Title

California State-wide Studies to Investigate Medical Conditions and Medication Uses in Relation to Macular Degeneration and Childhood Cancer

Author

Xu, Xiaoqing

Publication Date

2017

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA

Los Angeles

California State-wide Studies to Investigate Medical Conditions and Medication Uses in Relation to Macular Degeneration and Childhood Cancer

A dissertation submitted in partial satisfaction of the

requirements for the degree Doctor of Philosophy

in Epidemiology

by

Xiaoqing Xu

2017

ABSTRACT OF THE DISSERTATION

California Statewide Studies to Investigate Medical Conditions and Medication Uses in Relation to Childhood Cancer and Macular Degeneration

by

Xiaoqing Xu

Doctor of Philosophy in Epidemiology

University of California, Los Angeles, 2017

Professor Beate R. Ritz, Chair

Cancer is the second leading cause of mortality among children in the US, with very few well-established preventable causes. Since childhood cancers are diagnosed at an early age, it has been hypothesized that its pathogenesis is initiated during fetal development and possibly fueled by fetal growth. Indeed, population-based studies have linked various perinatal factors with childhood cancers. We investigated preeclampsia, a major cause of adverse effects on fetal health, as a possible risk factor of childhood cancers in a statewide case control study in California. We obtained childhood cancer cases diagnosed at five years old or younger between 1988 and 2012 from the California Cancer Registry and linked them to birth certificates. Controls were randomly selected from all California births and frequency matched to cases by birth year. We obtained information regarding preeclampsia during pregnancy, labor, and

delivery from the medical worksheet of the electronic birth record. We applied causal mediation analyses to assess the controlled direct effects of preeclampsia on childhood cancers, independent of preterm delivery and neonatal intensive care unit (NICU) admission. Our findings suggest that maternal preeclampsia increases risk of some rare childhood cancers, including seminomas, teratoma and hepatoblastoma, and may shed light on new etiologic factors for these cancers.

In addition, we examined associations between regular medication use and the risk of age-related macular degeneration (AMD) using a unique data source of the California Teachers Study (CTS), of which 133,479 participants were followed both actively with questionnaires from 1995-1996 until the most recent contact in 2012 and passively with linkage to vital statistics, cancer registries, and hospitalization records. We generated a linked hospital, vital status (including out of state mortality) and baseline questionnaire dataset for each CTS participant. We identified diagnoses of AMD from the California Office of Statewide Health Planning and Development (OSHPD) hospital discharge data. Regular consumption of aspirin, ibuprofen, acetaminophen and antihypertensive medications were collected in the self-administered questionnaire and information on specific antihypertensive drugs and anti-inflammatory drugs were available for subsamples who completed the followup questionnaires in 2000 and 2005 respectively.

The dissertation of Xiaoqing Xu is approved.

Marjan Javanbakht

Li Li

Wendie Robbins

Beate R. Ritz, Committee Chair

University of California, Los Angeles

2017

DEDICATION

Dedicated to the memories of my grandmother Sirong Li (1933-2014)

TABLE OF CONTENTS

LIST OF FIGURES

Chapter 2

LIST OF TABLES

Chapter 2

LIST OF ABBREVIATIONS

ACE	Angiotensin-converting enzyme
ACOG	American College of Obstetricians and Gynecologists
Ang II	Angiotensin II
ALL	Acute lymphoblastic leukemia
AMD	Age related macular degeneration
AML	Acute myeloid leukemia
AREDS	Age-Related Eye Disease Study
ATF	Activating Transcription Factor
BMI	Body mass index
BP	Blood pressure
CNS	Central nervous system
CTS	California Teachers Study
CI	Confidence interval
COX	Cyclooxygenase
CVD	Cardiovascular disease
DAG	Directed acyclic graph
DEHP	di-(2-ethylhexyl) phthalate
HIF-1	Hypoxia-inducible factor 1
HR	Hazard ratio
ICCC	International Classification of Childhood Cancer
IRB	Institutional review board

IRR	Incidence rate ratio
NHANES	National Health and Nutrition Examination Survey
NICU	Neonatal intensive care unit
NSAIDs	Non-steroidal anti-inflammatory drugs
OSHPD	Office of Statewide Health Planning and Development
OR	Odds Ratio
RAS	Renin-angiotensin system
RR	Risk Ratio
RPE	Retina pigment epithelium
SBP/DBP	Systolic/Diastolic blood pressure
SEER	Surveillance, Epidemiology, and End Results
SES	Socioeconomic status
sFlt-1	soluble fms-like tyrosine kinase 1
UKCCS	United Kingdom Childhood Cancer Study
VEGF	Vascular endothelial growth factor

ACKNOWLEDGEMENT

I would like to express my deepest gratitude to my mentor and the chair of my dissertation committee, Dr. Beate Ritz, for her constant inspiration, guidance and support. I would like to thank Dr. Ritz for giving me the opportunity to join her lab as a PhD trainee in 2014 and be able to pursue my dream of becoming an epidemiologist. Through these years, she has set an example for me by always striving for excellence in research, sharing knowledge without reservation, caring about each study participant, and being gracious in both the up and down moments in life. It has been the greatest fortune of my education to be able to work with and learn from her.

I am also very grateful to Dr. Julia Heck, my unofficial advisor and the principal investigator for my dissertation projects, for her constant encouragements, guidance, supports, and the many constructive comments she provided on the conception, analysis, and writing of my projects. My gratitude is extended to other committee members who are dedicated researchers or doctors, Drs. Marjan Javanbakht, Li Li, Wendie Robbins, and Tom Davidson, for their expertise, encouragement, invaluable advice and strong support. Special thanks to Dr. Zeyan Liew for providing me with guidance and encouragements throughout the training.

I would also like to thank my dear labmates and friends, for making my graduate study life much more enjoyable, and providing help and emotional support during challenging moments in both research and in life. I would like to acknowledge the wonderful professors and staffs at the UCLA Department of Epidemiology for teaching and supporting me.

Last but not least, my heartfelt appreciation goes to my parents, Baoguo Xu and Hongyan Sun, who always believed in me and made education possible for me; and to my boyfriend Le Shu, who met me part way through my graduate studies and gave me love and encouragement to finish.

Chapter 2 is incorporated from Xu, X., Ritz, B., Cockburn, M., Lombardi, C., & Heck, J. E. (2017). Maternal Preeclampsia and Odds of Childhood Cancers in Offspring: A California Statewide Case–Control Study. *Paediatric and Perinatal Epidemiology*, *31*(2), 157-164. © John Wiley and Sons, with kind permission from John Wiley and Sons.

VITA

EDUCATION

2014	Master of Public Health University of California, Los Angeles, CA, USA
2012	Bachelor of Science, Pharmaceutical Science Bachelor of Science, Economics Peking University, Beijing, China

RESEARCH EXPERIENCE

2014 – Present	PhD trainee Department of Epidemiology University of California, Los Angeles
2013 – Present	Research Associate Department of Research & Evaluation Kaiser Permanente Southern California
2013 – 2015	Student Researcher Department of Radiation Oncology UCLA David Geffen School of Medicine

PEER-REVIEWED PUBLICATIONS

Xu, X., Ritz, B., Cockburn, M., Lombardi, C., and Heck, J., 2016. Maternal Preeclampsia and Risk of Childhood Cancers in Offspring. ***Pediatric and Perinatal Epidemiology.*** DOI: 10.1111/ppe.12338

Haque, R., Shi, J., Schottinger, J.E., Chung, J., Avila, C., Amundsen, B., **Xu, X**., Barac, A. and Chlebowski, R.T., 2016. Cardiovascular disease after aromatase inhibitor use. ***JAMA oncology***. DOI: 10.1001/jamaoncol.2016.0429

Qi, X., Wang, J., Gomez, C.L., **Xu, X.**, Shao, W., King, C., Low, D., Steinberg, M. and Kupelian, P.A., 2016. Patient-reported outcome after prostate stereotactic body radiation therapy—an analysis of dosimetric correlation of minimally import difference for the Expanded Prostate Cancer Index Composite Short Form (EIPIC-26). ***International Journal of Radiation Oncology• Biology• Physics***, 96(2), p.E227.

Gomez, C.L.+, **Xu, X.,** Qi, X., Wang, P.C., Kupelian, P., Steinberg, M. and King, C., 2015.

Dosimetric parameters predict short-term quality-of-life outcomes for patients receiving stereotactic body radiation therapy for prostate cancer. ***Practical radiation oncology***, *5*(4), pp.257-262. (Co-first author)

Haque, R., **Xu, X.**, Yood, M.U., Cassidy-Bushrow, A.E., Van Den Eeden, S.K. and Potosky, A.L., 2015. Cardiovascular Disease Risk and Androgen Deprivation Therapy in Patients with Localized Prostate Cancer. ***Pharmacoepidemiology and Drug Safety***, 24, pp.323-324.

Ren, Z., **Xu, X.**, Bao, Y., He, J., Shi, L., Deng, J., Gao, X., Tang, H., Wang, Y., and Lu, L., 2015. The impact of genetic variation on sensitivity to opioid analgesics in patients with postoperative pain: a systematic review and meta-analysis. ***Pain physician***, 18, pp.131-152.

Qi, X., Wang, J., Gomez, C.L., Shao, W., **Xu, X.**, King, C., Low, D., Steinberg, M. and Kupelian, P., 2016. Plan quality and dosimetric association of patient-reported rectal and urinary toxicities for prostate stereotactic body radiotherapy. ***Radiotherapy and Oncology***, 121(1), pp.113-117.

Gomez, C.L., **Xu, X.**, Qi, X., Wang, P., Kupelian, P., Steinberg, M. and King, C., 2014. Dosimetric Parameters Predict Quality of Life Outcomes for Patients Receiving Stereotactic Body Radiation Therapy for Prostate Cancer. ***International Journal of Radiation Oncology• Biology• Physics***, 90(1), p.S419.

CONFERENCE PRESENTATIONS

Xu, X., Ritz, B., Cockburn, M., Lombardi, C., and Heck, J., 2016. Maternal Preeclampsia and Risk of Childhood Cancers in Offspring. ***Society for Epidemiologic Research***, June 2016, Miami (**Oral Presentation**).

Haque, R., **Xu, X**., UlcickasYood, M., Cassidy-Bushrow, A., Tsai, H., Keating, N., Van Den Eeden, E., and Potosky, A., 2015. Cardiovascular Disease Risk and Androgen Deprivation Therapy in Patients with Localized Prostate Cancer. ***International Society for Pharmacoepidemiology,*** August 2015, Boston (**Poster presentation**).

Chapter 1. Introduction

1.1 Preeclampsia

Definition of Preeclampsia

Preeclampsia/Eclampsia is a complicated multifactorial pregnancy-induced syndrome that occurs after the 20th week of gestation. Together with gestational hypertension, the continued presence of chronic hypertension, and superimposed preeclampsia on pre-existing hypertension, preeclampsia is one of the four categories of hypertensive disorders during pregnancy, which affect 5%-10% of all pregnancies.[1] Preeclampsia/Eclampsia is a leading cause of maternal mortality and morbidity, as well as a major cause of adverse effects on fetal well-being both worldwide and in the US. Specifically, one third of severe maternal morbidities, 10%-15% of maternal deaths in low-/middle- income countries and 30%-35% of preterm births worldwide are attributed to preeclampsia.[1-4] The diagnostic criteria of preeclampsia have been changing during the past two decades. The most recent guidelines for hypertension during pregnancy by the American College of Obstetricians and Gynecologists (ACOG, 2013) define preeclampsia as: new onset of hypertension (Systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg on two occasions at least four hours apart) and either proteinuria or end-organ dysfunction after 20 weeks of gestation in a previously normotensive woman. Two previous diagnostic criteria had been eliminated due to low specificity: edema, and "increases of 30 mmHg in systolic pressure and/or 15 mmHg in diastolic pressure". Likewise, proteinuria has also been eliminated from the diagnostic guideline, since it may occur late and patients should not wait until its manifestation to be diagnosed of preeclampsia and receive therapy.[2, 5] Although the definition of "severe preeclampsia" is still inconclusive, there is general consensus that severely

elevated blood pressure (>160 mmHg systolic or >110 mmHg diastolic) and the existence of deteriorating clinical features (proteinuria >5g/24h, neurologic disturbances, pulmonary edemas, hepatic dysfunction, renal compromise, thrombocytopenia) are factors that determine the need for prompt confirmation and treatment. [6] Eclampsia is defined as one or more convulsions happening to women with preeclampsia that cannot be attributed to any other cause.[7, 8]

Epidemiology of Preeclampsia

A population-based study in Washington State estimated the overall rate of preeclampsia as 3.11 per 100 singleton deliveries.[9] The rate is slightly higher (3.4%) in the whole United States, with a more diverse population, compared with Washington State alone.[10] A similar overall rate of preeclampsia was found in another population-based study using the Norway birth registry, in which the rate ranged from 3.6% in 2008 to 4.4% in 1999.[11] A recent cohort study (N=21,589) in Northern California observed a 4.5% overall rate of preeclampsia among mothers who gave birth between Jan.1st of 2010 to the end of 2012.[12] However, the observed higher rate of preeclampsia may be because the cohort is based on health plan setting and their blood pressure and fetal wellness were well monitored during pregnancy. Preeclampsia/Eclampsia cause approximately 50,000 maternal deaths worldwide each year of which the majority happens in developing countries. Although the disease-associated mortality rate among pregnant women is reduced in developed countries due to proper maternal health care and timely treatment, preeclampsia/eclampsia still accounts for a large proportion of maternal mortality.[3, 13]

Women with a history of preeclampsia in previous pregnancies were ten times more likely to have preeclampsia in subsequent pregnancies compared with women without a disease history.

The recurrence rate of preeclampsia can be as high as 65% among women who had preeclampsia onset during the second trimester of the previous pregnancy.[14, 15] The recurrence risk is even higher among women with previous preterm births associated with preeclampsia, because that indicates severe preeclampsia during the previous pregnancy.[11] Besides previous history of preeclampsia, preeclampsia has a broad array of risk factors including family history (family history of preeclampsia or cardiovascular disease: RR =1.70-4.93), maternal age older than 40 (RR = 1.10-1.68), nulliparity (RR = 1.28-6.61), multiple birth (RR = 2.10-4.19), longer interval between two pregnancies (RR = 1.12 with one year increase) and preexisting maternal conditions (chronic hypertension: RR = 1.15-1.40; pre-existing diabetes: RR = 1.10-1.35; BMI>35: RR = 1.56-2.88; chronic autoimmune disease: RR = 1.1-42.3).[7, 14, 16-18] Paradoxically, strong epidemiologic evidence including the dose response has shown a protective effect of smoking on preeclampsia (RR = 0.5-0.7).[19, 20] However, the biological mechanism or the specific cause of the protective effect is not clear.[19] Whether racial disparities exist in preeclampsia is inconsistent: A cohort study based on health plan data in California in the 1990s found increased preeclampsia rate in African American women (OR = 1.41) and decreased rate in Asian (OR = 0.79) and Latina (OR = 0.90) women;[21] The lower rate of preeclampsia in Hispanic women cannot be confirmed in another study in Massachusetts;[22] large population based study in New York State from 1993-2002 found the hospitalization rate due to preeclampsia was higher among African American women (3.3%) and Hispanic women (3.0%) compared with the Whites (2.0%) probably because of the higher obesity rate and lower rate of access to prenatal care in African American women and Hispanic women in New York State.[23] A case series in California observed the mortality rate due to preeclampsia is higher among foreign-born Hispanic women and African American women, probably due to delayed seeking of care and inconsistent care.[24]

Pathology and Pathophysiology of Preeclampsia

Maternal comorbid conditions arising from severe preeclampsia include thrombocytopenia, renal insufficiency, impaired liver function, pulmonary edema and newly-developed visual or cerebral changes.[25] A pathologic laboratory study showed that expanded endothelia cells present in renal biopsy samples from women with preeclampsia, which is different from the mechanistic damage that occurs from high blood pressure. This also implies that preeclampsia is a disorder beyond pregnancy-induced hypertension.[26, 27]

Previous research supports that preeclampsia is the consequence of reduced placental perfusion, which is suspected to be caused by abnormalities in implantation and vascular remodeling. As a result, there is reduced blood flow to organs other than the placenta, thus causing hemorrhage and necrosis to multiple organ systems including liver, kidney, brain and intervillous space.[26] Those pathological changes (organ hemorrhage and necrosis) to women with preeclampsia revert to normal soon after delivery, which suggests that unique features of pregnancy lead to the change.

During a normal pregnancy, spiral arteries connecting the maternal-fetal circulation experience significant remodeling as a result of trophoblastic invasion, an adaptive process which allows for the increasing blood flow necessary for pregnancy.[26, 28, 29] By contrast, this adaption does not occur to women with preeclampsia. Lacking these changes, abnormal trophoblastic implantation happens and leads to poor placental perfusion which subsequently releases factors (i.e. soluble

fms-like tyrosine kinase 1 (sFlt-1)) that changes the maternal endothelial activities and alters endothelial function. Biomedical research shows that endothelial dysfunction serves as an important link between reduced placental perfusion and systemic diseases.[26, 30]

Effects of Preeclampsia on the Fetus and Children's Long-term Health

Preeclampsia accounts for 12% of infants born small for gestational age, 25% of total stillbirths and neonatal deaths in developing countries.[3] A secondary analysis using clinical trial data showed that the perinatal outcomes are even worse among mothers with recurrent preeclampsia compared with nulliparous mothers who developed preeclampsia, with higher rates of preterm delivery, abruptio placentae, and neonatal death.[15] A population-based study in Washington State found that children who were exposed to preeclampsia during pregnancy were more likely to have low birth weight, lower Apgar score at 5 min, more likely to experience neonatal seizures, neonatal sepsis and neonatal intensive care unit (NICU) admission. The rate of adverse perinatal outcomes was much higher among women with early onset (<20 weeks) preeclampsia compared with women with late onset (20-34 weeks).[11]

A Danish cohort study of 1,618,481 singletons who were followed for up to 27 years observed elevated risks of hospitalization due to various diseases among children who exposed to preeclampsia during pregnancy, including infectious and parasitic diseases (IRR=1.2), neoplasms (IRR=1.3), diseases of blood and blood-forming organs and disorders involving immune mechanism (IRR=1.5), endocrine, nutritional, and metabolic diseases (IRR=1.6), respiratory diseases (IRR=1.2), and congenital malformations (IRR=1.1).[31] Higher levels of systolic and diastolic blood pressure and stroke have also been reported by studies using medical record

linkage.[32] In another follow-up study of neonatal outcomes within 35 weeks after delivery, no difference in incidence rates were found for neonatal death or respiratory distress syndrome between children born to mother with preeclampsia and those born to normotensive mothers.[33]

Management of Preeclampsia

Since preeclampsia onset can occur much earlier than the manifestation of clinical symptoms, various screening methods have been tested to identify the high-risk population in the last decade, but none has shown acceptable predicative accuracy.[34] Audibert et al. proposed a first-trimester screening method using "maternal serum biomarkers and uterine artery Doppler" combined with clinical characteristics, which can detect 75% early-onset preeclampsia with 10% false positive rate.[35] Investigators are still searching for biomarkers to detect preeclampsia with high sensitivity and specificity.[36]

Frequent clinical supervision of fetal and maternal well-being is required for mothers with preeclampsia during the whole pregnancy. For mild preeclampsia, there is not agreement on the optimal management with physicians typically recommending either hospitalization, complete bed rest, and/or antihypertensive prescriptions.[37-40] If the mother does not have symptoms of severe preeclampsia, outpatient care is sufficient for mothers at less than 37 weeks gestation. Once severe disease is present, prompt hospitalization is required to prevent further organ dysfunction and eclampsia.[39] Magnesium sulfate is usually recommended to prevent convulsions and prolong pregnancy.[38, 39] There is no definitive treatment for preeclampsia other than delivery, but the choice between delivery and treatment should be based on both maternal and fetal

conditions: indications for delivery in preeclampsia include later than 38 gestational weeks, platelet count <100,000 cells/mm,[3] progressive deterioration in organ function, suspected abruptio placentae, persistent severe headaches, visual changes, epigastric pain, severe fetal growth restriction, nonreassuring fetal testing results and oligohydramnios.[29]

1.2 Childhood Cancer

Childhood Cancer Classification

Although cancer is a relatively rare disease in children, its diagnosis could easily become a life-altering event for the patients and their families. Childhood cancer is not a single disease entity, but rather a combination of different groups and subgroups of malignancies. Childhood cancer is primarily classified based on tumor morphology, rather than by primary site, as is done for adult cancer. The International Classification of Childhood Cancer (ICCC) has classified childhood cancer into twelve groups, I) Leukemia, II) Lymphomas and reticuloendothelial neoplasms, III) CNS and miscellaneous intracranial and intraspinal neoplasms, IV) Neuroblastoma and other peripheral nervous cell tumors, V) Retinoblastoma, VI) Renal tumors, VII) Hepatic tumors, VIII) Malignant bone tumors, IX) Soft tissue and other extraosseous sarcoma, X) Germ cell & trophoblastic tumors & neoplasms of gonads, XI) Other malignant epithelial neoplasms and melanomas, XII) Other and unspecified malignant neoplasms.[41] The incidence of childhood cancer varies greatly by type of histology, site of cancer origin, sex, age and race.

Burden of Childhood Cancers

Globally, an estimated 175,000 cases of cancer are diagnosed yearly in children from the ages of 0 to 15.[42] Since 1975, the incidence rate of pediatric cancer in US has been increasing steadily at an annual rate of 0.6%, possibly due to changes in environmental or other risk factor distributions as well as improved diagnosis.[43] In Nordic countries including Denmark, the childhood cancer rate has stayed relatively stable at around 18 per 100,000 population.[44] In contrast, the overall 5-year survival rate of childhood cancer has increased to approximately 80%, thanks to advances in treatment and healthcare.[43] More than 24,000 persons in Nordic countries were survivors of childhood cancer at the end of 2005, accounting for approximately 0.1% of the population.[45] However, childhood cancer survivors are subject to higher risk of all-cause morbidity and mortality up to 30 years after diagnosis, according to the Childhood Cancer Survivor Study (CCSS).[46] Moreover, it is estimated that 60% to more than 90% of survivors develop chronic health conditions later in life.[47] The increased incidence of chronic health conditions was primarily attributed to recurrence of disease and subsequent neoplasms that are associated with the late effects of cancer treatment, as well as an underlying genetic predisposition related to the primary cancer.[48] Olsen *et al* reported a cumulative risk of 48% for a second primary cancer in Nordic childhood cancer survivors, with the highest rates for subjects in the era of intensive chemotherapy usage.[45] In addition, late effects could lead to problems in growth, organ function, reproductive capacity and negative psychosocial sequelae.[49] Due to the continuous developments in cancer biology, radiological science and supportive systems, the prevalence and spectrum of treatment effects is expected to drop.[50] Yet contemporary childhood cancer survivors are still expected to frequently experience higher treatment-related morbidities and mortalities across their lifespan.

Epidemiology (Incidence, Risk Factors, Survival Rate)

Following accidents, cancer is the second leading cause of pediatric mortality among children 1-14 years old in the US. The incidence rate of childhood cancer in the industrialized countries is approximately 140 per million, with a 0.6% increasing rate since 2007.[51, 52] It is estimated by American Cancer Society that there will be 10,380 incident childhood cancer cases and 1,250 deaths among children under 15 years old in 2015.[52] The most frequent diagnosis groups of all childhood cancers according to SEER (Surveillance, Epidemiology, and End Results) data are leukemia at 30%, brain and central nervous system (CNS) tumors at 26%, followed by neuroblastoma at 6%, soft tissue sarcomas at 6%, Wilms tumors at 5%, non-Hodgkin lymphomas at 5% and Hodgkin lymphomas at 3%. These are consistent with data from the German Childhood Cancer Registry (GCCR).[52, 53] In California, the most prevalent childhood cancer is also leukemia at 35%, followed by brain and CNS tumors at 22%.[54] Notably, the rate of childhood cancer in California is higher (170 per million) compared to the US average. This could be attributed to the difference in the racial/ethnic distribution in California: California has significantly higher proportions of Hispanics and Asians (US Census Bureau Website) among the non-white population, which have a higher incidence of childhood cancer compared with the African-American population (SEER).[55]

Overall survival rates for all types of cancer (75%) were similar between the US and European countries from 1985-1999. Five-year survival rates of childhood cancers ranged from 60%-90% according to the type of diagnosis, with the highest survival seen in retinoblastoma and Hodgkin lymphoma, and patients with AML experiencing the lowest survival. The death rate due to childhood cancer is decreasing significantly, with 2.1 per 100,000 in 2011 compared to 6.3 per

100,000 in 1970.[52] The improving survival rate is believed to result from better diagnostic technology, early detection, and more advanced treatment.[53]

Ionizing radiation acquired from either medical settings or the environment is the only consistently reported preventable risk factor for childhood cancers.[56, 57] Since pediatric tumors are diagnosed at an early age, the pathogenesis of childhood cancers has been hypothesized to originate from fetal development and fueled by fetal growth. Indeed, population-based studies are able to link various pregnancy factors with childhood cancer. For example, maternal age was linked to overall childhood cancer (5-year increase OR = 1.08).[58] High birth weight has been associated with acute lymphoblastic leukemia (OR = 1.18-1.26).[59, 60] Large for gestational age has been associated with Wilms tumor (OR: 2.1) and connective/soft tissue tumors (OR = 2.1). While small for gestational age has been associated with acute myeloid leukemia (OR =1.8) and neuroblastoma (HR = 2.6).[61, 62] Parity also has been associated with childhood cancer, yet the direction varies by specific cancer types.[63]

1.3 Age Related Macular Degeneration

Classification of AMD

Age-related macular degeneration is a chronic eye disease affecting the central retina, and can cause irreversible vision loss if left untreated. There are two clinical forms of AMD: "dry" AMD, which is usually asymptomatic at the earlier stage and develops into geographic atrophy if the condition worsens; and "wet" AMD, which is also called neovascular AMD and associated with a more rapid progression.[64] "Dry" AMD is a more prevalent subtype, accounting for 80% of

cases. Although several therapeutic options are available to slow the progression of dry AMD, there is no cure for the condition. A hallmark of dry AMD is the presence of drusen, a metabolic end product from degenerated retina pigment epithelium (RPE). Accumulating drusen disrupts photoreceptor cells, causing blind spots in the central vision, gradually progress to the atrophic stage (geographic atrophy). Over time, dry AMD can also develop into the less common "wet" form, which involves blood vessel generation. In the wet form of the disease, leaky blood vessels abnormally grow into the retina, causing swelling and bleeding of the retina. The "wet" form of AMD is more damaging and may lead to severe vision loss. However, the progression of neovascular AMD can be effectively stopped or slowed by anti-vascular endothelial growth factor (VEGF) treatment.[64, 65]

Prevalence and Incidence of AMD

A large population-based cohort study reported a 15-year cumulative incidence of 14.3% and 3.1% for early- and late-stage AMD respectively.[66] In the US, 11.5% of the population is suffering from AMD of any type. Specific to the late-stage disease, the prevalence among those 40 years or older is 1.5% and it is expected to double by 2020.[65] The Baltimore Eye Survey reported racial differences in AMD prevalence: AMD is four times more prevalent in Whites compared with African Americans.[67] Asians were found to have similar risk of late-stage AMD to Whites, but were disproportionately affected by polypoidal choroidal vasculopathy, a variant of AMD.[68] In the Rotterdam Study, one fourth of the subjects with early-stage AMD at baseline developed a more severe-stage disease in two years of follow-up.[69] The progression rate of late-stage AMD from patients with early-stage AMD was 5% over five years in the Blue Mountains Eye study,[70] and was 15% over 15 years in the Beaver Dam Eye study.[66]

Burden of AMD

AMD is becoming a public health issue globally with an expected growth of the older population (≥ 65 years old). The projected number of AMD cases will be 88.5 million in the US by 2050.[65] Following cataract and glaucoma, AMD is the third leading cause of blindness worldwide, a primary cause of vision deficiency in industrialized countries, and leads to significantly decreased quality of life and increased dependence on caregivers. The direct medical cost of AMD was US$575–733 million, not considering the cost of support service and productivity loss.[64, 65, 71]

Risk Factors for AMD

AMD is a multifactorial disorder which involves genetic factors, environmental factors and the aging retina in its etiology.[72] Family studies showed more than two times increased risk of AMD among people who had first-degree relatives having the disease history, as well as earlier onset of the condition,[73, 74] suggesting the contribution of genetic factors. Several genes have been associated with the risk of developing AMD, including *CFH, CFB* and *C2, ARMS2, APOE*, and *ABCA4-ABCE* genes. Susceptible genes found in complement pathway and oxidative stress pathways supported the presence of complement-mediated inflammation and oxidative damage in the pathogenesis of AMD.[64, 65]

Smoking is the strongest environmental risk factor for AMD. A pooled analysis of three population-based studies found the risk of AMD doubled among current smokers, and 1.3 times elevated among former smokers when comparing with non-smokers.[75] Increased amount of pack-years have also been found to increase AMD risk, for both neovascular AMD and

geographic atrophy.[76] Proposed mechanisms of the damaging effect on retina include the reduction of macular pigment, the activation of inflammatory mediators by nicotine and cotinine, and the increased oxidative stress.[77-79]

Previous cataract surgery was consistently found to increase AMD risk in prospective studies, case-control studies, and cross-sectional studies, with odds ratios, ranging from 1.5 to 3.0.[80] In a meta-analysis, other suggested risk factors were higher BMI (OR = 1.3-1.5), hypertension (OR = 1.0-1.5), cardiovascular disease (OR = 1.2-2.2), and plasma fibrinogen (OR = 1.0-1.4).[80] Although the AREDS study assessing risk factors for AMD did not find sun exposure as a risk factor for the progression to advanced AMD,[76] light-induced toxic effects on retina have been proven in animal studies and hypothesized to affect AMD risk.[81]

The Age-Related Eye Disease Study (AREDS), a multicenter randomized trial following 3,640 participants for 10 years, provided strong evidence of the protective effect of antioxidant supplements (Vitamin C, Vitamin E, beta-carotene) and mineral zinc for the progression of AMD.[82] The AREDS2 study reported beneficial effect of lutein/zeaxanthin comparing with beta-carotene in preventing AMD progression.[83] High intake of omega-3 fatty acids had also been proved to reduce risk for late-stage AMD.[84]

1.4 Literature Review

Preeclampsia and Childhood Cancer

A case-control study with 1867 cases and 2057 controls in Germany found no association between hypertension during pregnancy or edema with any type of childhood cancer with or

without any antihypertensive medication use. However, although hypertension and edema are two common characters of preeclampsia, this exposure definition is too broad to clearly define preeclampsia and may dilute the possible association between preeclampsia and childhood cancer.[85] In the same study, diuretics or other antihypertensive drug use has been found to be associated with increased risks of ALL (OR = 1.99, 95% CI: 1.01-3.93) and neuroblastoma (OR = 3.16, 95% CI: 1.03-9.70), which the author believed was caused by toxic effect of the drugs. Nevertheless, the observed association may be due to the underlying severe preeclampsia that required pregnant mothers to receive antihypertensive medications.[85] The same explanation can be applied to Stalberg et al.'s study, where maternal beta-blockers use were found to have a five-time risk of having a child with brain tumors (OR = 5.3, 95% CI 1.2-24.8).[86] In a study nested within the United Kingdom Childhood Cancer Study (UKCCS), a sharply elevated risk of hepatoblastoma was observed to be associated with fetal exposure to severe preeclampsia or eclampsia among children under 15 years old (OR = 52.5). However, the estimate was based on only 18 available hepatoblastoma cases. The authors stated that instead of being an etiological factor, very low birth weight may mediate the effect of preeclampsia/eclampsia on hepatoblastoma.[87] Heck et al. confirmed the adverse effect on hepatoblastoma (OR = 15.4, 95% CI 10.7–22.3) using California Cancer Registry data.[88] In an earlier study on hepatoblastoma by McLaughlin et al. in New York State, maternal hypertension was not indicated as a risk factor of hepatoblastoma. But the category of maternal hypertension combined mother with preeclampsia and those with chronic hypertension. Secondly, the authors included birth weight into their multivariate regression model for the hepatoblastoma risk estimate, which might block the causal pathway of maternal hypertension on cancer in offspring.[89] Roman et al. conducted another case control study on hematological malignancies nested within the UKCCS study and reported an

elevated risk of non-Hodgkin's lymphoma among women with preeclamptic pregnancies (OR = 3.8 for severe preeclampsia and OR = 1.7 for all preeclampsia diagnoses). No elevated risks were seen for Hodgkin's lymphoma (OR = 0.7), ALL (OR = 1.0) and AML (OR = 1.0). However, the authors did not account for other perinatal characteristics such as gestational age, fecundity and previous history of pregnancy complications for their analysis. The study might also suffer from self-selection problems since the risk of preeclampsia was 10% among the controls, much higher than the general population.[90]

In a Danish case-control study of prenatal risk factors of testicular cancer which used a self-administered questionnaire, preeclampsia was not found to be associated with testicular cancer, although whether confounding factors such as twin birth or history of previous preterm birth were adjusted was not stated by the authors.[91] The null association between preeclampsia and testicular germ cell tumor was also observed in another population based study in Nordic countries.[92] The effect of preeclampsia on neuroblastoma was controversial. Hamrick et al. did not found any adverse effect in a matched case control study, after adjusting for the race/ethnicity and socioeconomic status of the mothers,[93] yet Bjorge et al. indicated that preeclampsia increased the risk of early neuroblastoma (diagnosed before 18 months) by 2 times (RR=2.3).[94] No association has been found for CNS tumor or leukemia in California linked-registry studies.[95, 96]

The pathogenesis of preeclampsia and tumor development share some common features.[97, 98] Among the important pathogenic aspects of preeclampsia, placental hypoxia[99] and oxidative stress can induce tumorigenesis in offspring.[100] Hypoxia could contribute to subsequent

preeclampsia through disturbed placental development.[101] Placental insufficiency could further place the fetus of preeclamptic patient develop in a significantly hypoxia environment from early in pregnancy. An adaptive response to the long-term hypoxic fetal environment will cause increase in detrimental molecules such like Hypoxia-inducible factor 1 (HIF-1), whose expression level is associated with tumorigenesis processes including angiogenesis, cell survival, glucose metabolism and invasion.[102, 103] Moreover, hypoxia is capable of perturbing fetal epigenetic programming. Although no direct evidence is available that links hypoxia induced epigenetic dysregulation to tumorigenesis, abnormal epigenetic changes have been shown to be highly prevalent in pediatric tumors.[104, 105] On the other hand, traces of oxidative stress are detectable in both the placenta and maternal circulation.[106, 107] Preeclampsia-induced oxidative stress is sufficient to cause oxidative DNA damage and epigenetic dysregulation, independent of fetal growth restriction.[108, 109] Oxidative stress also mediates a series of transcription factors that participate directly in tumor growth, including growth factors, inflammatory cytokines and cell cycle regulatory molecules.[110] In addition, despite considerable heterogeneity of placenta sample of preeclampsia patients, Activating Transcription Factor 3 (ATF3) is consistently found to be upregulated, triggered by hypoxia and oxidative stress.[97, 111] Overexpression of ATF protects against apoptosis and could also predispose the developing fetus to cancer development.[112]

Medication Use and AMD

a. Anti-inflammatory drugs and AMD

Local inflammation is believed to be responsible for drusen formation, a typical feature of AMD. The inflammatory response is stimulated by cellular remnants and debris produced by degenerate RPE cells, analogue to the process of the accumulation of extracellular waste in Alzheimer's

disease and atherosclerosis.[113] NSAIDs has the potential to regulate vascular endothelial growth factor (VEGF) level through its suppression effect on COX-2, a pro-angiogenesis factor, thus inhibit the development of neovascular AMD.

In animal studies, oral administration of non-steroidal anti-inflammatory drugs (NSAIDs) inhibits retinal VEGF expression and vascular leakage.[114] A ten-fold lower prevalence of AMD was found in a cohort of patients with rheumatoid arthritis comparing to other cohorts with similar race composition, suggesting a protective effect of the long-term anti-inflammatory drugs taken for this condition.[115] In the AREDS study, regular anti-inflammatory drug use for five years largely reduced the risk of progression to central geographic atrophy (OR = 0.23), but not neovascular AMD (OR = 0.99).[76] A nested case-control study in veterans found reduced AMD risk in subjects filling prescription of any anti-inflammatory medications (OR = 0.15) and for each subtype of anti-inflammatory medication.[116] No association was observed between systematic NSAIDS use and five-year incidence of early- or late-stage AMD in the Blue Mountain Eye study or the Beaver Dam Eye Study, both of which were population-based prospective cohort studies.[117, 118]

b. Aspirin and AMD

A recent meta-analysis reported a pool odds ratio of 1.43 for early-AMD following aspirin use, ignoring the dose and duration of use. The strongest observed odds ratio was 2.5 in all the enrolled studies.[119] The Blue Mountain Eye Study observed a dose-response relationship of aspirin use in developing neovascular AMD over 15 years follow-up, but no increased risk was found in progression to neovascular AMD.[120] A dose-response was shown in early-stage AMD in

the European Eye Study.[121] Plausible biological explanations of the observed association include the reduced synthesis of prostacyclin by aspirin, which leads to hypoxia and consequently stimulates neovascularization, and increased lipid oxidation. In contrast to the prospective cohort studies and cross-sectional studies, a clinical trial in women suggests 20% decreased the risk of AMD over 10 years following 100 mg aspirin use on alternate days. The proposed mechanism of protection is through aspirin's beneficial effect on cardiovascular disease and atherosclerosis, the hypothesized risk factor for AMD development. Moreover, aspirin can up-regulate the production of a local endogenous anti-inflammatory mediator.[122]

c. Antihypertensive Medications and AMD

In a pooled analysis of three population-based cohort studies of AMD, a moderate elevated risk in early AMD was found in subjects ever exposed to antihypertensive medication (OR = 1.2), which was consistent with the finding from a mechanistic study assessing the role of antihypertensive drugs in lipofuscin deposition, a pathogenic factor of AMD.[123] Lipofuscin accumulation was shown in cultured human RPE cells after exposure to the β-blocker propranolol and calcium antagonists.[124] A case-control study using the United Kingdom General Practice Research Database found a slightly increased risk of AMD diagnoses patients with a history of hypertension, but not in Thiazide diuretics users.[125] In the Beaver Dam Eye study, taking antihypertensive medications was associated with impaired vision when reading regular newsprint, but not in other aspects of eye functions.[126] Any biological mechanism was not established.

1.5 Specific Aims for This Dissertation

We hypothesized that preeclampsia may affect cancer risk in the offspring either indirectly through adverse neonatal outcomes or directly as a consequence of an altered maternal-fetal circulation resulting from poor perfusion and conducted a large, population-based case control study to examine the association between preeclampsia and various childhood cancers including some rare subtypes (Chapter2).

In Chapter 3, we assumed that aspirin and other NSAIDs may have the potential to suppress the development of AMD through cardioprotective properties (from aspirin only) and their anti-inflammatory properties and tested the assumption in a prospective cohort of 88,481 female California teachers and school professionals who were followed since 1995-1996 until Dec.31st, 2012. In the same cohort, we also examined hypertension and its treatment by antihypertensive medications as potential risk factors for the development of AMD (Chapter 4).

Chapter 2. Maternal Preeclampsia and Odds of Childhood Cancers in Offspring — A California Statewide Case-Control Study

2.1 Abstract

Background: Preeclampsia is a major cause of adverse effects on fetal health. We examined associations between fetal exposure to preeclampsia and subsequent odds of childhood cancers.

Methods: We obtained childhood cancer cases (n=13,669) diagnosed at five years old or younger between 1988 and 2012 from the California Cancer Registry and linked them to birth certificates. Controls (n=271,383) were randomly selected from all California births and frequency matched to cases by birth year. We obtained data regarding preeclampsia during pregnancy, labour, and delivery from the medical worksheet of the electronic birth record. We used unconditional logistic regression models with stabilized inverse probability weights to estimate the effect of preeclampsia on each subtype of childhood cancer, taking into account potential confounding by pregnancy characteristics. Marginal structural models were fitted to assess the controlled direct effects of preeclampsia, independent of preterm delivery and NICU admission.

Results: Although a null association was observed for all cancer subtypes combined (odds ratio (OR) 1.0, 95% confidence interval (CI) 0.9, 1.2), preeclampsia was found to be associated with increased odds of two histologic subtypes of germ cell tumours: seminomas (OR 8.6, 95% CI 1.9, 38.4) and teratoma (OR 3.0, 95% CI 1.7, 5.4), but not yolk sac tumours in children. Odds remained elevated after adjusting for preterm delivery and NICU admission. Increases in odds were also observed for hepatoblastoma, however this association was attenuated in marginal structural models after accounting for NICU admission.

Conclusions: These findings suggest that maternal preeclampsia is associated with higher odds of some rare childhood cancers and may shed light on new aetiologic factors for these cancers.

2.2 Introduction

Preeclampsia, a subtype of hypertensive disorders during pregnancy, is a complex pregnancy-induced syndrome that occurs after the 20th week of gestation. Preeclampsia or eclampsia account for one third of severe maternal morbidities, 10%-15% of maternal deaths in low to middle- income countries and 30%-35% of preterm births worldwide.[2, 3] Preeclampsia is thought to be the consequence of reduced placental perfusion, and endothelial cell dysfunction, processes that causes persistent placental hypoxia, and subsequent release of antiangiogenic factors into the maternal system. These adaptive changes can alter placental development and even contribute to adverse health outcomes in offspring in the long run.[30]

By comparing placenta specimens from preeclampsia-complicated pregnancies with those from normotensive pregnancies, detrimental cellular signalling markers have been found to be overexpressed in umbilical cords from preeclamptic pregnancies and have been connected to chronic adverse health effects in offspring.[127] Whether placental transmission of detrimental factors can have tumorigenic effects on the fetus remains unknown.

Cancer is the second leading cause of mortality among children in the US, with very few well-established preventable causes. Since childhood cancers are diagnosed at an early age, it has been hypothesized that its pathogenesis is initiated during fetal development and possibly fueled by fetal growth. Indeed, population-based studies have linked various perinatal factors with childhood cancers.[58, 62, 88, 128, 129] Low birthweight and preterm delivery, both common consequences of preeclampsia, have been shown to be associated with increased childhood cancer risk.[88, 92, 128] Because of the rarity of cancers in young children, only a small number of

previous registry-based studies have evaluated the association between preeclampsia and some types of paediatric cancers with little discussion of potential biologic mechanisms. Some but not all studies have found positive associations.[87, 88, 90, 92, 129]

We hypothesise that preeclampsia may affect cancer risk in the offspring either indirectly through adverse neonatal outcomes or directly as a consequence of an altered maternal-fetal circulation resulting from poor perfusion and conducted a large, population-based case control study to examine the association between preeclampsia and various childhood cancers including some rare subtypes.

2.3 Methods

Childhood cancer cases (n = 13,677) who were born 1983-2011 and diagnosed at five years of age and younger between 1988-2012 were identified from the California Cancer Registry, as previously described.[128] Each case was matched to a California birth certificate by first and last name, date of birth, and social security number when available, using a probabilistic record linkage program with a successful linkage rate of 89%.[88] Given prior reports of rates of residential mobility in early childhood among California children, the remaining 11% of cases were likely born out of state.[130] Controls were randomly selected from among all California birth records during this period, frequency matching them by birth year (20:1 matching rate). All controls were alive and without a cancer diagnosis in California by age 5 according to CA state death files. As this was a record-linkage study, informed consent from each individual subject was not feasible. We excluded children whose mothers had unknown or unreported preeclampsia/eclampsia status (n = 188), controls who died before reaching age six (n = 1,792),

and controls who were likely not viable (n = 130 gestational age < 20 weeks and n = 65 birth weight < 500g), resulting in 13,669 cases and 271,383 controls for the final analyses.

We obtained maternal demographic and socioeconomic information, pregnancy history, pregnancy characteristics, and newborn abnormal conditions or clinical procedures from California birth certificates. Preeclampsia and eclampsia during pregnancy, labour, and delivery were based on the VS-10A medical data supplemental worksheet, an additional form attached to the live birth registry record, which is completed by hospital clerks based upon the medical record. Exposure to preeclampsia or eclampsia was first treated as a binary variable. Furthermore, we assessed the effect of preeclampsia/eclampsia according to the severity of the condition. When preeclampsia is worsening, the decision to induce birth will be made even if the baby is very premature. Thus, in mothers with preeclampsia we used preterm delivery as a marker of more severe preeclampsia. Mothers with preeclampsia and preterm delivery together with mothers who had eclampsia were classified as having “severe preeclampsia/eclampsia”. Otherwise mothers with preeclampsia were considered having “mild preeclampsia”.

Selection of potential confounding variables was based upon associations observed in our data as well as our review of the literature. As adjusting for post-exposure events in the causal pathway between exposures and outcome would block part of the total effect of preeclampsia on childhood cancers (i.e., act as an intermediate), any related condition occurring after preeclampsia onset such as delivery method, complications in labour and delivery, and adverse birth outcomes, could not be controlled when estimating the odds of cancer due to preeclampsia. Since the birth certificate stopped recording the child’s race/ethnicity in 1998, we used maternal

race/ethnicity instead. Gestational age was recorded as the number of weeks since the last menstrual period. An obstetric estimate of gestational age at delivery (in completed weeks) is estimated by a physician after a 20th-week ultrasound and was recorded only from 2007 onward. For subjects with missing gestational age information after 2006, we used the obstetric estimate to replace the missing value. Maternal education and primary payment method for prenatal care were used as proxies for maternal socioeconomic status, as described previously.[129] Other potential confounders such as multiple gestations, parity, and previous preterm births were recorded during the entire study period.

Maternal demographics, reproductive histories and perinatal characteristics of the index pregnancy were compared for childhood cancer cases and controls. We restricted our analysis to cancer subtypes with five or more exposed cases. Unconditional logistic regression analysis with stabilized inverse probability weights[131] was used to calculate odds ratios and 95% confidence intervals (CI) for each type of childhood cancer, accounting for the matching variable, birth year. Considering the heterogeneous aetiology of cancer subtypes, separate weights were generated for each subtype of cancer by generating the inverse probability weight for the foetus being exposed to preeclampsia based on various sets of covariates. Common variables used to generate the weights include maternal age at pregnancy, race/ethnicity and maternal birth place, which our group previously observed to be risk factors for childhood cancer[129, 132] and are previously reported risk factors for preeclampsia.[2, 17] Additional factors examined as potential confounders and effect modifiers include previous history of preterm birth, previous miscarriages, multiparity, principal payment method for prenatal care, and the number of prenatal care visits; they were retained in the model if effect estimates changed by 10% or more. Since childhood

cancers are very rare, odds ratios are good estimates of incidence rate ratios for childhood cancer.

To assess if preeclampsia has an adverse effect on childhood cancer odds beyond being mediated through preterm delivery, we applied a marginal structural model to estimate controlled direct effects[133] of preeclampsia on childhood cancers independent of preterm birth. Logistic regression with inverse probability weights was used, handling both confounding of the "preeclampsia—cancer" association and confounding of the "mediator—cancer" association. A directed acyclic graph showing the hypothesized underlying causal relationships is given in **Figure 2.1**. A similar approach was applied to estimate effects of preeclampsia on childhood cancers independent of NICU admission. Cases that were diagnosed within five days after birth were further excluded from the mediation analysis to prevent possible reverse causation of NICU attendance.

The coding for hypertensive conditions during pregnancy changed across the study period: in mothers whose children were born 1983-2005, chronic hypertension and preeclampsia/eclampsia were recorded separately; from 2006-2011, only one of the following hypertensive conditions during pregnancy was recorded: pre-existing hypertension, pregnancy-induced hypertension, and eclampsia. We used pregnancy-induced hypertension as a proxy for preeclampsia in 2006-2011. We separately analyzed the subset of children born before 2006 to evaluate the possibility of misclassification bias due to the changes in recording methods.

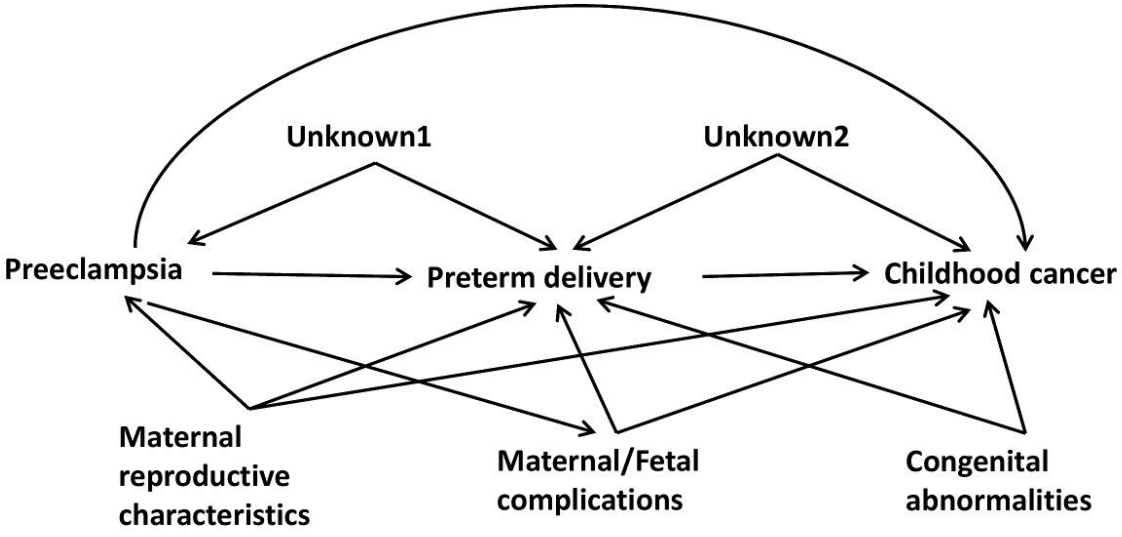

Figure 2.1 Directed acyclic graph (DAG). Hypothetical maternal reproductive characteristics that may confound preeclampsia – cancer association or preeclampsia – preterm birth association include: maternal age, race/ethnicity, maternal birth place, birth type, parity, principal method of payment for prenatal care, previous history of preterm birth, previous miscarriages and number of prenatal care visit; hypothetical factors confound preterm birth – cancer association include: maternal infections, congenital abnormalities, fetal distress and placental abruption.

2.4 Results

The study population consisted of a majority of Hispanics (45%), nulliparous pregnancies (40%), and women with at least 12 years of education (74%). Most women had at least more than five prenatal care visits (95%). Childhood cancer was more common among children born to mothers with advanced age at pregnancy, White non-Hispanic mothers, mothers with 16 or more years of education, mothers with less frequent prenatal care visits, and mothers whose prenatal care was paid by private insurance (**Tables 2.1 and 2.2**). Associations between demographic factors and specific cancer subtypes have been described previously.[88, 128, 129, 134, 135] Mothers of children with any type of childhood cancer and controls were similar in terms of parity and birth type (singleton vs. multiple gestation), but distributions varied by cancer subtype. Table 2 shows that case children had pregnancy characteristics and delivery outcomes distinct from control children. Consistent with previous reports,[58] cancer in all subtypes were more common in male children. Children with cancer had a higher propensity to be delivered preterm, had lower birth weight, and were more often transferred to the NICU after delivery.

Fetal exposure to preeclampsia was associated with a nearly two-fold increase in odds of germ cell tumours and hepatoblastoma, but no increased odds was observed for all cancer subtypes combined (OR 1.04, 95% CI 0.92, 1.18). Both germ cell tumor odds and hepatoblastoma odds were further elevated with fetal exposure to severe preeclampsia (**Table 2.3**).

As shown in stratified analyses presented in **Table 2.4**, the associations between preeclampsia and germ cell tumours were not uniform across histologic or morphological subtypes. Preeclampsia was strongly associated with seminomas and less so with non-seminomas. Only

one subtype of non-seminomas, teratoma, displayed an increased odds after preeclampsia exposure, while none of the 211 children who had yolk sac tumours were exposed to preeclampsia during gestation. Maternal preeclampsia doubled the odds of having an offspring with extracranial and extragonadal germ cell tumour even after restricting to children with a term birth. The direct effect of preeclampsia on teratoma after controlling for the intermediate effects of preterm delivery and NICU admission is nearly as strong as the total effect (**Table 2.5**). The controlled direct effect of preeclampsia on seminomas remained similar after controlling for preterm delivery, but strengthened further after the adjustment for NICU admission. The increased odds of hepatoblastoma, however, was attenuated after controlling for preterm delivery and NICU admission.

Table 2.1 Demographic characteristics in relation to childhood cancers, California Cancer Registry, birth year 1983-2011 (n=285,052).

Characteristic	Controls n (%)	All Cancers n (%)
Total	271383 (100%)	13669 (100%)
Maternal Age		
<20	28661 (10.6%)	1299 (9.5%)
20-29	140767 (51.9%)	6826 (49.9%)
30-34	63232 (23.3%)	3355 (24.5%)
>35	38681 (14.3%)	2187 (16%)
Missing	42 (0%)	2 (0%)
Maternal Race/Ethnicity		
White non-Hispanic	94945 (35%)	5231 (38.3%)
Hispanic	124636 (45.9%)	6125 (44.8%)
African American	18041 (6.6%)	703 (5.1%)
Asian/ PI	26518 (9.8%)	1246 (9.1%)
Other	7243 (2.7%)	364 (2.7%)
Maternal Birth Place		
US	68556 (25.3%)	3236 (23.7%)
Mexico	153381 (56.5%)	8068 (59%)
Other Foreign	49185 (18.1%)	2357 (17.2%)
Missing	261 (0.1%)	8 (0.1%)
Maternal Education (years) [a]		
8 or less	29392 (12.4%)	1355 (11.3%)
9-11	43020 (18.1%)	1995 (16.7%)
12	66092 (27.8%)	3456 (28.9%)
13-15	47429 (19.9%)	2355 (19.7%)
16 or more	47330 (19.9%)	2579 (21.5%)
Missing	4417 (1.9%)	229 (1.9%)
Principal method of payment for prenatal care [a]		
Government program/self	117782 (49.5%)	5370 (44.9%)
Private	117233 (49.3%)	6509 (54.4%)
Missing	2665 (1.1%)	90 (0.7%)
Parity		
0	106713 (39.3%)	5328 (39%)
1	84935 (31.3%)	4307 (31.5%)
>=2	79557 (29.3%)	4028 (29.5%)

Missing	178 (0.1%)	6 (0%)
Prior Miscarriages		
Yes	46811 (17.2%)	2468 (18.1%)
No	224318 (82.7%)	11191 (81.9%)
Missing	254 (0.1%)	10 (0.1%)

[a] This variable was not collected on birth certificates until 1989, therefore n(%) was based on data in existing years.

Table 2.2 Characteristics of index pregnancies and deliveries in relation to childhood cancers, California Cancer Registry, birth year 1983-2011 (n=285,052).

Characteristic	Controls n (%)	All Cases n (%)
Total	271383 (100%)	13669 (100%)
Child's Sex		
Male	138527 (51%)	7543 (55.2%)
Female	132853 (49%)	6126 (44.8%)
Unknown	3 (0%)	0 (0%)
Birth Type		
Single	264363 (97.4%)	13313 (97.4%)
Multiple	7020 (2.6%)	356 (2.6%)
No. of Prenatal Care Visit [a]		
5 times or fewer	13502 (5.7%)	567 (4.7%)
6 to 10 times	71341 (30.0%)	3520 (29.4%)
11 to 15 times	122999 (51.7%)	6275 (52.4%)
16 times or more	24277 (10.2%)	1330 (11.1%)
Missing	5560 (2.3%)	277 (2.3%)
Preeclampsia		
Yes	5686 (2.1%)	304 (2.2%)
No	265696 (97.9%)	13365 (97.8%)
Chronic hypertension		
Yes	902 (0.3%)	62 (0.5%)
No	270449 (99.7%)	13605 (99.5%)
Missing	32 (0%)	2 (0%)
Length of gestation		
≤ 37 weeks	26410 (9.7%)	1532 (11.2%)
38-42 weeks	222398 (81.9%)	11043 (80.8%)
≥ 42 weeks	9842 (3.6%)	477 (3.5%)
Missing	12733 (4.7%)	617 (4.5%)
Birthweight		
≤ 1499 g	2162 (0.8%)	172 (1.3%)
1500 - 2499 g	13885 (5.1%)	689 (5.0%)
2500 - 3999 g	227140 (83.7%)	11155 (81.6%)
≥ 4000 g	27968 (10.3%)	1637 (12.0%)
Missing	228 (0.1%)	16 (0.1%)
Size for gestational age		

Small	27301 (10.1%)	1272 (9.3%)
Normal	205747 (75.8%)	10260 (75.1%)
Large	38107 (14.0%)	2121 (15.5%)
Missing	228 (0.1%)	16 (0.1%)
NICU attendance [a]		
Yes	7951 (3.3%)	644 (5.4%)
No	229729 (96.6%)	11325 (94.6%)

[a] This variable was not collected on birth certificates until 1989, therefore n (%) was based on data in existing years.

Table 2.3 Maternal preeclampsia during pregnancy and the odds of childhood cancers by severity of preeclampsia.

Cancer Type	N	Preeclampsia Severity					
		All diagnoses		Mild Preeclampsia		Severe Preeclampsia	
		n	Adjusted [a] OR (95% CI)	n	Adjusted [a] OR (95% CI)	n	Adjusted [a] OR (95% CI)
All childhood cancers	13669	304	1.0 (0.9, 1.2)	206	1.0 (0.9, 1.2)	87	1.1 (0.9, 1.4)
ALL	4133	87	1.1 (0.9, 1.4)	66	1.1 (0.8, 1.4)	21	0.9 (0.6, 1.5)
AML	740	19	1.3 (0.8, 2.1)	13	1.2 (0.7, 2.0)	6	1.8 (0.9, 3.8)
Lymphoma	620	7	0.7 (0.3, 1.4)	5	0.5 (0.2, 1.3)	2	NA [b]
CNS tumor	2378	42	0.8 (0.6, 1.1)	31	1.0 (0.7, 1.4)	9	0.7 (0.4, 1.3)
Neuroblastoma and ganglioneuroblastoma	1385	37	1.2 (0.9, 1.8)	26	1.3 (0.9, 2.0)	11	1.3 (0.7, 2.4)
Retinoblastoma	746	16	1.1 (0.6, 1.7)	11	0.9 (0.5, 1.8)	5	1.2 (0.5, 2.9)
Wilms	1056	22	1.0 (0.7, 1.6)	14	1.0 (0.4, 2.2)	8	1.0 (0.6, 1.6)
Hepatoblastoma	346	13	1.7 (1.0, 3.0)	6	0.7 (0.3, 2.0)	7	4.9 (2.5, 9.5)
Soft tissue sarcomas	705	17	1.0 (0.6, 1.8)	14	1.2 (0.7, 2.1)	3	NA [b]
Germ Cell tumors	451	16	1.8 (1.1, 3.0)	9	1.3 (0.7, 2.6)	7	4.2 (2.2, 7.9)
Controls	271383	265697	1.0 (reference)	3987	1.0 (reference)	1468	1.0 (reference)

[a] For each type of childhood cancer we adjusted for a unique set of covariates.
[b] Not applicable due to less than five exposed cases.

Table 2.4 Maternal preeclampsia during pregnancy and the odds of germ cell tumors by histologic and morphological subtypes.

		Cases	Adjusted OR[a] (95% CI)
Germ Cell Tumors		451	1.8 (1.1, 3.0)
Stratified by Histological Types	**Seminomas**	16	8.6 (1.9, 38.4)
	Non-Seminomas[b]	431	1.7 (1.0, 2.8)
	Teratoma	211	3.0 (1.7, 5.4)
	Yolk sac tumor	181	NA [c]
	Other rare types	4	NA [c]
Stratified by Morphological Sites	**CNS germ cell tumor**	43	2.5 (0.6, 10.5)
	Extracranial germ cell tumor	408	1.8 (1.0, 3.0)
	Malignant gonadal tumor	192	0.9 (0.3, 2.7)
	Extracranial and extragonadal germ cell tumor	216	2.5 (1.3, 4.5)

[a] Adjusted for maternal age, race/ethnicity, maternal birth place, parity, birth type (multiple vs. single birth) and birth year

[b] Only more common subtypes were listed

[c] Not applicable due to less than five exposed cases

Table 2.5 Controlled direct effects of maternal preeclampsia during pregnancy and the odds of childhood cancers accounting for mediation by preterm delivery and neonatal NICU admission.

	Total Effects	**Controlled direct effect accounting for preterm birth**[a]	**Controlled direct effect accounting for NICU admission**[b]
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Hepatoblastoma	2.1 (1.2, 3.6)	1.8 (1.0, 3.2)	1.5 (0.8, 3.0)
Germ cell tumors			
Seminomas	7.2 (1.6, 31.6)	7.2 (1.7, 29.4)	10.2 (2.2, 46.6)
Teratoma	3.2 (1.6, 6.6)	3.2 (1.5, 6.9)	3.2 (1.5, 6.7)

[a] Marginal structural models were applied; adjusted for maternal age, race/ethnicity, parity, birth type, birth year, and congenital abnormalities using inverse probability weighting

[b] Marginal structural models were applied; adjusted for maternal age, race/ethnicity, parity, birth type, birth year, preterm delivery, congenital abnormalities, low birthweight using inverse probability weighting

2.5 Discussion

This study suggests that maternal preeclampsia during pregnancy is associated with increased odds of germ cell tumours and hepatoblastoma in offspring, while no increased odds for all paediatric tumours combined was observed. The association between preeclampsia exposure and germ cell tumours differs across histologic subtypes, with the strongest association observed in seminomas. Maternal preeclampsia did not increase the odds of germ cell tumours in a specific site except for extracranial and extragonadal germ cell tumours. These findings may point to different possible aetiologies of germ cell tumours and hepatoblastoma.

The increased odds of hepatoblastoma in children born to mothers who had severe preeclampsia/eclampsia during pregnancy has been previously reported in the United Kingdom Childhood Cancer Study (UKCCS), in which both preeclampsia and cancer diagnoses were abstracted from medical records.[87] The possible connection between preeclampsia and hepatoblastoma may have been supported by the finding of a lower level of apolipoprotein A-1 (Apo A-I) protein expression in the umbilical cord blood from pre-eclamptic pregnancies also identified in children with hepatoblastoma and this protein has been suggested as a serum biomarker for early diagnosis of hepatoblastoma.[136, 137]As reflected by our estimates from a marginal structural model that controlled for direct effects, the association between maternal preeclampsia and hepatoblastoma can be partly explained by mediation by NICU attendance and the intensive medical care associated with it. A previous investigation identified di-(2-ethylhexyl) phthalate (DEHP), a commonly applied plasticizer in medical devices and tubing, as a rodent hepatocarcinogen.[138, 139] Infants going through intensive and long-term medical

interventions such as mechanical ventilation and oxygen therapy are more likely to be exposed to high cumulative doses of DEHP which have tumorigenic effects on the immature liver.[139]

Most epidemiologic research has focused on adult rather than childhood germ cell tumours due to their much higher incidence; only a limited number of previous studies were designed to investigate the effect of perinatal factors on paediatric germ cell tumours. Different from our results, a null association between preeclampsia and paediatric testicular germ cell tumours was previously observed in a record linkage study in Nordic countries,[92] but the studies are difficult to compare since the Nordic study was restricted to boys only and included cases up to age 18; thus, the histologic types of cancer also differed due to the case ages as did the ethnic/racial composition of the two populations (our study included 45% Hispanics).

Germ cell tumours are presumed to arise from pluripotent primordial germ cells and show a broad range of possible histologies.[140] Both biologic features and clinical presentation differ by histologic subtype of germ cell tumours, which implies distinct aetiologies and necessitates subgroup analysis. Differential epigenetic changes, microRNA expression and signalling pathway activation were observed in certain subtypes of germ cell tumours, which provided potential biological evidence for distinct preeclampsia—germ cell tumor associations in epidemiologic studies. Interestingly, miR-182, that functions by downregulating immune response and antiapoptosis genes,[141] was found to be overexpressed in preeclamptic placentas and, was also suggested to be specifically linked to seminomas in contrast to other germ cell tumour subtypes.[142] This might partly explain the striking elevated odds for seminomas in preeclampsia patients observed in our study.

One typical pathophysiologic change in preeclampsia is placental hypoxia resulting from abnormal trophoblastic implantation,[30] which could lead to increased hypoxia-inducible factor (HIF)-1α and HIF-2α level in the placenta circulation as an adaptive response to the hypoxic environment. The controlled direct effect of preeclampsia on increased teratoma odds is possibly driven by HIF-2α's tumour promoting effect by altering stem cell differentiation through the activation of Oct-4, as proposed by Covello et al.[143]

Consistent with another case control study on haematological malignancies nested within the UKCCS study population,[90] no elevated odds were seen for ALL or AML. UKCCS also reported a doubling in odds for non-Hodgkin's lymphoma, for which the incidence in children peaks after the age of 10, outside the age range of our study population.

Strengths of the study

Our population-based study had a sufficient number of cases for many cancer subtypes. By using a record-linkage design, we did not rely upon interview-collected data for pregnancy characteristics and avoided recall bias. Furthermore, important confounding variables related to maternal demographic characteristics, perinatal characteristics and pregnancy history based on the birth certificate are reliably recorded on birth records lowering the chance of residual confounding.[144, 145] The novel findings for germ cell tumour subtypes provide the first epidemiologic evidence for distinct aetiologies of these cancers.

Limitations of the data

The results from our study should be interpreted with caution due to the following limitations. Firstly, our study is subject to misclassification bias. Underreporting of preeclampsia on the birth certificate was confirmed in our data. The rate of preeclampsia/eclampsia among controls was 2.20%, which is lower than the rate in California reported in a health plan database (4.5% in 2010-2012) [12] and in hospital records (3.1% in 2001-2006).[146] The sensitivity of preeclampsia exposure ascertainment thus is reduced with severe preeclampsia more likely to be recorded on the birth certificate.[147] Additionally, the change in recording of hypertensive conditions during pregnancy since 2006 attenuated estimates due to non-differential, independent under-reporting as shown in the restricted analysis in **Table A 2.1**. Secondly, the birth certificate does not record the date of onset of pregnancy complications. Researchers have suggested that the pathological mechanism differs for early and late onset of preeclampsia, but we were unable to consider timing of disease onset to assess potential effect measure modification.[9] Nor were we able to consider the effect of antihypertensives used to control the development of preeclampsia. Moreover, maternal smoking and maternal BMI were only available after 2006, which prevented us from adjusting for these two factors in the analysis and this may have caused residual confounding. An inherent problem of birth outcomes research other than neonatal death is that we can only assess the outcomes among live births. Since severe preeclampsia significantly increases the risk of fetal demise[3], the competing risk of death prevents some children from developing childhood cancer. Otherwise, stronger associations might have been observed.

2.6 Conclusions

We found a strong association between paediatric seminomas and fetal exposure to preeclampsia, a three-fold increased odds of teratomas in children born to mothers who

experienced preeclampsia, and our findings support a direct effect of preeclampsia on these germ cell tumours. The observation of distinct associations by histologic subtype of germ cell tumours provides epidemiologic evidence suggesting heterogeneous pathogenesis. The additional finding of elevated odds of hepatoblastoma in relation to severe preeclampsia exposure seemed to be due to an indirect effect through preeclampsia-associated preterm delivery and intensive neonatal medical care as well as having a direct effect. These findings underscore the importance of effective interventions targeting modifiable risk factors of maternal preeclampsia and close monitoring of high-risk women through antenatal health care to prevent the long-term health effects of in utero exposure to preeclampsia in children.

2.7 Appendix

Table A 2.1 Maternal preeclampsia during pregnancy and the odds of germ cell tumors by histologic and morphological subtypes, a subset of data of birth year before 2006.

		Cases	Adjusted [a] OR (95% CI)
Germ Cell Tumors		355	2.2 (1.3, 3.7)
Stratified by HistologicTypes	Seminomas	15	9.4 (2.1, 42.4)
	Non-Seminomas[b]	337	2.0 (1.1, 3.4)
	Teratoma	168	3.5 (1.9, 6.3)
	Yolk sac tumor	141	NA [c]
	Other rare types	4	NA [c]
Stratified by Morphological Sites	CNS germ cell tumor	36	3.2 (0.7, 13.3)
	Extracranial germ cell tumor	319	2.1 (1.2, 3.7)
	Malignant gonadal tumor	142	1.2 (0.4, 3.9)
	Extracranial and extragonadal germ cell tumor	177	2.7 (1.4, 5.1)

[a] Adjusted for maternal age, race/ethnicity, maternal birth place, parity, birth type and birth year
[b] Only more common subtypes were listed
[c] Not applicable due to less than five exposed cases

Table A 2.2 Maternal preeclampsia during pregnancy and the odds of childhood cancers by calendar years.

	Birthyear 1983 to 2005[a]		Birthyear 2006 to 2011[b]	
	N	OR (95% CI)	N	OR (95% CI)
All childhood cancers	10997	1.0 (0.9, 1.2)	2672	1.0 (0.8, 1.3)
ALL	3690	1.1 (0.8, 1.3)	443	1.3 (0.8, 2.4)
AML	562	1.5 (0.9, 2.5)	178	NA [c]
Lymphoma	540	0.8 (0.4, 1.7)	80	NA [c]
CNS tumor	1965	0.8 (0.5, 1.2)	413	0.7 (0.3, 1.6)
Neuroblastoma and ganglioneuroblastoma	1083	1.2 (0.8, 1.7)	302	1.1 (0.5, 2.3)
Retinoblastoma	582	0.9 (0.5, 1.7)	164	1.5 (0.6, 3.6)
Wilms' tumor	859	1.0 (0.6, 1.7)	197	1.2 (0.5, 2.9)
Hepatoblastoma	245	1.8 (1.0, 3.4)	101	NA [c]
Soft tissue sarcomas	572	0.8 (0.4, 1.7)	133	1.8 (0.7, 4.4)
Germ Cell tumors	355	2.2 (1.3, 3.7)	96	NA [c]
Controls	218244	1.0 Reference	53139	1.0 Reference

[a] Exposure was preeclampsia or eclampsia during pregnancy

[b] Exposure was hypertension during pregnancy (including pregnancy-induced hypertension and preeclampsia) or eclampsia

[c] Not applicable due to less than five exposed cases

Chapter 3. Non-steroidal Anti-inflammatory Drugs Use and Risk of Age-Related Macular Degeneration in California Teachers Cohort

3.1 Abstract

Background

Age-related macular degeneration (AMD) is a leading cause of blindness in the elderly. Aspirin and other NSAIDs may have the potential to suppress the development of AMD through cardioprotective properties (aspirin only) and anti-inflammatory properties, but previous evidence for AMD is inconclusive.

Methods

In the California Teachers Study (CTS) cohort (N = 88, 481) we identified diagnoses of AMD by linkage to California Office of Statewide Health Planning and Development (OSHPD) hospital discharge records between Dec.1990 and Dec.31, 2012. General aspirin, ibuprofen and acetaminophen use and comprehensive risk factor information was collected in the CTS via self-administered questionnaires at baseline in 1995-1996, and information on specific anti-inflammatory drug use (low-dose aspirin, COX-2 inhibitors, other NSAIDs) was provided by a cohort who completed a follow-up questionnaire in 2005. We employed Cox proportional hazard regressions models adjusting for a number of potential confounders to model AMD risk during follow-up.

Results

We identified 1,762 participants with AMD during 14.8 years of average follow-up in this cohort. We did not find any associations between AMD and frequency and duration of aspirin, ibuprofen, or acetaminophen (a negative control medication) use reported at baseline. For the 955 AMD cases that occurred after 2005, however, we observed a 20% decrease in risk of AMD

among low-dose aspirin users (HR = 0.81, 95% CI: 0.70, 0.95) and a 55% decrease among COX-2 inhibitors users (HR = 0.45, 95% CI: 0.26, 0.78) during 6.3 years of average follow-up.

Conclusions

Our study found no associations between use of regular standard-dose aspirin or ibuprofen and intermediate- or late-stage AMD. However, the decrease in risk among women who reported regular use of low-dose aspirin or specific COX-2 inhibitors in our 2005 subsample suggests a possible protective role for medications with COX-2 inhibitive properties or aspirin at doses used for cardiovascular disease prevention.

3.2 Introduction

Age-related macular degeneration (AMD) is a chronic eye disease affecting the central retina that can cause irreversible vision loss if left untreated.[64] Following cataract and glaucoma, AMD is the third leading cause of blindness worldwide, a primary cause of vision impairment in industrialized countries, and is associated with decreased quality of life and increased dependence on caregivers.[64, 148] Early-stage AMD is usually asymptomatic and can develop into one of two forms (dry- or wet-AMD) considered late-stage AMD: 1) geographic atrophy (dry form), in which accumulating drusen – i.e. yellowish deposits of lipid and proteins- disrupt photoreceptor cells, causing blind spots in the central vision; and 2) neovascular AMD (wet-AMD), which is characterized by leaky blood vessels abnormally growing into the retina and causing swelling and bleeding.[64, 65] In the US, 6.5% of the population over age 40 is suffering from AMD at any stage and the prevalence rises steeply to 30-45% of adults ages 75 years or more. For late-stage disease, the prevalence after age 40 is 1.5% and expected to double by 2020.[65]

Inflammation is proven to be a key component of drusen biogenesis,[113] and cyclooxygenase-2 (COX-2), an inducible enzyme involved in the process of inflammation, is highly expressed in choroidal neovascular membranes in wet-AMD patients.[149] These findings warrant research on a putative beneficial effect that anti-COX-2 medications may have for AMD. Aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit COX enzymes and may also have the potential to prevent the development of AMD. Along with acetaminophen, they are the most commonly used analgesics in the adult population used to treat chronic pain or manage inflammatory disorders with widespread availability from prescriptions as well as over-the-counter sales.[150, 151] Moreover, low-dose (baby) aspirin is widely used for its antithrombotic properties in the primary and secondary prevention of cardiovascular diseases, such as heart attack and stroke, both of which are clinical risk factors for AMD.[152-156]

Except for aspirin, few previous studies examined associations between different types of NSAIDs and AMD. A nested case-control study conducted among veterans found reduced AMD risk in subjects who had filled prescriptions for any anti-inflammatory medications (OR = 0.15) and also for each subtype of anti-inflammatory medication including aspirin.[116] Negative associations between long-term low-dose aspirin use and AMD were also reported in two clinical trials with an average of 5 years- and 10 years of follow-up respectively.[122, 157] In contrast, two large cohort studies with more than 10 years of follow-up concluded that self-reported regular aspirin users (at any dose) had a 2 to 2.5 times increased risk for developing wet-AMD compared with non-regular users, and this raised concern about the side effects of aspirin's inhibition of platelet-mediated release of vascular endothelial growth factor (VEGF) and subsequently induced hypoxia.[120, 121, 158] Long-term aspirin use may affect AMD through three mechanisms: 1)

reduce neovascularization through COX-2 inhibition; 2) reduce the development of AMD through its cardioprotective effects; or 3) stimulate neovascularization at the retina as a result of platelet inhibition and hypoxia. Non-aspirin NSAIDs are thought to affect AMD through the first mechanism only.

The primary aim of our study was to assess associations between long-term use of NSAIDs that are affecting the pathways described above and AMD in a cohort of California teachers who were followed over a 17-year period, accounting for frequency, duration, and co-medication use. Since acetaminophen is the most common pain medication used for similar indications as aspirin and other NSAIDs such as managing musculoskeletal pain and chronic headaches, but does not have anti-inflammatory or cardioprotective properties, here we used acetaminophen as a negative control to assess bias by indication. We refer to aspirin, ibuprofen and acetaminophen that were assessed at baseline as baseline analgesics use. We also assessed associations between low-dose aspirin, standard-dose aspirin, ibuprofen, other non-selective NSAIDs, and COX-2 inhibitors and AMD in the subsample that reported on these medications in the 2005 questionnaire. This subsample also had information on steroid medication use which allowed us to examine whether the COX pathway is most important for the anti-inflammatory action in preventing AMD.

3.3 Methods

A detailed description of the California Teachers Study (CTS) and the data available from it has been published.[159, 160] Briefly, a prospective cohort of 133,479 female California teachers and school professionals who completed and returned a baseline self-administered questionnaire in 1995-1996 were followed both actively with questionnaires and passively with linkage to vital

statistics, cancer registries, and hospitalization records. Comprehensive sociodemographic, life-style and health history information was collected at baseline and again in 1997, 2000, 2005, and 2011. The CTS cohort was annually linked to the California Office of Statewide Health Planning and Development (OSHPD) hospital discharge records and to the California Automated Mortality Linkage System, the Social Security Administration Death Master File and the National Death Index.

With institutional review board (IRB) approval, we generated a linked hospital, vital status (including out of state mortality) and baseline questionnaire dataset for each CTS participant. Eligibility was limited to women who were California residents at baseline and had at least one OSHPD record available (N = 89,877). Participants who, according to OSHPD records, suffered from AMD prior to completing the baseline questionnaire (N = 22) and those who did not report their regular analgesic medication use (n = 1,347) at baseline were excluded, leaving 88,481 participants for analysis.

Any AMD event was identified (ICD-9-CM codes 362.50, 362.51, 362.52, and 362.57) from three different OSHPD data sources: hospital discharge data (available from 12/1990--12/2012), ambulatory service data (01/2010—12/2012), and emergency department data (01/2010—12/2012); all of which captured up to 25 diagnoses and the date of service for the primary reason of the visit. The first AMD event (earliest date of AMD diagnosis in hospital record system) was considered the incident date. According to the guideline for secondary diagnoses in hospitals,[161] only co-existing conditions that affect current treatments should be recorded. Thus, we assume that the majority of AMD cases identified in this manner were intermediate to advanced and had

impaired central vision, because these patients are more likely to require additional therapeutic procedures, and increased nursing care and have an extended length of stay.[162] In contrast, patients with early stage AMD do not meet the criteria for a comorbid disorder relevant to current treatment and care during hospitalization; thus, we most likely did not capture as many of these with our passive linkage to hospital discharge records.

Follow-up started the day the baseline questionnaire was completed by a participant and ended at the earliest occurrence of one of the four events: 1) AMD diagnosis; 2) moving out of California; 3) death; 4) date of the most recent linkage of the CTS to OSHPD records (Dec. 31st, 2012). Address information was collected yearly from the U.S. Postal Service National Change of Address database, the California Department of Motor Vehicles, and/or was self-report in subsequent questionnaires. Participants who had moved out of California for more than a one year period were right-censored at the last time they were contacted in California since only inpatient visits in a hospital in California are captured in the OSHPD system.

At baseline, participants reported regular medication use (at least once per week), average frequency of use (1-3, 4-6, or 7 days per week), and total years of use (<1, 1, 2, 3-4, 5-9, or ≥10). Regular aspirin (Anacin, Bufferin, Excedrin), acetaminophen (Tylenol, Anacin-3, Panadol, Aspirin Free Excedrin, etc.), and ibuprofen (Advil, Motrin, Nuprin) consumption were asked separately. More detailed NSAIDs use was recorded in a subsequent questionnaire mailed to cohort members in 2005: 'baby' or low-dose aspirin, aspirin (Anacin, Bufferin, Excedrin), acetaminophen (Tylenol, Tempra, Aspirin Free Excedrin, etc.), ibuprofen (Advil, Motrin), COX-2 inhibitors (Celebrex, Vioxx), and naproxen, ketoprofen or other NSAIDs (Aleve, Feldene,

Indocin, Naprosyn, Orudis, Relafen). Regular steroid use, either in pill- or inhaled-form was also queried.

Potential confounder and effect modifier information was identified from questionnaire data or OSHPD hospital discharge records and were selected based on the literature on AMD.[65, 163] We included in our models age (continuous), race/ethnicity (Non-Latina white, African-American, Latina, Asian/Pacific Islander, Other), body mass index (<18.5, 18.5 to 25, 25 to <29, ≥30), smoking (never, former, current), alcohol use in the year prior to baseline (no use, less than 20 grams per day, greater than 20 grams per day), lifetime average moderate or strenuous physical activity per week (<2h, 2 to <4 h, 4 to <6 h, 6 h or more), history of high blood pressure (yes, no), and antihypertensive medication use; indications and contraindications for aspirin and other NSAIDs obtained from OSHPD hospital discharge data, including diagnoses of circulatory system diseases (ICD-9-CM: 390–459), diseases of the musculoskeletal system and connective tissue (ICD-9-CM: 710–739), diabetes (ICD-9-CM: 250), baseline asthma or allergic rhinitis (ICD-9-CM: 493 and 477), and baseline coagulation/hemorrhagic disease (ICD-9-CM: 286-289). We also assessed percent daily dietary calories from fat (continuous), and dietary antioxidant consumption (average daily dose) but we ultimately decided to not include them in our analyses because the less than 10% change in the point estimate of the association.

In the analyses of 2005 medication data, all the above-mentioned risk factors were updated to reflect the most recently available data (subsequent questionnaires and OSHPD hospital discharge data). We additionally adjusted for the type of health insurance as a proxy for family income and access to health care, regular consumption of medications to treat respiratory

conditions with anti-inflammatory properties (cromolyn, nedocromil, zileuton, zafirlukast, montelukast) and statin use in our analyses.

Statistical Analysis

Multivariable Cox proportional hazards regression was used to assess the association between pain medication use and AMD, using calendar time at start and end of follow-up (in days) to define person-time. We examined the baseline analgesics in categories of frequency and duration of use, and tested for trend using category midpoints. To account for potential effects from use of other classes of analgesics, we calculated the approximate intensity of each of the three medications by multiplying the average frequency and total years of use and mutually adjusting in our models for these variables. The proportional hazards assumption was checked using Kaplan-Meier survival curves and graphs of the log(-log(survival)) versus log of survival time; parallel lines indicated proportionality of hazards.[164] Stratified analyses were performed to assess possible confounding by indication and potential effect measure modification due to physical activity.

To distinguish low-dose and standard-dose of aspirin use and examine more categories of analgesic use, Cox proportional hazard regression with inverse probability weighting methods was applied in the subsample analyses that relied on the 2005 questionnaire information. After excluding women who developed AMD before 2005 or did not return this questionnaire, 50,202 subjects were eligible for the subsample. Separate weights for each type of analgesic intake were generated using logistic regression to address the potential confounding or censoring bias without impairing statistical power. The inverse weight was generated from two components, the

probability of taking a medication given risk factors discussed above and the probability of being censored prior to study end.

We performed sensitivity analyses by excluding women who had the first AMD diagnosis within the first two or five years of follow-up in the primary cohort and two years only in the subsample from 2005 onward to exclude prevalent AMD cases. Women who had a previous hospital record with no record of AMD within two years of the first AMD diagnosis were considered as incident AMD. Excluding the first two years of AMD diagnoses after 1995 or 2005 did not change our results more than minimally so here we report results only among the entire cohort without the exclusion.

To account for possible selection bias due to only including women with at least one OSHPD record (72% of eligible CTS subjects), we also conducted additional sensitivity analyses applying Cox models weighted by the inverse probability of selection (**Table A 3.1**). All analyses were performed using SAS Version 9.4 (SAS Institute, Cary, NC).

3.4 Results

Among 88,481 female teachers, we identified 1,762 subjects with AMD. During an average of 14.8 years follow-up, 6598 (7.5%) women moved out of California for a period of more than one year and 16,444 (18.6%) died, leaving 63,677 (72%) subjects who were right censored at end of follow-up (Dec.31, 2012). The median time to a first AMD diagnosis was 13.5 years (interquartile range: 10.2, 15.5). The distribution of demographic and life-style factors for the study population and AMD cases are presented in **Table 3.1**. AMD frequency increased sharply

with increasing age and was higher among Whites, overweight women (BMI 25-29.9 kg/m^2), women who exercised little (<2 hr/week moderate and strenuous physical activity), and women with a self-reported history of medical conditions at baseline that are known to contribute to AMD (high blood pressure, heart attack, stroke and diabetes). The current smoking rates were low in the cohort and comparable between AMD cases and controls, but total pack-years of smoking was higher among AMD cases due to a higher proportion of former smokers. A similar proportion of women drank alcohol, but heavier drinkers (≥20 g/day) were more common among AMD cases. Analgesic consumption in relation to demographic characteristics and health-related factors in the CTS has been previously described.[164, 165] Overall, women reporting aspirin, ibuprofen, and acetaminophen use were more often white, overweight and obese, and current or former smokers. Daily aspirin users were slightly older on average (65.3 vs. 60.0 and 63.3 for daily ibuprofen and acetaminophen) and acetaminophen users were more likely to report a lower alcohol intake (more than 20g per day).

Regular aspirin users were more likely to have a self-reported history of high blood pressure, stroke, heart attack, and were less active in terms of their reported moderate and strenuous physical activities compared with regular ibuprofen and acetaminophen users.
Regular aspirin users were more likely to take these medications for more days per week and a higher proportion were long-term (≥ 5 years) compared with ibuprofen and acetaminophen users (**Table 3.2**). The hazard ratio for AMD was above one for most of the use categories of aspirin and acetaminophen (between 1.00 and 1.29) but no trend was apparent for frequency or duration, while regular ibuprofen use was not associated with AMD. When we jointly examined frequency

and duration of use, the highest intensity use (more than 3 days per week and longer than 5 years) for the three baseline analgesics did not increase the risk of AMD (**Table 3.3**).

For women in the 2005 subsample, the median time to diagnosis of AMD was 4.3 years (interquartile range: 2.9, 7.1). We estimated negative hazard ratios for use of low-dose aspirin (HR = 0.81, 95% CI: 0.70, 0.95) and Cox-2 inhibitors (HR = 0.45, 95% CI: 0.26, 0.78) (**Table 3.4**). Regular acetaminophen use was positively associated with AMD risk (HR = 1.24, 95% CI: 1.06, 1.45) after accounting for the intensity of previous analgesic use and concurrent use of other classes of anti-inflammatory drugs or acetaminophen.

Table 3.1 Demographic characteristics and lifestyle factors of study population at baseline in the California Teachers Cohort, 1995-2012 (N=88,481).

Characteristics	All Participants	AMD Cases	Exposure to aspirin	Exposure to ibuprofen	Exposure to acetaminophen
Total	88481 (100%)	1762 (100%)	21212 (100%)	18335 (100%)	12399 (100%)
Age					
<50	35500 (40.1%)	53 (3.0%)	5637 (26.6%)	8289 (45.2%)	5865 (47.3%)
51-60	19916 (22.5%)	143 (8.0%)	5370 (25.3%)	4549 (24.8%)	2673 (21.6%)
61-70	16571 (18.7%)	498 (28.3%)	4929 (23.2%)	3033 (16.5%)	1940 (15.6%)
71-80	11825 (13.4%)	743 (42.2%)	3794 (17.9%)	1870 (10.2%)	1376 (11.1%)
>80	4669 (5.3%)	325 (18.4%)	1482 (7%)	594 (3.2%)	545 (4.4%)
Race/Ethnicity					
Non-Latina white	77079 (87.9%)	1662 (95.1%)	19185 (91.3%)	16313 (89.6%)	10822 (87.9%)
African American	2260 (2.6%)	24 (1.4%)	469 (2.2%)	431 (2.4%)	360 (2.9%)
Native American	3709 (4.2%)	20 (1.1%)	572 (2.7%)	712 (3.9%)	613 (5%)
Latina	829 (0.9%)	21 (1.2%)	242 (1.2%)	194 (1.1%)	114 (0.9%)
Asian/Pacific Islander	2796 (3.2%)	12 (0.7%)	376 (1.8%)	345 (1.9%)	263 (2.1%)
Other	1048 (1.2%)	8 (0.5%)	166 (0.8%)	213 (1.2%)	144 (1.2%)
Missing	760	15	202	127	83
BMI					
Underweight	2276 (2.7%)	40 (2.5%)	473 (2.4%)	359 (2%)	301 (2.5%)
Normal	47374 (56.1%)	495 (49.7%)	10477 (52.1%)	8874 (50.2%)	6081 (51%)
Overweight	21831 (25.8%)	525 (32.9%)	5719 (28.4%)	4830 (26.3%)	3236 (27.2%)
Obese	13012 (15.4%)	238 (14.9%)	3447 (17.1%)	3609 (20.4%)	2299 (19.3%)
Unknown	3988	164	1096	663	482
History of High blood pressure					
No	70874 (80.1%)	1140 (64.7%)	15106 (71.2%)	14580 (79.5%)	9571 (77.2%)
Yes	17607 (19.9%)	622 (35.3%)	6106 (28.8%)	3755 (20.5%)	2828 (22.8%)

History of Heart attack/MI					
No	87035 (98.4%)	1707 (96.9%)	20398 (96.2%)	18114 (98.8%)	12173 (98.2%)
Yes	1446 (1.6%)	55 (3.1%)	814 (3.8%)	221 (1.2%)	226 (1.8%)
History of Stroke					
No	87189 (98.5%)	1725 (97.9%)	20600 (97.1%)	18118 (98.8%)	12155 (98%)
Yes	1292 (1.5%)	37 (2.1%)	612 (2.9%)	217 (1.2%)	244 (2%)
History of Diabetes					
No	85492 (96.6%)	1659 (94.2%)	20260 (95.5%)	17741 (96.8%)	11904 (96%)
Yes	2989 (3.4%)	103 (5.8%)	952 (4.5%)	594 (3.2%)	495 (4%)
Smoking					
Never	16782 (19.2%)	270 (15.5%)	3059 (14.6%)	3050 (16.8%)	2082 (17%)
Passive	40368 (46.1%)	735 (42.2%)	9393 (44.8%)	8268 (45.6%)	5667 (46.2%)
Former	25751 (29.4%)	642 (36.8%)	7228 (34.4%)	5762 (31.8%)	3810 (31.1%)
Current	4590 (5.2%)	96 (5.5%)	1308 (6.2%)	1067 (5.9%)	709 (5.8%)
Missing	990	19	224	188	131
No. of smoking pack-years					
Never or passive smoker	57150 (67.4%)	1005 (59.9%)	12452 (61.3%)	11318 (64.4%)	7749 (65.1%)
≤ 10	14052 (16.6%)	246 (14.7%)	3611 (17.8%)	3231 (18.4%)	2112 (17.7%)
11-20	5187 (6.1%)	126 (7.5%)	1498 (7.4%)	1218 (6.9%)	794 (6.7%)
≥ 20	8464 (10.0%)	301 (17.9%)	2742 (13.5%)	1821 (10.4%)	1251 (10.5%)
Missing	3628	84	909	747	493
Daily alcohol intake (g)					
None	29086 (34.9%)	578 (35.1%)	6715 (33.4%)	5520 (31.7%)	4184 (35.6%)
<20	47340 (56.7%)	898 (54.5%)	11301 (56.1%)	10217 (58.7%)	6586 (56.1%)
≥20	7015 (8.4%)	173 (10.5%)	2117 (10.5%)	1666 (9.6%)	967 (8.2%)
Unknown	5040	113	1079	932	662

Lifetime moderate and strenuous physical activity (h/week)					
<2	29272 (33.3%)	804 (46%)	7616 (36.2%)	5604 (30.8%)	3990 (32.4%)
2 to 4	21822 (24.8%)	386 (22.1%)	5149 (24.5%)	4567 (25.1%)	3049 (24.8%)
4 to <6	14426 (16.4%)	240 (13.7%)	3283 (15.6%)	3088 (17%)	2036 (16.5%)
≥ 6	22319 (25.4%)	317 (18.1%)	5000 (23.8%)	4947 (27.2%)	3242 (26.3%)
Unknown	642	15	164	129	82

Table 3.2 Self-reported NSAIDs and acetaminophen use at baseline and age-related macular degeneration stratified by intensity and duration of use in California Teachers Cohort, 1995-2012 (N=88,481).

Frequency and duration of analgesics use	Aspirin		Ibuprofen		Acetaminophen	
	No. of participants	Adjusted-HR (95% CI) [a]	No. of participants	Adjusted-HR (95% CI) [a]	No. of participants	Adjusted-HR (95% CI) [a]
Frequency of regular use (days/week)						
None	67269 (76.6%)	1.00 (Ref.)	70146 (80.3%)	1.00 (Ref.)	76082 (86.7%)	1.00 (Ref.)
1 to 3	9539 (10.9%)	1.20 (0.93, 1.55)	9241 (10.6%)	0.97 (0.72, 1.31)	8130 (9.3%)	1.21 (0.85, 1.73)
More then 3	11011 (12.5%)	1.16 (0.95, 1.41)	7945 (9.1%)	1.17 (0.94, 1.45)	3513 (4.0%)	1.29 (0.94, 1.78)
P trend		0.48		0.22		0.69
Duration of regular use (yrs)						
Never	67269 (76.6%)	1.00 (Ref.)	70146 (79.5%)	1.00 (Ref.)	76082 (86.7%)	1.00 (Ref.)
<1 to 2	5270 (6.0%)	1.28 (0.94, 1.73)	7932 (9.0%)	1.03 (0.71, 1.48)	2908 (3.3%)	1.22 (0.80, 1.86)
3 to 4	2796 (3.2%)	1.26 (0.90, 1.76)	3920 (4.4%)	0.86 (0.57, 1.31)	1672 (1.9%)	1.00 (0.61, 1.66)
≥ 5	12650 (14.4%)	1.22 (0.96, 1.55)	6178 (7.0%)	1.01 (0.69, 1.48)	7556 (8.6%)	1.08 (0.77, 1.51)
P trend		0.77		0.97		0.58

[a] Multivariate model adjusted for age, smoking, diabetes, race/ethnicity, BMI, physical activities, alcohol use, hospitalization due to musculoskeletal system and connective tissue disease, hospitalization due to circulatory disease, asthma, coagulation/hemorrhagic conditions, antihypertensives use, frequency/duration of the index medication, and mutually adjusted for intensity of other classes of medication

Table 3.3 Joint analysis of dose and duration of regular NSAIDs and acetaminophen use at baseline and risk of age-related macular degeneration in California Teachers Cohort, 1995-2012 (N=88,481).

	Frequency (times/wk)	Year of Use		
		Never	<5 [a]	≥ 5 [a]
Aspirin	None	1.00 (ref.)		
	1 to 3		1.23 (0.90, 1.67)	1.14 (0.93, 1,40)
	4 or more		1.14 (0.96, 1.35)	1.12 (0.95, 1.33)
Ibuprofen	None	1.00 (ref.)		
	1 to 3		1.12 (0.85, 1.47)	0.93 (0.61, 1.40)
	4 or more		1.16 (0.96, 1.39)	1.23 (0.95, 1.60)
Acetaminophen	None	1.00 (ref.)		
	1 to 3		1.23 (0.87, 1.74)	1.07 (0.81, 1.42)
	4 or more		1.19 (0.88, 1.62)	1.24 (0.91, 1.69)

[a] Multivariate model adjusted for age, smoking, diabetes, race/ethnicity, BMI, physical activities, alcohol use, hospitalization due to musculoskeletal system and connective tissue disease, hospitalization due to circulatory disease, asthma, coagulation/hemorrhagic conditions, antihypertensives use, and mutually adjusted for intensity of other classes of medication.

Table 3.4 Self-reported NSAIDs and acetaminophen use in subsequent (2005) questionnaire and age-related macular degeneration in California Teachers Cohort, 2005-2012 (N=50,202).

Regular Medication use at 2005	No. of participants	Multivariable adjusted-HR [a]
Baby' or low-dose aspirin		
No	35834 (71.4%)	1.00 (ref.)
Yes	14368 (28.6%)	0.81 (0.70, 0.95)
Aspirin		
No	43594 (86.9%)	1.00 (ref.)
Yes	6596 (13.1%)	0.90 (0.73, 1.12)
Unknown	12	
Naproxen, ketoprofen or other NSAIDs		
No	45265 (90.2%)	1.00 (ref.)
Yes	4933 (9.8%)	1.00 (0.79, 1.27)
Unknown	4	
Ibuprofen		
No	40012 (79.7%)	1.00 (ref.)
Yes	10184 (20.3%)	0.90 (0.75, 1.07)
Unknown	6	
COX-2 Inhibitors		
No	48302 (96.2%)	1.00 (ref.)
Yes	1897 (3.8%)	0.45 (0.26, 0.78)
Unknown	3	
Steroid		
No	42859 (87.7%)	1.00 (ref.)
Yes	5993 (12.3%)	0.91 (0.73, 1.13)
Unknown		
Acetaminophen		
No	38697 (77.1%)	1.00 (ref.)
Yes	11504 (22.9%)	1.24 (1.06, 1.45)
Unknown	1	

[a] Multivariate model adjusted for updated age, smoking, diabetes, race/ethnicity, BMI, physical activities, alcohol use, hospitalization due to musculoskeletal system and connective tissue disease, hospitalization due to circulatory disease, asthma, coagulation/hemorrhagic conditions, antihypertensives use, previous analgesics use and mutually adjusted for other classes of medication.

3.5 Discussion

In a prospective cohort of California teachers followed on average for 14.8 years, regular consumption of aspirin or ibuprofen was not associated with increased risk of intermediate- or late-stage AMD. For the subsample of CTS participants who answered to the 2005 questionnaire, low-dose aspirin was assessed separately from standard-dose, and we observed a 20% decrease in the risk of AMD among women who regularly used low-dose aspirin. A potential beneficial effect was also seen in women who used selective COX-2 inhibitors, in fact, their risk of developing AMD was 55% lower.

Acetaminophen was presumed to have no association with AMD since it lacks proposed AMD-suppressive anti-inflammatory properties but due to its similar indications of use as NSAIDs it was used as a negative control. Thus, the hazard ratio greater than one for AMD among regular acetaminophen users implies that the underlying indications for analgesics use contribute to increased AMD risk may explain the estimated hazard ratios above 1 for aspirin and ibuprofen use at baseline. Also in 1995, regular aspirin users would have been taking a standard dose (325mg) rather than the cardioprotective low-dose aspirin (81mg) recommended later in the early 2000s by the American Heart Association guidelines[166], and the daily doses of NSAIDs may also have varied according to the indication of use:[167, 168] specifically the common dose at which ibuprofen is used for pain and anti-inflammatory purposes are 400-800 mg per day. Dose information, however, was not ascertained in the baseline questionnaire.

To assess potential residual confounding by indications, we conducted sensitivity analyses examining women with cardiovascular diseases or musculoskeletal disorders at baseline. Among

women with an OSHPD record of circulatory system disease at baseline, infrequent aspirin users (1-3 days/week) were at a higher risk of AMD compared with more frequent users (**Table A 3.2)**. In women with baseline hospital diagnoses of musculoskeletal system and connective tissue disease, regular aspirin and ibuprofen users did not increase or decrease their AMD risk. A slightly increased AMD risk among regular aspirin users was only observed among women who did not have cardiovascular diseases or severe musculoskeletal disorders at baseline, implying that either other unmeasured indications such as arthritis that did not require hospitalization or regular high dose aspirin use itself may contribute to an increased AMD risk.

Previous studies of aspirin use and AMD risk reported negative, null, as well as positive associations possibly due to the lack of a uniform definition of AMD, heterogeneous patterns of anti-inflammatory medication use, different lengths of follow-up, different degrees of residual confounding, and possibly confounding by indication. Two population-based longitudinal studies observed 2.2-2.5 times increased risk of incident neovascular AMD over 15 years of follow-up among participants who self-reported using aspirin more than one time per week, and a positive trend was seen with increasing frequency of use, however the authors did not find a trend with increasing dose. A null association was observed for geographic atrophy in these same studies.[120, 158] The positive association between aspirin use and wet AMD and the lack of an association for geographic atrophy was also seen in the European Eye Study, a cross-sectional assessment of frequent aspirin use and AMD prevalence.[121] Long-term aspirin use reduces synthesis of prostacyclin and leads to hypoxia with subsequent stimulation of neovascularization in the retina,[121] however, this positive association might also be due to selection bias from differential censoring by AMD status since these cohorts had high follow-up loss rates (44% and 48%).[120,

[158] In these cohorts standard retinal exams were performed at each follow-up visit, thus, subjects with existing maculopathies which require frequent monitoring of their retinal condition may have had higher incentives to participate. Estimates of the association between aspirin and AMD would be biased away from the null if those who were lost to follow-up were those without any retinal disorders and also those who had more medical conditions that necessitate aspirin use, thus making them more likely to drop-out due to severe health problems. The CTS follow-up we employed – OSHPD linkage - was passive in nature thus making a selective loss to follow-up among users less likely.

In contrast to these prospective cohort and cross-sectional studies, a clinical trial of female health professionals suggested a 20% decreased risk of AMD over ten years following 100 mg regular aspirin use (on alternate days i.e. ~3 days per week) to prevent cardiovascular diseases,[122] which is consistent with our findings of a 20% inverse association between 'baby' aspirin use and risk of AMD. Another randomized clinical trial of physicians followed for five years reported a comparable size risk reduction.[157] The proposed mechanism for aspirin are its beneficial effects on cardiovascular disease and atherosclerosis, which are risk factors for AMD development.[65, 169] Moreover, low dose aspirin can up-regulate the production of a local endogenous anti-inflammatory mediator.[122, 170] The inhibitive effects of aspirin on isoforms of the COX enzymes is irreversible and non-selective. Aspirin exerts its anti-thrombotic function through the acetylation of COX-1, a constitutive enzyme that is responsible for platelet aggregation. Long-term suppression of platelet aggregation is thought to decrease the progression to atherosclerosis, a common etiologic factor for cardiovascular disease and age-related macular degeneration.[171, 172] Deactivation of COX-2 by aspirin, on the other hand, reduces the production of

proinflammatory prostaglandins. Aspirin-triggered anti-inflammatory mediators have the potential to suppress drusen formation.[173] Another plausible biologic explanation is that the inhibition of COX-2 expression can regulate VEGF levels and prevent the development of neovascular AMD.[149]

Ignoring duration and frequency of aspirin use reported at baseline, the effect estimate for any aspirin use and risk of AMD in our data (HR = 1.12, 95% CI 1.00, 1.25) was similar to a previously reported pooled estimate [174] of risk for AMD in aspirin users that did not distinguish between low- and the standard-dose (RR = 1.09, 95% CI, 0.96, 1.24). In comparison, the HR for any acetaminophen use – our negative control medication - was 1.19 (95% CI: 1.09, 1.39). The discrepancy between the estimate for aspirin use in 1995 and as reported in 2005 in addition to being due to aspirin dose, may also be due to differences in residual confounding, lengths of follow-up, or a higher baseline risk of AMD among non-aspirin users in 2005 compared with 1995. As discussed earlier, certain underlying conditions that necessitate analgesic use may have confounded the association between baseline aspirin use and AMD.[163, 169] Since the hospitalization rate for arthritis patients is only 30%,[175] OSHPD records likely under record arthritis in the CTS women who were not hospitalized for other medical conditions, leading to potential residual confounding due to indication. However, this was partly addressed by adjusting for the use of other classes of anti-inflammatory medications i.e. information provided in the 2005 questionnaire. Moreover, a small group of women who were aspirin users in 1995 but had discontinued use in 2005 - probably due to side effects – and interestingly these women were also at a higher risk of developing AMD compared with never users **(Table A 3.3)**.

Previous studies reported inconclusive findings for non-aspirin NSAID use and AMD but few had information about the types of NSAIDs participants used. No association was observed between self-reported regular non-aspirin NSAIDs use and the five-year incidence of early- or late-stage AMD in two prospective cohort studies.[117, 118] In our 2005 subcohort analysis, a 60% decreased risk of AMD was detected specifically for COX-2 inhibitors but not for ibuprofen or other NSAIDs. This finding is further supported by an animal study in which oral administration of selective COX-2 inhibitors suppressed retinal VEGF expression and vascular leakage.[114] Although we cannot preclude the possibility of existing early-stage AMD at the time of completing the 2005 questionnaire, it was unlikely that women would stop taking NSAIDs after developing early-stage disease since a previous large multicenter study of AMD indicated a potentially protective effect of non-aspirin NSAIDs use on the risk of progression to geographic atrophy though not neovascular AMD.[76] The biologic explanations of potentially beneficial effects for COX-2 inhibitors are similar to aspirin, except that non-aspirin NSAIDs do not have anti-thrombotic or cardioprotective effects.

Our study has several strengths. This prospective study with routine passive follow-up via administrative hospital records provides us with a long average follow-up time and precludes self-selection out of this cohort. The large number of AMD cases observed enabled us to investigate effects for individual medications by duration and frequency and account for concurrent use of other types of medication. Furthermore, the self-reported medication use covered prescription and non-prescription anti-inflammatory medications and the use of OSHPD records allowed us to examine a comprehensive set of potential confounding factors, including indications and contraindications for aspirin and other NSAIDs use. However, there are also several limitations most notably, we had to rely on secondary diagnoses in hospitalization

records to identify AMD, thus the onset of early AMD could not be captured with this passive follow-up method. On the other hand, we did not have to rely on self-reported diagnoses and any outcome misclassification would be expected to be non-differential. Moreover, we were not able to disentangle any effects of medications on incident AMD from their effect on progression from early- to late-stage AMD. However, aspirin use has not been reported to contribute to AMD progression[76, 120] and in sensitivity analyses that excluded AMD cases within the first two or even five years of follow-up effect estimates did not change. Also, we were not able to separate geographic atrophy and neovascular AMD, which may have different pathophysiologies.[176] However, neovascular AMD is rare and can also be considered as the more severe and late stage form of AMD. Third, our study may suffer from bias due to non-differential misclassification of frequency and duration of medication use. Also, we had limited information to evaluate cumulative dose-response patterns since participants may have changed the frequency of their medication use over time. Lastly, we cannot rule out the possibility that the negative association between low-dose aspirin use was due to ophthalmologists' suggestion to avoid or discontinue regular use of aspirin to reduce AMD progression, since a number of subjects may have had early stage AMD by the time they completed the 2005 questionnaire; but as noted previously we excluded all cases diagnosed prior to baseline and in some of our analysis excluded all cases identified in the first five years of follow- up.[177]

3.6 Conclusions

The risk of intermediate- or late-stage AMD among women who reported regularly taking low-dose aspirin or COX-2 inhibitors was reduced in California teachers. However, standard-dose aspirin use and ibuprofen or other NSAIDs use was not protective, if anything our estimates

suggested an increased risk. The elevated risk we also estimated for acetaminophen use, our negative control medication, suggests that these increased risk estimates might be due to confounding by indication. Future prospective studies of AMD and pain medications should evaluate the dosage, type, and timing of analgesic use.[178]

3.7 Appendix

Table A 3.1 Demographic characteristics and lifestyle factors of participants at baseline in the California Teachers Cohort, 1995-2012 (N=122,629).

Characteristics	Participants with No hospitalization data (All CA residents)	Study population (CA resident with at least 1 OSHPD record)	Age-adjusted HR	Multivariable adjusted-HR [a]	Multivariable adjusted-HR weighted by 1/P(selection) [b]
Total	34148 (100%)	88481 (100%)	NA	NA	NA
Age					
<50	17615 (51.6%)	35500 (40.1%)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
51-60	10038 (29.4%)	19916 (22.5%)	5.11 (3.73, 7.00)	4.30 (3.07, 6.01)	4.30 (3.11, 5.94)
61-70	3868 (11.3%)	16571 (18.7%)	23.70 (17.86, 31.46)	19.60 (14.50, 26.55)	20.05 (14.92, 26.93)
71-80	1654 (4.8%)	11825 (13.4%)	71.80 (54.33, 94.90)	57.22 (42.39, 77.24)	58.46 (43.60, 78.39)
>80	973 (2.8%)	4669 (5.3%)	200.03 (149.28, 268.02)	160 (116.12, 220.81)	164 (119.68, 226.16)
Race/Ethnicity					
Non-Latina white	29157 (86.2%)	77079 (87.9%)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
African American	991 (2.9%)	2260 (2.6%)	0.56 (0.37, 0.84)	0.63 (0.41, 0.96)	0.68 (0.45, 1.04)
Native American	1446 (4.3%)	3709 (4.2%)	0.66 (0.42, 1.03)	0.61 (0.36, 1.01)	0.58 (0.34, 1.00)
Latina	283 (0.8%)	829 (0.9%)	0.99 (0.64, 1.52)	0.99 (0.58, 1.68)	0.98 (0.55, 1.74)
Asian/Pacific Islander	1488 (4.4%)	2796 (3.2%)	0.31 (0.18, 0.55)	0.24 (0.12, 0.49)	0.27 (0.13, 0.53)

Other	468 (1.4%)	1048 (1.2%)	0.80 (0.40, 1.61)	0.69 (0.28, 1.65)	0.69 (0.28, 1.71)
Missing	315	760			
BMI					
Underweight	921 (2.8%)	2276 (2.7%)	1.25 (0.91, 1.71)	1.12 (0.78, 1.61)	1.12 (0.77, 1.65)
Normal	21056 (63.9%)	47374 (56.1%)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Overweight	7499 (22.8%)	21831 (25.8%)	1.18 (1.05, 1.31)	1.14 (1.01, 1.28)	1.16 (1.02, 1.31)
Obese	3484 (10.6%)	13012 (15.4%)	1.21 (1.04, 1.39)	1.06 (0.90, 1.24)	1.08 (0.91, 1.29)
Unknown	1188	3988			
History of High blood pressure					
No	30239 (88.6%)	70874 (80.1%)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Yes	3909 (11.4%)	17607 (19.9%)	1.25 (1.13, 1.38)	1.17 (0.97, 1.40)	1.16 (0.96, 1.42)
History of Heart attack/MI					
No	33961 (99.5%)	87035 (98.4%)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Yes	187 (0.5%)	1446 (1.6%)	1.14 (0.87, 1.50)	1.05 (0.77, 1.42)	1.05 (0.75, 1.49)
History of Stroke					
No	33874 (99.2%)	87189 (98.5%)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Yes	274 (0.8%)	1292 (1.5%)	1.02 (0,74, 1.42)	0.95 (0.66, 1.36)	0.95 (0.63, 1.41)
History of Diabetes					

No	33615 (98.4%)	85492 (96.6%)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Yes	533 (1.6%)	2989 (3.4%)	1.75 (1.43, 2.13)	1.72 (1.37, 2.15)	1.73 (1.35, 2.22)
Smoking					
Never	6555 (19.4%)	16782 (19.2%)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Passive	16314 (48.4%)	40368 (46.1%)	0.95 (0.82, 1.09)	0.98 (0.84, 1.15)	0..99 (0.84, 1.17)
Former	9253 (27.4%)	25751 (29.4%)	1.16 (1.01, 1.34)	1.16 (0.99, 1.37)	1.16 (0.97, 1.38)
Current	1594 (4.7%)	4590 (5.2%)	1.41 (1.12, 1.78)	1.31 (1.00, 1.71)	1.33 (0.99, 1.79)
Missing	432	990			
No. of smoking pack-years					
Never or passive smoker	22869 (69.9%)	57150 (67.4%)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
≤ 10	5881 (18%)	14052 (16.6%)	1.00 (0.87, 1.15)	0.99 (0.86, 1.23)	1.00 (0.85, 1.18)
11-20	1901 (5.8%)	5187 (6.1%)	1.14 (0.95, 1.37)	1.09 (0.88, 1.33)	1.10 (0.88, 1.37)
≥ 20	2077 (6.3%)	8464 (10%)	1.62 (1.42, 1.84)	1.50 (1.30, 1.73)	1.50 (1.28, 1.76)
Missing	1420	3628			
Daily alcohol intake (g)					
None	10188 (31.3%)	29086 (34.9%)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
<20	19700 (60.5%)	47340 (56.7%)	0.97 (0.87, 1.08)	0.93 (0.83, 1.05)	0.93 (0.82, 1.05)
≥20	2651 (8.1%)	7015 (8.4%)	1.06 (0.90,	0.95 (0.79,	0.98 (0.80,

			1.26)	1.15)	1.20)
Unknown	1609	5040			
Lifetime moderate and strenuous physical activity (h/week)					
<2	10390 (30.6%)	29272 (33.3%)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
2 to 4	9019 (26.6%)	21822 (24.8%)	0.93 (0.82, 1.05)	0.91 (0.79, 1.04)	0.91 (0.78, 1.04)
4 to <6	5853 (17.3%)	14426 (16.4%)	0.95 (0.82, 1.10)	0.89 (0.76, 1.05)	0.87 (0.73, 1.04)
≥ 6	8667 (25.5%)	22319 (25.4%)	0.83 (0.73, 0.94)	0.81 (0.70, 0.93)	0.80 (0.69, 0.94)
Unknown		642			
Aspirin use					
No	27464 (80.4%)	67269 (76.0%)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Yes	6684 (19.6%)	21212 (24.0%)	1.14 (1.03, 1.26)	1.10 (0.99, 1.24)	1.10 (0.97, 1.24)
Ibuprofen use					
No	27991 (82%)	70146 (79.3%)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Yes	6157 (18%)	18335 (20.7%)	1.22 (1.08, 1.37)	1.10 (0.95, 1.25)	1.07 (0.92, 1.24)
NSAIDs					
Missing	1 (.%)				
No	23617 (69.2%)	55967 (63.2%)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Yes	10530 (30.8%)	32514 (36.7%)	1.19 (1.09, 1.31)	1.13 (1.01, 1.39)	1.12 (1.00, 1.26)

Acetaminophen use					
No	30253 (88.6%)	76082 (86.0%)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Yes	3895 (11.4%)	12399 (14.0%)	1.28 (1.12, 1.48)	1.19 (1.01, 1.39)	1.18 (1.00, 1.40)
Antihypertensives					
No	30156 (88.3%)	77706 (87.8%)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Yes	3992 (11.7%)	10775 (12.2%)	1.25 (1.14, 1.38)	1.03 (0.86, 1.23)	1.02 (0.84, 1.25)

[a] Multivariable model adjusted for all variables listed in the table.
[b] Weighted multivariable model is weighted by the inverse probability of having at least one OSHPD records throughout the study period.

Table A 3.2 Self-reported NSAIDs and acetaminophen use and age-related macular degeneration stratified by indications in California Teachers Cohort, 1995-2012 (N=88,481).[a]

	History of circulatory system disease at baseline		History of musculoskeletal system and connective tissue disease at baseline		Moderate or strenuous physical activity	
	No	**Yes**	**No**	**Yes**	**<2 hrs/week**	**≥ 2 hrs/week**
Aspirin (days/week)						
None	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
1 to 3	1.19 (0.89, 1.58)	1.41 (0.76, 2.60)	1.29 (0.98, 1.68)	0.60 (0.23, 1.56)	1.15 (0.86, 1.53)	1.44 (0.79, 2.64)
More then 3	1.25 (1.01, 1.56)	0.89 (0.56, 1.43)	1.22 (0.99, 1.50)	0.76 (0.38, 1.52)	1.12 (0.90, 1.39)	1.34 (0.84, 2.15)
Ibuprofen (days/week)						
None	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
1 to 3	0.87 (0.62, 1.22)	1.65 (0.83, 3.28)	1.04 (0.76, 1.43)	0.62 (0.23, 1.67)	0.94 (0.67, 1.33)	1.04 (0.55, 1.95)
More then 3	1.23 (0.98, 1.56)	0.82 (0.45, 1.52)	1.20 (0.95, 1.51)	1.00 (0.54, 1.85)	1.17 (0.92, 1.48)	1.17 (0.70, 1.96)
Acetaminophen (days/week)						
None	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
1 to 3	1.22 (0.82, 1.83)	1.36 (0.64, 2.87)	1.36 (0.93, 1.98)	0.72 (0.26, 2.00)	1.05 (0.69, 1.58)	1.19 (0.83, 1.70)
More then 3	1.38 (0.96, 1.99)	1.11 (0.57, 2.13)	1.38 (0.98, 1.96)	0.85 (0.37, 1.97)	1.23 (0.86, 1.76)	1.30 (0.95, 1.79)

[a] Multivariate model adjusted for age, smoking, diabetes, race/ethnicity, BMI, physical activities, alcohol use, hospitalization due to musculoskeletal system and connective tissue disease, hospitalization due to circulatory disease, asthma, coagulation/hemorrhagic conditions, antihypertensives use, and mutually adjusted for intensity of other classes of medication.

Table A 3.3 Self-reported NSAIDs and acetaminophen use and age-related macular degeneration stratified by time of initiation in California Teachers Cohort, 2005-2012 (N=50,202).

	No. of participants	Adjusted-OR[a] (95% CI)
Aspirin		
No use at baseline or in 2005	22635 (51.9%)	1.00 (ref.)
Use at baseline, no use in 2005	4446 (10.2%)	1.24 (0.98, 1.56)
No use at baseline, use in 2005	10713 (24.6%)	0.98 (0.83, 1.17)
Use at baseline and in 2005	5817 (13.3%)	0.86 (0.70, 1.07)
Non-aspirin NSAIDs		
No use at baseline or in 2005	2558 (58.6%)	1.00 (ref.)
Use at baseline, no use in 2005	4363 (10.0%)	1.14 (0.91, 1.43)
No use at baseline, use in 2005	8702 (19.9%)	0.84 (0.69, 1.03)
Use at baseline and in 2005	4993 (11.4%)	0.87 (0.66, 1.15)
Acetaminophen		
No use at baseline or in 2005	30781 (70.6%)	1.00 (ref.)
Use at baseline, no use in 2005	3174 (7.3%)	1.15 (0.83, 1.60)
No use at baseline, use in 2005	6891 (15.8%)	1.15 (0.96, 1.37)
Use at baseline and in 2005	2772 (6.4%)	1.50 (1.13, 1.96)

[a] Multivariate model adjusted for age, smoking, diabetes, race/ethnicity, BMI, physical activities, alcohol use, hospitalization due to musculoskeletal system and connective tissue disease, hospitalization due to circulatory disease, asthma, coagulation/hemorrhagic conditions, antihypertensives use, frequency/duration of the index medication, and mutually adjusted for intensity of other classes of medication.

Chapter 4. Hypertension, Antihypertensive Medications Use and Risk of Age-Related Macular Degeneration

4.1 Abstract

Background

Sustained and badly controlled hypertension has the potential to promote the development of age-related macular degeneration (AMD) through multiple biologic pathways. Epidemiologic studies of high blood pressure, antihypertensive therapies, and the risk of AMD thus far have been inconclusive. However, few studies evaluated risks according to use of different classes of antihypertensive drugs separately or took combinations of use into account.

Methods

In the California Teachers Study (CTS) cohort (N = 88, 481) we identified diagnoses of AMD by linkage to California Office of Statewide Health Planning and Development (OSHPD) hospital discharge records between Dec.1990 and Dec.31, 2012. History of high blood pressure, regular use of diuretics and other antihypertensive medications and comprehensive risk factor information were reported in self-administered CTS questionnaire in 1995-1996 at cohort enrolment, and information on specific antihypertensive drugs (diuretics, ACE inhibitors, calcium channel blockers, or others) was available for a subsample of CTS participants who completed a followup questionnaire in 2000. We employed Cox proportional hazard regressions models adjusting for a number of potential confounders to model AMD risk during follow-up.

Results

We identified 1,762 teachers with AMD during the 14.8 years of average follow-up in this cohort. We estimated increased risks of AMD among women with a history of high blood pressure and were using at least one antihypertensive drug at baseline (HR = 1.15, 95% CI: 1.03, 1.30); the magnitude of the association increased with longer duration of antihypertensive treatment. Among 1,156 AMD cases identified after 2000 and followed for 11.1 years on average, we estimated a 45% increased risk of AMD among women receiving diuretics as monotherapy in 2000 (HR = 1.45, 95% CI 1.10, 1.90). While proper control of hypertension with a combination of drugs did not result in an increase in AMD.

Conclusion

Our study found an increased risk of intermediate- or late-stage AMD among women with badly controlled hypertension while those treated with a combination of antihypertensive drugs or consistently treated with medications more potent than diuretics did not have an increased risk of AMD.

4.2 Introduction

Both age-related macular degeneration (AMD) and the prevalence of high blood pressure increase with advancing age. Approximately 30% of those aged 40-60 years in the US have high blood pressure, and the prevalence increases to 65% in the over 60 year old population.[179] These high rates make antihypertensives the most commonly prescribed medications in the US with 678.2 million prescriptions filled in 2010.[174] Among the blood pressure lowering drugs, beta-

blockers, angiotensin-converting enzyme (ACE) inhibitors, diuretics, calcium-channel blockers, and angiotensin receptor blockers were the most used agents since 1990s, when used either alone or in combination.[174, 180]

Hypertension is thought to increase the risk of AMD through damaging retinal vessels.[181] Narrowed retinal arteries resulting from chronic hypertension may reduce choroidal blood flow and disturb the vascular homeostasis of the retina, thus stimulating choroidal neovascularization, a key feature of wet-AMD.[182] Previous epidemiologic studies of elevated blood pressure, history of hypertension and its treatment and AMD risk reported inconsistent findings. The Framingham Study found increased risk of moderate to severe age-related maculopathy in 25 years follow up among participants who had hypertension at baseline and the strength of the associations increased with longer duration of chronic hypertension.[183] This finding has been confirmed in other prospective studies with both short- or long-term follow up,[184, 185] but not in some other population-based studies for AMD at any stage.[186, 187]

Due to the heterogeneous pathophysiology of hypertension, subclasses of antihypertensive medications with distinct pharmacological effects have been developed to manage the disorder. For example, renin-dependent hypertension can be effectively controlled by ACE inhibitors, beta-blockers, or angiotensin II receptor blockers.[188] While in circumstances when plasma renin levels are suppressed, diuretics or calcium channel blockers are the more appropriate medication

to lower the blood pressure.[188] Those medications are also commonly used complementarily to improve the efficacy of the treatment and to prevent dose-dependent side effects from managing hypertension.[189] Antihypertensive treatment effects on AMD have also been investigated, but these studies have not supported a dose-dependent effect.[125, 190-192] A case-control study reported that a history of hypertension doubled the risk of neovascular AMD, and physician-reported antihypertensive medication use increased the risk of neovascular AMD by 2.5 times.[193] Increased risk of AMD with the use of specific types of antihypertensive drugs was observed for calcium channel blockers,[190, 192] beta blockers,[124, 191] and thiazide diuretics,[163] in some studies but not others.[124, 163, 190-192] Moreover, previous studies targeting antihypertensive medications did not consider the duration of the treatment nor the combined use of blood pressure lowering drugs of different classes.

Given the high prevalence of hypertension and its treatment, even a modestly increased AMD risk would have considerable public health implications. Thus, it is imperative to better understand the association between antihypertensive medication use and the risk of developing AMD. The purpose of this study is to investigate whether hypertension and regular use of specific antihypertensive medications is associated with the risk of AMD accounting for the duration of use. Also, specific types including diuretics, calcium channel blockers, ACE-inhibitors, and other antihypertensive drugs will be examined separately.

4.3 Methods

Detailed information on the study population, data sources for the California Teachers Study and the ascertainment of AMD cases has been described in the last chapter. Briefly, eligibility for this study was limited to women who were California residents at baseline and had at least one OSHPD record available (N = 89,877). Participants who, according to OSHPD records, suffered from AMD prior to completing the baseline questionnaire (N = 22) and those who did not report their regular antihypertensive medication use (n = 1,347) at baseline were excluded, leaving 88,481 participants for analysis.

Any AMD event was identified (ICD-9-CM codes 362.50, 362.51, 362.52, and 362.57) from three different OSHPD data sources: hospital discharge data (available from 12/1990--12/2012), ambulatory service data (01/2010—12/2012), and emergency department data (01/2010—12/2012); all of which captured up to 25 diagnoses and the date of service for the primary reason of the visit. The first AMD event (earliest date of AMD diagnosis in hospital record system) was considered the incident date. According to the guideline for secondary diagnoses in hospitals,[161] only co-existing conditions that affect current treatments should be recorded. Thus, we assume that the majority of AMD cases identified in this manner were intermediate to advanced and had impaired central vision, because these patients are more likely to require additional therapeutic procedures, and increased nursing care and have an extended length of stay.[162] In contrast, patients with early stage AMD do not meet the criteria for a comorbid disorder relevant to

current treatment and care during hospitalization; thus, we most likely did not capture as many of these with our passive linkage to hospital discharge records.

Follow-up started the day the baseline questionnaire was completed by a participant and ended at the earliest occurrence of one of the four events: 1) AMD diagnosis; 2) moving out of California; 3) death; 4) date of the most recent linkage of the CTS to OSHPD records (Dec. 31st, 2012). At baseline, participants reported history of high blood pressure (ever or never), regular medication use (at least once per week), average frequency of use (1-3, 4-6, or 7 days per week), and total years of use (<1, 1, 2, 3-4, 5-9, or ≥10). Intake of regular "reserpine (Raudixin, Ser-Ap-Es, Hydropres, Rauwolfia, Metatensin)", "Water pills for high blood pressure (Diuril, Hydrodiuril, Dyazide, etc.)", and "other high blood pressure medications" were asked separately. More detailed antihypertensive medication use was recorded in a subsequent questionnaire mailed to cohort members in 2000-2001 assessing daily medication use for at least two months within 2 years prior to the survey: "Thiazide diuretic (for example: Diuril, Hydrodiuril, Dyazide)", "Lasix", "Calcium blocker (for example: Calan, Procardia, Cardizem)", "ACE inhibitors (for example: Capoten, Vasotec, Zestril)" and "Other high blood pressure medication". Regular antidiabetics use (insulin or oral hypoglycemic medication) and cholesterol-lowering drugs (Questran, Mevacor, Lopid) use were also queried.

Potential confounder and effect modifier information was identified from questionnaire data or OSHPD hospital discharge records and were selected based on the literature on AMD.[65, 163] We included in our models age (continuous), race/ethnicity (Non-Latina white, African-American, Latina, Asian/Pacific Islander, Other), body mass index (<18.5, 18.5 to 25, 25 to <29, ≥30), diabetes (ever/never), smoking (never, former, current), alcohol use in the year prior to baseline (no use, less than 20 grams per day, greater than 20 grams per day), lifetime average moderate or strenuous physical activity per week (<2h, 2 to <4 h, 4 to <6 h, 6 h or more), acetaminophen and non-steroidal anti-inflammatory medication use; baseline diagnostic history of heart failure (ICD-9-CM: 428) or other circulatory system diseases (ICD-9-CM: 390-427, 429–459) were obtained from OSHPD hospital discharge data. We also assessed percent daily dietary calories from fat (continuous), and dietary antioxidant consumption (average daily dose) but we ultimately decided to not include them in our analyses due to less than 10% change in the point estimates of hazard ratios.

In the analyses of 2000 medication data, all the above-mentioned risk factors were updated to reflect the most recently available data (subsequent questionnaires and OSHPD hospital discharge data). We additionally adjusted for the type of diagnoses of cardiac diseases that may affect the choice of antihypertensive agents: cardiac failure (ICD-9-CM: 428), cardiac arrhythmia (ICD-9-CM: 427), coronary artery disease (ICD-9-CM: 414), and other circulatory

system diseases (ICD-9-CM: 390-413, 415-425, 429–459). Cholesterol-lowering medication use was also accounted for in the analyses.

Statistical Analysis

Multivariable Cox proportional hazards regression was used to assess the association between hypertension (reflected by self-reported history of having high blood pressure or antihypertensive medication use) and AMD, using calendar time at start and end of follow-up (in days) to define person-time. A composite variable of treated hypertension was generated and assessed for the association with AMD risk: women who self-reported having a history of high blood pressure and taking at least one type of antihypertensive medications were considered as treated hypertension, those who had a history but not taking any antihypertensive medications were considered as untreated hypertension and the reference category was women with no history of high blood pressure. We examined the baseline antihypertensive medications (any antihypertensive drugs use, diuretics, or other high blood pressure drugs) in categories of duration of use (never, <1, 2-4, 5-9, ≥10 years) and tested for trend using category midpoints. The longest duration of reserpine, diuretics, or other high blood pressure medication use was considered as the duration of any antihypertensive medication use. Since reserpine was taken by a very small number of participants and is rarely prescribed nowadays, it was co-adjusted for in the analyses of other classes of drugs but was not separately. The proportional hazards

assumption was checked using Kaplan-Meier survival curves and graphs of the log(-log(survival)) versus log of survival time; parallel lines indicated proportionality of hazards.[164] To examine the strength of associations according to the severity of underlying hypertension, the adequacy of the high blood pressure control, and different classes of antihypertensive medication use, Cox proportional hazard regression was applied in the subsample analyses that relied on the 2000 questionnaire information. After excluding women who developed AMD, moved out of California, died before 2000, or who did not return this 2nd questionnaire or answering questions on medication use, 56,628 subjects were eligible for the subsample. We further excluded 3% of women (N=1,655) who reported using antihypertensive drugs at baseline but not in the 2000 questionnaire for analyses in the subsample. We divided women who reported using antihypertensive medications in the follow-up questionnaire into newly initiated users and consistent users, and did two separate comparisons of them to a common reference group of women who were never exposed to antihypertensive drugs at baseline or in 2000 to assess the association between hypertension (as reflected by combinations of antihypertensive medication use) and the risk of AMD.

According to hypertension treating guidelines, at least two drugs are recommended at the initiation of antihypertensive therapy when the hypertension is severe (>20 mm Hg above systolic goal or >10 mm Hg above diastolic goal),[194] when there are comorbidities such as chronic kidney diseases and cardiovascular diseases, or when evidence shows the presence of

asymptomatic organ damage. Since more extensive initial treatment of hypertension is required for high-risk patients to achieve a lower target of blood pressure,[195] we used the number of different classes of antihypertensive agents initiated as a proxy for the difficulty in achieving BP control and compared the risks of AMD across categories of the severity indicator among women with newly developed hypertension in 2000.

Adjustments to the antihypertensive regimen, including adding a new agent to the existing therapeutic combination or switch to new combinations, are required whenever the blood pressure target is not achieved.[195] In a previous large prospective study, starting with combination regimens for hypertension and maintained using more than two types of drugs in the follow-up demonstrated a benefit effects on lowering incident cardiovascular events compared to patients who initiated with monotherapy and moved to combination therapy, or who failed to maintain the combination therapy.[196] Thus, among women with long-term hypertension in 2000, we generated an indicator for the adequacy of blood pressure control according to the modification of the therapeutic combination since baseline and assessed the risk of AMD in each category of the adequacy of control indicator. Specifically, long-term antihypertensive drug users were categorized as 1) consistently used, or newly switched to diuretics as monotherapy; 2) added a new type of agent to the existing combination or switched to a new class of agent since baseline; 3) consistently used medication(s) more potent than diuretics since baseline with or without an adjustment for the treatment strategy since baseline.

To evaluate if a specific class of antihypertensives was inferior or superior to others with regard to the AMD toxicity, regular use of major types of antihypertensive medications including any diuretics (thiazide or loop diuretics), calcium channel blockers, ACE inhibitors, and other antihypertensive medications was examined separately among women who reported using at least one type of antihypertensive medications.

Stratified analyses were conducted to assess any potential modifying effect by the history of hospitalization due to circulartory system diseases, cholesterol-lowering drug use, diabetes, average lifetime strenuous or moderate physical activities, and BMI.

We performed sensitivity analyses by excluding women who had the first AMD diagnosis within the first five years of follow-up to exclude prevalent AMD cases. Excluding the first five years of AMD diagnoses after 1995 or 2000 did not change our results more than minimally (**Table A 4.1**).

4.4 Results

Among 88,481 female teachers enrolled in the CTS, we identified 1,762 subjects with AMD. During an average of 14.8 years of follow-up, 6,598 (7.5%) women moved out of California for a period of more than one year and 16,444 (18.6%) died, leaving 63,677 (72%) subjects who were right censored at end of follow-up (Dec.31, 2012). The median time to a first AMD

diagnosis was 13.5 years (interquartile range: 10.2, 15.5). The distribution of demographic and lifestyle factors among women who did or did not use antihypertensive medications is presented in **Table 4.1**. The crude self-reported rate of having a history of high blood pressure in 1995 was 20%. The frequency of antihypertensive medication use increased with age and was higher among overweight or obese women (BMI $\geq$ 30 kg/m^2), women who exercised little (<2 hr/week moderate and strenuous physical activity), and women with a self-reported history of medical conditions at baseline that are highly correlated with high blood pressure (heart attack, stroke and diabetes). The current smoking rates in this cohort were low but slightly higher among antihypertensive drug users, and the total pack-years of smoking were higher among women who regularly took antihypertensive medication due to a higher proportion of former smokers amongst them. A slightly lower proportion of women drank alcohol among antihypertensive drug users, but heavier drinkers (≥20 g/day) were more common in users.

In those with a history of high blood pressure at baseline 85% reported taking at least one antihypertensive drug with 37% taking them for more than 10 years (**Table 4.2**). Compared with teachers without high blood pressure, the risk of AMD was slightly increased in women with treated hypertension (HR = 1.15, 95% CI: 1.03, 1.30), but not in women with untreated hypertension. A positive trend was suggested with increasing duration of antihypertensive medications use (P-trend=0.08). No difference was shown when evaluating diuretics use or other antihypertensive drugs use respectively. When stratifying by severity of a newly developed

hypertension in 2000 (according to the number of treatments they took), such that we estimated a 70% increased risk among women who started with two types of anti-hypertensives, and the risk of AMD doubled in women who used three types or more (**Table 4.3**). Evaluating the adequacy of blood pressure control and AMD risk (**Table 4.3**), the highest risk of AMD was seen in women using the least potent therapy long-term (HR=1.80, 95% CI: 1.32, 2.47) while associations between high blood pressure treatment and AMD were null in women who experience modifications of the combination of therapeutic strategy since baseline.

For women in the year 2000 subsample, the median time to diagnosis of AMD was 9.6 years (interquartile range: 7.0, 11.2). Compared to baseline, a higher proportion of women reported using at least one type of antihypertensive drugs (27.5% vs. 21.3%). Among women with combination therapy, we saw no increased risk of AMD for each individual class of drugs. In contrast, among antihypertensive women with monotherapy, diuretics increased AMD risk (HR=1.45, 95% CI: 1.10, 1.90) (**Table 4.4**). There was however no increased risk of AMD with use of ACE inhibitors, calcium channel blockers, or other antihypertensive medications.

We found AMD risk associated with daily antihypertensive medication use to be modified by circulatory system disease status, cholesterol-lowering drugs use, diabetes, BMI, physical activities and antihypertensive medication use at baseline (**Table 4.5).** Larger effect estimates in

those not using cholesterol-lowering drugs, with diabetes or cardiovascular disease, BMI over 30 kg/m^2, and physical inactivity.

Table 4.1 Demographic characteristics and life style factors of study population at baseline in the California Teachers Cohort, 1995-2012 (N=88,481).

Characteristics	All participants	Exposure to antihypertensive medications
Total	88481 (100%)	18812 (100%)
Age		
<50	35500 (40.1%)	2411 (12.8%)
51-60	19916 (22.5%)	4230 (22.5%)
61-70	16571 (18.7%)	5277 (28.1%)
71-80	11825 (13.4%)	4797 (25.5%)
>80	4669 (5.3%)	2097 (11.1%)
Race/Ethnicity		
Non-Latina white	77079 (87.9%)	16482 (88.5%)
African American	2260 (2.6%)	829 (4.5%)
Native American	3709 (4.2%)	379 (2%)
Latina	829 (0.9%)	234 (1.3%)
Asian/Pacific Islander	2796 (3.2%)	554 (3%)
Other	1048 (1.2%)	150 (0.8%)
Missing	760	184
BMI		
Underweight	2276 (2.7%)	310 (1.8%)
Normal	47374 (56.1%)	7132 (40.6%)
Overweight	21831 (25.8%)	5540 (29.4%)
Obese	13012 (15.4%)	4589 (24.4%)
Unknown	3988	1241
History of High blood pressure		
No	70874 (80.1%)	3799 (20.2%)
Yes	17607 (19.9%)	15013 (79.8%)
History of Heart attack/MI		
No	87035 (98.4%)	17955 (95.4%)
Yes	1446 (1.6%)	857 (4.6%)
History of Stroke		
No	87189 (98.5%)	18148 (96.5%)
Yes	1292 (1.5%)	664 (3.5%)
History of Diabetes		
No	85492 (96.6%)	17251 (91.7%)
Yes	2989 (3.4%)	1561 (8.3%)
Smoking		
Never	16782 (19.2%)	2641 (14.2%)

Passive	40368 (46.1%)	8517 (45.8%)
Former	25751 (29.4%)	6388 (34.3%)
Current	4590 (5.2%)	1053 (5.7%)
Missing	990	213
No. of smoking pack-years		
Never or passive smoker	57150 (67.4%)	11158 (62.1%)
≤ 10	14052 (16.6%)	2763 (15.4%)
11-20	5187 (6.1%)	1338 (7.4%)
≥ 20	8464 (10%)	2722 (15.1%)
Missing	3628	831
Daily alcohol intake (g)		
None	29086 (34.9%)	6914 (39.1%)
<20	47340 (56.7%)	9038 (51.1%)
≥20	7015 (8.4%)	1735 (9.8%)
Unknown	5040	1125
Lifetime moderate and strenuous physical activity (h/week)		
<2	29272 (33.3%)	6914 (39.1%)
2 to 4	21822 (24.8%)	9038 (51.1%)
4 to <6	14426 (16.4%)	1735 (9.8%)
≥ 6	22319 (25.4%)	3761 (20%)
Unknown	642	192

Table 4.2 Hypertension, antihypertensive medications use at baseline and age-related macular degeneration in California Teachers Cohort, 1995-2012 (N=88,481).

Regular medication use at baseline	No. of participants[b]	Age-adjusted HR	Multivariable adjusted-HR[a]	P-trend
Self-reported high blood pressure				
None	70874 (80.1%)	1.00 (ref.)	1.00 (ref.)	
Treated hypertension	15013 (17.0%)	1.26 (1.13, 1.39)	1.15 (1.03, 1.30)	
Untreated hypertension	2594 (2.9%)	1.14 (0.89, 1.45)	0.97 (0.73, 1.30)	
Regular use of any antihypertensive medications				
No	69669 (78.7%)	1.00 (ref.)	1.00 (ref.)	
Yes	18812 (21.3%)	1.23 (1.12, 1.36)	1.09 (0.98, 1.22)	
Duration of any antihypertensive medications use				0.08
No regular use	70162 (79.3%)	1.00 (ref.)	1.00 (ref.)	
Less than 1 year	2022 (2.3%)	1.13 (0.85, 1.51)	0.92 (0.66, 1.29)	
1-4 years	5606 (6.3%)	1.20 (1.02, 1.41)	1.05 (0.88, 1.26)	
5-9 years	3801 (4.3%)	1.21 (1.00, 1.45)	1.08 (0.88, 1.33)	
More than 10 years	6884 (7.8%)	1.28 (1.12, 1.45)	1.13 (0.98, 1.31)	
Regular use of diuretics				
No	77706 (87.8%)	1.00 (ref.)	1.00 (ref.)	
Yes	10775 (12.2%)	1.18 (1.06, 1.33)	1.05 (0.91, 1.20)	
Duration of diuretic use				0.25
No regular use	77706 (88.1%)	1.00 (ref.)	1.00 (ref.)	
Less than 1 year	1331 (1.5%)	1.02 (0.72, 1.44)	0.86 (0.59, 1.27)	

1-4 years	3196 (3.6%)	1.11 (0.90, 1.36)	1.01 (0.80, 1.27)	
5-9 years	1972 (2.2%)	1.21 (0.95, 1.55)	1.12 (0.86, 1.47)	
More than 10 years	4026 (4.6%)	1.27 (1.09, 1.49)	1.09 (0.90, 1.30)	
Regular use of other antihypertensive drugs				
No	74652 (84.4%)	1.00 (ref.)	1.00 (ref.)	
Yes	13829 (15.6%)	1.20 (1.08, 1.33)	1.05 (0.92, 1.19)	
Duration of other antihypertensive drugs use				0.16
No regular use	74652 (84.8%)	1.00 (ref.)	1.00 (ref.)	
Less than 1 year	1426 (1.6%)	1.20 (0.87, 1.85)	0.93 (0.64, 1.35)	
1-4 years	4476 (5.1%)	1.19 (0.98, 1.39)	1.01 (0.83, 1.23)	
5-9 years	2922 (3.3%)	1.17 (0.95, 1.45)	1.03 (0.81, 1.30)	
More than 10 years	4526 (5.1%)	1.26 (1.08, 1.46)	1.14 (0.95, 1.36)	

[a] Multivariate model adjusted for age, smoking, diabetes, race/ethnicity, BMI, physical activities, history of cardiovascular disease, acetaminophen use, NSAIDS use and mutually adjusted for other categories of antihypertensive drugs.

[b] Number of participants may not add up to total due to unknown value.

Table 4.3 Self-reported antihypertensive medication use among follow-up respondents (2000) and age-related macular degeneration stratified by drug combinations in California Teachers Cohort, 2000-2012 (N=55,768).

Combination of antihypertensive medication use in 2000	No. of participants	No. of AMD	HR (95% CI)
Never used	40206	565	1.00 (ref.)
Used for newly developed hypertension (N=6,529)			
Single type	5162	158	1.16 (0.94, 1.43)
2 types	1137	47	1.70 (1.20, 2.40)
3 types or more	229	14	1.93 (1.02, 2.40)
Used for long-term hypertension (N=9,033)			
Consistent use of, or reduced to diuretics monotherapy[*ł]	898	53	1.80 (1.32, 2.47)
Adjustment to drug combinations compared to baseline[£]	2748	105	1.20 (0.93, 1.54)
Consistent use of medications more potent than diuretics[ǂ]	5374	187	1.07 (0.87, 1.32)

[*] The diuretics category includes both thiazide and loop diuretics

[ł] Consistent use of diuretics as monotherapy or reduced the combination at baseline to diuretics monotherapy in 2000

[£] Added medication(s) to the baseline combination or switch to another type in 2000

[ǂ] With or without adjustment in 2000

Table 4.4 Self-reported antihypertensive medications use among follow-up respondents with hypertension and age-related macular degeneration stratified by type of treatment in California Teachers Cohort, 2000-2012 (N=15,562).

	Women with Monotherapy		Women with Combination Therapy	
	No. of participants	**Adjusted-HR [ł] (95% CI)**	**No. of participants**	**Adjusted-HR [ł] (95% CI)**
Any diuretic*				
No regular use	8154	1.00 (Ref.)	1258	1.00 (Ref.)
Regular use	2120	1.45 (1.10, 1.90)	4016	1.14 (0.79, 1.65)
Calcium channel blocker				
No regular use	9285	1.00 (Ref.)	3485	1.00 (Ref.)
Regular use	989	1.05 (0.70, 1.56)	1789	0.81 (0.59, 1.12)
ACE inhibitor				
No regular use	8816	1.00 (Ref.)	3012	1.00 (Ref.)
Regular use	1458	0.98 (0.68, 1.40)	2262	1.03 (0.76, 1.40)
Other antihypertensive medication				
No regular use	4567	1.00 (Ref.)	1349	1.00 (Ref.)
Regular use	5707	0.75 (0.59, 0.96)	3925	1.27 (0.88, 1.84)

[ł] For specific types, multivariate model adjusted for age, smoking, diabetes, race/ethnicity, BMI, physical activities, history of cardiovascular disease, asthma, acetaminophen use, NSAIDS use, cholesterol lowering drug use

[*] The diuretics category includes both thiazide and loop diuretics

Table 4.5 Self-reported antihypertensive medication use among follow-up respondents (2000) and age-related macular degeneration stratified by potential effect modifiers in California Teachers Cohort, 2000-2012 (N=55,768).

	No. of participants [a]	No. of AMD	HR (95% CI)
History of hospitalization due to circulatory system diseases			
No	9570 (61.5%)	289 (51.2%)	1.17 (0.97, 1.40)
Yes	5992 (38.5%)	275 (48.7%)	1.27 (1.00, 1.61)
Regular Cholesterol-lowering drug use in the past two years			
No	10505 (74.0%)	380 (74.1%)	1.28 (1.09, 1.50)
Yes	3693 (26.0%)	133 (25.9%)	0.92 (0.65, 1.30)
Diabetes			
No	12228 (78.6%)	412 (73.0%)	1.15 (0.98, 1.35)
Yes	3334 (21.4%)	152 (26.9%)	1.44 (1.02, 2.03)
Average lifetime strenuous or moderate physical activity per week			
<2 hours	6403 (34.6%)	271 (48.5%)	1.32 (1.06, 1.64)
≥ 2 hours	9033 (65.4%)	288 (51.55)	1.12 (0.93, 1.37)
BMI			
Normal weight or under	6208 (42.2%)	218 (42.2%)	1.16 (0.97, 1.39)
Overweight	4797 (32.6%)	176 (34.1%)	1.28 (1.00, 1.64)
Obese	3705 (25.2%)	122 (23.6%)	1.70 (1.18, 2.44)

[a] Numbers may not add up to the total due to missing values.

4.5 Discussion

In this prospective cohort study of California teachers badly controlled hypertension was associated with an increased risk of developing intermediate- or late-stage AMD. Those treated for hypertension were at a slightly increased risk of AMD (HR = 1.15, 95% CI: 1.03, 1.30), but the risk of AMD was strongest among chronically hypertensive women who reported diuretics only use –which we consider the least effective type of treatment – while no increased risk was seen for well controlled hypertension i.e. women remained being treated with antihypertensive agents more potent than diuretics or women who were treated with combination therapies. We also observed an increasing trend risk with severity of the initial hypertension. In our subcohort of women who responded to the questionnaire in the year 2000, we were able to assess major subclasses of antihypertensive agents. There was a 45% increase in risk of AMD in women who reported daily diuretics use alone compared with women who used other antihypertensive agents as monotherapy, but not in users of any other subtype of antihypertensive agents. At the time data collection in the CTS (1995 and 2000), Congress had not yet passed the Medicare prescription drug benefit, and the medication coverage was not extended to the retired teachers until 2003, some CTS participants may have opted for the most affordable hypertension medications, which at the time were diuretics.

According to the third National Health and Nutrition Examination Survey (NHANES III), less than half of all women treated with antihypertensive medications are able to adequately control their blood pressure (SBP <140 mm Hg and DBP <90 mm Hg).[197] We were not able to disentangle association between antihypertensive medications and AMD from the potential adverse effect of residual high blood pressure, a phenomenon commonly referred to as

confounding by indication. Thus, the observed associations between treated high blood pressure and AMD risk may reflect either pharmacologic effects of the antihypertensive drugs or the chronic pathophysiologic effects of badly controlled high blood pressure.

Previous studies evaluating the association between high blood pressure and AMD risk have been inconclusive.[182, 183, 185, 186] A large prospective cohort study reported that a 10 mmHg systolic blood pressure increase at baseline exam was associated with a 20% increased risk of wet-AMD after 10 years.[185] Another similar study estimated a 10% risk increase and associations strengthened with increasing severity of hypertension.[184] A history of mild or temporarily elevated blood pressure, found in 13% of hypertensives,[195] that requires only life-style modification and not therapeutic drug use may explain the lack of an association with AMD among women with "untreated hypertension". The 20% increased risk of AMD among hypertensive women (as reflected by daily antihypertensive use) in our subsample of women who responded to the year 2000 questionnaire was consistent with a pooled estimate for any antihypertensive drug use in three population-based prospective cohorts that covered almost all Northern European populations (HR = 1.20, 95% CI 1.0-1.5).[124] Similar strength and direction of associations were also seen in two other studies in wet-AMD but not in early AMD.[191, 193]

Biological mechanisms linking hypertension and AMD involve the increased activity of the renin-angiotensin system (RAS) and specifically its major component angiotensin II (Ang II), the level of which is affected by elevated plasma renin activity that occurs in about 70% of all hypertensives.[188] The promoted activation of systemic RAS in hypertension interacts with the local RAS in the retina and contributes to inflammation and neovascularization, both of which

are key components of AMD pathogenesis. Specifically, through the activation of angiotensin II type 1 receptors expressed in the retina, Ang II exerts its vasoconstriction effect on retinal capillary network and induces endothelial cell apoptosis, which may contribute to neovascularization from disturbed retinal circulation.[182, 198] Moreover, Ang II can potentiate neovascularization through upregulations of the vascular endothelial growth factor.[182] An inhibitive effect of angiotensin II type 1 receptors blockers on the development of choroidal neovascularization was demonstrated in animal studies but has not been confirmed in humans,[199] probably because the inherent limitation in epidemiologic studies to disentangle the association from elevated blood pressure and the drug itself.

Our results suggested effect measure modification by diabetes (**Table 4.5**). In parallel to the vascular damaging effect of hypertension, diabetes increases the activation of the local RAS in the retina and increases the vitreous concentration of Ang II, which may exaggerate the adverse effect of hypertension.[200] We also found null hazard ratios for anti-hypertensive drug use among women with healthy BMI, more frequent physical activity, or controlling their lipid levels with statins indicated that all of these additional factors may make adequate blood pressure control that prevents AMD less likely.

Few studies investigated more than one subtype of antihypertensive agents separately possibly masking effects from use of specific types of antihypertensive agents. In our evaluation, only diuretics but not more aggressive combination treatments were associated with an increased risk of AMD when comparing with other classes of medication use among women with monotherapy. The Age-Related Eye Disease Study Research Group (AREDS) also reported

increased frequency of intermediate- or large drusen among people who used hydrochlorothiazide diuretics (OR =1.51),[163] but this was not reported in other studies that assessed diuretics separately, although elevated hazard ratios were estimated among diuretic users in a prospective cohort study with 20 years follow-up.[125, 191, 192] The discrepancy between the present and previous studies may reflect different degrees of residual confounding, the various lengths and cumulative dose of drug use, and the lack of consideration of the concurrent use of other types of drug use while assessing each class individually in previous studies. Although some studies accounted for measured blood pressure, a one-time measure may not sufficiently reflect the degree of blood pressure control in the long term. Furthermore, indications and contraindications such as heart failure, chronic kidney disease, cardiac arrhythmia or coronary artery disease which may affect selection of therapeutic agents were not taken into account.[201]

The especially high risk of AMD observed in women who consistently used diuretics as monotherapy, or who moved from a more extensive combination therapy to diuretics only may indicate that the lack of an adequate treatment strategy[202] and the resulting potential failure of high blood pressure control plays a role in the pathogenesis of AMD. Moreover, treatment with the most inexpensive medication alone may be an indicator of lower socioeconomic status and inadequate medical access. However, a potentially harmful effect attributed to the drug aside from the effect of the underlying high blood pressure could not be ruled out. There is no generally accepted biologic mechanism for a harmful effect of diuretics use but lack of adequate blood pressure control in the long term and hyperlipidemia may play a role. Diuretics function by inhibiting renal sodium reabsorption, thus decreasing the extracellular volume and reducing

cardiac output.[201] One of the well-acknowledged side effects of diuretics therapy is hyperlipidemia,[203, 204] a proposed risk factor for AMD.[205, 206] Moreover, thiazide diuretic use is associated with decreased serum zinc levels.[207] Zinc plays an essential role in the function of antioxidant enzymes that can suppress AMD progression, and the aging retina is inherently susceptible to zinc deficiency.[208, 209]

In a sensitivity analysis incorporating the time-varying use of diuretics and censoring due to death or moving out of California, women who reported diuretics use both at baseline and in 2000 had a higher risk of developing AMD (**Table A 4.2**). The lack of association between diuretic use and the development of AMD at baseline or in the subsample of new users in 2000may be explained by the around 14% of women who did not report using "water pills" initiated diuretics later elevated the AMD risk in the reference group. Additionally, side effects of diuretics with regard to the long-term activation of RAS that are associated with AMD pathogenesis may only manifest when diuretics are used for a long time.[210]

Our study has several strengths. This prospective study with routine passive follow-up via administrative hospital records provides us with a uniquely long follow-up time. The large number of AMD cases observed enabled us to investigate individual medications by duration, account for concurrent use of other types of medication, and evaluation both the antihypertensive monotherapy and polytherapy. Furthermore, the linkage of self-reported questionnaires and OSHPD records allowed us to examine a comprehensive set of potential confounding factors, including indications and contraindications for the selection of different antihypertensive drugs. However, there are also several limitations, most notably, we had to rely on secondary diagnoses

found in hospitalization records to identify AMD, thus the onset of early AMD could not be captured with this passive follow-up method. On the other hand, we did not have to rely on self-reported diagnoses and any outcome misclassification would be expected to be non-differential. Moreover, we were not able to disentangle any effects of medications on incident AMD from their effect on progression from early- to late-stage AMD. However, in sensitivity analyses that excluded AMD cases within the first five years of follow-up effect estimates did not change. Also, we were not able to separate geographic atrophy and neovascular AMD, which may have different pathophysiologies.[176] However, neovascular AMD is rare and can also be considered as the more severe and late stage form of AMD. Third, the prevalence of high blood pressure was under-reported in our study even after considering the unawareness of the condition. NHANES III estimated an over 50% prevalence of hypertension among female non-Hispanic Whites who were greater than 60 years old in 1988-1991,[197] higher than the self-reported rate (34%) in our study. Also, we did not have sufficient information to evaluate cumulative dose-response patterns since participants may have changed their frequency of medication use, or switch to a new class of drugs over time.[211] Fifth, two major subclasses of antihypertensive drugs, beta-blockers and angiotensin II receptor blockers were not queried separately. Lastly, for the assessment of combination medication use, we were not able to distinguish sequential use or concurrent use of different types of antihypertensive medications.

4.6 Conclusions

In summary, hypertension and antihypertensive treatments were associated with an increased risk of intermediate- or late-stage AMD. However, increased risk of AMD was mainly due to possible inadequate blood pressure control in diuretics only users with chronic hypertension, but

not aggressively combination treated high blood pressure. Future prospective studies of AMD and antihypertensive medications should evaluate the dosage and type and timing of use.

4.7 Appendix

Table A 4.1 Self-reported antihypertensive medications use among follow-up respondents with hypertension and age-related macular degeneration with a 5-year lagging stratified by type of treatment in California Teachers Cohort, 2000-2012 (N=13,489).

	Women with Monotherapy		Women with Combination Therapy	
	No. of participants	**Adjusted-HR [†] (95% CI)**	**No. of participants**	**Adjusted-HR [†] (95% CI)**
Any diuretic*				
No regular use	7245	1.00 (Ref.)	1087	1.00 (Ref.)
Regular use	1804	1.46 (1.08, 1.98)	3345	1.18 (0.79, 1.46)
Calcium channel blocker				
No regular use	8179	1.00 (Ref.)	2949	1.00 (Ref.)
Regular use	870	1.03 (0.67, 1.60)	1483	0.77 (0.54, 1.11)
ACE inhibitor				
No regular use	7730	1.00 (Ref.)	2511	1.00 (Ref.)
Regular use	1319	0.91 (0.61, 1.35)	1921	1.09 (0.78, 1.51)
Other antihypertensive medication				
No regular use	3993	1.00 (Ref.)	1134	1.00 (Ref.)
Regular use	5056	0.78 (0.59, 1.02)	3298	1.47 (0.97, 2.24)

[†] For specific types, multivariate model adjusted for age, smoking, diabetes, race/ethnicity, BMI, physical activities, history of cardiovascular disease, asthma, acetaminophen use, NSAIDS use, cholesterol lowering drug use

[*] The diuretics category includes both thiazide and loop diuretics

Table A 4.2 Self-reported antihypertensive medication use in 2000 and age-related macular degeneration stratified by time of treatment initiation in California Teachers Cohort, 2000-2012 (N=56,628).[a]

Regular antihypertensive medication use[*]	No. of participants	Adjusted-OR (95% CI)
Any antihypertensive medications		
Never used any antihypertensive medication	39411 (69.6%)	1.00 (ref.)
Used both at baseline and in 2000	9952 (17.6%)	1.22 (1.09, 1.38)
Used at baseline but not in 2000	1655 (2.9%)	0.97 (0.89, 1.07)
Not used at baseline but used later in 2000	5610 (9.9%)	1.26 (1.13, 1.40)
Diuretics		
Never used diuretics	47300 (83.5%)	1.00 (ref.)
Used both at baseline and in 2000	3441 (6.1%)	1.32 (1.20, 1.44)
Used at baseline but not in 2000	3181 (5.6%)	0.97 (0.88, 1.06)
Not used at baseline but used later in 2000	2704 (4.8%)	1.36 (1.24, 1.50)

[a] Time varying analysis with inverse-probability weighting method was applied (accounted for censoring)

[*] The diuretics category includes both thiazide and loop diuretics

Chapter 5. Public Health Relevance

The incidence of childhood cancer is increasing by 1% each year.[212] Although the survival of childhood cancer has improved dramatically thanks to more advanced diagnostic and treatment techniques, childhood cancer survivors are at an increased risk of developing adverse health outcomes associated with cancer treatments some of which are even life-threatening or disabling.[213] Understanding the risk factors for childhood cancer can facilitate uncovering the etiology and the development of preventive interventions. Since the fetal origin of childhood cancer has been proposed, a number of epidemiologic studies were conducted linking perinatal characteristics to cancer in offspring. Nevertheless, investigations addressing the association between maternal preeclampsia and the risk of childhood cancer remained scarce, and specific types of cancer were not separately examined due to the rarity of the disease. Our large population-based study provided an opportunity to examine in utero exposure to preeclampsia and some rare types of childhood cancer. Our findings of increased risks in hepatoblastoma and certain subtypes of germ cell tumors may shed light on potential etiologic factors of these cancers. Moreover, our study highlighted the importance of lifestyle intervention, sufficient maternal care, and closely monitoring of blood pressure in women at high risk of having preeclampsia to prevent adverse health outcomes in offspring.

Age-related macular degeneration accounts for nearly 9% of blindness worldwide, and the prevalence of AMD is increasing due to the growth of the aged population.[214] The impaired vision as a result of AMD leads to substantial reduction in the quality of life of the affected patients and a considerable economic loss to the country.[148] Although vision loss can be halted by intraocular injection of anti-VEGF medication for neovascular AMD patients, the treatment is

costly, and there is no effective pharmacotherapy method for dry-AMD. Our finding of a reduced risk of intermediate- or late-stage AMD among COX-2 inhibitor users provided epidemiologic evidence for COX-2 as a potential therapeutic target for preventing the development of AMD. In addition, our finding of an increased risk of intermediate- or late-stage AMD among regular antihypertensive users underscores the importance of adequate blood pressure control as a modifiable factor in AMD prevention. The elevated AMD risk among women with long-term diuretics monotherapy warrants future observational research to assess a potential dose-response of diuretics use on AMD risk. If the association between diuretics use and AMD risk is proven to be causal, recommendations of antihypertensive treatment need to be modified to guide rational prescriptions for blood pressure control.

Reference

1. Vest A, Cho L. Hypertension in Pregnancy. *Current Atherosclerosis Reports*. 2014; 16:1-11.

2. Roberts JM, Pearson G, Cutler J, Lindheimer M. Summary of the NHLBI Working Group on Research on Hypertension During Pregnancy. *Hypertension*. 2003; 41:437-445.

3. Duley L. The Global Impact of Pre-eclampsia and Eclampsia. *Seminars in Perinatology*. 2009; 33:130-137.

4. Wu J, Ren C, Delfino RJ, Chung J, Wilhelm M, Ritz B. Association between local traffic-generated air pollution and preeclampsia and preterm delivery in the south coast air basin of California. *Environmental Health Perspectives*. 2009; 117.

5. Killion M. New hypertension in pregnancy guidelines. *MCN Am J Matern Child Nurs*. 2015; 40:128.

6. Tranquilli AL, Brown MA, Zeeman GG, Dekker G, Sibai BM. The definition of severe and early-onset preeclampsia. Statements from the International Society for the Study of Hypertension in Pregnancy (ISSHP). *Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health*. 2013; 3:44-47.

7. Jeyabalan A. Epidemiology of preeclampsia: impact of obesity. *Nutr Rev*. 2013; 71 Suppl 1:S18-25.

8. Abalos E, Cuesta C, Grosso AL, Chou D, Say L. Global and regional estimates of preeclampsia and eclampsia: a systematic review. *European Journal of Obstetrics & Gynecology and Reproductive Biology*. 2013; 170:1-7.

9. Lisonkova S, Joseph KS. Incidence of preeclampsia: risk factors and outcomes associated with early- versus late-onset disease. *American Journal of Obstetrics and Gynecology*. 2013; 209:544.e541-544.e512.

10. Ananth CV, Keyes KM, Wapner RJ. Pre-eclampsia rates in the United States, 1980-2010: age-period-cohort analysis. *BMJ*. 2013; 347.

11. Klungsøyr K, Morken NH, Irgens L, Vollset SE, Skjærven R. - Secular trends in the epidemiology of pre-eclampsia throughout 40 years in Norway: prevalence, risk factors and perinatal survival. 2012; - 26:- 198.

12. Avalos LA, Chen H, Li D-K. Antidepressant medication use, depression, and the risk of preeclampsia. *CNS Spectrums*. 2015; 20:39-47.

13. Ghulmiyyah L, Sibai B. Maternal Mortality From Preeclampsia/Eclampsia. *Seminars in Perinatology*. 2012; 36:56-59.

14. Barton JR, Sibai BM. Prediction and prevention of recurrent preeclampsia. *Obstet Gynecol*. 2008; 112:359-372.

15. Hnat MD, Sibai BM, Caritis S, Hauth J, Lindheimer MD, MacPherson C, et al. Perinatal outcome in women with recurrent preeclampsia compared with women who develop preeclampsia as nulliparas. *American Journal of Obstetrics and Gynecology*. 2002; 186:422-426.

16. Conde-Agudelo A, Belizán JM. Risk factors for pre-eclampsia in a large cohort of Latin American and Caribbean women. *BJOG: An International Journal of Obstetrics & Gynaecology*. 2000; 107:75-83.

17. Ros HS, Cnattingius S, Lipworth L. Comparison of risk factors for preeclampsia and gestational hypertension in a population-based cohort study. *American journal of epidemiology*. 1998; 147:1062-1070.

18. Duckitt K, Harrington D. Risk factors for pre-eclampsia at antenatal booking: systematic review of controlled studies. *BMJ*. 2005; 330:565.

19. England L, Zhang J. Smoking and risk of preeclampsia: a systematic review. *Frontiers in Bioscience*. 2007:2471-2483.

20. Wikström A-K, Stephansson O, Cnattingius S. Tobacco use during pregnancy and preeclampsia risk effects of cigarette smoking and snuff. *Hypertension*. 2010; 55:1254-1259.

21. Caughey AB, Stotland Ne Fau - Washington AE, Washington Ae Fau - Escobar GJ, Escobar GJ. Maternal ethnicity, paternal ethnicity, and parental ethnic discordance: predictors of preeclampsia.

22. Wolf M, Shah A, Jimenez-Kimble R, Sauk J, Ecker JL, Thadhani R. Differential risk of hypertensive disorders of pregnancy among Hispanic women. *Journal of the American Society of Nephrology*. 2004; 15:1330-1338.

23. Tanaka M, Jaamaa G, Kaiser M, Hills E. Racial disparity in hypertensive disorders of pregnancy in New York State: a 10-year longitudinal population-based study. *American Journal of Public Health*. 2007; 97:163.

24. Main EK, McCain CL, Morton CH, Holtby S, Lawton ES. Pregnancy-related mortality in California: causes, characteristics, and improvement opportunities. *Obstetrics & Gynecology*. 2015; 125:938-947.

25. American College of O, Gynecologists, Task Force on Hypertension in P. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. *Obstet Gynecol*. 2013; 122:1122-1131.

26. Roberts JM, Redman CWG. Preeclampsia - More Than Pregnancy-Induced Hypertension (Vol 341, Pg 1447, 1993). *Lancet*. 1993; 342:504-504.

27. Packham DK, Mathews DC, Fairley KF, Whitworth JA, Kincaid-Smith PS. Morphometric analysis of pre-eclampsia in women biopsied in pregnancy and post-partum. *Kidney Int*. 1988; 34:704-711.

28. Osol G, Moore LG. Maternal Uterine Vascular Remodeling During Pregnancy. *Microcirculation*. 2014; 21:38-47.

29. Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. *Am J Obstet Gynecol*. 2000; 183:S1-S22.

30. Redman CW, Sargent IL. Latest Advances in Understanding Preeclampsia. *Science*. 2005; 308:1592-1594.

31. Wu CS, Nohr EA, Bech BH, Vestergaard M, Catov JM, Olsen J. Health of children born to mothers who had preeclampsia: a population-based cohort study. *American Journal of Obstetrics and Gynecology*. 2009; 201:269.e261-269.e210.

32. Vatten LJ, Romundstad PR, Holmen TL, Hsieh C-c, Trichopoulos D, Stuver SO. Intrauterine exposure to preeclampsia and adolescent blood pressure, body size, and age at menarche in female offspring. *Obstetrics & Gynecology*. 2003; 101:529-533.

33. Friedman SA, Schiff E, Kao L, Sibai BM. Neonatal outcome after preterm delivery for preeclampsia. *American Journal of Obstetrics and Gynecology*. 1995; 172:1785-1792.

34. Conde-Agudelo A, Villar J Fau - Lindheimer M, Lindheimer M. World Health Organization systematic review of screening tests for preeclampsia.

35. Audibert F, Boucoiran I, An N, Aleksandrov N, Delvin E, Bujold E, et al. Screening for preeclampsia using first-trimester serum markers and uterine artery Doppler in nulliparous women. *American Journal of Obstetrics and Gynecology*. 2010; 203:383.e381-383.e388.

36. Kolialexi A, Gourgiotis D, Daskalakis G, Marmarinos A, Lykoudi A, Mavreli D, et al. Validation of Serum Biomarkers Derived from Proteomic Analysis for the Early Screening of Preeclampsia. *Disease markers*. 2015; 2015.

37. Barton JR, Stanziano GJ, Sibai BM. Monitored outpatient management of mild gestational hypertension remote from term. *American Journal of Obstetrics and Gynecology*. 1994; 170:765-769.

38. Cahill AG, Macones GA, Odibo AO, Stamilio DM. Magnesium for Seizure Prophylaxis in Patients With Mild Preeclampsia. *Obstetrics & Gynecology*. 2007; 110.

39. Sibai BM. Diagnosis and management of gestational hypertension and preeclampsia. *Obstet Gynecol*. 2003; 102:181-192.

40. Sibai BM, Barton JR, Sherif A, Sarinoglu C, Mercer BM. A randomized prospective comparison of nifedipine and bedrest versus bed rest alone in the management of preeclampsia remote from term. *American Journal of Obstetrics and Gynecology*. 1992; 167:879-884.

41. Steliarova-Foucher E, Stiller C, Lacour B, Kaatsch P. International Classification of Childhood Cancer, third edition. *Cancer*. 2005; 103:1457-1467.

42. Center M, Siegel R, Jemal A. Global cancer facts & figures. *Atlanta, Georgia: American Cancer Society*. 2011.

43. Howlader N NA, Krapcho M, Garshell J, Miller D, Altekruse SF, Kosary CL, Yu M, Ruhl J, Tatalovich Z, Mariotto A, Lewis DR, Chen HS, Feuer EJ. SEER Cancer Statistics Review, 1975-2012. *National Cancer Institute*. 2015.

44. Steliarova-Foucher E, Stiller C, Kaatsch P, Berrino F, Coebergh JW, Lacour B, et al. Geographical patterns and time trends of cancer incidence and survival among children and adolescents in Europe since the 1970s (the ACCISproject): an epidemiological study. *Lancet*. 2004; 364:2097-2105.

45. Olsen JH, Möller T, Anderson H, Langmark F, Sankila R, Tryggvadóttír L, et al. Lifelong cancer incidence in 47 697 patients treated for childhood cancer in the Nordic countries. *J Natl Cancer Inst*. 2009; 101:806-813.

46. Armstrong GT, Liu Q, Yasui Y, Neglia JP, Leisenring W, Robison LL, et al. Late mortality among 5-year survivors of childhood cancer: a summary from the Childhood Cancer Survivor Study. *J Clin Oncol*. 2009; 27:2328-2338.

47. Armstrong GT, Kawashima T, Leisenring W, Stratton K, Stovall M, Hudson MM, et al. Aging and risk of severe, disabling, life-threatening, and fatal events in the childhood cancer survivor study. *J Clin Oncol*. 2014; 32:1218-1227.

48. Henderson TO, Friedman DL, Meadows AT. Childhood cancer survivors: transition to adult-focused risk-based care. *Pediatrics*. 2010; 126:129-136.

49. Hudson MM, Mertens AC, Yasui Y, Hobbie W, Chen H, Gurney JG, et al. Health status of adult long-term survivors of childhood cancer: a report from the Childhood Cancer Survivor Study. *JAMA*. 2003; 290:1583-1592.

50. Inskip PD, Robison LL, Stovall M, Smith SA, Hammond S, Mertens AC, et al. Radiation dose and breast cancer risk in the childhood cancer survivor study. *J Clin Oncol*. 2009; 27:3901-3907.

51. Siegel DA, King J, Tai E, Buchanan N, Ajani UA, Li J. Cancer Incidence Rates and Trends Among Children and Adolescents in the United States, 2001–2009. *Pediatrics*. 2014.

52. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. *CA: A Cancer Journal for Clinicians*. 2015; 65:5-29.

53. Kaatsch P. Epidemiology of childhood cancer. *Cancer Treat Rev*. 2010; 36:277-285.

54. Cook SN GB, Morris CR, Parikh-Patel A, Kizer KW, Kwong SL, Bates JH, Snipes KP. Annual Report on the Status of Cancer in California, 1988-2010: Sacramento, CA: California Department of Public Health, Cancer Surveillance Section2014.

55. *U.S. Censue Bureau*. (2015, July 23). Retrieved from https://www.census.gov/quickfacts/table/PST045214/00,06.

56. Mathews JD, Forsythe AV, Brady Z, Butler MW, Goergen SK, Byrnes GB, et al. Cancer risk in 680,000 people exposed to computed tomography scans in childhood or adolescence: data linkage study of 11 million Australians. *BMJ*. 2013; 346:f2360.

57. Pearce MS, Salotti JA, Little MP, McHugh K, Lee C, Kim KP, et al. Radiation exposure from CT scans in childhood and subsequent risk of leukaemia and brain tumours: a retrospective cohort study. *Lancet*. 2012; 380:499-505.

58. Johnson KJ, Carozza SE, Chow EJ, Fox EE, Horel S, McLaughlin CC, et al. Parental age and risk of childhood cancer: a pooled analysis. *Epidemiology (Cambridge, Mass)*. 2009; 20:475.

59. Caughey RW, Michels KB. Birth weight and childhood leukemia: A meta-analysis and review of the current evidence. *International Journal of Cancer*. 2009; 124:2658-2670.

60. Hjalgrim LL, Westergaard T, Rostgaard K, Schmiegelow K, Melbye M, Hjalgrim H, et al. Birth weight as a risk factor for childhood leukemia: a meta-analysis of 18 epidemiologic studies. *American journal of epidemiology*. 2003; 158:724-735.

61. Bjørge T, Sørensen HT, Grotmol T, Engeland A, Stephansson O, Gissler M, et al. Fetal growth and childhood cancer: a population-based study. *Pediatrics*. 2013; 132:e1265-e1275.

62. Johnson KJ, Puumala SE, Soler JT, Spector LG. Perinatal characteristics and risk of neuroblastoma. *International journal of cancer*. 2008; 123:1166-1172.

63. Von Behren J, Spector LG, Mueller BA, Carozza SE, Chow EJ, Fox EE, et al. Birth order and risk of childhood cancer: a pooled analysis from five US States. *International Journal of Cancer*. 2011; 128:2709-2716.

64. Lim LS, Mitchell P, Seddon JM, Holz FG, Wong TY. Age-related macular degeneration. *The Lancet*. 2012; 379:1728-1738.

65. Velez-Montoya R, Oliver SCN, Olson JL, Fine SL, Quiroz-Mercado H, Mandava N. Current knowledge and trends in age-related macular degeneration: genetics, epidemiology, and prevention. *Retina*. 2014; 34:423-441.

66. Klein R, Klein BEK, Knudtson MD, Meuer SM, Swift M, Gangnon RE. Fifteen-year cumulative incidence of age-related macular degeneration: the Beaver Dam Eye Study. *Ophthalmology*. 2007; 114:253-262.

67. Friedman DS, Katz J, Bressler NM, Rahmani B, Tielsch JM. Racial differences in the prevalence of age-related macular degeneration: The Baltimore eye survey11The authors have no proprietary interest in any of the instruments used in this study. *Ophthalmology*. 1999; 106:1049-1055.

68. Laude A, Cackett PD, Vithana EN, Yeo IY, Wong D, Koh AH, et al. Polypoidal choroidal vasculopathy and neovascular age-related macular degeneration: same or different disease? *Progress in retinal and eye research*. 2010; 29:19-29.

69. Klaver CCW, Assink JJM, van Leeuwen R, Wolfs RCW, Vingerling JR, Stijnen T, et al. Incidence and progression rates of age-related maculopathy: the Rotterdam Study. *Investigative ophthalmology & visual science*. 2001; 42:2237-2241.

70. Mitchell P, Wang JJ, Foran S, Smith W. Five-year incidence of age-related maculopathy lesions: the Blue Mountains Eye Study. *Ophthalmology*. 2002; 109:1092-1097.

71. Brown GC, Brown MM, Sharma S, Stein JD, Roth Z, Campanella J, et al. THE BURDEN OF AGE-RELATED MACULAR DEGENERATION: A VALUE-BASED MEDICINE ANALYSIS. *Transactions of the American Ophthalmological Society*. 2005; 103:173-186.

72. de Jong PTVM. Age-Related Macular Degeneration. *New England Journal of Medicine*. 2006; 355:1474-1485.

73. Klaver CCW, Wolfs RCW, Assink JJM, van Duijn CM, Hofman A, de Jong PTVM. Genetic risk of age-related maculopathy: population-based familial aggregation study. *Archives of Ophthalmology*. 1998; 116:1646-1651.

74. Seddon JM, Ajani UA, Mitchell BD. Familial aggregation of age-related maculopathy. *American Journal of Ophthalmology*. 1997; 123:199-206.

75. Tomany SC, Wang JJ, van Leeuwen R, Klein R, Mitchell P, Vingerling JR, et al. Risk factors for incident age-related macular degeneration: pooled findings from 3 continents. *Ophthalmology*. 2004; 111:1280-1287.

76. Age-Related Eye Disease Study Research G. Risk factors for the incidence of advanced age-related macular degeneration in the Age-Related Eye Disease Study (AREDS): AREDS report no. 19. *Ophthalmology*. 2005; 112:533-539.

77. Hammond BR, Wooten BR, Snodderly DM. Cigarette smoking and retinal carotenoids: implications for age-related macular degeneration. *Vision research*. 1996; 36:3003-3009.

78. Sastry BVR, Hemontolor ME. Influence of nicotine and cotinine on retinal phospholipase A2 and its significance to macular function. *Journal of ocular pharmacology and therapeutics*. 1998; 14:447-458.

79. Espinosa-Heidmann DG, Suner IJ, Catanuto P, Hernandez EP, Marin-Castano ME, Cousins SW. Cigarette smoke–related oxidants and the development of sub-RPE deposits in an experimental animal model of dry AMD. *Investigative ophthalmology & visual science*. 2006; 47:729-737.

80. Chakravarthy U, Wong TY, Fletcher A, Piault E, Evans C, Zlateva G, et al. Clinical risk factors for age-related macular degeneration: a systematic review and meta-analysis. *BMC ophthalmology*. 2010; 10:1.

81. Remé CE. The dark side of light: rhodopsin and the silent death of vision the proctor lecture. *Investigative ophthalmology & visual science*. 2005; 46:2672-2682.

82. Age-Related Eye Disease Study Research G. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. *Archives of Ophthalmology*. 2001; 119:1417.

83. Chew EY, Clemons TE, SanGiovanni JP, Danis RP, Ferris FL, Elman MJ, et al. Secondary analyses of the effects of lutein/zeaxanthin on age-related macular degeneration progression: AREDS2 report No. 3. *JAMA ophthalmology*. 2014; 132:142-149.

84. Merle B, Delyfer M-N, Korobelnik J-F, Rougier M-B, Colin J, Malet F, et al. Dietary omega-3 fatty acids and the risk for age-related maculopathy: the Alienor Study. *Investigative ophthalmology & visual science*. 2011; 52:6004-6011.

85. Schüz J, Weihkopf T, Kaatsch P. Medication use during pregnancy and the risk of childhood cancer in the offspring. *European Journal of Pediatrics*. 2007; 166:433-441.

86. Stålberg K, Haglund B, Strömberg B, Kieler H. Prenatal exposure to medicines and the risk of childhood brain tumor. *Cancer Epidemiology*. 2010; 34:400-404.

87. Ansell P, Mitchell CD, Roman E, Simpson J, Birch JM, Eden TOB. Relationships between perinatal and maternal characteristics and hepatoblastoma: a report from the UKCCS. *European Journal of Cancer*. 2005; 41:741-748.

88. Heck JE, Meyers TJ, Lombardi C, Park AS, Cockburn M, Reynolds P, et al. Case-control study of birth characteristics and the risk of hepatoblastoma. *Cancer epidemiology*. 2013; 37:390-395.

89. McLaughlin CC, Baptiste MS, Schymura MJ, Nasca PC, Zdeb MS. Maternal and infant birth characteristics and hepatoblastoma. *American journal of epidemiology*. 2006; 163:818-828.

90. Roman E, Simpson J, Ansell P, Lightfoot T, Mitchell C, Eden TOB. Perinatal and reproductive factors: a report on haematological malignancies from the UKCCS. *European Journal of Cancer*. 2005; 41:749-759.

91. Møller H, Skakkeb\sgmaelig, k N. Testicular cancer and cryptorchidism in relation to prenatal factors: case-control studies in Denmark. *Cancer Causes & Control*. 1997; 8:904-912.

92. Stephansson O, Wahnström C, Pettersson A, Sørensen HT, Tretli S, Gissler M, et al. Perinatal risk factors for childhood testicular germ-cell cancer: A Nordic population-based study. *Cancer epidemiology*. 2011; 35:e100-e104.

93. Hamrick SEG, Olshan AF, Neglia JP, Pollock BH. Association of pregnancy history and birth characteristics with neuroblastoma: a report from the Children's Cancer Group and the Pediatric Oncology Group. *Paediatric and perinatal epidemiology*. 2001; 15:328-337.

94. Bjorge T, Engeland A, Tretli S, Heuch I. Birth and parental characteristics and risk of neuroblastoma in a population-based Norwegian cohort study. *Br J Cancer*. 2008; 99:1165-1169.

95. Oksuzyan S, Crespi CM, Cockburn M, Mezei G, Kheifets L. Birth weight and other perinatal characteristics and childhood leukemia in California. *Cancer Epidemiology*. 2012; 36:e359-e365.

96. Oksuzyan S, Crespi CM, Cockburn M, Mezei G, Kheifets L. Birth weight and other perinatal factors and childhood CNS tumors: A case–control study in California. *Cancer Epidemiology*. 2013; 37:402-409.

97. Moslehi R, Mills JL, Signore C, Kumar A, Ambroggio X, Dzutsev A. Integrative transcriptome analysis reveals dysregulation of canonical cancer molecular pathways in placenta leading to preeclampsia. *Sci Rep*. 2013; 3:2407.

98. Louwen F, Muschol-Steinmetz C, Reinhard J, Reitter A, Yuan J. A lesson for cancer research: placental microarray gene analysis in preeclampsia. *Oncotarget*. 2012; 3:759-773.

99. Julian CG. High altitude during pregnancy. *Clin Chest Med*. 2011; 32:21-31, vii.

100. Hung TH, Burton GJ. Hypoxia and reoxygenation: a possible mechanism for placental oxidative stress in preeclampsia. *Taiwan J Obstet Gynecol*. 2006; 45:189-200.

101. Genbacev O, Joslin R, Damsky CH, Polliotti BM, Fisher SJ. Hypoxia alters early gestation human cytotrophoblast differentiation/invasion in vitro and models the placental defects that occur in preeclampsia. *J Clin Invest*. 1996; 97:540-550.

102. Semenza GL. Targeting HIF-1 for cancer therapy. *Nat Rev Cancer*. 2003; 3:721-732.

103. Semenza GL. HIF-1 and tumor progression: pathophysiology and therapeutics. *Trends Mol Med*. 2002; 8:S62-67.

104. Lawlor ER, Thiele CJ. Epigenetic changes in pediatric solid tumors: promising new targets. *Clin Cancer Res*. 2012; 18:2768-2779.

105. Huether R, Dong L, Chen X, Wu G, Parker M, Wei L, et al. The landscape of somatic mutations in epigenetic regulators across 1,000 paediatric cancer genomes. *Nat Commun*. 2014; 5:3630.

106. Raijmakers MT, Dechend R, Poston L. Oxidative stress and preeclampsia: rationale for antioxidant clinical trials. *Hypertension*. 2004; 44:374-380.

107. Siddiqui IA, Jaleel A, Tamimi W, Al Kadri HM. Role of oxidative stress in the pathogenesis of preeclampsia. *Arch Gynecol Obstet*. 2010; 282:469-474.

108. Rahman I, Marwick J, Kirkham P. Redox modulation of chromatin remodeling: impact on histone acetylation and deacetylation, NF-kappaB and pro-inflammatory gene expression. *Biochem Pharmacol*. 2004; 68:1255-1267.

109. Fujimaki A, Watanabe K, Mori T, Kimura C, Shinohara K, Wakatsuki A. Placental oxidative DNA damage and its repair in preeclamptic women with fetal growth restriction. *Placenta*. 2011; 32:367-372.

110. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: how are they linked? *Free Radic Biol Med*. 2010; 49:1603-1616.

111. Hoetzenecker W, Echtenacher B, Guenova E, Hoetzenecker K, Woelbing F, Bruck J, et al. ROS-induced ATF3 causes susceptibility to secondary infections during sepsis-associated immunosuppression. *Nature Medicine*. 2012; 18:128-134.

112. Thompson MR, Xu D, Williams BR. ATF3 transcription factor and its emerging roles in immunity and cancer. *J Mol Med (Berl)*. 2009; 87:1053-1060.

113. Anderson DH, Mullins RF, Hageman GS, Johnson LV. A role for local inflammation in the formation of drusen in the aging eye. *American journal of ophthalmology*. 2002; 134:411-431.

114. Ayalasomayajula SP, Kompella UB. Celecoxib, a selective cyclooxygenase-2 inhibitor, inhibits retinal vascular endothelial growth factor expression and vascular leakage in a streptozotocin-induced diabetic rat model. *European journal of pharmacology*. 2003; 458:283-289.

115. McGeer PL, Sibley J. Sparing of age-related macular degeneration in rheumatoid arthritis. *Neurobiology of Aging*. 2005; 26:1199-1203.

116. Swanson MW, McGwin Jr G. Anti-inflammatory drug use and age-related macular degeneration. *Optometry & Vision Science*. 2008; 85:947-950.

117. Wang JJ, Mitchell P, Smith W, Gillies M, Billson F. Systemic use of anti-inflammatory medications and age-related maculopathy: the Blue Mountains Eye Study. *Ophthalmic epidemiology*. 2003; 10:37-48.

118. Klein R, Klein BK, Jensen SC, et al. Medication use and the 5-year incidence of early age-related maculopathy: The beaver dam eye study. *Archives of ophthalmology*. 2001; 119:1354-1359.

119. Kahawita SK, Casson RJ. Reprint of: Aspirin use and early age-related macular degeneration: a meta-analysis. *Canadian Journal of Ophthalmology / Journal Canadien d'Ophtalmologie*. 2015; 50, Supplement 1:S29-S33.

120. Liew G, Mitchell P, Wong TY, Rochtchina E, Wang JJ. The association of aspirin use with age-related macular degeneration. *JAMA internal medicine*. 2013; 173:258-264.

121. de Jong PTVM, Chakravarthy U, Rahu M, Seland J, Soubrane G, Topouzis F, et al. Associations between aspirin use and aging macula disorder: the European Eye Study. *Ophthalmology*. 2012; 119:112-118.

122. Christen WG, Glynn RJ, Chew EY, Buring JE. Low-dose aspirin and medical record–confirmed age-related macular degeneration in a randomized trial of women. *Ophthalmology*. 2009; 116:2386-2392.

123. Petrukhin K. Pharmacological inhibition of lipofuscin accumulation in the retina as a therapeutic strategy for dry AMD treatment. *Drug Discovery Today: Therapeutic Strategies*. 2013; 10:e11-e20.

124. van Leeuwen R, Tomany SC, Wang JJ, Klein R, Mitchell P, Hofman A, et al. Is medication use associated with the incidence of early age-related maculopathy?: Pooled findings from 3 continents. *Ophthalmology*. 2004; 111:1169-1175.

125. Douglas IJ, Cook C, Chakravarthy U, Hubbard R, Fletcher AE, Smeeth L. A case–control study of drug risk factors for age-related macular degeneration. *Ophthalmology*. 2007; 114:1164-1169.

126. Klein BEK, Klein R, Knudtson MD, Lee KE, Danforth LG, Reinke JO, et al. Associations of selected medications and visual function: the Beaver Dam Eye Study. *British journal of ophthalmology*. 2003; 87:403-408.

127. Afroze SH, Kalagiri RR, Reyes M, Zimmerman JD, Beeram MR, Drever N, et al. Apoptotic and stress signaling markers are augmented in preeclamptic placenta and umbilical cord. *BBA Clinical*. 2016; 6:25-30.

128. Heck JE, Lombardi CA, Cockburn M, Meyers TJ, Wilhelm M, Ritz B. Epidemiology of rhabdoid tumors of early childhood. *Pediatric blood & cancer*. 2013; 60:77-81.

129. Marcotte EL, Ritz B, Cockburn M, Clarke CA, Heck JE. Birth characteristics and risk of lymphoma in young children. *Cancer epidemiology*. 2014; 38:48-55.

130. Urayama KY, Von Behren J, Reynolds P, Hertz A, Does M, Buffler PA. Factors associated with residential mobility in children with leukemia: implications for assigning exposures. *Annals of epidemiology*. 2009; 19:834-840.

131. Cole SR, Hernán MA. Constructing inverse probability weights for marginal structural models. *American journal of epidemiology*. 2008; 168:656-664.

132. Heck JE, Park AS, Contreras ZA, Davidson TB, Hoggatt KJ, Cockburn M, et al. Risk of Childhood Cancer by Maternal Birthplace: A Test of the Hispanic Paradox. *JAMA pediatrics*. 2016; 170:585-592.

133. VanderWeele TJ. Marginal structural models for the estimation of direct and indirect effects. *Epidemiology*. 2009; 20:18-26.

134. Heck JE, Lombardi CA, Meyers TJ, Cockburn M, Wilhelm M, Ritz B. Perinatal characteristics and retinoblastoma. *Cancer Causes & Control*. 2012; 23:1567-1575.

135. Clint Hall BR, Meyles Cockburn, Tom Davidson, Julia Heck. . Risk of malignant childhood germ cell tumors in relation to demographic, gestational, and perinatal characteristics. *Cancer epidemiology*. 2016.

136. Zhao W, Li J, Zhang Y, Gao P, Zhang J, Guo F, et al. Screening and identification of apolipoprotein AI as a potential hepatoblastoma biomarker in children, excluding inflammatory factors. *Oncology letters*. 2015; 10:233-239.

137. Catarino C, Rebelo I, Belo L, Rocha-Pereira P, Rocha S, Bayer Castro E, et al. Fetal lipoprotein changes in pre-eclampsia. *Acta obstetricia et gynecologica Scandinavica*. 2008; 87:628-634.

138. Turcotte LM, Georgieff MK, Ross JA, Feusner JH, Tomlinson GE, Malogolowkin MH, et al. Neonatal medical exposures and characteristics of low birth weight hepatoblastoma cases: A report from the Children's Oncology Group. *Pediatric blood & cancer*. 2014; 61:2018-2023.

139. Latini G, Gallo F, De Felice C. Birth characteristics and hepatoblastoma risk in young children. *Cancer*. 2004; 101:210-210.

140. Shaikh F, Murray MJ, Amatruda JF, Coleman N, Nicholson JC, Hale JP, et al. Paediatric extracranial germ-cell tumours. *The Lancet Oncology*. 2016; 17:e149-e162.

141. Pineles BL, Romero R, Montenegro D, Tarca AL, Han YM, Kim YM, et al. Distinct subsets of microRNAs are expressed differentially in the human placentas of patients with preeclampsia. *American Journal of Obstetrics and Gynecology*. 2007; 196:261.e261-261.e266.

142. Palmer RD, Murray MJ, Saini HK, van Dongen S, Abreu-Goodger C, Muralidhar B, et al. Malignant germ cell tumors display common microRNA profiles resulting in global changes in expression of messenger RNA targets. *Cancer research*. 2010; 70:2911-2923.

143. Covello KL, Kehler J, Yu H, Gordan JD, Arsham AM, Hu C-J, et al. HIF-2α regulates Oct-4: effects of hypoxiaon stem cell function, embryonic development, and tumor growth. *Genes & Development*. 2006; 20:557-570.

144. Braveman P, Pearl M, Egerter S, Marchi K, Williams R. Validity of insurance information on California birth certificates. *American journal of public health*. 1998; 88:813-816.

145. Baumeister L, Marchi K, Pearl M, Williams R, Braveman P. The validity of information on "race" and "Hispanic ethnicity" in California birth certificate data. *Health Services Research*. 2000; 35:869-883.

146. Haghighat N, Hu M, Laurent O, Chung J, Nguyen P, Wu J. Comparison of birth certificates and hospital-based birth data on pregnancy complications in Los Angeles and Orange County, California. *BMC pregnancy and childbirth*. 2016; 16:1.

147. Li DK, Wi S. Maternal pre-eclampsia/eclampsia and the risk of sudden infant death syndrome in offspring. *Paediatric and perinatal epidemiology*. 2000; 14:141-144.

148. Brown GC, Brown MM, Sharma S, Stein JD, Roth Z, Campanella J, et al. The burden of age-related macular degeneration: a value-based medicine analysis. *Transactions of the American Ophthalmological Society*. 2005; 103:173.

149. Maloney SC, Fernandes BF, Castiglione E, Antecka E, Martins C, Marshall J-C, et al. Expression of cyclooxygenase-2 in choroidal neovascular membranes from age-related macular degeneration patients. *Retina*. 2009; 29:176-180.

150. Paulose-Ram R, Hirsch R, Dillon C, Losonczy K, Cooper M, Ostchega Y. Prescription and non-prescription analgesic use among the US adult population: results from the third National Health and Nutrition Examination Survey (NHANES III). *Pharmacoepidemiology and drug safety*. 2003; 12:315-326.

151. Colwell JA. Antiplatelet agents for the prevention of cardiovascular disease in diabetes mellitus. *American Journal of Cardiovascular Drugs*. 2004; 4:87-106.

152. Curhan GC, Bullock AJ, Hankinson SE, Willett WC, Speizer FE, Stampfer MJ. Frequency of use of acetaminophen, nonsteroidal anti-inflammatory drugs, and aspirin in US women. *Pharmacoepidemiology and drug safety*. 2002; 11:687-693.

153. Carney RM, Freedland KE, Eisen SA, Rich MW, Skala JA, Jaffe AS. Adherence to a prophylactic medication regimen in patients with symptomatic versus asymptomatic ischemic heart disease. *Behavioral Medicine*. 1998; 24:35-39.

154. O'Connor PJ, Pronk NP, Tan AW, Rush WA, Gray RJ. Does professional advice influence aspirin use to prevent heart disease in an HMO population. *Eff Clin Pract*. 1998; 1:26-32.

155. Ajani UA, Ford ES, Greenland KJ, Giles WH, Mokdad AH. Aspirin use among US adults: behavioral risk factor surveillance system. *American journal of preventive medicine*. 2006; 30:74-77.

156. Chakravarthy U, Wong TY, Fletcher A, Piault E, Evans C, Zlateva G, et al. Clinical risk factors for age-related macular degeneration: a systematic review and meta-analysis. *BMC ophthalmology*. 2010; 10:31.

157. Christen WG, Glynn RJ, Ajani UA, Schaumberg DA, Chew EY, Buring JE, et al. Age-related maculopathy in a randomized trial of low-dose aspirin among US physicians. *Archives of ophthalmology*. 2001; 119:1143-1149.

158. Klein BEK, Howard KP, Gangnon RE, Dreyer JO, Lee KE, Klein R. Long-term use of aspirin and age-related macular degeneration. *JAMA*. 2012; 308:2469-2478.

159. Bernstein L, Allen M, Anton-Culver H, Deapen D, Horn-Ross PL, Peel D, et al. High breast cancer incidence rates among California teachers: results from the California Teachers Study (United States). *Cancer Causes and Control*. 2002; 13:625-635.

160. Duan L, Xu X, Koebnick C, Lacey JV, Sullivan-Halley J, Templeman C, et al. Bilateral oophorectomy is not associated with increased mortality: the California Teachers Study. *Fertility and sterility*. 2012; 97:111-117.

161. Foley SM, Daley J, Hughes J, Fisher ES, Heeren T. Comorbidities, complications, and coding bias: does the number of diagnosis codes matter in predicting in-hospital mortality? *JAMA*. 1992; 267:2197-2203.

162. Halpern MT, Schmier JK, Covert D, Venkataraman K. Resource utilization and costs of age-related macular degeneration. *Health care financing review*. 2006; 27:37.

163. Age-Related Eye Disease Study Research G. Risk factors associated with age-related macular degeneration: a case-control study in the age-related eye disease study: age-related eye disease study report number 3. *Ophthalmology*. 2000; 107:2224-2232.

164. Marshall SF, Bernstein L, Anton-Culver H, Deapen D, Horn-Ross PL, Mohrenweiser H, et al. Nonsteroidal anti-inflammatory drug use and breast cancer risk by stage and hormone receptor status. *Journal of the National Cancer Institute*. 2005; 97:805-812.

165. Zell JA, Ziogas A, Bernstein L, Clarke CA, Deapen D, Largent JA, et al. Nonsteroidal anti-inflammatory drugs. *Cancer*. 2009; 115:5662-5671.

166. Pearson TA, Blair SN, Daniels SR, Eckel RH, Fair JM, Fortmann SP, et al. AHA guidelines for primary prevention of cardiovascular disease and stroke: 2002 update. *Circulation*. 2002; 106:388-391.

167. Iso H, Hennekens CH, Stampfer MJ, Rexrode KM, Colditz GA, Speizer FE, et al. Prospective study of aspirin use and risk of stroke in women. *Stroke*. 1999; 30:1764-1771.

168. Wilcox CM, Cryer B, Triadafilopoulos G. Patterns of use and public perception of over-the-counter pain relievers: focus on nonsteroidal antiinflammatory drugs. *The Journal of rheumatology*. 2005; 32:2218-2224.

169. Keenan TDL, Goldacre R, Goldacre MJ. Associations between age-related macular degeneration, osteoarthritis and rheumatoid arthritis: Record Linkage Study. *Retina*. 2015; 35:2613-2618.

170. Machado FS, Johndrow JE, Esper L, Dias A, Bafica A, Serhan CN, et al. Anti-inflammatory actions of lipoxin A4 and aspirin-triggered lipoxin are SOCS-2 dependent. *Nature medicine*. 2006; 12:330-334.

171. Ridker PM, Manson JE, Buring JE, Goldhaber SZ, Hennekens CH. The effect of chronic platelet inhibition with low-dose aspirin on atherosclerotic progression and acute thrombosis: clinical evidence from the Physicians' Health Study. *American heart journal*. 1991; 122:1588-1592.

172. Vingerling JR, Dielemans I, Bots ML, Hofman A, Grobbee DE, de Jong PTVM. Age-related macular degeneration is associated with atherosclerosis the Rotterdam Study. *American journal of epidemiology*. 1995; 142:404-409.

173. Nowak JZ. Aspirin and age-related macular degeneration: positives versus negatives. Taylor & Francis; 2014.

174. Zhu W, Wu Y, Xu D, Li Y-H, Jun B, Zhang X-L, et al. Aspirin use and risk of age-related macular degeneration: a meta-analysis. *PLoS One*. 2013; 8:e58821.

175. Michet Iii CJ, Achenbach SJ, Crowson CS, Matteson EL. Hospitalization rates and utilization among patients with giant cell arteritis: A population-based study from 1987 to 2012. 1 ed: Elsevier. p. 70-74.

176. Kahawita SK, Casson RJ. Aspirin use and early age-related macular degeneration: a meta-analysis. *Canadian Journal of Ophthalmology/Journal Canadien d'Ophtalmologie*. 2014; 49:35-39.

177. Ying G-s, Maguire MG, Daniel E, Grunwald JE, Ahmed O, Martin DF, et al. Association between antiplatelet or anticoagulant drugs and retinal or subretinal hemorrhage in the comparison of age-related macular degeneration treatments trials. *Ophthalmology*. 2016; 123:352-360.

178. Keller DL. Is 81-mg aspirin associated with age-related macular degeneration risk? *JAMA internal medicine*. 2013; 173:1476-1476.

179. Yoon SS, Burt V, Louis T, Carroll MD. Hypertension among adults in the United States, 2009–2010. NCHS data brief, no 107. Hyattsville, MD: National Center for Health Statistics. 2012. 2013.

180. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, et al. Seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure. *hypertension*. 2003; 42:1206-1252.

181. Liew G, Mitchell P, Leeder SR, Smith W, Wong TY, Wang JJ. Regular aspirin use and retinal microvascular signs: the Blue Mountains Eye Study. *Journal of hypertension*. 2006; 24:1329-1335.

182. Katsi VK, Marketou ME, Vrachatis DA, Manolis AJ, Nihoyannopoulos P, Tousoulis D, et al. Essential hypertension in the pathogenesis of age-related macular degeneration: a review of the current evidence. *Journal of hypertension*. 2015; 33:2382-2388.

183. Sperduto RD, Hiller R. Systemic hypertension and age-related maculopathy in the Framingham Study. *Archives of ophthalmology*. 1986; 104:216-219.

184. van Leeuwen R, Ikram MK, Vingerling JR, Witteman JCM, Hofman A, de Jong PTVM. Blood pressure, atherosclerosis, and the incidence of age-related maculopathy: the Rotterdam Study. *Investigative ophthalmology & visual science*. 2003; 44:3771-3777.

185. Klein R, Klein BEK, Tomany SC, Cruickshanks KJ. The association of cardiovascular disease with the long-term incidence of age-related maculopathy: the Beaver Dam Eye Study. *Ophthalmology*. 2003; 110:636-643.

186. Tan JSL, Mitchell P, Smith W, Wang JJ. Cardiovascular risk factors and the long-term incidence of age-related macular degeneration: the Blue Mountains Eye Study. *Ophthalmology*. 2007; 114:1143-1150.

187. Park SJ, Lee JH, Woo SJ, Ahn J, Shin JP, Song SJ, et al. Age-related macular degeneration: prevalence and risk factors from Korean National Health and Nutrition Examination Survey, 2008 through 2011. *Ophthalmology*. 2014; 121:1756-1765.

188. Blumenfeld JD, Laragh JH. Renin system analysis: a rational method for the diagnosis and treatment of the individual patient with hypertension. *American journal of hypertension*. 1998; 11:894-896.

189. Gu Q, Burt VL, Dillon CF, Yoon S. Trends in Antihypertensive Medication Use and Blood Pressure Control Among United States Adults With HypertensionClinical Perspective. *Circulation*. 2012; 126:2105-2114.

190. Klein R, Klein BEK, Jensen SC, Cruickshanks KJ, Lee KE, Danforth LG, et al. Medication use and the 5-year incidence of early age-related maculopathy: the Beaver Dam Eye Study. *Archives of ophthalmology*. 2001; 119:1354-1359.

191. Klein R, Myers CE, Klein BEK. Vasodilators, blood pressure-lowering medications, and age-related macular degeneration: the Beaver Dam Eye Study. *Ophthalmology*. 2014; 121:1604-1611.

192. Klein R, Deng Y, Klein BEK, Hyman L, Seddon J, Frank RN, et al. Cardiovascular disease, its risk factors and treatment, and age-related macular degeneration: Women's Health Initiative Sight Exam ancillary study. *American journal of ophthalmology*. 2007; 143:473-483.

193. Hyman L, Schachat AP, He Q, Leske MC. Hypertension, cardiovascular disease, and age-related macular degeneration. *Archives of ophthalmology*. 2000; 118:351-358.

194. Wang T-D, Chen Y-H, Huang C-H, Chen W-J, Chen M-F. Bidirectional Adherence Changes and Associated Factors in Patients Switched From Free Combinations to Equivalent Single-Pill Combinations of Antihypertensive DrugsNovelty and Significance. *hypertension*. 2014; 63:958-967.

195. Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Böhm M, et al. 2013 ESH/ESC guidelines for the management of arterial hypertension: the Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *Blood pressure*. 2013; 22:193-278.

196. Corrao G, Nicotra F, Parodi A, Zambon A, Heiman F, Merlino L, et al. Cardiovascular protection by initial and subsequent combination of antihypertensive drugs in daily life practice. *hypertension*. 2011; 58:566-572.

197. Burt VL, Whelton P, Roccella EJ, Brown C, Cutler JA, Higgins M, et al. Prevalence of hypertension in the US adult population. *hypertension*. 1995; 25:305-313.

198. Barro-Soria R, Stindl J, Müller C, Foeckler R, Todorov V, Castrop H, et al. Angiotensin-2-mediated Ca 2+ signaling in the retinal pigment epithelium: role of angiotensin-receptor-associated-protein and TRPV2 channel. *PLoS One*. 2012; 7:e49624.

199. Hikichi T, Mori F, Takamiya A, Sasaki M, Horikawa Y, Takeda M, et al. Inhibitory effect of losartan on laser-induced choroidal neovascularization in rats. *American journal of ophthalmology*. 2001; 132:587-589.

200. Steckelings UM, Rompe F, Kaschina E, Unger T. The evolving story of the RAAS in hypertension, diabetes and CV disease–moving from macrovascular to microvascular targets. *Fundamental & clinical pharmacology*. 2009; 23:693-703.

201. Sarafidis PA, Georgianos PI, Lasaridis AN. Diuretics in clinical practice. Part I: mechanisms of action, pharmacological effects and clinical indications of diuretic compounds. *Expert opinion on drug safety*. 2010; 9:243-257.

202. Amar J, Chamontin B, Genes N, Cantet C, Salvador M, Cambou JP. Why is hypertension so frequently uncontrolled in secondary prevention? *Journal of Hypertension*. 2003; 21:1199-1205.

203. Ames R. Hyperlipidemia of diuretic therapy. *Archives des maladies du coeur et des vaisseaux*. 1998; 91:23-27.

204. Ames RP, Hill P. Elevation of serum lipid levels during diuretic therapy of hypertension. *The American journal of medicine*. 1976; 61:748-757.

205. Ratnapriya R, Chew EY. Age-related macular degeneration—clinical review and genetics update. *Clinical genetics*. 2013; 84:160-166.

206. Seddon JM, Cote J, Rosner B. Progression of age-related macular degeneration: association with dietary fat, transunsaturated fat, nuts, and fish intake. *Archives of ophthalmology*. 2003; 121:1728-1737.

207. Khedun SM, Naicker T, Maharaj B. Zinc, hydrochlorothiazide and sexual dysfunction. 1995.

208. Zarbin MA. Current concepts in the pathogenesis of age-related macular degeneration. *Archives of ophthalmology*. 2004; 122:598-614.

209. Beatty S, Koh H-H, Phil M, Henson D, Boulton M. The role of oxidative stress in the pathogenesis of age-related macular degeneration. *Survey of ophthalmology*. 2000; 45:115-134.

210. Ernst ME, Moser M. Use of diuretics in patients with hypertension. *New England Journal of Medicine*. 2009; 361:2153-2164.

211. Bloom BS. Continuation of initial antihypertensive medication after 1 year of therapy. *Clinical therapeutics*. 1998; 20:671-681.

212. Kaatsch P. Epidemiology of childhood cancer. *Cancer treatment reviews*. 2010; 36:277-285.

213. Geenen MM, Cardous-Ubbink MC, Kremer LCM, van den Bos C, van der Pal HJH, Heinen RC, et al. Medical assessment of adverse health outcomes in long-term survivors of childhood cancer. *JAMA*. 2007; 297:2705-2715.

214. Wong WL, Su X, Li X, Cheung CMG, Klein R, Cheng C-Y, et al. Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. *The Lancet Global Health*. 2014; 2:e106-e116.