Given the extensive data on this NAS cohort, the following variables were analyzed: age at baseline (years), race/ethnicity (white or other), body mass index (BMI, varying at each follow-up visit, kg/m^2), educational attainment (equal to or less than high school, high school, some college, and

higher) and job types (blue collar, white collar or mixed). Cigarette smoking status is an inconsistent risk factor for POAG, but meta-analyses and systemic reviews show heavy smoking, not simply a positive smoking status, is associated with POAG (Bonovas et al. 2004; Jain et al. 2016; Prum et al. 2016; Zhou et al. 2016). Thus, we used categorized cigarette consumption data based on pack-years (0, 0-19 pack-years and ≥20 pack-years) to adjust for smoking behavior. In addition, we also controlled for diabetes mellitus status (yes/no, identified by either had been diagnosed as diabetes mellitus, or had used insulin or other diabetes medication/treatment, or had blood fasting glucose level ≥ 126 mg/dl), systemic hypertension (yes/no, identified by systolic blood pressure ≥ 140 mm/Hg or diastolic blood pressure ≥ 90 mm/Hg or had used hypertension medication/treatment) and ocular hypertension (yes/no, identified by either the highest intraocular pressure (untreated) value ≥ 23 mm/Hg at that visit, or the highest intraocular pressure (treated) value ≥ 23 mm/Hg after being divided by 0.7 at that visit; criteria was made according to Jun Li's IOP GWAS study (Ozel et al. 2014)). The covariates were collected by the time of bone lead measurement.

3.5. Handling Selection Bias and Inverse Probability Weighting

Our bone lead study conducted several decades after the inception of the NAS is subject to selection bias due to loss to follow-up (Weisskopf et al. 2015), which is common to observational prospective cohort studies (Howe et al. 2016). We have 2 types of selection bias: selection bias due to restriction of analysis to the KXRF sub-study, and selection bias due to survivorship from glaucoma diagnosis (i.e., no development of glaucoma) at the later follow-up period or loss to follow-up that could have been influenced by lead exposure (directed acyclic graphs depicting these 2 types of selection bias in Figure II.2).

Among the original 2280 NAS participants enrolled in the 1960s, nonparticipation in the

subsequent KXRF bone lead sub-study in the 1990s is likely to be related to past lead exposure and other confounders (e.g., socioeconomic status) that could affect participation. Restricting to the subset of those who participated in the bone lead substudy (i.e., conditioning on a collider) could therefore bias the exposure-outcome association (Hernán et al. 2004). To reduce this potential selection bias, we applied inverse probability weighting (IPW) to our models (Weisskopf et al. 2015). Briefly, we ran a logistic regression model to predict the probability of KXRF enrollment for all NAS participants, and calculated IPW from this probability. For those in our sub-study, in this model we used all observations from NAS recruitment to the time of KXRF measurement and each visit was treated as a single observation. For those who were not in the sub-study, we used observations until the last visit before year 1999, which is the last year of bone lead measurement in our study. IPW of our study population ranged from 1.0 to 6.1, with the mean of 1.18 (data not shown). This approach simulates a pseudo-population similar to the original NAS population and therefore can account for potential selection bias that may have happened before our bone lead sub-study.

Selection bias due to survivorship from glaucoma diagnosis at the later follow-up period or loss to follow-up is also likely to occur. Those who were more susceptible of lead toxicity could develop POAG earlier or be dropped out earlier. Although IPW is again a standard recommendation to account for this selection bias, because we already included the aforementioned IPW and it is challenging to include two IPWs in analysis. Such selection bias may result in time-varying hazard ratios (HRs), which have been commonly reported in prospective observational studies (Hernán 2010). Simply reporting the average HR during the whole follow-up time may result in the underestimation of association. Thus, instead of IPW, we created adjusted survival curves as the solution. This approach was suggested to address two key

limitations of the use of average HR using Cox proportional hazard modeling, the time-varying HR and a built-in selection bias (Hernán 2010). See analytical approach used below.

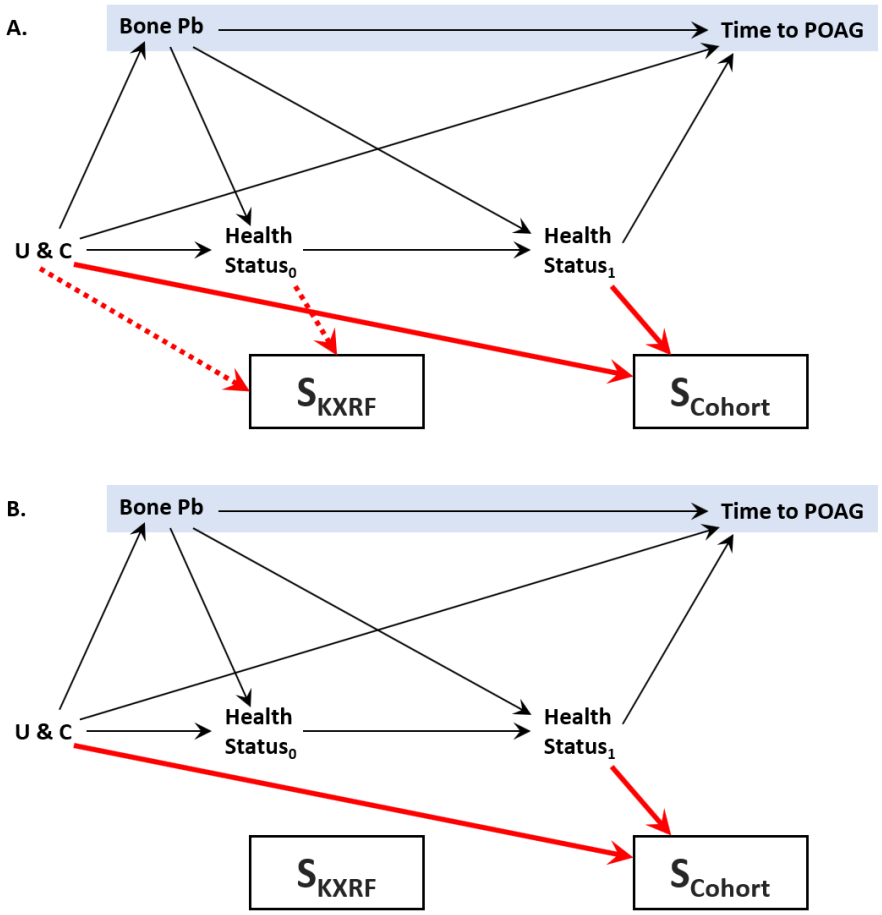

Figure II.2. Causal diagram representing the impact of two types of selection bias at the baseline of KXRF measurement and during follow-ups in the unweighted (A) and IPW-weighted (B) models. S_{KXRF} is the selection bias caused by participation of KXRF sub-study from the NAS inception; S_{Cohort} is the selection bias due to survivorship from either POAG diagnosis or loss to follow-up during current study. U and C refer to unmeasured and measured covariates at the baseline. Health $Status_0$ and Health $Status_1$ are severe health conditions which may affect the attendance of study at baseline and during the follow-ups. Bone Pb refer to the bone lead concentration at baseline, which reflects past cumulative lead exposure (by tibia lead) and baseline endogenous source of stored lead (by patella lead). The rectangular blue shade illustrates the main association we investigated in this study. The inverse probability weighting (IPW) applied in the current study has already accounted for the effect of S_{KXRF}; this IPW removed the dash red lines in A. Standard solution for the 2nd selection bias occurred during the follow-up time (S_{Cohort}) was still the application of another IPW (which can remove the bold red lines). However, since it was challenging to combine two IPWs into one model, S_{Cohort} was hard to be avoided.

3.6. Statistical analysis

We compared baseline population characteristics (means (standard deviations (SDs)) for continuous variables and frequencies for categorical variables) by POAG status. We also performed bivariate analysis between baseline covariates and bone lead concentrations.

We used Cox proportional hazard models to evaluate the association between bone lead and incident POAG. Three sequential covariate models were performed: Model 1 adjusted for age; Model 2 further adjusted for BMI, educational levels, job types, and categorical pack-years which are known risk factors for POAG; Model 3 further adjusted for diabetes mellitus, hypertension, and ocular hypertension, systemic or ocular diseases that are related to the pathogenesis of POAG. We show Model 3 as a separate model because those diseases may play as mediators rather than confounders in the lead-POAG association.

The proportional hazard assumption was tested by creating Schoenfeld residual plots. Because the assumption is often violated and HRs are not constant over time in prospective observational studies (Hernán 2010), we evaluated whether HRs are time-varying in our study by using the adjusted survival curves. We illustrated the risks of POAG of participants in different bone lead quartiles throughout the entire 23 years' follow-up using adjusted survival curves. The procedures of creating adjusted survival curves was adopted from Hernan, briefly fitted discrete-time models with adjustment of covariates, and then estimated the conditional survivals under different exposure levels using a manipulated counterfactual data (Hernán 2010).

In Cox regression, we restricted our analysis with the follow-up visits to 15 years after baseline because selection bias may have increased with longer follow-ups, since those who tended to live longer were healthier than the baseline population and less susceptible to the lead

toxicity. We chose 15 years because Schoenfeld residual plots for bone lead verses time using the entire follow-ups of up to 23 years showed flat fit lines centered at zero during the 15 years' follow-up and then inclined afterwards, which suggests that HRs were consistent across the first 15 years and then declined over time; such characteristics of time-varying HRs was confirmed by the adjusted survival curves.

We treated the lead variables in two ways in Cox proportional hazard models: 1) we log-transformed the lead variables on the natural scale and calculated HRs together with 95% confidence interval (CI) for the occurrence of POAG for a 10-fold increase in each lead variable (5 participants for tibia lead and 3 participants for patella lead were excluded due to negative values); 2) we categorized the lead variables into four quartiles, calculated HRs for POAG by each quartile, and tested the significance of a linear trend across the quartiles (ordinally coded each quartile by 1, 2, 3, 4). To evaluate nonlinear dose-response relationships, we fit the lead variable using natural splines with knots at the 25^{th}, 50^{th}, and 75^{th} percentiles.

As a sensitivity analysis, we additionally ran all models without the application of IPW. We also restricted the models within white race, or extended the follow-up time beyond 15 years by using all follow-ups (range=1 to 23 years) to test the robustness of the association.

All analyses were performed using SAS system version 9.4 (Cary, NC) and RStudio version 1.0.136.

4. Results

In total, 634 individuals with 1868 observations were eligible to be included in the study after excluding those who had missing covariate data. During follow up, (median=10.6 years, range=2 to 15), 44 incident POAG cases were identified (incidence rate=74 per 10,000 person-years). The mean baseline age at the date of bone lead measurement was 66.8 years (SD 6.7, range from 49.9 to 94.0 years) (Table II.2). The concentration of tibia lead ranged from -5 to 126 μg/g (median was 19 μg/g), while patella lead ranged from -10 to 165 μg/g (median was 27 μg/g). The Pearson correlation coefficient comparing the two bone lead measures was 0.78 ($p<0.001$). Baseline ocular hypertension ($p<0.001$) was associated with POAG identification (Table II.2).

Higher tibia lead levels were associated with older baseline age ($p<0.001$), non-white ($p=0.03$), baseline diabetes history ($p=0.04$), baseline systemic hypertension history ($p=0.05$), lower education levels ($p<0.001$), and blue collar jobs ($p<0.001$) (Table II.3). Higher patella lead levels were associated with older baseline age ($p<0.001$), non-white ($p=0.04$), history of systemic hypertension ($p=0.02$), history of ocular hypertension ($p=0.02$), lower educational attainment ($p<0.001$), a greater number of baseline pack-years of cigarette smoking ($p=0.01$), and blue collar jobs ($p<0.001$) (Table II.3).

Log-transformed bone lead was associated with incident POAG (Table II.4). After adjustment for age, educational level, job types, BMI and cumulative cigarette smoke, a 10-fold increase in patella lead was significantly associated with an HR of 5.30 (95% CI: 1.71, 16.43, $p=0.004$), and a 10-fold increase in tibia lead was positively but not significantly associated with an HR of 2.78 (95% CI: 0.83, 9.31, $p=0.10$) (Table II.4, Model 2). The HRs comparing participants in the third and fourth quartiles with the lowest quartile were 3.90 (95% CI: 1.52,

9.97) and 3.60 (95% CI: 1.34, 9.65) with a positive linear trend (p-for-trend=0.007) for patella lead; and 3.95 (95% CI: 1.59, 9.86) and 2.44 (95% CI: 0.87, 6.83) for tibia lead (p-for-trend=0.03) (Table II.4, Model 2). The associations remained significant even after further controlling for ocular hypertension, diabetes mellitus and systemic hypertension. A 10-fold increase in patella lead was significantly associated with an HR of 5.06 (95% CI: 1.61, 15.88, p=0.005), and a 10-fold increase in tibia lead was positively but not significantly associated with an HR of 3.07 (95% CI: 0.94, 10.0, p=0.06) (Table II.4, Model 3). The HRs comparing participants in the third and fourth quartiles with the lowest quartile were 3.41 (95% CI: 1.34, 8.66) and 3.24 (95% CI: 1.22, 8.62) for patella lead (p-for-trend=0.01); and 3.84 (95% CI: 1.54, 9.55) and 2.61 (95% CI: 0.95, 7.21) for tibia lead (p-for-trend=0.02) (Table II.4, Model 3). Smoothing plots based on natural splines support these findings that the associations linearly increased until the third quartile and plateaued in the range of the fourth quartile (Figure II.3).

The survival curves comparing 4 quartiles of bone lead illustrated that the absolute risks started to get closer and cross over between 15-20 years. This suggests that the HRs in our study were not constant and changed over time (Figure II.4). This observation is consistent with the Schoenfeld residual plots, the assessment of proportional hazard assumption.

We performed several sensitivity analyses to assess the robustness of the findings (data not shown). In fully adjusted models, restricting the study population to whites only (n=613 for patella lead and n=611 for tibia lead) did not change main findings, with a 10-fold increase in patella lead significantly associated with an HR of 4.18 (95% CI: 1.29, 13.57, p=0.02), and a 10-fold increase in tibia lead positively but not significantly associated with an HR of 3.00 (95% CI: 0.89, 10.15, p=0.08). The association was attenuated when follow-up time extended up to 23 years: a 10-fold HR for a fully adjusted model for patella lead became 2.59 (95% CI: 1.00, 6.68,

p=0.049) and the association between tibia lead and POAG became insignificant. Results were similar without the application of IPW: the associations were attenuated, with a 10-fold HR of 4.29 for a fully adjusted model for patella lead (95% CI: 1.18, 15.55, p=0.03) and non-significant association for tibia lead.

Table II.2. Baseline Characteristics of Study Population Comparing Participants with POAG vs participants with Non-POAG

Characteristics	Total Population (n=634)	Non-POAG (n=590)	POAG (n=44)	*P* value[a]
Bone lead levels				
Tibia Lead, mean±SD, µg/g	21.7±13.7	21.6±13.8	23.5±12.4	0.37
Patella Lead, mean±SD, µg/g	31.0±20.2	30.6±20.1	36.3±21.4	0.08
Age at baseline, mean±SD, years	66.8±6.7	66.8±6.8	67.7±6.1	0.36
Age at end of 15 years' follow-up, mean±SD, years	76.8±6.7	76.8±6.7	75.8±6.4	0.30
BMI, mean±SD, kg/m^2	27.9±3.7	27.9±3.8	27.5±3.4	0.44
Diabetes mellitus, n (%)	89 (14.0)	81 (13.7)	8 (18.2)	0.41
Systemic hypertension, n (%)	346 (54.6)	320 (54.2)	26 (59.1)	0.53
Ocular hypertension, n (%)	21 (3.3)	13 (2.2)	8 (18.2)	<0.001
White population, n (%)	616 (97.2)	575 (97.5)	41 (93.2)	0.11
Educational levels, n (%)				
≤ High school	65 (10.3)	62 (10.5)	3 (6.8)	
High school	230 (36.3)	214 (36.3)	16 (36.4)	
Some college	157 (24.8)	144 (24.4)	13 (29.6)	
≥ 4 years' college	182 (28.7)	170 (28.8)	12 (27.3)	0.71
Pack-years, n (%)				
0	204 (32.2)	190 (32.2)	14 (31.8)	
1-19	171 (27.0)	159 (27.0)	12 (27.3)	
≥20	259 (40.9)	241 (40.9)	18 (40.9)	0.97
Job type, n (%)				
Blue collar	265 (41.8)	247 (41.9)	18 (40.9)	
Mix	139 (21.9)	129 (21.9)	10 (22.7)	
White collar	230 (36.3)	214 (36.3)	16 (36.4)	0.99

Abbreviations: POAG, primary open-angle glaucoma; SD, standard deviation; KXRF, K x-ray fluorescence; BMI, body mass index.

[a] *P* values were calculated using logistic regression; educational levels and pack-years were treated as ordinal variables.

Table II.3. Bivariate Analysis of Lead Concentration by Baseline Characteristics

		Bone Lead Concentration, mean (SD), µg/g		*P* value[a]	
Characteristics	**No. (%)**	**Tibia Lead**	**Patella lead**	**Tibia Lead**	**Patella lead**
Overall	634 (100)	21.7 (13.7)	31.0 (20.2)	-	-
Age at baseline (years)					
45-59	95 (15.0)	14.7 (8.0)	22.5 (12.9)		
60-70	351 (55.4)	20.7 (11.8)	29.3 (17.6)		
70+	188 (29.7)	27.1 (16.9)	38.5 (24.9)	<.001	<.001
Race/ethnicity					
White	616 (97.2)	21.5 (13.5)	30.7 (19.7)		
Non-white	18 (2.8)	28.5 (19.2)	40.5 (33.2)	0.03	0.04
Diabetes mellitus					
Yes	89 (14.0)	24.5 (14.1)	34.1 (21.5)		
No	545 (86.0)	21.2 (13.6)	30.5 (20.0)	0.04	0.12
Systemic hypertension					
Yes	346 (54.6)	22.7 (15.3)	32.7 (22.8)		
No	288 (45.4)	20.5 (11.3)	28.9 (16.3)	0.05	0.02
Ocular hypertension					
Yes	21 (3.3)	25.8 (18.4)	41.0 (27.7)		
No	613 (96.7)	21.6 (13.5)	30.6 (19.8)	0.17	0.02
BMI ((kg/m^2))					
<25	135 (21.3)	20.4 (11.5)	29.9 (15.6)		
25-30	342 (53.9)	22.1 (13.3)	31.1 (19.2)		
≥30	157 (24.8)	21.8 (16.1)	31.6 (25.3)	0.40	0.47
Educational levels					
≤ High school	65 (10.3)	28.1 (17.9)	39.5 (24.3)		
High school	230 (36.3)	24.2 (15.4)	35.3 (23.0)		
Some college	157 (24.8)	20.6 (11.4)	28.7 (17.2)		
≥ 4 years' college	182 (28.7)	17.2 (9.4)	24.4 (14.0)	<.001	<.001
Pack-years					
0	204 (32.2)	21.2 (14.1)	29.6 (20.2)		
1-19	171 (27.0)	20.1 (12.4)	27.9 (17.0)		
≥20	259 (40.9)	23.2 (14.1)	34.1 (21.7)	0.10	0.01
Job type					
Blue collar	265 (41.8)	26.2 (16.4)	37.0 (4.0)		
Mix	139 (21.9)	19.1 (10.8)	27.3 (15.3)		
White collar	230 (36.3)	18.1 (9.8)	26.2 (15.8)	<.001	<.001

Abbreviations: SD, standard deviation; KXRF, K x-ray fluorescence; BMI, body mass index.

[a] *P* values were calculated using linear regression. Educational levels and pack-years were treated as ordinal variables.

Table II.4. Hazard Ratio (95% CI) of POAG by Bone Lead Concentrations with Application of IPW

				Model 1[a]		Model 2[b]		Model 3[c]	
Exposure	**N total**	**N case**	**Range (μg/g)**	**10-fold HR (95% CI)**	***P* value**	**10-fold HR (95% CI)**	***P* value**	**10-fold HR (95% CI)**	***P* value**
Tibia									
Continuous[d]	629	44	1 to 126	2.73 (0.92, 8.16)	0.07	2.78 (0.83, 9.31)	0.10	3.07 (0.94, 10.0)	0.06
Quartiles[e]									
Quartile 1	148	6	-5 to 12	Reference		Reference		Reference	
Quartile 2	154	7	13 to 18	1.48 (0.51, 4.24)		1.56 (0.54, 4.55)		1.76 (0.60, 5.13)	
Quartile 3	169	20	19 to 27	3.75 (1.55, 9.05)		3.95 (1.59, 9.86)		3.84 (1.54, 9.55)	
Quartile 4	163	11	28 to 126	2.34 (0.89, 6.19)	0.02	2.44 (0.87, 6.83)	0.03	2.61 (0.95, 7.21)	0.02
Patella									
Continuous[d]	631	44	1 to 165	4.68 (1.65, 13.30)	0.004	5.30 (1.71, 16.43)	0.004	5.06 (1.61, 15.88)	0.005
Quartiles[e]									
Quartile 1	162	6	-10 to 18	Reference		Reference		Reference	
Quartile 2	150	10	19 to 26	2.29 (0.88, 6.01)		2.52 (0.95, 6.73)		2.23 (0.83, 5.98)	
Quartile 3	165	14	27 to 38	3.47 (1.40, 8.58)		3.90 (1.52, 9.97)		3.41 (1.34, 8.66)	
Quartile 4	157	14	39 to 165	3.35 (1.32, 8.53)	0.006	3.60 (1.34, 9.65)	0.007	3.24 (1.22, 8.62)	0.01

Note: We applied un-stabilized IPW into our models; IPW of all participants ranged from 1.0 to 6.1.

Abbreviations: POAG, primary open-angle glaucoma; IPW, inverse probability weighting; HR, hazard ratio; CI, confidence interval.

[a] Model 1 was adjusted for age.

[b] Model 2 was further adjusted for body mass index, educational levels, job types, and categorical pack-years.

[c] Model 3 was further adjusted for diabetes mellitus, systemic hypertension, ocular hypertension.

[d] To calculate 10-fold HR for POAG using continuous bone lead levels, we natural-log-transformed the values, excluded 5 participants for negative levels in tibia lead and 3 participants for negative levels in patella lead. Bone lead levels measured by KXRF can have negative values since the instrument provided an unbiased point estimate that may oscillate around the true value.

[e] *P*-values represented trend *P*-values calculated by applying ordinal values (1,2,3,4) to bone lead quartiles.

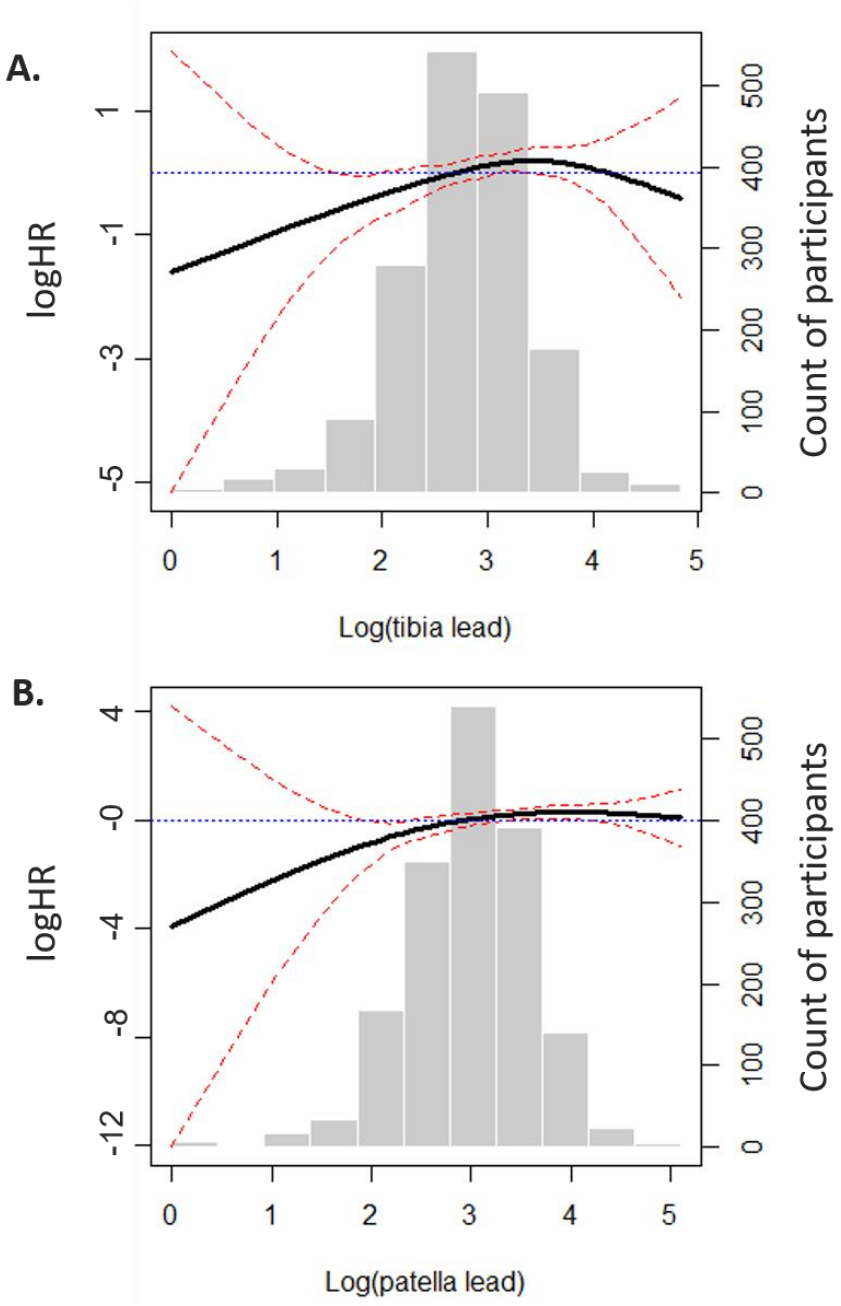

Figure II.3. Splines Illustrating non-linear association between bone lead levels and log of Hazard Ratio (logHR) for incident POAG adjusted for baseline age, BMI, educational levels, job types, smoking, systemic hypertension, diabetes mellitus, and ocular hypertension. IPW was applied. Dark black line illustrated the Natural Splines with knots at 25th, 50th, and 75th percentiles, together with the 95% CI (red dash lines). X-axis is log(bone lead). Histogram illustrates the distribution of log(bone lead) of all participants by count (right y-axis). The left y-axis is logHR, with the reference horizontal blue dash line illustrating logHR=0 at the mean of log(bone lead) (21.8 µg/g for tibia lead and 31.0 µg/g for patella lead). A. Spline for tibia lead; B. Spline for patella lead.

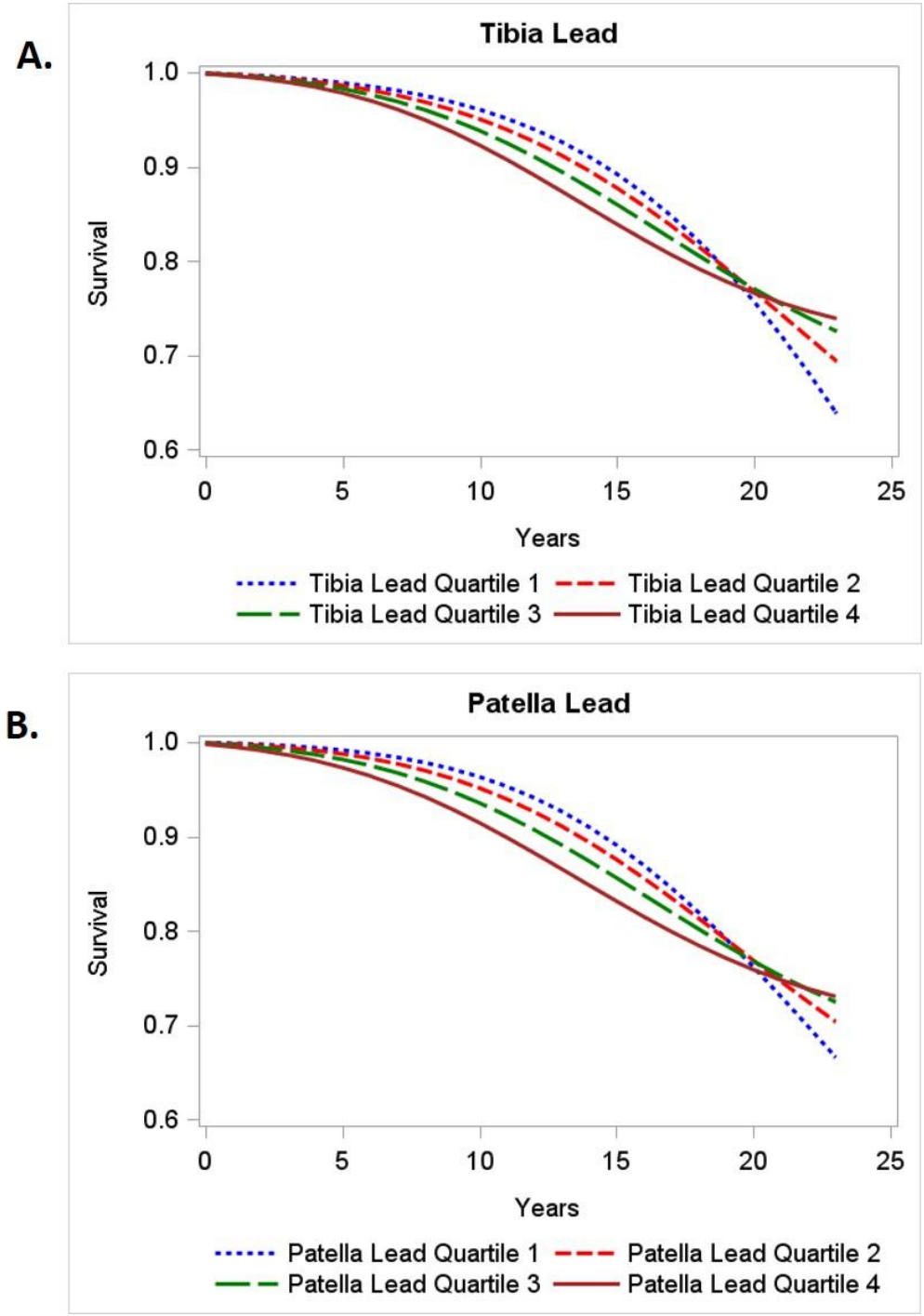

Figure II.4. Adjusted survival curves illustrating changes of survival of different bone lead quartiles during follow-up. X-axis indicates years since baseline, y-axis indicates the survival calculated by discrete-time hazard models with adjustment for baseline age, BMI, educational levels, job types, smoking, diabetes mellitus, systemic hypertension, ocular hypertension. A. Tibia lead; B. Patella lead.

5. Discussion

Our study provided longitudinal evidence that bone lead may be an important risk factor of POAG. Men in the third and fourth quartiles of patella lead levels had a more than 3-fold higher risk of POAG compared to those in the lowest quartile during 15 years of follow-up. A 10-fold increase in patella lead level was associated with more than 5-fold higher risk of POAG during 15 years of follow-up. Similar but slightly weak associations were observed for tibia lead.

Previous studies suggested that lead and other heavy metals may be associated with glaucoma pathogenesis in different Asian populations. Although the end organ damage of glaucoma is at the level of the optic nerve, there are diverse phenotypes based on anatomy and clinical findings that vary widely based on different populations (Jonas et al. 2017). The various phenotypes include POAG, normal tension glaucoma, and primary angle closure glaucoma. As the epidemiology for the various forms of glaucoma varies among different populations (Chan et al. 2016; Cheng et al. 2014; Kapetanakis et al. 2016; Tham et al. 2014), it is essential that epidemiology studies be interpreted in the context of the study population and not generalized to different populations. In addition, it is important to not over interpret findings from cross-sectional study designs regarding causal inferences and reverse causality. Two major strengths of our study are the longitudinal study design and a predominantly white study population.

Another strength of our study was the utilization of bone lead levels as biomarkers. Tibia bone lead can better indicate cumulative lead dose compared with blood or hair lead levels measured in previous studies, while patella bone lead mainly reflects a source of cumulatively stored lead that is bioavailable (Hu et al. 2007). Blood lead reflects a combination of recent exogenous exposure and endogenous exposure by the cumulative lead body burden; it has a half-life at approximately one month, which limits inferences regarding chronic effects of cumulative

exposure (Hu et al. 1995, 2007). As POAG is an age-related disease, any biomarkers with a relatively short half-life should be interpreted cautiously as a risk of chronic conditions (Lin et al. 2015). Hair lead levels used in the Japanese case-control study were also poor indicators of cumulative lead exposure, since hair lead can be greatly affected by the frequency and method of hair washing and cutting (Barbosa et al. 2005). Bone lead has a much longer half-life, which makes it a better indicator of cumulative exposure. Studies showed that more than 90% of lead body burden are stored in bone with a half-life from years to decades: half-life of tibia lead can be up to 48.6 years assuming a constant decline rate (Wilker et al. 2011).

We observed a stronger association with patella lead than tibia lead. We hypothesize that this discordance may reflect the different metabolic activity of these two kinds of bones. Bones are the major storage site of lead in the body, but also are an important source of endogenous lead (Hu et al. 1998). Lead in bone can be mobilized gradually into the plasma, and transferred into other target tissues through the circulatory system (Wilker et al. 2011). Trabecular bone, such as patella bone, has a higher rate of metabolic activity compared with cortical bone such as mid-tibia bone (Rabinowitz 1991; Wilker et al. 2011). Tibia lead slowly declines about 1.4% per year after the cessation of exogenous exposure to lead, while patella lead follows a piecewise log-linear decline with a rapid initial rate more than twice as fast as tibia lead and then go to a plateau (Wilker et al. 2011). Thus patella lead may be more likely to reflect biologically available endogenous lead which can affect the development of age-related diseases in other tissue, such as glaucoma in eyes.

The mechanisms of lead on the pathogenesis of glaucoma may involve oxidative stress. Lead can increase oxidative stress through the depletion of the glutathione and thiol pools, as well as disrupting the antioxidant defense system (Ercal et al. 2001; Jomova and Valko 2011; Valko et

al. 2016). Excessive oxidative stress and lipid peroxidation may lead to the accumulation of free radicals and their derivatives (reactive oxygen species, ROS), overwhelming the antioxidant defense system, cause the loss of cell adhesion and changes in the cytoskeletal structure of trabecular meshwork cells, induce the dysfunction of the aqueous humor drainage system, disrupt the outflow of aqueous humor from the eyeball, result in the increase of intraocular pressure, and finally cause the development of glaucomatous neuropathy (Babizhayev 2012; Saccà et al. 2016; Zhao et al. 2016). In addition, oxidative stress may directly damage the head of optic nerves through the similar cell dysfunction mechanism to induce the development of glaucoma (Tezel et al. 2010). Our results showed that after controlling for ocular hypertension, the association between patella lead and POAG was attenuated but remained significant, suggesting that lead could directly affect glaucoma pathogenesis other than through the dysfunction of aqueous humor drainage system.

Further investigation on lead-gene interaction may help reveal the mechanisms of lead's effect on POAG as well as the unclear pathogenesis of POAG. The heritability of POAG is polygenic, and usually related to genes having incomplete penetrance (Wiggs 2015). Current known genes associated with POAG includes Myocilin (*MYOC*), Atonal BHLH transcription Factor 7 (*ATOH7*), Transmembrane And Coiled-Coil Domains 1 (*TMCO1*), SIX Homeobox 1/SIX Homeobox 6 (*SIX1/SIX6*), Growth Arrest Specific 7 (*GAS7*), (Abu-Amero et al. 2015) etc. These genes may interact with the lead metabolic pathways and affect the development of glaucoma. For instance, mutation in some genes such as *MYOC* may change the sensitivity of oxidative stress (Joe and Tomarev 2010), thus change the susceptibility of lead poisoning.

Our study has several limitations. Those who were eligible at the baseline might be healthier than the original NAS cohort recruited in 1960s. We applied IPW to reduce such

selection bias at the time of bone lead measurements (baseline). We also have another selection bias during the follow-up time. Follow-up time varied greatly among our participants, and as expected with an aging population, the rate of loss to follow-up was relatively high. Those who remained in the study for long follow-up may be even healthier than the baseline study sample, and consequently may have been less susceptible to glaucoma. This was reflected by the time-varying HRs. Such selection bias could result in underestimation of the association, which means it would not change our conclusion. Besides, baseline bone lead levels may not capture the environmental lead exposure during the follow-up. Since the usage of lead in gasoline and paint was generally banned in the U.S. since 1990s, we assumed that the environmental exposure of lead largely decreased after our baseline. Thus, we hypothesized that participants' tibia lead levels may not change a lot, while patella lead levels may reflect the gradually decreased exogenous exposures during the follow-up time. A natural limitation for costly cohort epidemiology studies is reduced power to detect age-related diseases, such as POAG; the sample size for incident cases was relatively small. We did not include family history of POAG in to our analysis, although it is an important risk factor of POAG. Family history of glaucoma was self-reported in NAS with lots of missing and uncertain description. Since there is no previous study reported that family history of POAG was associated with bone lead levels, it may not confound the lead-POAG association. NAS only recruited male veterans living in the Boston area (97% were white). Therefore, our results may not be generalizable to other populations, although the incident POAG rate in our population (15-year incidence of 7%) is comparable to other populations (i.e., the 26-year incidence of 9.7% in the Health Professional Follow-up Study) (Kang et al. 2018).

Lead is related to multiple age-related health problems, such as cognitive decline (Fishbein et al. 2008), hearing loss (Park et al. 2010), cataract (Mosad et al. 2010; Schaumberg et al. 2004),

and AMD (Erie et al. 2009; Hwang et al. 2015). Recent concerns about the widespread exposure of the residents of Flint, Michigan to elevated lead levels in their drinking water was an important reminder that lead continues to be a dangerous environmental toxicant (Gómez et al. 2018; Hanna-Attisha et al. 2016; Zahran et al. 2017). Concerns are especially high in urban environments with aging infrastructure and public awareness of lead exposure needs to be reinforced. Aging populations are at greater risk to lead toxicity given the cumulative nature of lead. Further, the older population is growing and age-related glaucoma-induced blindness will impose a huge economic burden on the whole society. In 2013, the number of glaucoma patients worldwide (aged 40-80 years old) was estimated to be 64.3 million, and this number is projected to increase to 76.0 million in 2020 and 111.8 million in 2040 (Tham et al. 2014). In the U.S., the National Eye Institute reported that approximately 1.9% of the population aged 40 years and older was suffering from POAG in 2010. The number of cases rose from 2.22 million in 2000 to 2.72 million in 2010 (Glaucoma, Open-angle | National Eye Institute). Glaucoma is a neurodegenerative disease; the loss of visual function is irreversible once symptomatic, and there is no cure (Prum et al. 2016; WHO | Glaucoma is second leading cause of blindness globally). In order to minimize the burden of glaucoma-related blindness, it is important to identify risk factors that can implemented to clinical practice for pre-symptomatic prevention and earlier detection. Our finding contributes additional evidence on the chronic health effects of environmental lead exposure, which might help strengthen the public awareness of lead-related ocular diseases and blindness.

In conclusion, this is the first epidemiologic study indicating the association between bone lead levels and risk of POAG at a longitudinal scale. We show that bone lead may be an important risk factor of POAG in a U.S. population of men. Further studies for replication and in women are needed to validate our findings. We expect our study to increase the public awareness

of cumulative environmental lead exposure, provide new points of view for the exploration of the pathogenesis of glaucoma, give new ideas for glaucoma interventions such as mitigating the oxidative stress consequence of lead in ocular tissues, and therefore provide new avenues to effectively decrease the global burden of blindness.

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CHAPTER III

Effect Modification by Dietary Patterns and Dietary Vitamin Intake in the Association between Bone Lead Levels and Risk of Incident Primary Open-Angle Glaucoma: the VA Normative Aging Study

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1. Abstract

Background: Elevated bone lead level may be a risk factor for age-related primary open-angle glaucoma (POAG). High intake of nutrients has been associated with lower POAG risk. We examined effect modification by dietary intake of vitamins and dietary patterns in the association between bone lead and POAG.

Methods: A total of 620 POAG free males (mean baseline age=66.9 years (SD=6.7)) from the Normative Aging Study were followed for 15 years since their bone lead measurements by K X-ray fluorescence between 1991 and 1999. Those who had consistent observed enlarged or asymmetric cup-to-disc ratio together with visual field defect or existence of disc hemorrhage were identified as POAG cases. Two dietary patterns were identified by principal component analysis: a 'prudent' diet was highly correlated with high intake of legumes, vegetables, seafood, onions, tomatoes, fruit and poultry, while a 'Western' diet was highly correlated with high intake of eggs, red and processed meat, high-fat dairy products, butter, beers, chowder, fries, refined grains and mayonnaise. Cox proportional hazards regressions were performed to estimate hazard ratios (HRs) of incident POAG comparing low (<median dietary scores) vs. high (≥median) dietary groups.

Results: Forty-four incident POAG were identified during a median of 10.4 years' (incidence rate=72 per 10,000 person-years) follow-up. In a fully adjusted model, an HR of POAG was 2.03 (95% CI: 1.25, 3.29) per 2-fold increase in patella lead among participants with low adherence to prudent diet, whereas it was 1.33 (95% CI: 0.86, 2.05) among participants with high adherence to prudent diet. Similar effect modification was found for provitamin A carotenoid intake: HRs per 2-fold increase in patella lead between those with low intake versus high intake of carotenoid

were 2.04 (95% CI: 1.26, 3.29) and 1.26 (95% CI: 0.90, 2.11), respectively. No significant effect modification was found by western diet or dietary intake of vitamins C, D and E.

Conclusions: High adherence of prudent diet and high dietary intake of carotenoids may help reduce POAG risk related to bone lead. These results need to be interpreted cautiously due to the small sample size and generalization issues.

Key words: Bone lead, primary open-angle glaucoma, diet, vitamin A

2. Introduction

Glaucoma is the second leading cause of blindness and the leading cause of irreversible vision loss globally (Pascolini and Mariotti 2012). In the United States, approximately 1.9% of the population aged 40 and older are suffering primary open-angle glaucoma (POAG) (Prevalence of Open-Angle Glaucoma Among Adults in the United States 2004). Despite the increasing patient population and severe health consequence with largely effects on quality of life, the etiology of glaucoma remains unclear. As a neurodegenerative disease, the established pathophysiology of glaucoma includes pathological or anatomic change in the complex tissues in the anterior chamber of eye ball that control the dynamics of aqueous humor fluid, regulate the intraocular pressure (IOP), and induce the progressive end-organ optic neuropathy in the posterior segment (Jonas et al. 2017). Commonly known risk factors for age-related glaucoma includes older age, ocular hypertension, race/ethnicity, positive family history, optic nerve susceptibility and myopia, according to previous clinical trials (Jonas et al. 2017).

As mentioned in Chapter II, increased oxidative stress may play an important role in the development of glaucoma (Babizhayev 2012a; Goyal et al. 2014; Majsterek et al. 2011; Zhao et al. 2016; McMonnies 2018). The threaten of none-occupational lead exposure, an important source of environmental oxidative stress, has not vanished even though lead was phased out of paint in 1978 and gasoline in the 1990's by the U.S. government. Lead's toxic ocular effects in aging populations was noted in a previous investigation in the Normative Aging Study (NAS): bone lead levels were associated with the risk of age-related cataract (Schaumberg et al. 2004). Our group's study in Chapter II using an NAS subsample found a significant positive association between bone lead levels and incident POAG on a longitudinal scale. We utilized tibia lead level as a biomarker of cumulative environmental lead exposure, and patella lead level as an indicator

of endogenous exposure from lead body burden. We founded that patella lead level was positively associated with the risk of incident POAG.

Dietary intervention can be a more applicable and acceptable treatment for lead poisoning compared with the traditional chelation therapy (Aposhian et al. 1995; Kalia and Flora 2005). Some previous studies have suggested that antioxidant nutrients, such as vitamins C and E intake, can attenuate the toxicity of lead by balancing the lead-induced oxidative stress (Al-Attar 2011; Calabrese et al. 1987; Hsu and Guo 2002; Rendón-Ramírez et al. 2014; Simon and Hudes 1999). Traditional glaucoma therapies mostly emphasize in decreasing the IOP. Novel treatments focus on reduction of intraocular oxidative stress using antioxidants and have been tested in mouse models (Dong et al. 2016; Inman et al. 2013; Kimura et al. 2017; Yang et al. 2016). Dietary intake of vitamins A and C has a protective association with OAG (D. Ramdas et al. 2018). Lipid-soluble antioxidant vitamins D and E may be associated with risk of glaucoma (Kim et al. 2016; Ko et al. 2010; Yoo et al. 2014).

Single nutrient intake styduesm without the consideration of a combination of multiple food and nutrients, may be limited in their ability to establish a practical dietary intervention of POAG. The traditional single nutrient approach has multiple limitations: it cannot reflect the complex interactive effects among nutrients; it can hardly distinguish separate effect of each nutrient due to high intercorrelation among some nutrients; the statistical significance of association between single nutrient and chronic disease may simply being detected by chance when analyzing large number of nutrients; when the effect of single nutrient is too small to be detected, investigators cannot conclude that the cumulative or combined effect of multi-nutrient is null (Hu 2002). Dietary patterns can solve these problems through a more comprehensive view of nutrient intakes. Dietary pattern analysis is a common method for investigating the association

between diet and chronic disease (Hu 2002; Kant 2010; Tucker 2010).

To the best of our knowledge, no previous epidemiologic study has ever investigated the potential effect modification by dietary intakes on the lead-POAG association. In this study, we aimed to examine the effect modification by dietary patterns as well as single dietary vitamin intake on the association between bone lead levels and incident POAG. Our study sample was male participants lived around the Great Boston Area, who were derived from the VA Normative Aging Study (NAS). This study is a further exploration of potential intervention of the lead toxicity on POAG development.

3. Methods

3.1. Ethical Declaration

This study has been reviewed and approved by Institutional Review Boards (IRB) of the following participating institutes: 1) University of Michigan School of Public Health, 2) Harvard School of Public Health, and 3) Department of Veterans Affairs Boston Healthcare System.

3.2. Study Population

The overall structure of NAS has been described in Chapter II.

Among the initial NAS participants, 868 individuals attended the bone lead measurement via K x-ray fluorescence (KXRF) from January 1st, 1991 to December 31th, 1999. In our study, we used the date of the first bone lead measurement as the baseline of our prospective cohort. We restricted the follow-up time within 15 years in order to reduce the cumulative survival bias since our study population was relatively old at baseline. Among this bone lead sub-group, 702 participants had sufficient information for glaucoma identification via ocular evaluation. We further excluded 82 individuals (more details described in Figure III.1); among them 6 lacking complete Food Frequency Questionnaire (FFQ) information for effective dietary evaluation. Finally, we established a population of 620 participants for the analysis of dietary patterns and vitamins A and C. For vitamins D and E, the total sample size was 405 due to missingness in vitamin intake.

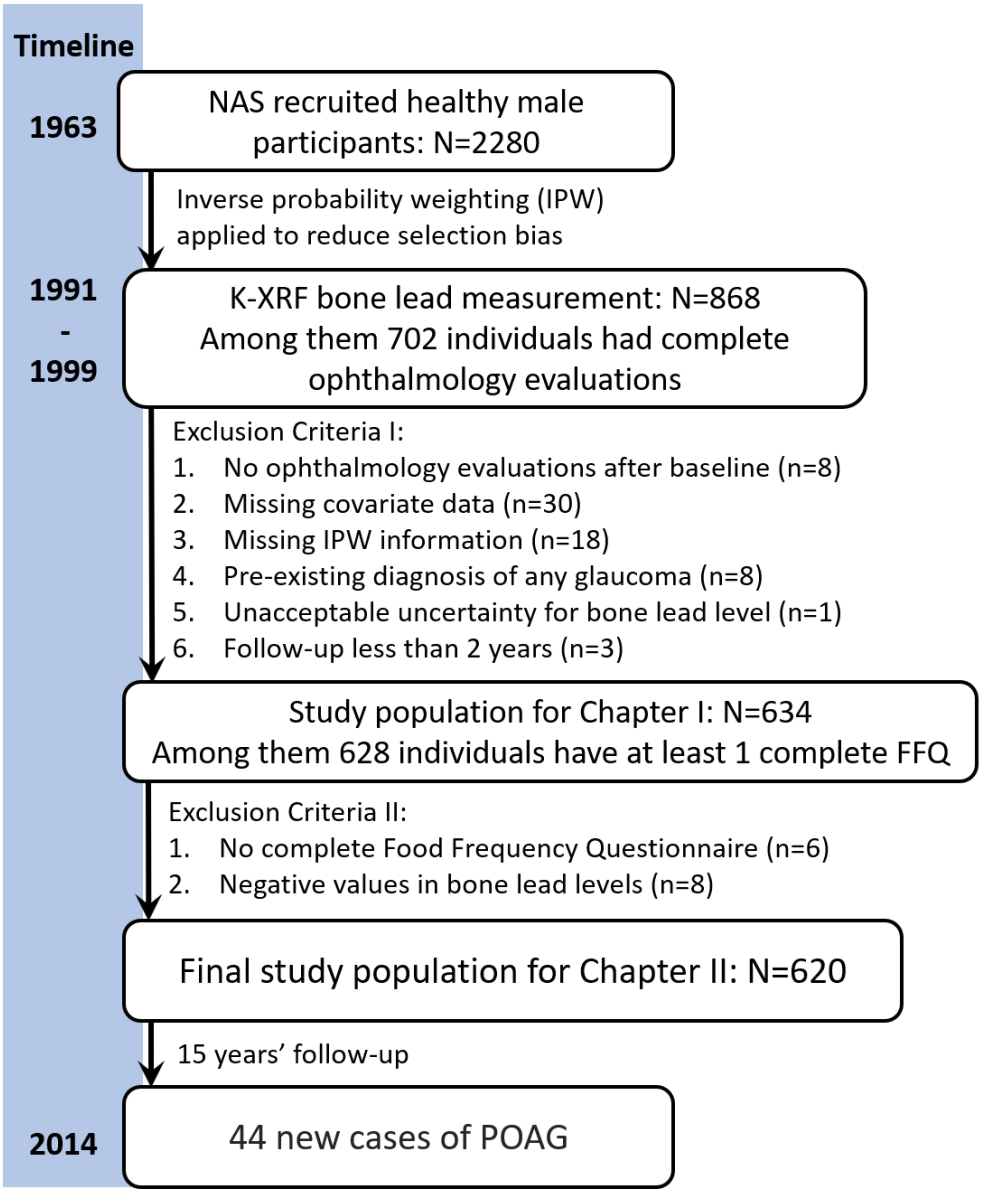

Figure III.1. Diagram illustrates the establishment of study population.

3.3. Primary open-angle glaucoma identification

During the routine NAS standard ocular evaluation, participants were asked about their family and personal systemic or ocular disease history, medical history, and underwent visual acuity test, biomicroscopy, tonometry and ophthalmoscopy for a comprehensive evaluation (Schaumberg et al. 2004). Details of how we reviewed and extracted information for glaucoma

identification were described in our previous study (Chapter II).

We adopted the definition of glaucoma phenotype from the National Eye Institute Glaucoma Human genetics collaBORation (NEIGHBOR) Consortium for the identification of POAG in our study (Wiggs et al. 2013). The criteria were also described in detail in Chapter II. Briefly, POAG were ascertained for participants who showed any one of the following characteristics with an open angle between cornea and iris in the anterior segment: 1) enlarged vertical cup-to-disc ratios (CDR) (either eye's CDR≥0.7); 2) asymmetric CDR (the difference of two eyes' CDRs≥0.2); 3) relatively large CDR together with other typical glaucomatous phenotypes (any eye's CDR≥0.6 with either disc hemorrhage or visual field defect); 4) glaucomatous blindness (caused by loss of nerve fiber layer). Individuals with re-existing glaucoma were treated as baseline glaucoma cases and were ineligible for our study (n=8).

All 620 eligible participants were followed until the date of first ocular evaluation reporting POAG onset, or the end of 15 years' follow-up, or the last return visit before loss to follow-up. Individuals with incident glaucoma types other than POAG (narrow angle glaucoma or secondary glaucoma) were treated as lost to follow-up which stopped at the onset visit (Chapter II, Table II.1).

3.4. Bone lead measurements

We used the K x-ray fluorescent instrument to measure the bone lead levels with the unit of microgram of lead per gram of bone mineral (μg/g), at the mid-tibial shaft and patella for each participant. Tibia bone is a representative of cortical bone while patella bone is trabecular bone (Hu et al. 1995). As mentioned in Chapter II, the low decline rate of tibia lead makes it a good biomarker for cumulative environmental lead exposure, while patella lead may represent

endogenous lead exposure due to a higher rate of metabolic activity of trabecular bone (Rabinowitz 1991; Wilker et al. 2011).

Since we used log-transformed bone lead levels in the following statistical analysis, 8 individuals had negative values for either tibia or patella lead were excluded for simplicity. The KXRF instrument also provides measurement uncertainty (1 standard deviation of replicate measurements) to evaluate the quality of estimated bone lead level (Hu et al. 1995). Those who had unacceptable uncertainty (higher than 10 μg/g for tibia and 15 μg/g for patella) were ineligible for our study (n=1). Details for the KXRF bone lead measurement were described elsewhere (Burger et al. 1990) and in Methods of Chapter II.

3.5. Food frequency questionnaires and single dietary nutrient intakes

The NAS adapted a self-reported semi-quantitative FFQ from the Nurses' Health Study for the evaluation of dietary nutrient intake (Willett et al. 1985). Participants were asked to report serving counts for each of 135 food items per month, week or day during the past year through mailed FFQ on about every four years since 1987. Intake frequency of each food item was categorized into nine possible responses, ranging from "never or less than one serving per month" to "more than six servings per day". Single nutrient intake was calculated by the sum of each food intake frequency multiplied by the specific nutrient content of the specific serving size. Nutrient intake estimation of this FFQ was reported to be reproducible and valid by previous studies (Rimm et al. 1992; Wang et al. 2017; Willett et al. 1985).

In this study, we extracted the dietary intakes of vitamins A, C, D and E, which are reported to be related with either health effect of lead toxicity or POAG development. Vitamin A can be absorbed from diet by two forms: preformed vitamin A, which includes retinols, and

provitamin A, which includes different carotenoids (Ross et al. 2010). Retinols are found in animal sources food, such as meat, fish and dairy products, while carotenoids are mostly converted from plant pigments by human body (Ross et al. 2010; Office of Dietary Supplements - Vitamin A). Previous meta-analysis showed that dietary vitamin A intakes may have beneficial association with POAG risk (D. Ramdas et al. 2018); however, most previous studies regarding vitamin A intakes and glaucoma did not separate preformed vitamin A and provitamin A. Fruit and vegetable based provitamin A more frequently have protective effect on POAG (Coleman et al. 2008; Giaconi et al. 2012; Kang et al. 2016; Ramdas et al. 2012; Wang et al. 2013), while effect of animal-sourced retinols is rarely evaluated independently. To determine the exact dietary source of potential effective vitamin A, we used total dietary vitamin A intake, dietary retinols intake, and dietary carotenoid intake into our study. Since dietary carotenoid has lots of missing, we estimated the carotenoid intake by calculating the difference between total vitamin A intake and retinol intake (carotenoids = vitamin A – retinols). Vitamin C, D and E were also reported to have protective effect on the development of glaucoma, however the association is inconsistent across different studies (Giaconi et al. 2012; Ramdas et al. 2012; Veach 2004; Wang et al. 2013; Goncalves et al. 2015; Yoo et al. 2014).

For each individual, we calculated the “baseline daily vitamin intake” by averaging intake reported by all following FFQs since year 1987 till “1 year after the baseline” (date of KXRF measurement + 365.25 days).

Our study only focused on the dietary intakes of single vitamins, without the inclusion of nutritional supplementary intakes.

3.6. Dietary pattern scores

Commonly used methods of measuring dietary patterns include data-driven approaches such as principal component analysis (PCA) and cluster analysis (Tucker 2010). The procedure of creating dietary pattern scores by PCA using FFQ of NAS has been introduced by previous study (Wang et al. 2017). Briefly, we first created 40 food groups by aggregating 135 food items based on the similarities of food species, components, and cooking styles (Wang et al. 2017). We then used PCA to create factors, which are highly correlated with certain food groups. Orthogonal rotation (varimax rotation) was adopted to maximize the variance of food groups' coefficients, centralize the effect of correlated food groups, simplify the structure of factors, and enhance their interpretability (O'Rourke and Hatcher 2013). We extracted two factors with the highest two eigenvalues, which means they captured the highest two proportion of dietary variance of the study population. The first factor was highly correlated with legumes, vegetables, seafood, onions, tomatoes, fruits and poultry (Table III.1); we defined this factor as "prudent" dietary pattern. The second factor was characterized by eggs, processed meat, high-fat dairy products, butter, red meat, beers, chowder, fires, refined grains, and mayonnaise (Table III.1); we defined this factor as "Western" dietary pattern. Dietary pattern scores, which were adopted from factor scores, were calculated by summing the weighted values of each food groups. The scores quantitatively reflect the adherence to the dietary pattern.

Similar to single nutrient intake, baseline dietary pattern scores were calculated for our study: we averaged dietary pattern from the beginning of FFQs to one year after the baseline date. We defined those with baseline dietary pattern scores lower than the median of study population as "low adherence group", while the others are identified as "high adherence group" (median for prudent diet= - 0.14, median for Western diet= - 0.19). We then stratified the study

population into four dietary subgroups: low prudent and low Western group (n=156), low prudent and high Western group (n=154), high prudent and low Western group (n=154), high prudent and high Western (n=156) (Figure III.2, details explained later).

Table III.1. Factor Loading Matrix[a] for the Calculation of Dietary Patterns using FFQ Data of the Normative Aging Study (*n*=620).

Food groups	Prudent dietary pattern	Western dietary pattern
Legumes	0.71	-
Other vegetables	0.71	-
Dark-yellow vegetables	0.70	
Cruciferous vegetables	0.65	-
Leafy vegetables	0.58	-
Seafood	0.43	-
Onions	0.39	-
Tomatoes	0.37	-
Fruits	0.32	-
Poultry	0.26	-
Eggs	-	0.63
Processed meat	-	0.59
High-fat dairy products		0.57
Butter	-	0.54
Red meat	-	0.52
Beers	-	0.32
Chowder	-	0.30
Fries		0.29
Refined grains	-	0.29
Mayonnaise	-	0.26

[a] Only food groups with absolute values of factor loading score > 0.25 were shown.

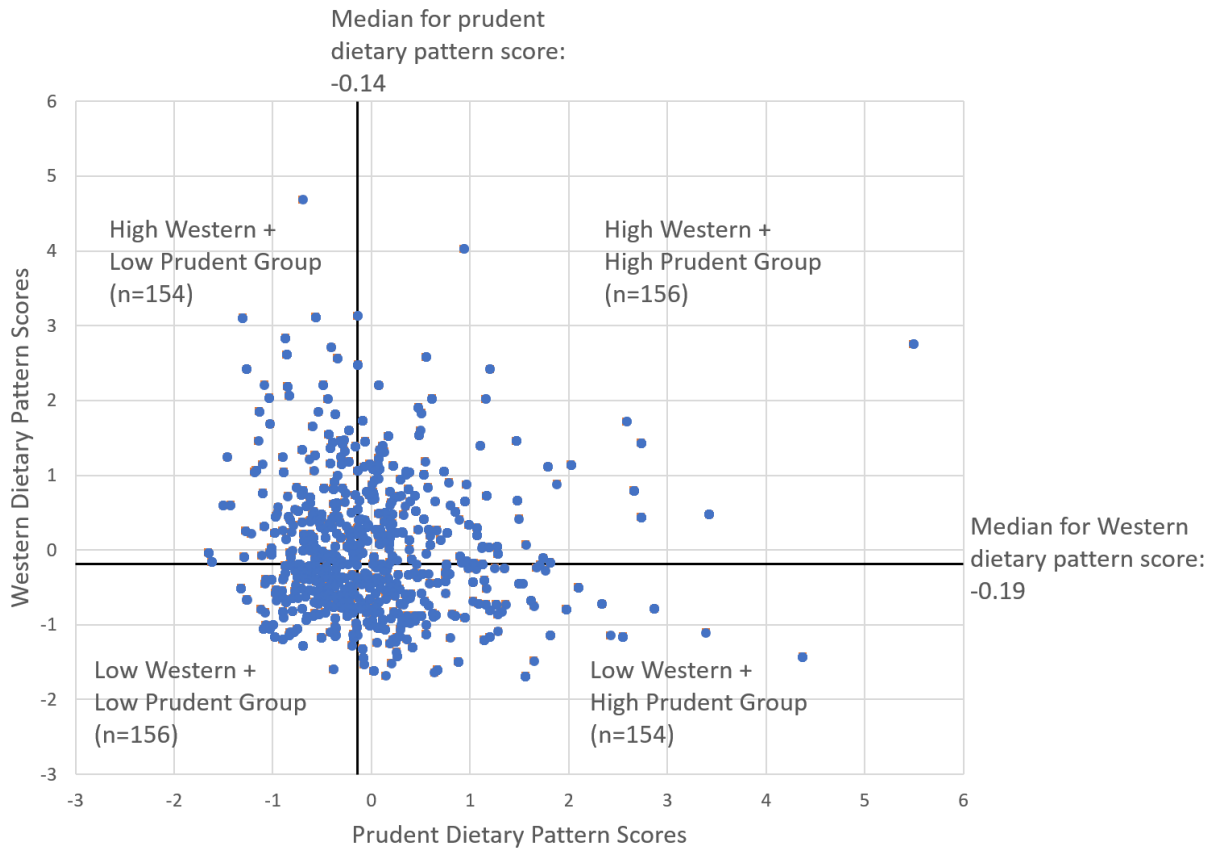

Figure III.2. Plot illustrating the dietary pattern scores of participants in four dietary sub groups.

3.7. Covariates

We included baseline age (years), race/ethnicity (white, non-white), body mass index (BMI, varying at each return visit, kg/m^2), educational attainment (≤high school, high school, some college, and higher), job types (blue collar, white collar or mixed), cigarette consumption in pack-years (0, 0-19, ≥20) in our models. Inclusion criteria of above covariates has been explained by previous paper (Chapter II, Methods). We further adjusted for diabetes mellitus status (yes/no), systemic hypertension (yes/no) and ocular hypertension (yes/no). Identification criteria of those three symptoms were also described in Chapter II. Additionally, we adjusted for total energy intake (kCal) as a marker of individual's comprehensive health status (physical

activity, body size, and metabolic efficiency), as well as to mitigate the measurement error caused by self-report bias (Neuhouser et al. 2008; Willett et al. 1997).

3.8. Statistical analysis

We conducted bivariate analyses to compare the baseline characteristics of our study population by POAG status at the end of follow-up. Means with standard deviations (SDs) were provided for continuous variables while frequencies and percentages were provided for categorical variables. Pearson correlation coefficients were calculated among bone lead levels, dietary pattern scores, and single nutrients.

We used Cox proportional hazards regression to assess the association between bone lead levels and incident POAG in our 15 years' prospective cohort. We computed hazard ratios (HRs) and 95% confidence intervals (CIs) to quantitatively evaluate the risk of POAG related with either patella or tibia lead. The Cox regression we performed for the main association between bone lead and POAG was:

$$\lambda(t) = \lambda_0(t) \times exp(\widehat{\beta_1} Lead + \hat{\beta} Covariates) \qquad \text{(Model 0)}$$

Here $\lambda(t)$ and $\lambda_0(t)$ represents the hazard of POAG at time *t* and baseline, respectively; *Lead* indicates log-transformed patella or tibia lead concentrations (base-2 logarithms); *Covariates* included total energy intake, age at baseline, BMI, job types, educational levels, pack-year of cigarettes, systemic hypertension, diabetes mellitus, and ocular hypertension. The proportional hazard assumption was tested and discussed in Chapter II. The HRs were reported based on a two-fold increase in bone lead levels.

In order to reduce selection bias of those who had attended the bone lead measurement

from the initial NAS recruitment, we applied inverse probability weighting (IPW) into our Cox models to assign weights to each participant (Weisskopf et al. 2015), as we've done previously in Chapter II.

We used two methods to evaluate effect modification effect by dietary patterns or individual vitamin intake on the lead-POAG association:

1) Interaction analysis: test the interaction effect on multiplicative scale by adding interaction terms between bone lead and dietary pattern or single vitamin intake, using the following model:

$$\lambda(t) = \lambda_0(t) \times exp(\widehat{\beta_1} Lead + \widehat{\beta_2} dietary\ pattern\ (or\ single\ vitamin) + \widehat{\beta_3} Lead \times dietary\ pattern\ (or\ single\ vitamin) + \hat{\beta} Covariates)$$ (Model 1a); Here *dietary pattern (or single vitamin)* refers to the dichotomized dietary pattern scores or single nutrient intake which cut at median.

2) Stratified analysis: stratified the total study population by dichotomized dietary pattern scores or single vitamin intake and run Model 0 within each sub-group to intuitively observe the change of HRs. This stratified analysis is equivalent to the following model:

$$\lambda(t) = \lambda_0(t) \times exp(\widehat{\beta_1} Lead + \widehat{\beta_2} dietary\ pattern\ (orsingle\ vitamin) + \widehat{\beta_3} Lead \times dietary\ pattern\ (or\ single\ vitamin) + \hat{\beta} Covariates + \hat{\beta} Covariates \times dietary\ pattern\ (or\ single\ vitamin)$$ (Model 1b), which included the interactions of dietary pattern/vitamins with not only bone lead levels, but also covariates.

We also ran both interaction analysis and stratified analysis using continuous dietary pattern scores and vitamin intake to avoid the loss of information caused by dichotomization.

The stratified analysis considered complicated interactions, which is more comparable to the real situation; however, due to the limited sample size and low incidence rate of POAG, the power of stratified analysis was reduced. We adopted the interaction analysis as the main strategy, and stratified analysis as tests for sensitivity.

To test the complicated interactions among prudent diet, Western diet, and bone lead, we further stratified the total population into 4 dietary pattern groups. Participants were classified into low prudent and low Western diet (Group 1), low prudent and high Western diet (Group 2), high prudent and low Western diet (Group 3), and high prudent and high Western diet (Group 4), according to their adherence to two dietary patterns.

For interaction analysis, we used four dietary pattern groups to analyze complicate cross-product interactions: $\lambda(t) = \lambda_0(t) \times exp(\widehat{\beta_1}Lead + \widehat{\beta_2}Group_2 + \widehat{\beta_3}Group_3 + \widehat{\beta_4}Group_4 + \widehat{\beta_5}Lead \times Group_2 + \widehat{\beta_6}Lead \times Group_3 + \widehat{\beta_7}Lead \times Group_4 + \hat{\beta}Covariates)$ (Model 2a). This model is equivalent to the follow one: $\lambda(t) = \lambda_0(t) \times exp(\widehat{\beta_1}Lead + \widehat{\beta_2}Prudent + \widehat{\beta_3}Western + \widehat{\beta_4}Lead \times Prudent + \widehat{\beta_5}Lead \times Western + \widehat{\beta_6}Prudent \times Western + \widehat{\beta_7}Lead \times Prudent \times Western + \hat{\beta}Covariates)$ (Model 3). Here *Prudent* and *Western* indicate dichotomized variables of prudent and Western dietary pattern scores, which were cut at the median. To evaluate the separate effect of modification by prudent or Western diet with bone lead, we used likelihood ratio test (with covariates) to compare the difference of fitness between Model 3 and the following reduced models:

1) For effect of prudent diet, we compared Model 3 with reduced Model 4:

$\lambda(t) = \lambda_0(t) \times \exp(\widehat{\beta_1}Lead + \widehat{\beta_2}Western + \widehat{\beta_3}Prudent + \widehat{\beta_4}Lead \times Western + \widehat{\beta_5}Prudent \times Western + \hat{\beta}Covariates)$ (Model 4).

2) For effect of Western diet, we compared Model 3 with reduced Model 5:

$\lambda(t) = \lambda_0(t) \times \exp(\widehat{\beta_1} Lead + \widehat{\beta_2} Western + \widehat{\beta_3} Prudent + \widehat{\beta_4} Lead \times Prudent + \widehat{\beta_5} Prudent \times Western + \hat{\beta} Covariates)$ (Model 5).

Again, we also conducted stratification analysis, running Model 0 within each group to intuitively compare the HRs of POAG among different diet, which was equivalent to the following model including interactions of four dietary groups with both bone lead levels and covariates: $\lambda(t) = \lambda_0(t) \times exp(\widehat{\beta_1} Lead + \widehat{\beta_2} Group_2 + \widehat{\beta_3} Group_3 + \widehat{\beta_4} Group_4 + \widehat{\beta_5} Lead \times Group_2 + \widehat{\beta_6} Lead \times Group_3 + \widehat{\beta_7} Lead \times Group_4 + \hat{\beta} Covariates + \hat{\beta} Covariates \times Group_2 + \hat{\beta} Covariates \times Group_3 + \hat{\beta} Covariates \times Group_4)$ (Model 2b).

We used SAS system version 9.4 (SAS Institute, Inc., Cary, North Carolina) and R version 3.5.0 to perform all analysis.

4. Results

In total 620 individuals with 1817 observations (6132 person-years) were eligible to be included in our study for the evaluation of effect modification by dietary patterns and vitamins A (total, carotenoids, retinols) and C. For vitamin D and E, the sample size was 405 with 1184 observations (3963 person-years). The baseline characteristics of the study population (n=620) were shown in Table III.2. During the 15 years' follow-up, we identified 44 incident POAG cases with an incidence rate at 72 per 10,000 person-years (median follow-up=10.4 years). The average age of participants at baseline was 66.9 years (SD 6.7, range from 49.9 to 66.9 years). The mean concentration of tibia lead was 21.9 μg/g (SD 13.6, range from 1 to 126 μg/g, median=19 μg/g), while the mean concentration of patella lead was 31.3 μg/g (SD 20.2, range from 1 to 165 μg/g, median=27 μg/g). Pearson correlation coefficient showed that tibia lead was highly correlated with patella lead (coefficient=0.79, $p<0.001$). Those who developed incident POAG were more likely to have ocular hypertension at the baseline ($p<0.001$). The characteristics of covariates were similar to what we reported in Chapter II.

We created two dietary patterns using PCA with orthogonal rotation (Table III.1). The mean score of prudent dietary pattern was -0.02 (SD 0.81, range from -1.65 to 5.50), while the mean score of Western dietary pattern was 0.01 (SD 0.90, range from -1.69 to 4.68) (Table III.2). The baseline average value for total energy intakes was 1987.2 kCal (SD 571.5 kCal). Dietary intake of total vitamin A was 10851.0 IU (SD 5486.6 IU), carotenoids was 8453.2 IU (SD 5161.2 IU), retinoids was 2397.3 IU (SD 1817.6 IU), vitamin C was 155.0 mg (SD 67.5 mg), vitamin D was 220.8 IU (SD 135.9 IU), and vitamin E was 8.20 mg (SD 6.66 mg) (Table III.2). Pearson correlation test showed that prudent diet is highly correlated with vitamin A (coefficient for total vitamin A=0.72, coefficient for carotenoid=0.73, both $p<0.001$), and positively

correlated with other vitamins (Figure III.2). On the contrast, Western diet was negatively correlated with vitamins A, C, D and E (Figure III.2).

For interaction analysis, we intuitively compared the HRs of POAG between low or high adherence of dietary patterns, as well as low or high dietary intake of single vitamins, respectively. After adjusting for total energy intake, age at baseline, BMI, job types, educational levels, cigarette consumption, systemic hypertension, diabetes mellitus, and ocular hypertension, comparing those who had low adherence versus high adherence to prudent diet, the HR of POAG per 2-fold increase in patella lead dropped from 2.03 (95% CI: 1.25, 3.29) to 1.33 (95% CI: 0.86, 2.05, p-for-interaction=0.18, Table III.3). Similar effect modification was also found for dietary vitamin A intake, especially the provitamin A (carotenoids): the 2-fold HRs for patella lead comparing those who had low intake versus high intake of carotenoid decreased from 2.04 (95% CI: 1.26, 3.29) to 1.37 (95% CI: 0.90, 2.11) (p-for-interaction=0.20, Table III.3).

Although the p-for-interaction terms were not significant when using dichotomized dietary pattern and vitamin intake. In analysis using continuous ones, the interaction effects showed significance between patella lead with prudent dietary scores (p-for-interaction=0.03), total vitamin A intake (p-for-interaction=0.02) and carotenoid intake (p-for-interaction=0.02), and between tibia lead with carotenoid intake (p-for-interaction=0.04) (Table III.4). We found that the effect of patella lead on the risk of POAG changed when we held prudent dietary pattern score at different levels. When we increased prudent dietary pattern score from 1st quartile (-0.54) to 3rd quartile (0.31), the slope of lead-POAG association decreased from 1.04 to 0.47, and the 2-fold HRs decreased from 2.05 (95% CI: 3.10, 1.36) to 1.39 (95% CI: 2.00, 0.96) (Table III.5). This suggested a higher susceptibility of patella lead toxicity for those who have low adherence to prudent diet, and a higher resistance for lead toxicity for those who have high

adherence to prudent diet. We did not observe significant interactions on the multiplicative scale between bone lead and Western diet or dietary intakes of vitamins C, D and E.

In the four group analysis of dietary patterns, we found that participants with low prudent dietary score and low Western dietary score showed a HR per 2-fold increase in patella and tibia lead at 2.03 (95%CI: 1.13, 3.66) and 2.09 (1.03, 4.20), respectively (Figure III.4 and Figure III.5). Those who had low prudent dietary score and high Western dietary score showed a HR per 2-fold increase in patella lead at 2.13 (95%CI: 0.98, 4.62), which was marginally significant (Figure III.4). Our findings suggested that prudent diet may be protective to the risk of POAG caused by patella lead, no matter of the adherence of Western dietary pattern (p-for-interaction of prudent diet=0.03).

For sensitive analysis, we found that the results were similar when utilized the stratified analysis instead of interaction analysis (data not shown).

Table III.2. Baseline Characteristics of Study Population Comparing Participants with POAG vs participants with Non-POAG

Characteristics	Total Population (n=620)	Non-POAG (n=576)	POAG (n=44)	*P* value[a]
Bone lead levels				
Tibia lead, mean±SD, μg/g	21.9±13.6	21.8±13.6	23.5±12.4	0.44
High tibia lead (>19 μg/g), n (%)		278 (51.7)	30 (68.2)	
Patella lead, mean±SD, μg/g	31.3±20.2	31.0±20.0	36.3±21.4	0.09
High patella lead (>27 μg/g), n (%)		290 (50.4)	28 (63.6)	
Age at baseline, mean±SD, years	66.9±6.7	66.9±6.7	67.7±6.1	0.40
Age at end of 15 years' follow-up, mean±SD, years	77.3±6.9	77.4±6.9	75.8±6.4	0.13
BMI, mean±SD, kg/m^2	27.8±3.7	27.9±3.8	27.5±3.4	0.48
Diabetes mellitus, n (%)	88 (14.2)	80 (13.9)	8 (18.2)	0.43
Systemic hypertension, n (%)	339 (54.7)	313 (54.3)	26 (59.1)	0.54
Ocular hypertension, n (%)	21 (3.4)	13 (2.3)	8 (18.2)	<0.001
White population, n (%)	602 (97.1)	561 (97.4)	41 (93.2)	0.12
Educational levels, n (%)				
≤ High school	64 (10.3)	61 (10.6)	3 (6.8)	
High school	225 (36.3)	209 (36.3)	16 (36.4)	
Some college	152 (24.5)	139 (24.1)	13 (29.6)	
≥ 4 years' college	179 (28.9)	167 (29.0)	12 (27.3)	0.71
Pack-years, n (%)				
0	198 (31.9)	184 (31.9)	14 (31.8)	
1-19	167 (26.9)	155 (26.9)	12 (27.3)	
≥20	255 (41.1)	237 (41.2)	18 (40.9)	0.99
Job type, n (%)				
Blue collar	261 (42.1)	243 (42.2)	18 (40.9)	
Mix	136 (21.9)	126 (21.9)	10 (22.7)	
White collar	223 (36.0)	207 (35.9)	16 (36.4)	0.99
Total energy intakes, kCal	1987.2±571.5	1991.4±569.2	1931±606.1	0.50
Dietary pattern				
Prudent diet score, mean±SD	-0.02±0.81	-0.01±0.81	-0.05±0.78	0.79
Western diet score, mean±SD	-0.01±0.90	-0.00±0.90	-0.08±0.99	0.57
Dietary intakes of single nutrient [b]				
Total Vitamin A, mean±SD, IU	10851.0±5486.6	10836.3±5468.1	11043.5±5785.9	0.74
Carotenoid, mean±SD, IU	8453.2±5161.2	8422.1±5113.0	8860.0±5804.6	0.59
Retinol, mean±SD, IU	2397.3±1817.6	2412.8±1852.5	2194.5±1275.4	0.43
Vitamin C, mean±SD, mg	155.0±67.5	154.9±68.7	156.6±51.0	0.87
Vitamin D [c], mean±SD, IU	220.8±135.9	222.0±133.6	203±167.7	0.51
Vitamin E [c], mean±SD, mg	8.20±6.66	8.23±6.84	7.81±3.17	0.76
Selected food group intakes (servings/day)				
Legumes, mean±SD	0.41±0.27	0.41±0.27	0.39±0.31	0.70
Other vegetables, mean±SD	0.38±0.28	0.39±0.29	0.34±0.25	0.28
Dark-yellow vegetables, mean±SD	0.39±0.37	0.39±0.37	0.40±0.45	0.96
Cruciferous vegetables, mean±SD	0.37±0.35	0.37±0.35	0.36±0.26	0.74
Leafy vegetables, mean±SD	0.57±0.46	0.58±0.46	0.50±0.40	0.28
Seafood, mean±SD	0.39±0.28	0.39±0.28	0.35±0.20	0.37
Onions, mean±SD	0.15±0.21	0.16±0.21	0.14±0.22	0.64

Tomatoes, mean±SD	0.57±0.40	0.57±0.40	0.55±0.39	0.77
Fruit, mean±SD	1.52±1.07	1.51±1.07	1.68±1.00	0.32
Poultry, mean±SD	0.36±0.26	0.36±0.24	0.39±0.43	0.43
Eggs, mean±SD	0.23±0.25	0.23±0.25	0.24±0.30	0.67
Processed meat, mean±SD	0.34±0.33	0.34±0.33	0.30±0.28	0.38
High-fat dairy products, mean±SD	0.76±0.72	0.76±0.72	0.75±0.77	0.94
Butter, mean±SD	0.25±0.47	0.25±0.47	0.28±0.45	0.67
Red meat, mean±SD	0.51±0.31	0.51±0.31	0.45±0.31	0.23
Beers, mean±SD	0.46±0.92	0.48±0.93	0.28±0.64	0.19
Chowders, mean±SD	0.07±0.08	0.07±0.08	0.06±0.05	0.26
Fries, mean±SD	0.09±0.10	0.09±0.10	0.08±0.09	0.47
Refined grains, mean±SD	1.45±0.96	1.46±0.97	1.32±0.85	0.34
Mayonnaise, mean±SD	0.18±0.21	0.18±0.21	0.20±0.24	0.66

Abbreviations: POAG, primary open-angle glaucoma; SD, standard deviation; KXRF, K x-ray fluorescence; BMI, body mass index.

[a] *P* values were calculated using logistic regression; educational levels and pack-years were treated as ordinal variables.

[b] Single nutrient intake were adjusted for total energy intake.

[c] Sample size for vitamins D and E was 405 (including 26 POAG cases) due to missing in vitamin intake.

Table III.3. Hazard Ratio (with 95% CI) of POAG by Bone Lead Concentrations, Stratified by Dichotomized Dietary Pattern Scores and Single Vitamin Dietary Intakes, using interaction analysis[a], with Application of IPW (*n*=620).

		Patella lead		Tibia lead	
	N case/ Person-years	**2-fold HR (95% CI)**	***P* for interaction[b]**	**2-fold HR (95% CI)**	***P* for interaction[b]**
Total Study Population (n=620)	44/6132	1.62 (1.15, 2.29)		1.40 (0.98, 2.01)	
Dietary Patterns					
Prudent Diet					
Low	24/3055	2.03 (1.25, 3.29)	0.18	1.65 (1.02, 2.69)	0.31
High	20/3077	1.33 (0.86, 2.05)		1.20 (0.75, 1.92)	
Western Diet					
Low	26/2013	1.67 (1.10, 2.55)	0.83	1.60 (0.97, 2.64)	0.43
High	18/3119	1.56 (0.94, 2.60)		1.23 (0.77, 1.96)	
Single Nutrients[c]					
Total Vitamin A (IU)					
≤ 9871.5	24/3056	1.85 (1.19, 2.86)	0.31	1.44 (0.90, 2.28)	0.83
9875.3 - 90506.8	20/3076	1.36 (0.85, 2.16)		1.34 (0.81, 2.19)	
Carotenoid (IU) [d]					
≤ 7344.2	20/3083	2.04 (1.26, 3.29)	0.20	1.64 (0.98, 2.74)	0.48
7354.3 - 87086.2	24/3049	1.37 (0.90, 2.11)		1.31 (0.83, 2.05)	
Retinol (IU)					
≤ 1999.9	26/3090	1.74 (1.13, 2.69)	0.53	1.58 (0.98, 2.53)	0.44
2016.8 – 29498.2	18/3042	1.42 (0.86, 2.36)		1.22 (0.74, 2.00)	
Vitamin C (mg)					
≤ 148.4	23/3115	1.85 (1.19, 2.86)	0.32	1.61 (0.99, 2.63)	0.44
148.5 – 660.9	21/3017	1.36 (0.84, 2.21)		1.25 (0.78, 2.00)	
Total population (n=405)	26/3963	1.90 (1.19, 3.04)		1.58 (0.96, 2.61)	
Vitamin D (IU) [f]					
≤ 192.5	17/2037	1.89 (1.10, 3.25)	1.00	1.53 (0.88, 2.64)	0.75

194.5 – 1252.5	9/1925	1.89 (0.94, 3.80)		1.78 (0.77,4.07)	
Vitamin E (mg) [f]					
≤ 6.78	12/1899	2.00 (1.08, 3.72)	0.89	1.60 (0.85, 3.02)	0.95
6.84 – 53.73	14/2063	1.90 (1.03, 3.50)		1.56 (0.79, 3.08)	

Abbreviations: POAG, primary open-angle glaucoma; IPW, inverse probability weighting; HR, hazard ratio; CI, confidence interval.

[a] Cox regression model was adjusted for total energy intake, age at baseline, BMI, job types, educational levels, pack-year of cigarettes, systemic hypertension, diabetes mellitus, and ocular hypertension. The interaction analysis adopted the following model, which only include interaction between diet/vitamin and lead: $\lambda(t) = \lambda_0(t) \times exp(\widehat{\beta_1} Lead + \widehat{\beta_2} dietary/vitamin + \widehat{\beta_3} Lead \times dietary/vitamin + \hat{\beta} Covariates)$.

[b] *P*-values for interaction terms of dichotomized dietary pattern scores/single nutrient intake on the lead-POAG association were calculated by Wald test, which were p for $\widehat{\beta_3}$.

[c] Single nutrient intake were dietary, which did not include supplement intake.

[d] Carotenoid intake was estimated by subtracting retinol intake from total vitamin A intake.

[f] Sample size for vitamins D and E was 405 (including 26 POAG cases) due to missing in vitamin intake.

Table III.4. Interaction between Bone Lead Concentrations and Continuous Dietary Pattern Scores or Single Vitamin Dietary Intakes on the Lead-POAG Association, using Interaction Analysis[a] (*n*=620).

		Patella lead		Tibia lead	
	N case/ Person-years	**Beta for interaction (se)**	***P* for interaction[b]**	**Beta for interaction (se)**	***P* for interaction[b]**
Total Study Population (n=620)	44/6132				
Dietary Patterns					
Prudent Diet		-0.67 (0.31)	0.03	-0.22 (0.31)	0.49
Western Diet		-0.27 (0.26)	0.31	-0.50 (0.29)	0.09
Single Nutrients[c]					
Total Vitamin A (IU)		-0.88 (0.37)	0.02	-0.77 (0.40)	0.05
Carotenoid (IU) [d]		-0.73 (0.32)	0.02	-0.71 (0.34)	0.04
Retinol (IU)		-0.55 (0.38)	0.15	-0.16 (0.36)	0.65
Vitamin C (mg)		-0.36 (0.40)	0.37	-0.25 (0.43)	0.57
Total population (n=405)	26/3963				
Vitamin D (IU) [f]		-0.00 (0.30)	0.99	0.02 (0.37)	0.96
Vitamin E (mg) [f]		0.14 (0.53)	0.80	0.22 (0.55)	0.69

Abbreviations: POAG, primary open-angle glaucoma; IPW, inverse probability weighting; HR, hazard ratio; CI, confidence interval.

[a] Cox regression model was adjusted for total energy intake, age at baseline, BMI, job types, educational levels, pack-year of cigarettes, systemic hypertension, diabetes mellitus, and ocular hypertension. The interaction analysis adopted the following model, which only include interaction between diet/vitamin and lead: $\lambda(t) = \lambda_0(t) \times exp(\widehat{\beta_1} Lead + \widehat{\beta_2} dietary/vitamin + \widehat{\beta_3} Lead \times dietary/vitamin + \hat{\beta} Covariates)$.

[b] *P*-values for interaction terms between continuous dietary pattern scores/single nutrient intake and bone lead levels were calculated by Wald test, which were p for $\widehat{\beta_3}$.

[c] Single nutrient intakes were dietary, which did not include supplement intakes; all single nutrient intakes were log-transformed by natural base.

[d] Carotenoid intake was estimated by subtracting retinol intake from total vitamin A intake.

[f] Sample size for vitamins D and E was 405 (including 26 POAG cases) due to missing in vitamin intake.

Table III.5. The Effect of Bone Lead Concentrations on the Risk of POAG, Holding Continuous Dietary Pattern Scores at 25% (1st Quartile), Median and 75% (3rd Quartile) of the Total Population, using Interaction Analysisa (n=620).

		Patella lead			Tibia lead		
	Dietary pattern scores	Slope/Beta[b]	2-Fold HR (95%CI)[c]	*P* for interaction[d]	Slope/Beta[b]	2-Fold HR (95%CI)[c]	*P* for interaction[d]
Prudent Diet							
1st Quartile: 25%	-0.54	1.04	2.05 (3.10, 1.36)	0.03	0.60	1.52 (2.30, 1.00)	0.49
Median: 50%	-0.14	0.77	1.71 (2.42, 1.20)		0.51	1.42 (2.05, 1.00)	
3rd Quartile: 75%	0.31	0.47	1.39 (2.00, 0.96)		0.42	1.33 (1.96, 0.91)	
Western Diet							
1st Quartile: 25%	-0.62	0.86	1.82 (2.74, 1.21)	0.31	0.80	1.74 (2.71, 1.12)	0.09
Median: 50%	-0.17	0.74	1.67 (2.38, 1.17)		0.58	1.49 (2.16, 1.03)	
3rd Quartile: 75%	0.47	0.57	1.48 (2.20, 1.00)		0.26	1.19 (1.79, 0.80)	

Abbreviations: POAG, primary open-angle glaucoma; IPW, inverse probability weighting; HR, hazard ratio; CI, confidence interval.

[a] Cox regression model was adjusted for total energy intake, age at baseline, BMI, job types, educational levels, pack-year of cigarettes, systemic hypertension, diabetes mellitus, and ocular hypertension. The interaction analysis adopted the following model, which only include interaction between diet/vitamin and lead: $\lambda(t) = \lambda_0(t) \times exp(\widehat{\beta_1} Lead + \widehat{\beta_2} dietary + \widehat{\beta_3} Lead \times dietary + \hat{\beta} Covariates)$.

[b] Slopes/Betas of bone lead levels on risk of POAG were calculated by combining the beta of main effect term of lead and the beta of interaction term: $\widehat{\beta_1} + \widehat{\beta_3}$.

[c] The HRs and 95% CIs were calculated by delta method.

[d] *P*-values for interaction terms between continuous dietary pattern scores/single nutrient intake and bone lead levels were calculated by Wald test, which were p for $\widehat{\beta_3}$.

Pearson Correlation Coefficients, N = 620										
Patella Lead Level, µg/g	**Patella Lead**									
Tibia Lead Level, µg/g	0.79*	**Tibia Lead**								
Prudent Dietary Index	-0.01	-0.04	**Prudent Diet**							
Western Dietary Index	0.07	0.04	-0.05	**Western Diet**						
Vitamin A[a], IU	-0.01	-0.04	0.72*	-0.17*	**Vitamin A**					
Vitamin C[a], mg	-0.04	-0.04	0.28*	-0.23*	0.33*	**Vitamin C**				
Carotenoid[a], IU	-0.01	-0.05	0.73*	-0.20*	0.94*	0.37*	**Carotenoid**			
Retinol[a], IU	-0.01	0.03	0.05	0.02	0.34*	-0.04	0.00	**Retinol**		
Vitamin D[b], IU	-0.08	-0.01	0.01	-0.26*	0.09	0.13*	0.03	0.21*	**Vitamin D**	
Vitamin E[b], mg	-0.05	-0.01	0.04	-0.13*	0.07	0.28*	0.06	0.04	0.08	**Vitamin E**

* P-values <0.05, which indicated that the correlation test was significant.

[a] Single dietary nutrient intakes were adjusted for total energy intake.

[b] Sample size for vitamins D and E was 405 due to missing in vitamin intake.

Figure III.3. Pearson correlation coefficients among bone lead concentrations, dietary patterns, and selected single dietary vitamin intake.

Dietary Pattern Sub-Groups	Sample size	No. case/person-year	HR (95%CI) for 2-Fold Increase in Patella Lead Level
Low Prudent + Low Western	156	15/1545	2.03 (1.13, 3.66)
Low Prudent + High Western	154	9/1511	2.13 (0.98, 4.62)
High Prudent + Low Western	154	11/1468	1.38 (0.76, 2.50)
High Prudent + High Western	156	9/1608	1.28 (0.70, 2.35)
Total Study Population	620	44/6132	1.62 (1.15, 2.29)

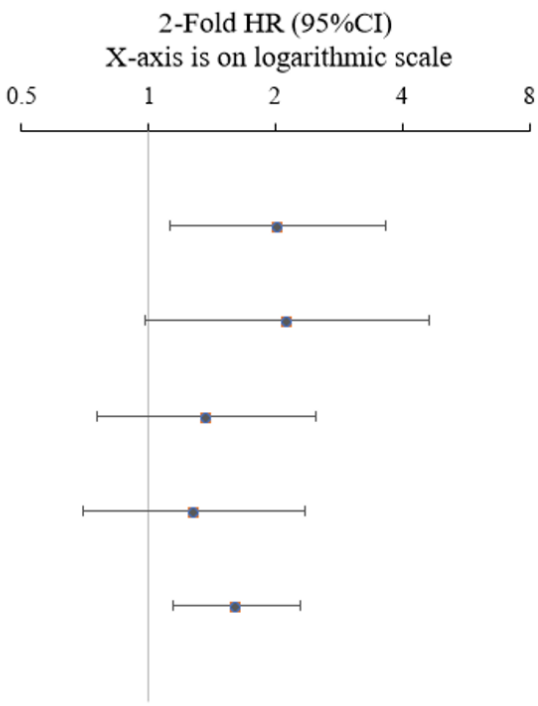

Figure III.4. HR (95%CI) of POAG per 2-fold increase in patella lead level, among different dietary pattern sub-groups, and the total study population (n=620), in Cox regression models using interaction analysis method adjusted for total energy intake, age at baseline, BMI, job types, educational levels, pack-year of cigarettes, systemic hypertension, diabetes mellitus, and ocular hypertension. Participants were classified into low prudent and low Western diet (Group 1), low prudent and high Western diet (Group 2), high prudent and low Western diet (Group 3), and high prudent and high Western diet (Group 4), according to their adherence to two patterns. The interaction analysis adopted the following model, which only include interaction between four dietary groups and lead: $\boldsymbol{\lambda(t) = \lambda_0(t) \times exp(\widehat{\beta_1} Lead + \widehat{\beta_2} Group_2 + \widehat{\beta_3} Group_3 + \widehat{\beta_4} Group_4 + \widehat{\beta_5} Lead \times Group_2 + \widehat{\beta_6} Lead \times Group_3 + \widehat{\beta_7} Lead \times Group_4 + \widehat{\beta} Covariates)}$**.**

Dietary Pattern Sub-Groups	Sample size	No. case/person-year	HR (95%CI) for 2-Fold Increase in Tibia Lead Level
Low Prudent + Low Western	156	15/1545	2.09 (1.03, 4.20)
Low Prudent + High Western	154	9/1511	1.35 (0.70, 2.58)
High Prudent + Low Western	154	11/1468	1.22 (0.61, 2.41)
High Prudent + High Western	156	9/1608	1.11 (0.59, 2.08)
Total Study Population	620	44/6132	1.40 (0.98, 2.01)

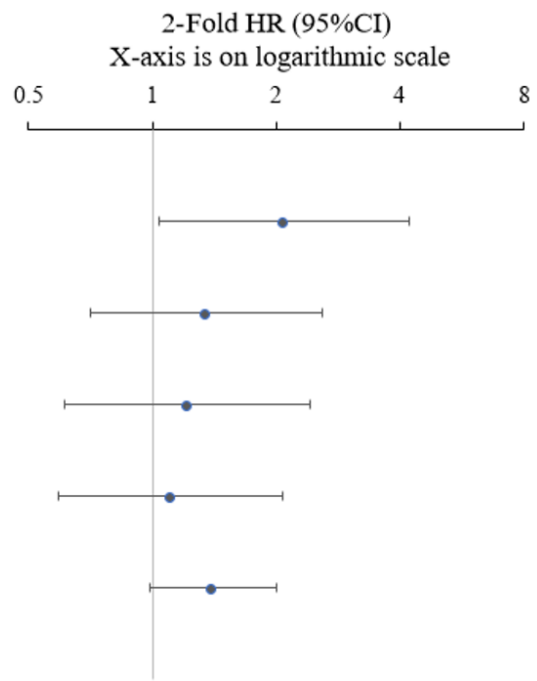

Figure III.5. HR (95%CI) of POAG per 2-fold increase in tibia lead level, among different dietary pattern sub-groups, and the total study population (n=620), in Cox regression models using interaction analysis method adjusted for total energy intake, age at baseline, BMI, job types, educational levels, pack-year of cigarettes, systemic hypertension, diabetes mellitus, and ocular hypertension. Participants were classified into low prudent and low Western diet (Group 1), low prudent and high Western diet (Group 2), high prudent and low Western diet (Group 3), and high prudent and high Western diet (Group 4), according to their adherence to two patterns. The interaction analysis adopted the following model, which only include interaction between four groups and lead: $\lambda(t) = \lambda_0(t) \times exp(\widehat{\beta_1} Lead + \widehat{\beta_2} Group_2 + \widehat{\beta_3} Group_3 + \widehat{\beta_4} Group_4 + \widehat{\beta_5} Lead \times Group_2 + \widehat{\beta_6} Lead \times Group_3 + \widehat{\beta_7} Lead \times Group_4 + \hat{\beta} Covariates)$**.**

5. Discussion

In a 15 years' longitudinal cohort of older men, we observed that those who had high adherence with prudent dietary pattern, which is plentiful of legumes, vegetables, seafood, onions, tomatoes, fruits and poultry, were less susceptible to the toxicity of patella lead on the risk of POAG. The HR of POAG was more than 2-fold higher per 2-fold increase in patella lead among participants with low adherence to prudent diet, whereas it dropped to almost null among participants with high adherence to prudent diet. The attenuation effect of prudent diet on the lead toxicity may be due to the abundance of vitamin A, especially the provitamin A carotenoid which is plant-sourced. Moreover, this protective effect of high adherence of prudent dietary pattern on lead-associated POAG was independent of the simultaneous adherence to Western diet.

As an irreversible neural degenerative disease, glaucoma currently has no widely-used effective cure, and treatments mainly focus on the cessation of deterioration (Cohen and Pasquale 2014; Prum et al. 2016). Hence, pre-symptomatic prevention and intervention are very important. Dietary modifications are commonly used by the general public as applicable interventions for chronic diseases. Multiple studies have investigated the associations between single nutrient intakes and the risk of glaucoma. However, single nutrient analysis may not have sufficient reference value for the establishment of an effective dietary intervention, due to its built-in limitations (as mentioned in introduction, (Hu 2002)). Furthermore, previous studies regarding the effects of dietary patterns on glaucoma were very limited. One study among type 2 diabetic patients in Africa reported that, regular intake of Mediterranean diet, which includes high consumption of fruit and vegetable sourced foods, significantly reduced the risk of glaucoma (Moïse et al. 2012). Higher consumption of fruits and vegetables was also reported to

reduce the risk of glaucoma among African-American women (Giaconi et al. 2012). Among Caucasians, only one previous study using the Willet food frequency questionnaire has investigated the association between glaucoma and six food groups (including green leafy vegetables, all fruits, and all vegetables), and there were no observed associations (Kang et al. 2003). However, unlike the previous two studies, that study used the self-reported glaucoma which may underestimate the risk. Even in high income countries, nearly 50% of glaucoma patients are unaware of their condition (Giaconi et al. 2012; Gupta et al. 2016). The effect of dietary patterns other than single nutrient intake on the development of glaucoma demands more attention.

To the best of our knowledge, our study is the first to investigate the effect of dietary patterns, which were derived from a data-driven PCA approach, on the risk of POAG, and the first to reveal a protective effect of prudent dietary pattern on the lead-POAG association. We found that this association may be due to the abundance of provitamin A carotenoid intake caused by the high adherence to prudent diet. Our study further suggested that it is the plant-sourced vitamin A which can attenuate the lead toxicity on the development of POAG, not the animal-sourced vitamin A. This is consistent with a previous study that showed diets including high abundance of plant-sourced foods can reduce the risk of glaucoma (Giaconi et al. 2012; Moïse et al. 2012).

Lead induced oxidative stress may play an important role in the pathogenesis of glaucoma. As mentioned in Chapter II, lead can disrupt the antioxidant defense system, increase oxidative stress, accumulate reactive oxygen species (ROS), induce the dysfunction of the aqueous humor drainage system, block the outflow of aqueous humor from the eye, elevate the intraocular pressure, and cause the glaucomatous neuropathy (Babizhayev 2012b; Saccà et al. 2016; Zhao et al. 2016).

Previous studies and our results from Chapter II also suggest that oxidative stress may directly damage the optic nerve head other than through the dysfunction of aqueous humor drainage system (Tezel et al. 2010). In our study, dietary intake of provitamin A carotenoid had a protective effect against the lead toxicity. The plant-sourced vitamin A may be an effective antioxidant which protects the optic nerve from damage due to lead-induced oxidative stress. It may help to balance the antioxidant defense system from excessive oxidative stress and reduce the oxidative damage on trabecular meshwork, retinol ganglion cells, and vessels around the optic nerve head (Giaconi et al. 2012). The animal-sourced preformed vitamin A retinol also acts as an antioxidant, however its protective effect may be attenuated by the combined effect of a high intake of animal-sourced food. Previous studies have reported that high-cholesterol or high-fat diet, such as with a highly animal based diet, may increase the risk of glaucoma (Kang et al. 2004; Kashiwagi et al. 2012).

We did not observe interaction effects for vitamins C, D and E. These findings should be interpreted cautiously. A limitation of single nutrient analysis makes is that it can be difficult to separate and detect the effect of a single vitamin. In addition, analyse of vitamins D and E were restricted in a smaller study population with only 26 incident POAG patients. The small sample size reduced the power of statistical analysis.

We detect differences in HRs for the association of lead and POAG between the low and high adherence to prudent diet, as well as the low and high intake of provitamin A carotenoids. The interaction terms between bone lead and diet/vitamin were significant only when we used the continuous forms of diet/vitamin intake. No interactions were found when used dichotomized forms. This may be simply due to the loss of information after the transformation of a continuous variable into a dichotomized variable. Besides, in the interaction analysis which ignored the comprehensive effects between diet/vitamin and covariate, the difference of HRs between two

groups may be due to a combined effect of main diet/vitamin term plus the interaction term. In the stratified analysis which includes the interaction terms of lead with both diet/vitamin and covariates, the interaction between both lead and covariates contributed to the difference of HRs between two groups.

Our study also has several limitations. Aside from the limitations discussed in Chapter II, this further exploration was based on the information provided in the self-reported FFQ, which lists foods and their serving size, as well as details of any supplement intake. Recall bias as well as measurement error are possible, since the questionnaire cannot cover all kinds of intake situations. Even the same food which underwent different cooking styles could result in different nutrient intake. In addition to this, we only incorporated baseline diet/vitamin into the analysis. Dietary habits may change over time and baseline may not reflect the intake levels during the long follow-up time. The data-driven PCA approach has been widely used in generating the dietary pattern scores, however, the definition or classification of food items/groups may be arbitrary since there is no gold standard.

Environmental low-dose exposure to lead has declined for a long time, yet the threat has not vanished. Recent reports of increasing proportions of high blood lead levels among children due to change in water source in Flint, Michigan, reflect lead contamination from corrosion of aging water pipes (Gómez et al. 2018; Hanna-Attisha et al. 2016). Peeling of wall paint in old houses built before 1978 is also a major source of environmental lead exposure (Leighton et al. 2003). Due to the cumulative nature of lead metabolism, older people were exposed for a longer term and to a higher dose of lead (Vig and Hu 2000). Our study provides evidence that prudent dietary pattern, which is abundant of fruits and vegetables and provitamin A carotenoids, may attenuate lead toxicity on the development of age-related POAG. The results suggest that

modified diet may be a practical approach to effectively prevent and reduce the risk of POAG caused by lead toxicity. We hope our study can add reference on the effect of diet in the development of POAG, help to further understand the etiology of this irreversible ocular disease, and provide an applicable approach of intervention to reduce the global burden of blindness.

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CHAPTER IV

A Two-Stage Environment-Wide Association Study (EWAS) to Discover Environmental Risk Factors for Cataract Surgery in U.S. Adults, using NHANES 1999-2008

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1. Abstract

Background and Objective: The etiology of age-related cataract (ARC), which is a leading cause of vision loss and visual impairment, is not yet fully understood. A few individual environmental pollutants, such as lead, cadmium, and biomass fuel product, are associated with ARC, there has been no systematic evaluation of various pollutants as potential risk factors for ARC. We conducted a two-stage environment-wide association study (EWAS) to identify potential environmental risk factors for ARC.

Methods: We examined 104 biomarkers of environmental pollutants from the National Health and Nutrition Examination Survey (NHANES) between 1999 and 2008. The sample sizes ranged from 1161 to 21641 for different pollutants. Self-reported cataract surgery was a surrogate for the presence of clinically significant ARC. We performed survey weighted logistic regressions associating each pollutant with cataract surgery using a two-stage approach, discovery then validation. Because half-lives of pollutant biomarkers are related to the degrees of measurement error, which in turn affect the probability of being detected by EWAS, we further attempted a weighted approach by weighting the significance thresholds inversely proportional to maximum composite half-lives.

Results: Five biomarkers were identified (false discovery rate<0.10) after adjusting for age, gender, education attainment, race/ethnicity, diabetes mellitus, smoking status, BMI and NHANES cycle number: serum polychlorinated biphenyls 44 (PCB 44) (Odds ratio (OR) per 2-fold increase=1.67 (95% confidence interval, 1.06, 2.62)), PCB 49 (OR=1.74 (1.13, 1.67)), urinary cadmium (OR=1.30 (1.11, 1.52)), urinary cobalt (OR=1.15 (1.05, 1.25)), and urinary tungsten (OR=1.15 (1.04, 1.27)). Additional pollutants with relatively short half-lives were

identified from the weighted approach: urinary N-acetyl-S-(3-hydroxypropyl-1-methyl)-L-cysteine (OR=0.92 (0.75, 1.13)), urinary N-acetyl-S- (3-hydroxypropyl)-L-cysteine (OR=0.93 (0.73, 1.17)), and urinary mono-(3-carboxypropyl) phthalate (OR=1.06 (0.97, 1.16)).

Conclusion: Our data-driven EWAS approach suggests unrecognized environmental pollutants, such as cobalt, tungsten, PCB 44 and 48, N-acetyl-S-(3-hydroxypropyl-1-methyl)-L-cysteine, N-acetyl-S- (3-hydroxypropyl)-L-cysteine, and mono-(3-carboxypropyl) phthalate, as potential risk factors for ARC. Causal links need to be validated using hypothesis-driven, targeted approaches.

Key words: EWAS, cataract, heavy metal, PCB, half-life

2. Introduction

Cataract, defined as any opacification or clouding of lens tissue which affects vision (Bobrow et al. 2015; Cataracts | National Eye Institute), is the leading cause of vision loss and a major cause of visual impairment (Jick and American Academy of Ophthalmology. 2016). Previous meta-analysis reported that the proportion of blindness attributed to cataract among individuals aged 50 and older ranged from less than 22% in developed region to more than 44% in Southeast Asia and Oceania in year 2015 (Flaxman et al. 2017). It is estimated that approximately 13.4 million people will suffer from blindness attributed to cataract by the year 2020 (Flaxman et al. 2017). In the United States, approximately 24 million individuals were affected by cataract in 2010, and it is estimated to rise to 30.1 million by 2020 (Jick and American Academy of Ophthalmology. 2016). The only effective treatment for visually significant cataract is surgery, which removes the patient's cataract and replaces it with an artificial lens. A retrospective study using data from 2001 to 2011 reported that approximately 23.1% of cataract patients in the U.S. underwent at least one surgery (Kauh et al. 2016).

Despite the high prevalence and severe consequences, the etiology of age-related cataract (ARC) is not completely understood. Commonly known risk factors other than genetic factors for ARC include increasing age, smoking, obesity, hyperglycemia, UV light/radiation exposure, and intake of specific pharmaceuticals (such as corticosteroids) (Bobrow et al. 2015).

Oxidative stress may play an important role in the development of cataract. UV filter compounds can protect the lens from damage of photo-oxidation (Jick and American Academy of Ophthalmology. 2016). Increased oxidative stress can generate excessive reactive oxygen species (ROS) which disrupt the synthesis of these compounds, disrupt the antioxidant defense system, cause oxidative damage to functional DNA, enhance apoptosis of epithelial cells of the lens, and

induce protein and lipid aggregation in the lens (Babizhayev 2012; Bobrow et al. 2015; Spector 1995; Truscott 2005; Tweeddale et al. 2016).

Environmental heavy metal exposures can lead to oxidative stress through the depletion of the glutathione and thiol pools, hence increase the risk of ARC. Lead and cadmium were positively associated with ARC risk (Schaumberg et al. 2004; Wang et al. 2016). In the cataractous lens among smokers, researchers also found elevated levels of lead and cadmium (Cekic 1998; Harding 1995; Mosad et al. 2010; Rácz and Erdöhelyi 1988; Ramakrishnan et al. 1995). In addition to heavy metals, several studies have reported that indoor smoke generated from household use of biomass cooking fuels can increase the risk of cataract, especially among women (Mishra et al. 1999; Mohan et al. 1989; Pokhrel et al. 2005; Ravilla et al. 2016; Smith et al. 2014). The risk may be attributed to increased $PM_{2.5}$ levels and polycyclic aromatic hydrocarbon (PAH) levels generated by biomass fuels combustion (Ravilla et al. 2016). Animal studies and clinical observations have also suggested an association between cataract and exposure to naphthalene and formaldehyde, which can be released in large amounts during the burning of biofuels (Hayasaka et al. 2001; Pokhrel et al. 2005; Xu et al. 1992).

Many other pollutants may also affect cataract risk given that oxidative stress is a common underlying biological mechanism of ARC. A comprehensive and systematic approach would be useful to identify those potential pollutants as modifiable risk factors for ARC. An environment-wide association study (EWAS) which adopts the framework of genome-wide association study (GWAS) to search for health-related environmental factors in a much broader range than traditional targeted environmental epidemiological studies (Patel et al. 2010). Like GWAS, EWAS treats each individual pollutant as a single environmental “locus”, and tests the associations between each pollutant and the health outcome of interest. The pollutants that meet the criteria of

significance (i.e. false discovery rate) are subsequently validated either internally or in external independent populations. EWAS methodology is now widely used to discover environmental risk factors as well as nutrients related to multiple health outcomes (McGinnis et al. 2016; Patel et al. 2010, 2013, 2014).

Previous EWAS studies have shown that those pollutants with longer half-lives, such as persistent heavy metals and persistent organic pollutants, were more likely to be associated with chronic diseases (McGinnis et al. 2016; Park et al. 2014; Patel et al. 2010, 2014). The temporal variability of a biomarker depends on its half-life and temporal variation in exposure, which can introduce measurement error (García-Closas et al. 2006). Biomarkers with relatively short half-lives have larger temporal variability, which may introduce more measurement error into the analysis compared to biomarkers with relatively long half-lives (García-Closas et al. 2006; White 2011). Biomarkers with relatively long half-lives are generally more recommended in epidemiological study design, especially in cross-sectional studies with measurement at a single but not optimal time point (García-Closas et al. 2006). In order to address this issue, we propose a weighted approach based on the half-lives of biomarkers and applied it into the typical EWAS framework.

In short, few studies have evaluated environmental risk factors other than radiation, heavy metals and indoor smoke from biomass fuel as risk factors for ARC. Our study used EWAS to identify potential environmental pollutants for cataract surgery in U.S. adults, in NHANES 1999-2008. To address measurement error related to half-lives of pollutants, we adopted a two-stage EWAS into our study: in Stage One we conducted a conventional two-step EWAS, in Stage Two we further applied a new approach on EWAS with hypothesis testing weighted by half-lives.

3. Methods

3.1. Study population

This study used NHANES, a population-based cross-sectional study designed to analyze the physical status of the U.S. general population (NHANES - Questionnaires, Datasets, and Related Documentation). Each cycle of NHANES is an independent cross-sectional survey which includes different representative samples. Survey protocols were approved by the National Center for Health Statistics Research Ethics Review Board, and all participants have provided written informed consent. We limit our study to adult participants aged 20 and older over five cycles from year 1999-2000 to 2007-2008, to reduce the prevalence of congenital cataract (cataract presents at birth) in the sample.

3.2. Identification of ARC surgery

Eligible participants of NHANES were asked whether they have had eye surgery for cataracts, before undertaking detailed vision examination according to the NHANES' Vision Procedures Manual (US Dept of Health and Human Services 2005). Due to the advanced cataract detection strategy and increasing rate of cataract surgery in the U.S. (Bobrow et al. 2015; Lundström et al. 2015), self-reported cataract surgery can be viewed as a robust indicator of the presence of clinically significant cataract. This method was also used in previous studies (Wang et al. 2016; Zhang et al. 2012). Participants who answered "yes" were considered as cataract cases. Those who were blind or had severe eye infections were excluded.

3.3. Environmental Risk Factors

We extracted as many biomarkers of environmental risk factors from the NHANES'

laboratory data as feasible. We excluded those biomarkers for which the data are not completely available in those aged 20 and older, as well as all pooled and surplus datasets, datasets unrelated to biomarkers of external exposure, virus antibodies, all hormone levels, variables have total sample size fewer than 1000 individuals after excluding those who missing in covariates, and variables with more than 35% values below or above detection limits (Figure IV.1). Inorganic arsenic in the urine was manually calculated by subtracting urinary arsenobetaine and arsenocholine from total urinary arsenic.

We identified 104 biomarkers for environmental pollutants in NHANES's laboratory data. These included blood acrylamide and glycidamide (2), blood brominated flame retardants (BFRs) (4), blood cotinine (1), blood polychlorinated biphenyls (PCBs)/dioxins/furans (14), urinary N,N-Diethyl-meta-toluamide (DEET) (1), blood and urinary heavy metals (17), urinary herbicides (2), urinary organophosphate insecticides (1), urinary polycyclic aromatic hydrocarbons (PAHs) (10), urinary perchlorate/nitrate/thiocyanate (3), environmental pesticides (2), urinary phenols and parabens (5), urinary phthalates (12), urinary phytoestrogens (6), perfluorinated compounds (PFCs) (4), blood and urinary biomarkers of volatile organic compounds (VOC) (20) (Table IV.1). Study sample sizes for the 104 biomarkers ranged from 1161 to 21641. We used lipid-adjusted biomarkers for PCBs and BFRs to control the measurement error caused by serum lipids' variation (O'Brien et al. 2016, 2018).

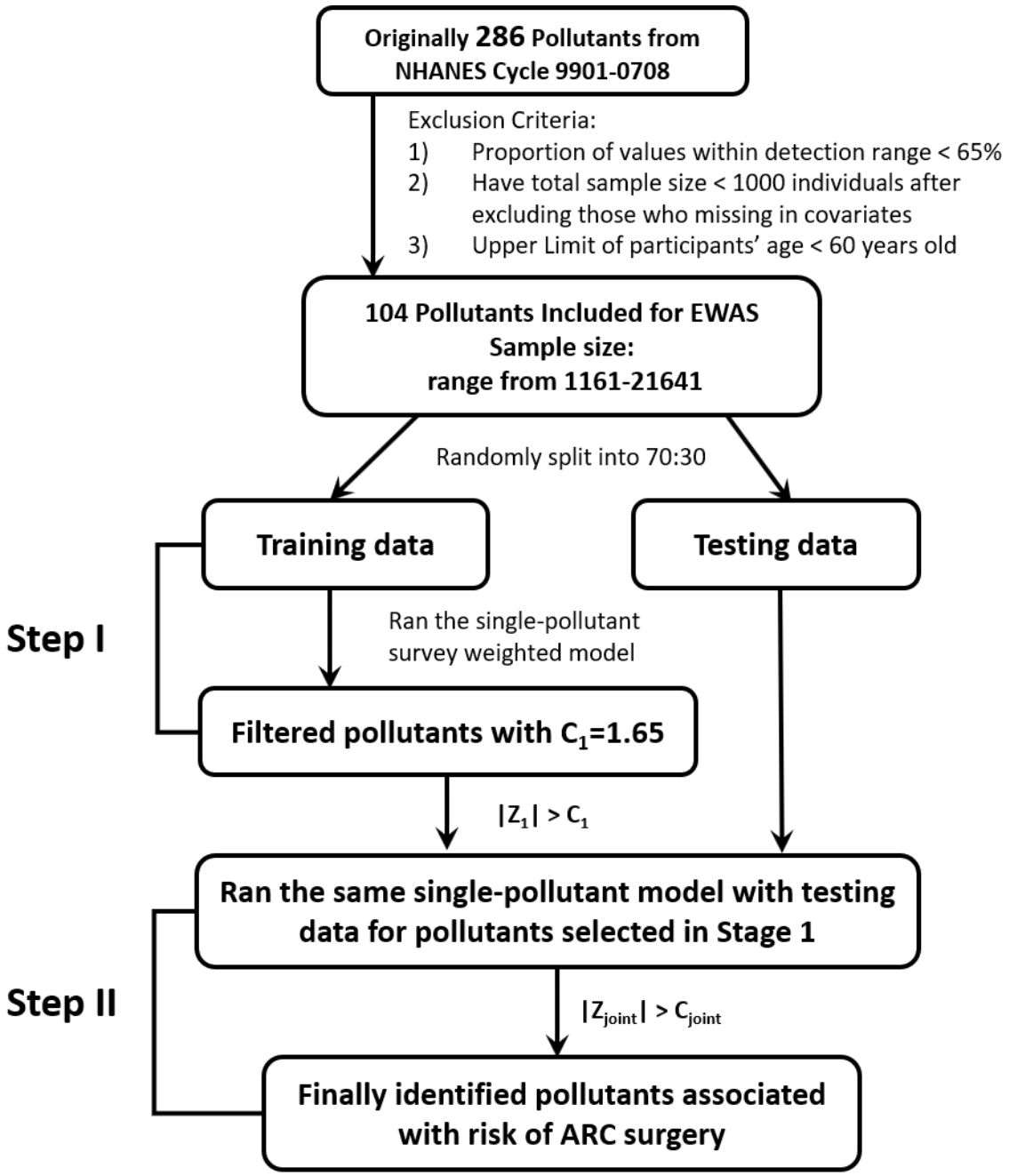

Figure IV.1. Diagram illustrates the establishment of the study population and the procedure of two-step EWAS.

Table IV.1. Counts of eligible biomarkers for environmental pollutants, by NHANES cycle.

Cycle #	1	2	3	4	5	sum
Acrylamide and Glycidamide	0	0	2	2	0	2
BFRs - Polybrominated diphenyl ethers (PBDEs)	0	0	4	0	0	4
Cotinine	1	1	1	1	1	1
Dioxins, Furans, PCBs	11	10	13	0	0	14
DEET	0	0	0	0	1	1
Heavy Metals	13	15	17	17	17	17
Herbicides	2	2	0	0	2	2
Organophosphate Insecticides	1	1	1	1	1	1
PAHs	0	8	10	10	9	10
Perchlorate/Nitrate/Thiocyanate	0	0	1	3	3	3
Environmental Pesticides	0	0	2	2	2	2
Phenols and Parabens	0	0	2	5	5	5
Phthalates	5	8	9	11	11	12
Phytoestrogens	6	6	6	6	6	6
PFCs	0	0	4	4	4	4
Volatile Organic Compounds	4	4	4	20	4	20
sum	43	55	76	82	66	104

3.4. Covariates

We performed independent regression models for each pollutant. We included commonly known risk factors for ARC such as age (years), race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, other), gender, body mass index (BMI) (kg/m^2), smoking status (current/former/none), and diabetes mellitus status (yes/no) into the regressions as confounders. Education (elementary, middle, high, college and higher) collected from the NHANES demographic dataset was adjusted for as an indicator of socioeconomic status (SES). We also adjusted for cycle number (ranging from 1-5) to account for cycle-to-cycle variations.

3.5. Statistical Analysis

All biomarkers were log-transformed with base 2 to reduce the skewness of their respective

distributions. Base 2 was selected to easily standardize the beta coefficient as the change in the outcome per doubling increases in each pollutant. They will then be centered at 0 for easy interpretation. NHANES survey-specific weights will be created for each class of pollutants for each individual.

3.5.1. Stage 1. Conventional EWAS

We adopted a two-step EWAS framework using joint analysis to filter the potential environmental risk factors for ARC; the detail of procedure was described by previous literature (Park et al. 2014; Skol et al. 2006). We randomly split the total population into 2 groups, where 70% was assigned as training data and 30% was assigned as testing data (Figure IV.1).

In Step One, we fit a fully adjusted single-pollutant survey weighted logistic model for each pollutant *i* ($log\ (p/(1-p))_i = \beta_0 + \beta_1 E_i + \beta_2' Z_i$, where *p* is the probabilty of having ARC surgery, E_i indicates an environmental pollutant, and Z_i are co-variates. β_1 is our coefficient of primary interest and it represents log(OR)). Based on the Wald test statistices from the training data (z_1) for each polluant, those with p-values less than the false discovery rate (*FDR*=0.1) were selected. Thus, the threshold of z-score at Step One is 1.65 (C_1). Setting the FDR at a threshold of 0.1 enabled us to capture small effects of environmental pollutants under an acceptible expected rate of false positives for all significant risk factors.

We then ran the same model using the testing data in Step Two. Wald test statistics from the training data (z_1) and testing data (z_2) for the selected pollutants were used to calculate a joint z_{joint} score evaluating the between-step heterogeneity ($z_{joint} = \sqrt{\pi_{samples}} z_1 + \sqrt{1-\pi_{samples}} z_2$, where $\pi_{samples}$ indicates the proportion of training samples (0.7)). If the $|\ z_{joint}\ |$ value was larger than a pre-defined threshold (C_{joint}, used for FDR control), then this pollutant was identified as a

risk factor for ARC surgery. The pre-defined threshold of C_{joint} was calculated via CaTS-Power Calculator provided online by Center for Statistical Genetics of University of Michigan School of Public Health (http://csg.sph.umich.edu).

In order to reduce the type I error, the probability of making false discoveries in the EWAS, we adopted two methods to calculate a corrected significance threshold T_i used for C_{joint} calculation in Step Two. We rejected all hypothesis with $P_i \leq T_i$, where P_i is corresponding to the z_{joint}:

A) The false discovery rate (FDR) control using the Benjamini-Hochberg (BH) procedure, briefly ordered the z_{joint} of pollutant selected by Step I descendingly and consecutively calculated $T_i = FDR \times i/m$. Here FDR=0.1, i refers to the order of z_{joint}, m refers to the total number of hypothesis tested in the EWAS (m=104). Hence different pollutants have different T_i and C_{joint}.

B) The family-wise error rate (FWER) control using Bonferroni correction, which is more stringent. We calculated constant threshold T for all pollutants at $T = \alpha$/m=0.00096. Here α refers to the significance level 0.05. In this circumstance C_{joint} after Bonferroni correction was constant at 3.299 (calculated by CaTS-Power Calculator).

For sensitivity analysis, we further adjusted for current job types for the pollutants selected by conventional EWAS, to test the robustness of associations. Job types were classified as "UV highly exposed group" and "UV low exposed group". Outdoor jobs and jobs related with welding were classified as "UV highly exposed", such as agriculture, forestry, fishing, mining, construction, utility, and armed forces.

3.5.2. Stage 2. Application of weighted approach based on half-lives in EWAS

In order to adjusted for the variable measurement errors due to different half-lives of

pollutants, we've adopted the biological half-lives in humans of all 104 biomarkers, either collected from previous literature, or calculated by a quantitative structure activity relationship (QSAR) approach (Arnot et al. 2014; Brown et al. 2012).

Adding weights which can control for measurement error to the threshold of each hypothesis' test has been increasingly utilized in GWAS (Li and Ghosh 2014; Roeder and Wasserman 2009; Zhao and Zhang 2014). Here we adopted a weighted approach described in detail elsewhere (Genovese et al. 2006). First, for each null hypothesis test where the pollutant is not associated with ARC surgery, we created a weight based on the half-life of the pollutant (W_i) using the following equation:

$$\frac{W_i}{m} = \frac{1/\log_{10}(\text{half-life}_i)}{\sum_{i=1}^{m}[1/\log_{10}(\text{half-life}_i)]}$$

Briefly, we log-transformed the maximum composite half-life of each chemical at base 10 ($\log_{10}(\text{half-life}_i)$), then inversed the log-transformed half-life. We set the mean of all weights equals to 1 ($\frac{1}{m}\sum_{i=1}^{m}(W_i) = 1$, m=104). Those chemicals with shorter half-lives tended to have larger weights.

Similar to conventional EWAS, the weight W_i can be used with two methods for the calculation of weighted thresholds for Step Two (Table IV.2):

1) In the weighted FDR control using BH procedure, we defined $Q_i = P_i/W_i$. We rejected all hypothesis with $Q_i < T_i = FDR \times i/m$, which was equivalent to $P_i < W_i \times FDR \times i/m$. The weighted thresholds were $T_i \times W_i$.
2) In the weighted FWER control using Bonferroni correction, again we rejected all

hypothesis with $Q_i < T = \alpha/m$, which was equivalent to $P_i < W_i \times \frac{\alpha}{m}$. The weighted thresholds were $T \times W_i$.

When all pollutants have the same W_i (equal to one), the weighted thresholds are the same thresholds in the unweighted conventional EWAS (Table IV.2).

This two-step cross-validation method is an approach to increase the validity of our study (Figure IV.1). Finally, odds ratios (ORs) per 2-fold increase in pollutant concentration and 95% confidence intervals (CIs) for selected pollutants were calculated using the total study population (no split) for easier interpretation.

All analysis other than C_{joint} calculation was performed via SAS system version 9.4 (SAS Institute, Inc., Cary, North Carolina) and R version 3.5.0.

Table IV.2 Demonstration of the weighted controls in EWAS.

	Weighted FWER Control Bonferroni Correction	**Weighted FDR Control BH Procedure**
P_i[a] for each null hypothesis test	$P_1<P_2<\ldots.<P_{m-1} < P_m$	$P_1<P_2<\ldots.<P_{m-1} < P_m$
Defination of Q_i	$Q_i = P_i/W_i$	$Q_i = P_i/W_i$
Condition to reject the null hypothesis	$Q_i < \alpha/m$	$Q_i < \alpha \times i/m$
α	$\alpha = 0.05$	$\alpha = FDR = 0.10$
Weighted thresholds	$W_i \times \alpha/m$	$W_i \times \alpha \times i/m$
Threshold of pollutants if the W_i were all equal to one	α/m	$\alpha \times i/m$

Abbreviations: FWER, family-wise error rate; FDR, false discovery rate; BH procedure, Banjamini-Hochberg procedure; W_i, weights for pollutants based on their maximum composite half-lives.
[a] These *p*-values P_i were corresponding to z_{joint} calculated in Step Two.

4. Results

After excluding those missing covariates, 22602 individuals were included in our EWAS, among them 1938 (5.65%) reported a cataract surgery experience (Table IV.3). Specific sample size for each biomarker ranged from 1161 to 21641 (Table IV.4). The half-lives among our 104 items ranged from 0.4 hours (urinary methyl paraben) to 70.0 years (blood 1,2,3,6,7,8-hxcdd, a dioxin-like chemical), which were quite diverse (Table IV.4). The survey weighted mean age of total population was 45.9 years (Std.E=0.12). Those who had cataract surgery were more likely to be older, non-hispanic white women, with lower education attainment (all p-values<0.001) (Table IV.3). They were also more likely to have had diabetes, with a bit lower BMI, and more likely to be former smokers (all p-values <0.001 due to large sample sizes) (Table IV.3).

In the two-step conventional EWAS analysis, after the filter in Step One, 19 biomarkers had $|z_1|>1.65$, which passed the threshold C_1. They were six biomarkers for heavy metal (urinary cadmium, urinary cobalt, urinary mercury, urinary tungsten, urinary thallium, and blood total mercury), four PCBs or furans (lipid adjusted blood trans-nonachlor and 1,2,3,4,6,7,8-heptachlorodibenzofuran, PCB 49 and PCB 44), two phenols (urinay bisphenol A and urinary benzophenone-3), two VOCs (urinary N-Acetyl-S-(4-hydroxy-2-butenyl)-L-Cysteine, and urinary phenylglyoxylic acid), one PFC (blood perfluorohexane sulfonic acid), one PAH (urinary 1-hydroxynaphthalene), one BFR (lipid adjusted blood 2,4,4'-tribromodiphenyl ether), urinary nitrate, and urinary DEET acid (z_1 data not shown).

In Step Two, via a BH procedure for FDR control, we identified five biomarkers which had $|z_{joint}|>C_{joint}$: PCB 44 and PCB 49, urinary cadmium, urinary cobalt and urinary tungsten (Table IV.5). All five pollutants showed positive associations with cataract surgery. In a fully adjusted

survey weighted logistic regression using total study population (training+testing), the OR per 2-fold increase in serum PCB 44 was 1.67 (95%CI: 1.06, 2.62; p=0.026), the OR per 2-fold increase in PCB 49 was 1.74 (95%CI: 1.13, 1.67; p=0.012), the OR per 2-fold increase in urinary cadmium was 1.30 (95%CI: 1.11, 1.52; p=0.0009), the OR per 2-fold increase in urinary cobalt was 1.15 (95%CI: 1.05, 1.25; p=0.0019), and the OR per 2-fold increase in urinary tungsten was 1.15 (95%CI: 1.04, 1.27; p=0.0060) (Table IV.5). The mean half-lives of those five pollutants were 5.1 years. Only urinary cadmium remained selected when we changed into FWER control using Bonferroni correction in Step Two. Further adjustment for job types did not change the significance of these associations (data not shown).

The tendency that those chemicals with longer half-lives have higher significance were confirmed by our results in the Stage 1 conventional EWAS. The linear association between half-lives and p-values of the pollutant-cataract surgery association was significant, for every 1 unit increase in log-transformed half-life (base 10), the p-value dropped for 0.035 (95%CI: -0.069, 0.001; p=0.048).

In Stage 2, we then created weights based on half-lives by Genovese's method. The weights W_i ranged from 0.24 to 4.70 (median=0.96). Under the weighted FDR control, we identified six biomarkers associated with cataract surgery, via BH method: serum PCB 49, serum PCB 44, urinary cobalt, urinary N-acetyl-S-(3-hydroxypropyl-1-methyl)-L-cysteine, urinary N-acetyl-S-(3-hydroxypropyl)-L-cysteine, and urinary mono-(3-carboxypropyl) phthalate (Table IV.6). The latter three with relatively short half-lives were newly identified via more liberal weighted thresholds for p-values. In a fully adjusted model, the OR per 2-fold increase in urinary N-acetyl-S-(3-hydroxypropyl-1-methyl)-L-cysteine was 0.92 (95%CI: 0.75, 1.13; p=0.44), the OR per 2-fold increase in urinary N-acetyl-S- (3-hydroxypropyl)-L-cysteine was 0.93 (95%CI: 0.74, 1.17;

p=0.52), the OR per 2-fold increase in urinary mono-(3-carboxypropyl) phthalate is 1.06 (95%CI: 0.97, 1.16; p=0.20) (Table IV.6). Mean of half-lives dropped to only 14.9 weeks. Figure IV.2 illustrates the change of half-lives of selected pollutants from the Stage 1 conventional EWAS to the Stage 2 weighted version. Those pollutants with much shorter half-lives, such as VOCs and PAHs, were successfully identified after accounting for weights (Figure IV.2). However, no pollutant was identified with the weighted FWER control in EWAS.

Table IV.3. Survey-Weighted Characteristics of Study Participants by Cataract Surgery Status, NHANES, 1999-2008.

Characteristics	total samples	Ever had cataract operation		*p*-value[b]
		Yes	No	
Total sample, N (%)	22602 (100)	1938 (5.65)	20664 (94.4)	
Age, years	45.9 ± 0.12	73.4 ± 0.3	44.1 ± 0.1	<.001
Female, n (%)	11689 (51.6)	1063 (61.7)	10626 (51.0)	<.001
Race/ethnicity, n (%)				
Non-Hispanic White	11301 (71.8)	1327 (84.3)	9974 (71.0)	<.001
Mexican American	4491 (10.8)	244 (6.7)	4247 (11.0)	
Non-Hispanic Black	4675 (7.4)	227 (2.6)	4448 (7.7)	
Other	2135 (10.1)	140 (6.4)	1995 (10.3)	
Education, n (%)				
<high school	6858 (19.3)	790 (31.9)	6068 (18.5)	<.001
High school	5465 (25.8)	466 (27.7)	4999 (25.7)	
Some college+	10279 (54.9)	682 (40.3)	9597 (55.8)	
Diabetes, n (%)	2803 (8.8)	532 (25.4)	2271 (7.9)	<.001
Body mass index, kg/m^2	28.3 ± 0.1	28.2 ± 0.2	28.3 ± 0.1	<.001
Smoking status, n (%)				
None smoker	11662 (50.7)	893 (45.5)	10769 (51.0)	<.001
Former smoker	5946 (25.0)	848 (43.8)	5098 (23.9)	
Current smoker	4994 (24.3)	197 (10.7)	4797 (25.1)	
Urinary creatinine, mg/dL	100.5 (99.6, 101.5)	79.0 (77.5, 80.6)	102.0 (101.3, 102.6)	<.001

[a]Geometric mean (95% confidence interval) is presented because of skewness.
[b]P-value based on t-tests for continuous variables and Rao-Scott Chi-squared tests for categorical variables.

Table IV.4. Half-Lives of Biomarkers Included in EWAS Analysis (m=104).

Variable Name in NHANES	Label	Half-Life (hours)	Sample Size (n)	# ARC Surgery (n)	Source of Half-Live
LBXD03LA	**1,2,3,6,7,8-hxcdd (fg/g)**	613620	3464	290	Knudsen and Merlo 2011
LBX209LA	**PCB209 (ng/g)**	303598.5	1161	106	Nguyen et al., unpublished data
URXUCD	**Cadmium, urine (ng/mL)**	205124	7213	636	Ishizaki et al. 2015
LBXPFNA	**Perfluorononanoic acid**	175320	4358	419	Gleason et al. 2015
LBX153LA	**PCB153 (ng/g)**	143467	3782	303	Ritter et al. 2011
LBXBCD	**Cadmium (ug/L)**	140256	21641	1860	Järup et al. 1983
LBX138LA	**PCB138 (ng/g)**	110627	3779	302	Ritter et al. 2011
LBX180LA4	**PCB180 (ng/g)**	100809	3778	302	Viluksela et al. 2014
LBXBHCLA	**Beta-hexachlorocyclohexane (ng/g)**	63115	3825	315	ATSDR 2005
LBXD05LA	**1,2,3,4,6,7,8-hpcdd (fg/g)**	57856	3453	290	Knudsen and Merlo 2011
LBXBR7LA	**2,2',4,4',5,5'-hexabromodiphenyl ether**	57120	1301	127	Geyer et al. 2004
LBXD07LA	**1,2,3,4,6,7,8,9-ocdd (fg/g)**	49090	3406	285	Knudsen and Merlo 2011
LBXPFHS	**Perfluorohexane sulfonic acid**	46460	4358	419	Li et al. 2017
LBXPFOS	**Perfluorooctane sulfonic acid**	29804	4358	419	Li et al. 2017
LBXBR2LA	**2,4,4'-tribromodiphenyl ether**	26297	1277	126	Makey et al. 2014
LBXBR5LA	**2,2',4,4',5-pentabromodiphenyl ether**	24960	1272	126	Geyer et al. 2004
LBXPFOA	**Perfluorooctanoic acid**	23668	4358	419	Li et al. 2017
LBXPCBLA	**3,3',4,4',5-pncb (fg/g)**	23668	3454	289	Ogura 2004
LBXF08LA	**1,2,3,4,6,7,8-hpcdf (fg/g)**	22792	3321	279	Knudsen and Merlo 2011
LBXPDELA	**p,p'-DDE (ng/g)**	21240	3871	320	Ferreira et al. 2011
URXUCO	**Cobalt, urine (ng/mL)**	17520	7322	644	Nguyen et al., unpublished data
LBX044LA	**PCB44 (ng/g)**	14025	1177	108	Shirai and Kissel 1996
LBXBR6LA	**2,2',4,4',6-pentabromodiphenyl ether**	13752	1301	127	Geyer et al. 2004

URXUCS	**Cesium, urine (ng/mL)**	2616	7322	644	ATSDR 2004
URXUHG	**Mercury, urine (ng/mL)**	2160	15796	1238	Nuttall 2004
LBXTNALA	**Trans-nonachlor (ng/g)**	2112	3849	317	Toxicology 1982
URXUTU	**Tungsten, urine (ng/mL)**	1608	7243	642	Radcliffe et al. 2010
LBXTHG	**Mercury, total (ug/L)**	1368	7043	434	Yaginuma-Sakurai et al. 2012
URXUPB	**Lead, urine (ng/mL)**	1080	21641	1860	ATSDR 2007
LBX049LA	**PCB49 (ng/g)**	1046.9	1171	108	Nguyen et al., unpublished data
URXUBA	**Barium, urine (ng/mL)**	820.8	7188	635	Rundo 1968
LBXBPB	**Lead (ug/dL)**	672	7322	644	ATSDR 2007
URXUTL	**Thallium, urine (ng/mL)**	520.8	7286	641	U.S. Environmental Protection Agency 1980
URXUUR	**Uranium, urine (ng/mL)**	360	5999	539	Bhattacharyya et al. 1992
URXUIO	**Iodine, urine (ng/mL)**	192	9340	814	Marwaha and Gopalakrishnan 2011
URXUAS	**Urinary total Arsenic (µg/L)**	96	4570	424	Hughes 2006
URXUSB	**Antimony, urine (ng/mL)**	95	7217	638	Kentner et al. 1995
URXSCN	**Urinary thiocyanate (ng/mL)**	72	2174	150	Schulz et al. 1979
URXUDMA	**Urinary Dimethylarsonic acid (µg/L)**	60	4562	424	Crecelius 1977
URX14D	**2,5-dichlorophenol (ug/L) result**	39.9	4491	394	Somani and Khalique 1982
URXDCB	**2,4-dichlorophenol (ug/L) result**	35.8	4491	394	Nguyen et al., unpublished data
LBXVXY	**blood m-/p-xylene (ng/ml)**	32.8	6717	370	Matsumoto et al. 1992
URX2MH	**2-Methylhippuric acid (ng/mL)**	30.1	2174	150	Engström et al. 1978
URXUMO	**Molybdenum, urine (ng/mL)**	30	7221	636	Werner et al. 2000
urxtrs	**Urinary Triclosan (ng/mL)**	29	3119	272	Olaniyan et al. 2016
URXCPM	**3,5,6-trichloropyridinol (ug/L) result**	26.9	3969	283	Satoh and Gupta 2011
URX34M	**3-methipurc acd & 4-methipurc acd(ng/mL)**	20.1	2174	150	Engström et al. 1978
URXCNP	**Mono(carboxynonyl) phthalate (ng/mL)**	18	3119	272	Wittassek and Angerer

					2008
URXCOP	**Mono(carboxyoctyl) phthalate(ng/mL)**	18	3119	272	Hines et al. 2012
LBXCOT	**Cotinine (ng/mL)**	16	21329	1826	Benowitz and Jacob 1994
URXECP	**Mono-2-ethyl-5-carboxypentyl phthalate**	15	4528	407	Wittassek and Angerer 2008
URXETL	**Enterolactone (ng/mL)**	12.6	7265	598	Kuijsten et al. 2005
URXOP3	**Dimethylthiophosphate(µg/L)**	12	6788	504	Barr and Angerer 2006
URXUP8	**Perchlorate, urine (ng/mL)**	12	10697	928	ATSDR 2008
URXDEA	**DEET acid (ug/L)**	11.1	1689	172	ATSDR 2017
URXPHG	**Phenylglyoxylic acid(ng/mL)**	10.5	2174	150	Guillemin and Bauer 1979
URXMOH	**Mono-(2-ethyl-5-oxohexyl) phthalate**	10	7266	598	Wittassek and Angerer 2008
URXMHH	**Mono-(2-ethyl-5-hydroxyhexyl) phthalate**	10	6025	504	Wittassek and Angerer 2008
URXEQU	**Equol (ng/mL)**	9.7	7392	608	Lu et al. 1995
URXOPM	**3-phenoxybenzoic (ug/L) acid result**	8.7	3900	278	Ferland et al. 2015
URXBP3	**Urinary Benzophenone-3 (ng/mL)**	8.04	4490	394	Kasichayanula et al. 2007
URXNO3	**Urinary nitrate (ng/mL)**	8	9327	806	Bondonno et al. 2015
URXP03	**3-hydroxyfluorene (ng/L)**	6.1	5877	493	Li et al. 2012
URXHP2	**N-Ace-S-(2-hydroxypropyl)-L-cys(ng/mL)**	6	2174	150	de Rooij et al. 1996
URXBMA	**N-Acetyl-S-(benzyl)-L-cysteine(ng/mL)**	6	2174	150	de Rooij et al. 1996
URXBPM	**N-Acetyl-S-(n-propyl)-L-cysteine(ng/mL)**	6	2174	150	de Rooij et al. 1996
URXMB3	**N-A-S-(4-hydrxy-2butn-l-yl)-L-cys(ng/mL)**	6	9327	806	de Rooij et al. 1996
URXAAM	**N-Ace-S-(2-carbamoylethyl)-L-cys(ng/mL)**	6	2174	150	de Rooij et al. 1996
URXAMC	**N-Ace-S-(N-methlcarbamoyl)-L-cys(ng/mL)**	6	2174	150	de Rooij et al. 1996
URXCYM	**N-acetyl-S-(2-cyanoethyl)-L-cyst(ng/mL)**	6	2174	150	de Rooij et al. 1996
URXPMM	**N-A-S-(3-hydrxprpl-1-metl)-L-cys(ng/mL)**	6	2174	150	de Rooij et al. 1996
URXCEM	**N-Acetyl-S-(2-Carbxyethyl)-L-Cys(ng/mL)**	6	2174	150	de Rooij et al. 1996
URXHPM	**N-Ace-S-(3-Hydroxypropyl)-L-Cys(ng/mL)**	6	2174	150	de Rooij et al. 1996
URXDHB	**N-Ace-S- (3,4-Dihidxybutl)-L-Cys(ng/mL)**	6	2174	150	de Rooij et al. 1996

URXMIB	**Mono-isobutyl pthalate**	6	6025	504	Seckin et al. 2009
URXGNS	**Genistein (ng/mL)**	5.5	7112	591	Lu et al. 1995
URXBPH	**Urinary Bisphenol A (ng/mL)**	5.4	4490	394	Völkel et al. 2002
URXP06	**1-hydroxyphenanthrene (ng/L)**	5.1	5836	488	Li et al. 2012
URXMHP	**Mono-(2-ethyl)-hexyl phthalate (ng/mL)**	5	6025	504	Wittassek and Angerer 2008
LBXGLY	**Glycideamide (pmoL/G Hb)**	4.6	7927	694	Calleman 1996
LBXACR	**Acrylamide (pmoL/G Hb)**	4.6	7931	686	Calleman 1996
URXETD	**Enterodiol (ng/mL)**	4.4	7392	608	Kuijsten et al. 2005
URXP01	**1-hydroxynaphthalene (ng/L)**	4.3	5843	487	Li et al. 2012
URXP05	**3-hydroxyphenanthrene (ng/L)**	4.1	5897	496	Li et al. 2012
URXATC	**2-amnothiazolne-4-carbxylic acid(ng/mL)**	4.00	2174	150	Bhandari et al. 2014
URXP07	**2-hydroxyphenanthrene (ng/L)**	3.9	5842	487	Li et al. 2012
URXP10	**1-hydroxypyrene (ng/L)**	3.9	5855	488	Li et al. 2012
URXMAD	**Mandelic acid(ng/mL)**	3.9	2174	150	Guillemin and Bauer 1979
URXDAZ	**Daidzein (ng/mL)**	3.9	7392	608	Lu et al. 1995
URXDMA	**o-Desmethylangolensin (O-DMA) (ng/mL)**	3.9	7387	608	Lu et al. 1995
URXP19	**4-hydroxyphenanthrene (ng/L)**	3.5	2508	213	Li et al. 2012
URXP17	**9-hydroxyfluorene (ng/L)**	3.1	4403	396	Li et al. 2012
URXMEP	**Mono-ethyl phthalate (ng/mL)**	3	7056	576	Calafat and McKee 2006
URXP04	**2-hydroxyfluorene (ng/L)**	2.9	5847	490	Li et al. 2012
URXP09	**3-fluoranthene (ng/L)**	2.8	1175	84	Motorykin et al. 2015
URXP02	**2-hydroxynaphthalene (ng/L)**	2.5	5880	490	Li et al. 2012
LBXVTO	**blood toluene (ng/ml)**	2	6564	352	Nise et al. 1989
URXMBP	**Mono-n-butyl phthalate (ng/mL)**	1.9	7254	597	Mittermeier et al. 2016
LBXVCF	**blood chloroform (pg/ml)**	1.5	6288	356	Ekwall et al. 1998
URXPPB	**Propyl paraben (ng/ml)**	1.155	3119	272	Abbas et al. 2010
URXMZP	**Mono-benzyl phthalate (ng/mL)**	1.1	7267	598	Nguyen et al., unpublished data

URXMC1	**Mono-(3-carboxypropyl) phthalate**	0.84	6025	504	Nguyen et al., unpublished data
LBXVBM	**blood bromodichloromethane (pb/ml)**	0.78	6694	384	Leavens et al. 2007
URXMPB	**Methyl paraben (ng/ml)**	0.36	3119	272	Abbas et al. 2010

Table IV.5. Odds Ratios (95% Confidence Intervals) of Cataract Operation History by Five Biomarkers Selected via Conventional EWAS under FDR Control

	Median (IQR)	# cases/all	Half-life[a] (years)	2-fold OR (95% CI)[b]	*p*-values[c]
Urinary cadmium, ng/mL	0.30 (0.41)	633/7213	23.4	1.30 (1.11, 1.52)	0.0009
Serum PCB 49[d], ng/g	1.32 (1.10)	108/1171	0.12	1.74 (1.13, 1.67)	0.0115
Urinary cobalt, ng/mL	0.35 (0.33)	644/7322	2.00	1.15 (1.05, 1.25)	0.0019
Serum PCB 44[d], ng/g	2.00 (1.70)	108/1171	1.60	1.67 (1.06, 2.62)	0.0259
Urinary Tungsten, ng/mL	0.073 (0.11)	642/7243	0.18	1.15 (1.04, 1.27)	0.0060

[a] Using maximum composite half-lives.
[b] Survey weighted model adjusted for age, race/ethnicity, gender, BMI, smoking status, education attainment, diabetes mellitus status, and NHANES cycle number.
[c] P-values and ORs were calculated via survey weighted logistic model running in total population, not split ones.
[d] Serum PCB 44 and 49 were lipid adjusted.

Table IV.6. Odds Ratios (95% Confidence Intervals) of Cataract Operation History by Six Biomarkers Selected via EWAS under Weighted FDR Control

	Median (IQR)	# cases/all	Half-life[a] (years)	2-fold OR (95% CI)	*p*-values[c]
Serum PCB 49[d], ng/g	1.32 (1.10)	108/1171	0.12	1.74 (1.13, 1.67)	0.0115
Urinary cobalt, ng/mL	0.35 (0.33)	644/7322	2.00	1.15 (1.05, 1.25)	0.0019
Serum PCB 44[d], ng/g	2.00 (1.70)	108/1171	1.60	1.67 (1.06, 2.62)	0.0259
Urinary N-acetyl-S-(3-hydroxypropyl-1-methyl)-L-cysteine, ng/mL	418 (626)	150/2174	0.0007	0.92 (0.75, 1.13)	0.4371
Urinary N-acetyl-S- (3-hydroxypropyl)-L-cysteine, ng/mL	215 (399)	150/2174	0.0007	0.93 (0.74, 1.17)	0.5200
Urinary mono-(3-carboxypropyl) phthalate, ng/mL	2.35 (3.40)	504/6025	0.0001	1.06 (0.97, 1.16)	0.1966

[a] Using maximum composite half-lives.
[b] Survey weighted model adjusted for age, race/ethnicity, gender, BMI, smoking status, education attainment, diabetes mellitus status, and NHANES cycle number.
[c] P-values and ORs were calculated via survey weighted logistic model running in total population, not split ones.
[d] Serum PCB 44 and 49 were lipid adjusted.

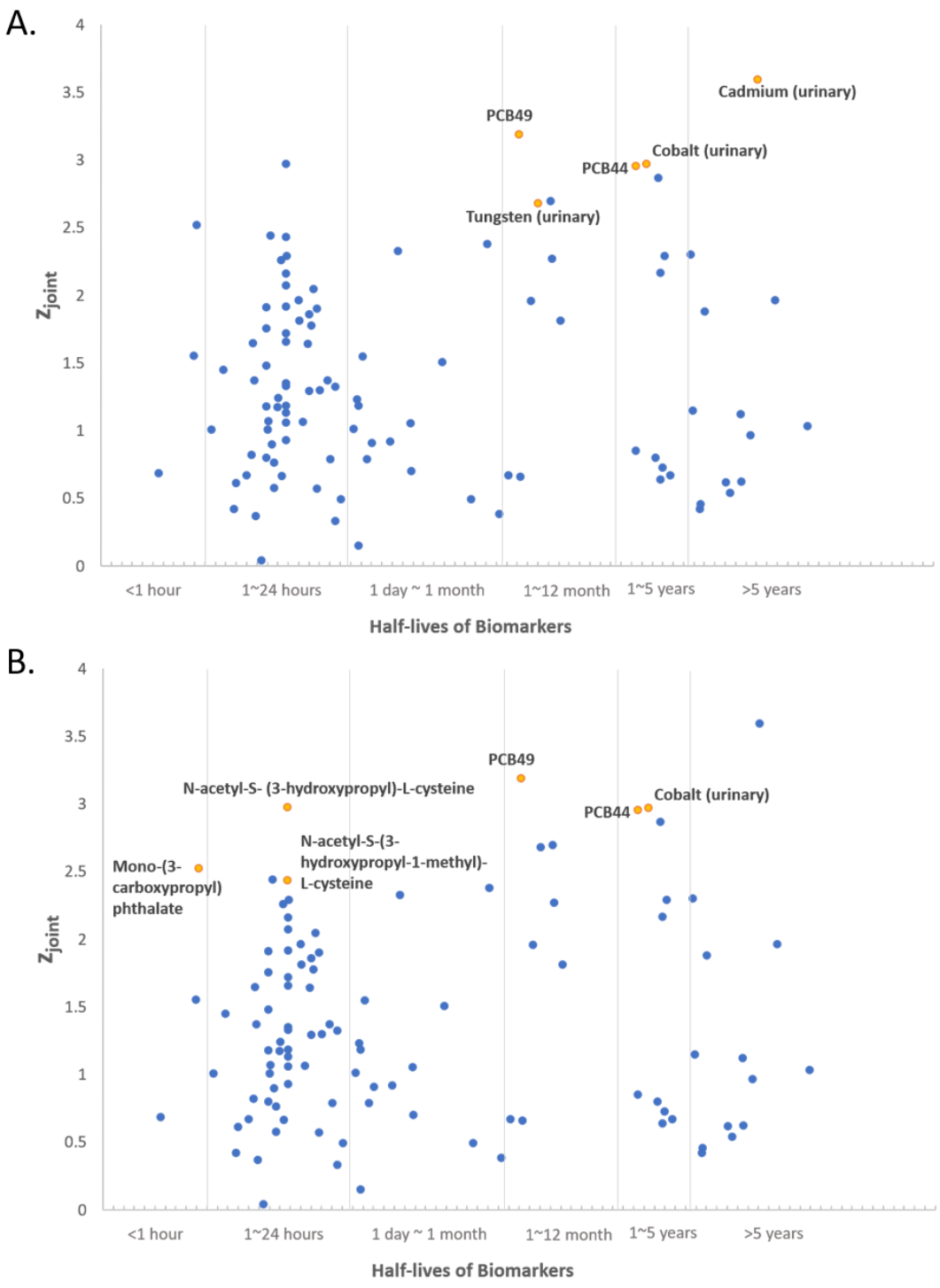

Figure IV.2. Plots illustrating change of selected pollutants by conventional EWAS and weighted EWAS using FDR control. The y-axis showed z_{joint}. The x-axis illustrates half-lives. Plots in orange indicate selected pollutants, while plots in blue refer to unselected pollutants. A. Conventional EWAS. B. Weighted EWAS.

5. Discussion

Using the conventional EWAS approach, we found that elevated levels of urinary heavy metals (cadmium, cobalt and tungsten), and serum PCBs 44 and 49, were positively associated with the risk of cataract surgery. Using our new approach accounting for the biological half-lives of pollutants, we further identified urinary mono-(3-carboxypropyl) phthalate and two VOCs: urinary N-acetyl-S-(3-hydroxypropyl-1-methyl)-L-cysteine and urinary N-acetyl-S- (3-hydroxypropyl)-L-cysteine. The present study suggests that the weighted approach could be a useful tool for discovering potential false negative associations that may have not been captured in conventional approaches due to the relatively short half-lives and hence high measurement errors.

The development of cataract is affected by both genetic and environmental factors. Previous epidemiological studies concerning the association between environmental risk factors and cataract were very limited. Researchers have reported that heavy metals, such as lead and cadmium can be risk factors for cataract (Schaumberg et al. 2004; Wang et al. 2016). Cumulative evidence also suggested that elevated PM2.5 and PAH generated by indoor biomass cooking fuel may also increase the risk of cataract (Mishra et al. 1999; Mohan et al. 1989; Pokhrel et al. 2005; Ravilla et al. 2016). To the best of our knowledge, our study is the first to utilize a non-targeted EWAS strategy to systematically and efficiently identify potential environmental risk factors for ARC.

Heavy metals, as an important environmental source of oxidative stress, may promote the formation of cataract in lens. Heavy metal exposure can disrupt the antioxidant defense system by depleting the glutathione and thiol pools, causing oxidative DNA damage in lens cells and

enhancing apoptosis of lens epithelial cells, thus inducing protein and lipid aggregation in the lens (Babizhayev 2012; Bobrow et al. 2015; Ercal et al. 2001; Jomova and Valko 2011; Spector 1995; Truscott 2005; Tweeddale et al. 2016; Valko et al. 2016). In our previous research, a hypothesis-driven targeted study of cadmium and lead in relation to cataract surgery using data from NHANES, we found significant association between urinary cadmium and the risk of cataract surgery (Wang et al. 2016). In the current nontargeted EWAS, we additionally identified cobalt and tungsten as potential risk factors for cataract surgery. Our study provides evidence that cadmium, tungsten and cobalt may also play a role in the pathogenesis of ARC.

The overall exposure of cadmium in the U.S. population has decreased significantly during 1988 and 2008 (Tellez-Plaza et al. 2012). Cigarette smoking is a major source of environmental exposure of cadmium for smokers: each cigarette contains about 2.0 µg of cadmium, of which about 2-10% emits into air in the form of smoke (Mannino et al. 2004). In addition to inhalation, oral ingestion is also a main source of environmental cadmium exposure, especially in areas where food is produced in severely contaminated soil (Sulfide 1997). There is no known biological function of cadmium in higher organisms.

Tungsten is the heaviest element having bioactivity in some bacteria and archaea (Koribanics et al. 2015). As a material with one of the highest melting points of all known elements (>3400°C, second to carbon) (Langmuir 1915), tungsten has been widely used in industrial and military production, such as producing hard materials, bulb filaments, heavy metal alloys, radiation shielding, etc. Although tungsten has been extensively used in various areas, toxicological studies of tungsten were relatively limited. Some studies have shown that chronic health problems such as chronic inflammation, histological lesion and leukemia may be related with low-dose tungsten exposure (Witten et al. 2012). Nevertheless, there is no conclusive

evidence.

As an important component of vitamin B_{12}, cobalt is a well-known essential trace mineral for all animals. It has been used in the production of alloys, batteries, painting pigment, and so on. Tungsten carbide-cobalt alloy is a widely used hard material (Tien et al. 1980), while nickel-cadmium batteries often included cobalt to improve the oxidation reaction (Armstrong et al. 1988), implying the possibility of cobalt-tungsten and cobalt-cadmium co-exposure. The toxicity of cobalt has been noticed within patients who have undergone hip arthroplasties/implants, and is associated with the risk of several chronic health effects such as cognitive function decline, hearing loss, visual impairment, cardiomyopathy, etc. (Pizon et al. 2013)

Our study suggests that noncoplanar PCBs 44 and 49 may be potential risk factors for ARC. PCBs 44 and 49 are degradation byproducts of larger PCBs (Grimm et al. 2015), which implies that they may not be the causal PCB species. PCBs, a group of 209 organic chlorine compounds, can act as another environmental source of oxidative stress in the formation of cataract. Studies have suggested that PCBs can increase the intracellular superoxide dismutase (SOD) activity and disrupt the thiol antioxidant system (Zhu et al. 2009). The toxicity of PCBs has long been noticed by the U.S. government and the use of PCBs has been banned since 1978. However, as a long-lasting chemical that is resistant to biodegradation, PCBs still exist in the environment. It can be absorbed by the human body of the general public through ingestion, inhalation and dermal exposure (ATSDR 2000; Beyer and Biziuk 2009). There are two categories of PCBs with distinct toxicological characteristics, coplanar (non-ortho) PCBs and noncoplanar PCBs, differentiated by their molecular chlorine substitution position (Fischer et al. 1998). Coplanar PCBs have no more than one chlorine atom at the ortho-position of the biphenyl rings, while noncoplanar PCBs have more chlorine atoms (Fischer et al. 1998). Coplanar PCBs

show dioxin-like toxicity which may cause various severe chronic health effects (ATSDR 2000). By contrast, noncoplanar PCBs, such as PCBs 44 and 49 identified in our EWAS, have relatively lower toxicity than coplanar PCBs (Fischer et al. 1998). However, the toxicity of noncoplanar PCBs should not be underestimated since they comprise the major fraction of the PCB body burden in humans (Fischer et al. 1998).

Using our new weighted approach, we further identified two VOCs and one phthalate as risk factors for ARC. Our weighted approach successfully identified pollutants with relatively short half-lives. On the other hand, cadmium and tungsten, which have relatively long half-lives, were excluded by a more stringent threshold. This result should be interpreted cautiously that it does not overturn the previous literature that cadmium may be a risk factor for ARC. The exclusion of pollutants with relatively long half-lives was a trade-off with the inclusion of pollutants with relatively short half-lives under the weighted approach. Generally, those pollutants with relatively long half-lives have smaller measurement error, which is genuinely preferred in association studies and should not be abruptly excluded (White 2011). Therefore, this new approach can be a complementary to the conventional approach.

The weighted approach added mono-(3-carboxypropyl) phthalate as a potential risk factor for ARC. Mono-(3-carboxypropyl) phthalate, which is a metabolite of di-n-octyl phthalate (DnOP), is a kind of plasticizer composites in flooring, carpet products and toys (Calafat et al. 2006). Previous studies have suggested that phthalates have endocrine disrupting effects (Frederiksen et al. 2007) as well as the ability to induce oxidative stress, resulting in DNA damage (Franken et al. 2017). A study using NHANES 2001-2010 found that urinary phthalates, especially mono-n-octyl phthalate (MOP), may be associated with self-reported eye affliction or retinopathy (Mamtani et al. 2016). Our results may add reference on a possible link between

phthalate and ocular diseases.

We also found associations between two VOCs (N-acetyl-S-(3-hydroxypropyl-1-methyl)-L-cysteine ($C_9H_{17}NO_4S$, commonly called HPMMA) and N-acetyl-S- (3-hydroxypropyl)-L-cysteine ($C_8H_{15}NO_4S$, commonly called 3HPMA)) and cataract surgery through the survey logistic regression (Table IV.5). VOCs emit from all homes and workplaces, and can be absorbed by the human body through ingestion, inhalation and dermal contact (Wallace et al. 1989). HPMMA is a metabolite of crotonaldehyde, which is mainly found in tobacco smoke (Urban et al. 2003; NHANES laboratory procedure manual 2011-2012). 3HPMA is a metabolite of acrolein, which is generated during heating foods, burning fuels and biomass, and tobacco smoking (Stevens et al. 2008, NHANES laboratory procedure manual 2011-2012). Several studies have indicated the genotoxicity and neurotoxicity of crotonaldehyde and acrolein (UI Islam et al. 2014; Moghe et al. 2015).

Further investigation is required for our weighted approach. In order to get a rational estimation of the beta coefficients, we need to implant the half-life-based weight into the regression. Our weighted approach also has other limitations. We only controlled the measurement error caused by half-lives of pollutants. Measurement error caused by power (related with the estimated beta, sample size, and variability of pollutants' values) of each hypothesis test is not controlled in our EWAS. Besides, in future study, we need to test our weighted approach in simulated data to validate our results and horizontally compare it with other weighted methods.

Although our study benefits from a large total sample size and a nationally representative study population, a number of limitations deserve consideration. The cross-sectional nature of the NHANES study design raises concerns about the temporality of exposure and cataract

development. Since the exposure levels were measured after the occurrence of cataract surgery, high levels of chemicals with relatively short half-lives may not be necessarily relevant to cataract surgery happened long ago. One potential solution is to exclude pollutants with short half-lives from the EWAS. We identified 23 biomarkers with biological half-lives ≥ 1 year. They may be more likely to have effect on the development of chronic diseases, such as age-related cataract. Conducting the conventional EWAS among these 23 biomarkers, we identified four pollutants associated with cataract surgery via FDR control: urinary cadmium and cobalt, serum PCB 44, and 1,2,3,4,6,7,8-Heptachloro dibenzofuran (Table IV.7). 1,2,3,4,6,7,8-Heptachloro dibenzofuran is a furan congener (chlorinated dibenzofuran, PCDF), usually generated as a byproduct during the manufacturing of iron/steel and other chlorinated chemicals (phenols, diphenyl ethers and PCBs) (World Health Organization 2000). PCDF can be absorbed by the human body through inhalation, ingestion, and contact (World Health Organization 2000). Once being absorbed, PCDF can be excreted into human milk (World Health Organization 2000).

Additionally, self-report bias and recall-bias may have been introduced into the study. The etiology and risk factors for different subtypes of ARC may differ, but the NHANES vision examination provides neither the age at onset nor the subtype of ARC. We included current job types as a surrogate of UV radiation exposure in the sensitivity analysis. Due to a large amount of missingness, we did not adjust for current job types in the logistic regressions used within EWAS. Family history of ARC was not considered in our analysis since it was not available in NHANES. There is no concrete evidence supporting the association between family history and the exposure to environmental chemicals, thus they can reasonably be considered as non-confounders on the association between chemical exposure and ARC surgery. Finally, the sample sizes for some pollutants were relatively small since they were only measured in one cycle of NHANES (e.g.

brominated flame retardants (BFRs), with the corresponding sample size of only about 1500). Such small sample size may not provide enough power for a valid conclusion. Since no previous study has investigated the association of these newly-recognized pollutants with ARC development, the results can still be valuable as pilot implications for further analysis.

In conclusion, our data-driven EWAS suggests unrecognized environmental pollutants, such as cobalt, tungsten, PCB 44 and 48, as potential risk factors for ARC. By adopting a weighted approach based on the half-lives of pollutants on the conventional EWAS, we further identified two VOCs (N-acetyl-S-(3-hydroxypropyl-1-methyl)-L-cysteine and N-acetyl-S- (3-hydroxypropyl)-L-cysteine) and one phthalate (mono-(3-carboxypropyl) phthalate) associated with cataract surgery. Our weighted approach is a pilot attempt, which requires further modification and validation. Causal links of these unrecognized risk factors with ARC need to be validated using hypothesis-driven, targeted approaches.

Table IV.7. Odds Ratios (95% Confidence Intervals) of Cataract Operation History by Four Biomarkers Selected via Conventional EWAS under FDR Control, among 23 Pollutants with Half-Lives ≥ 1 year.

	Median (IQR)	# cases/all	Half-life[a] (years)	2-fold OR (95% CI)	*p*-values[c]
Urinary cadmium, ng/mL	0.30 (0.41)	633/7213	23.4	1.30 (1.11, 1.52)	0.0009
Urinary cobalt, ng/mL	0.35 (0.33)	644/7322	2.00	1.15 (1.05, 1.25)	0.0019
Serum PCB 44[d], ng/g	2.00 (1.70)	108/1171	1.60	1.67 (1.06, 2.62)	0.0259
1,2,3,4,6,7,8-Heptachloro dibenzofuran, fg/g	7.6 (6.8)	279/3321	2.60	0.75 (0.63, 0.90)	0.0024

[a] Using maximum composite half-lives.
[b] Survey weighted model adjusted for age, race/ethnicity, gender, BMI, smoking status, education attainment, diabetes mellitus status, and NHANES cycle number.
[c] P-values and ORs were calculated via survey weighted logistic model running in total population, not split ones.
[d] Serum PCB 44 and 49 were lipid adjusted.

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CHAPTER V

Conclusions

1. Conclusions

Cataract and glaucoma are two leading causes of visual impairment and blindness (Bourne et al. 2013; Flaxman et al. 2017). Their pathogeneses were not completed understood (Bobrow et al. 2015; Cioffi and American Academy of Ophthalmology. 2015; Gupta et al. 2014). The development of these two ocular diseases is known to be affected by both genetic and environmental risk factors. Despite their high prevalence and severe consequences, epidemiologic studies focusing on the associations of environmental risk factors with cataract or glaucoma were limited.

This dissertation systematically investigated the association between potential environmental risk factors with these two ocular diseases. We used comprehensive epidemiologic and statistic approaches, including a longitudinal prospective study design and a survey weighted cross-sectional study design, a hypothesis-driven targeted approach as well as exposure-untargeted environment-wide association study (EWAS), various regressions and statistic methods (logistic regression, Cox proportional hazard model, inverse probability weighting, adjusted survival curve, effect modification analysis, dietary pattern score derived from principal component analysis, weighted

hypothesis testing framework, etc.). We identified unrecognized environmental risk factors, such as bone lead for primary open-angle glaucoma (POAG), and cobalt, tungsten, PCBs 44 and 49, two VOCs and one phthalate for age-related cataract (ARC). We also found that a prudent dietary pattern which is abundant in plant-sourced food may attenuate the toxicity of bone lead on the development of POAG, where plant-sourced provitamin A carotenoid may play an important role in this interaction.

The risks of ARC and POAG increase with age. As more cures for acute lethal diseases are available and more affordable, with the rise of awareness of the benefits of healthy lifestyle, the expected lifespan will continue to grow worldwide. Together with a diminishing birthrate, population aging is unavoidable. As life expectancy increases, increasing numbers of ARC and POAG patients will mean a significant economic burden to society, especially for developing countries where the accessibility of effective treatment is relatively low. The productivity loss due to blindness has been estimated to be $2.5 billion (minimum wage assessment) and $7.8 billion (gross national income per capita assessment) in the U.S., whereas for moderate to severe visual impairment, the cost is $5.3 billion and $16.5 billion, respectively (Eckert et al. 2015). Not only is productivity loss an issue, medical cost plays an even bigger role in the financial burden. Therefore, pre-symptomatic prevention, especially for irreversible glaucoma, plays a very important role in dealing with these serious issues. Identifying the environmental risk factors is the first step towards the establishment of preventive interventions of ARC and POAG. Our studies identified unrecognized environmental risk factors for these two ocular diseases. We also evaluated the effectiveness of intervention through adjustment of dietary patterns. Our study provided evidence that controlling for certain diet could be

an applicable preventive intervention for lead toxicity. Future study is required to examine the effectiveness of dietary intervention for other chemicals.

Awareness of these severe ocular diseases should also be emphasized among young people. As we enter the digital age, the bytes of information processed by individual consumers have increased at an annual rate of 5.4% during 1980 to 2008 in the U.S., which is greater than the GDP growth over the same period (Bohn and Short 2012b). Of all media formats, visual information such as video and text makes up 71.2% time-wise, 99.4% byte-wise, and 83% word-wise (Bohn and Short 2012a). Younger generations are more likely to be exposed to visual information via various digital instruments: the exposure is long-term, high-dose, and the effect is yet unclear. It is observed that working on computers longer than 0.8 hours/day can increase the occurrence of myopia, whereas reading and writing need longer than 2 hours/day to have the same effect (Czepita et al. 2010). The prevalence of smartphones and the rise of near-field optical instruments like virtual reality headsets may pose even higher risk of myopia for younger generations. Myopia is clinically identified as a risk factor for glaucoma (Cioffi and American Academy of Ophthalmology. 2015). Awareness must be raised in today's society in order to prevent tomorrow's tragedies.

Lead has been a prevalent source of toxicity since ancient times. It is believed that lead beverage vessels and lead water pipes may have induced wide lead poisoning in ancient Rome, which eventually led to the fall of the Roman Empire (Needleman 2004). Even though lead-based paint and gas additives have been banned since 1970's, the risk of lead poisoning still exists in today's life by means of corroded water pipes (Maas et al. 2005) and residual lead paint (Jacobs et al. 2002). In recent years, many large-scale lead

pollution incidents have been reported. Water contamination is one of the most severe: Flint water crisis in 2014, which affected over 100,000 residents, including 6000-12,000 children (Gómez et al. 2018; Hanna-Attisha et al. 2016), and Washington D.C. drinking water contamination in 2001, which resulted in dangerous lead levels in over 15,000 homes and caused lasting health risks for thousands of children (Edwards et al. 2009).

Although recent news regarding lead toxicity mainly focused on children, older population is also highly susceptible. Low dose environmental lead exposure is associated with multiple age-related chronic diseases, such as decline in cognitive function (Bandeen-Roche et al. 2009; Shih et al. 2007), hearing loss (Choi et al. 2012; Park et al. 2010), cardiovascular disease (Navas-Acien et al. 2007; Schwartz 1995), systemic hypertension (Schwartz 1995), chronic renal disease (Lin et al. 2003, 2006; Staessen 1995), etc. Several studies have reported the effect of environmental lead exposure on age-related ocular diseases. Elevated lead levels were found to be associated with a higher risk of ARC and age-related macular disease (AMD), and may increase the blood-retina permeability, which itself is a risk factor for retinal vascular diseases (Erie et al. 2009; Hwang et al. 2015; Mosad et al. 2010; Schaumberg et al. 2004; Shen et al. 2016). Our studies added reference on the health consequences of environmental lead exposure for older population. Lead can deposit in bones for decades and gradually degenerate into the circulatory system as age increases. Hence, endogenous lead poisoning may continue even though exogenous exposure has ceased. As life expectancy increases, aging population susceptible for not only environmental but also endogenous lead exposure increases. The threat of lead poisoning to older population demands more public awareness.

Besides known pollutants like lead, contemporary chemical engineering and industry are also creating new challenges for environmental protection and public health by creating new substances. It is estimated that new substances are synthesized at a rate of every 2.6 seconds in 2009 (American Chemical Society 2009). Most, if not all, of the newly invented substances will eventually enter the environment and people's life, but little is known about their toxicology and health effect on humans.

Together with the traditional hypothesis-driven targeted approach, data-driven untargeted approach such as EWAS can provide an effective and systematic way to identify unknown health effects of those chemicals. Further efforts on exploring updated or advanced untargeted approach are definitely worthwhile. One potential method may be using multi-layer feed-forward neural networks to quickly filter the potential risk factors. Deep neural networks (deep learning), which is a newly developed technique in machine learning, are a family of universal function approximators; nonlinearity and layered structure are the key factors that enable their expressive power to grow exponentially with respect to depth (LeCun et al. 2015). Recently, artificial intelligence based on deep learning framework was adopted in clinical diagnosis (Nishio et al. 2018; Suzuki 2017). Its application in epidemiological studies may also be expectable.

In conclusion, this dissertation provides new points of view for the exploration of the pathogenesis of ARC and POAG, gives new ideas for interventions targeting these two highly prevalent and debilitating ocular diseases, and therefore provides new avenues to effectively decrease the global burden of blindness.

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