A Lipid-Centric Model of Alzheimer's Dementia: Exploring the Role of PCSK9 and the LDL Receptor Family in Neurodegeneration

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DECLARATION

I, Joshua Wageman, verify that the work presented in this dissertation is my own. This research has been conducted independently of any corporate entity, employer, or healthcare system. Appropriate credit has been given where information has been derived from other sources.

Although I have previously or currently been employed as a Physical Therapist, practitioner in Endocrinology with certification as a Clinical Lipid Specialist, and Medical Science Liaison, this research is independent of these vocational endeavors. These experiences have shaped my perspectives, but this research is independent of any clinical or occupational affiliation.

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ABSTRACT

Although Alois Alzheimer originally noted lipid abnormalities in the brains of those with Alzheimer's Dementia, much research has been directed towards amyloid beta (Aβ), phosphorylated tau, and the various ways in which these entities are processed. These pathologic hallmarks are almost certainly not inconsequential, but neurodegenerative disease is incredibly complex. Thus, this dissertation seeks to offer a unifying model based on the intersection of substrate utilization and central nervous system (CNS) cholesterol homeostasis that will reconcile the core defects noted in Alzheimer's Disease. These include glucose hypometabolism, impaired amyloid clearance, and deficient lipid trafficking; the overlapping pathophysiology with atherosclerotic vascular disease and its well-documented risk factors is striking.

Community clinicians are often tasked with the burden of caring for those with Alzheimer's Disease, and yet significant gaps in understanding remain regarding the association of lipids with cognitive impairment. These health care professionals desire to intervene in a manner that could possibly mitigate cognitive decline in their patients, and yet their selection of lipid-modifying interventions may not be rooted in what is currently understood about the relationships described in the lipid-centric model of AD. Identifying this potential issue is another aspect of this research.

The discussion requires a basic foundation of glucose homeostasis, insulin signaling, and lipid transport. A detailed explanation of peripheral and central lipid transport along with the history of lipid-lowering therapy establishes the context for discussing proprotein convertase subtilisin/kexin type 9 (PCSK9) and its putative role in Alzheimer's Disease.

Expanding far beyond the canonical role of PCSK9 in degrading the low-density lipoprotein receptor (LDLr), evidence from laboratory experiments, genome wide association studies, and clinical trials will highlight the pleiotropic functions of PCSK9 peripherally and centrally.

The project culminates in a multifaceted approach to this complex discussion, commencing with a more specific literature review of PCSK9's role in the central nervous system. Additionally, a survey administered to a sampling of community clinicians throughout the United States elucidates the discordance between clinician knowledge of lipid-related factors and use of lipid-modifying treatments in the context of neurodegeneration. A patient case report will also provide additional fuel for the discussion. This hybridized methodology, incorporating qualitative analysis, semi-quantitative data compilation, and dashes of interspersed phenomenology will highlight the current, albeit limited, understanding of the interaction between PCSK9 and members of the LDLr family in the CNS. This research will foster a discussion of gaps that must be filled to better ascertain whether or not centrally expressed PCSK9 and its interactions with its litany of ligands is a bystander or causative agent in the context of neurodegeneration. By addressing these gaps

in knowledge, this will hopefully address the significant chasm revealed by this research between genuine understanding and pharmacologic intervention among community clinicians.

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LIST OF ACRONYMS & ABBREVIATIONS

7-DHCR	7-Dehydrocholesterol Reductase
24-DHCR	24-Dehydrocholesterol Reductase
24s-OHC	24s-hydroxycholesterol
ABCA1	ATP-Binding Cassette Transporter A1
ABCA7	ATP-Binding Cassette Transporter A7
ABCG1	ATP-Binding Cassette Transporter G1
ABCG4	ATP-Binding Cassette Transporter G4
ABCG5/G8	ATP-Binding Cassette Transporter G5/G8
ACAT	AcylCoA Acyltransferase
ACE	Angiotensin Converting Enzyme
AD	Alzheimer's Disease
ADAS-Cog	Alzheimer's Disease Assessment Scale-Cognitive
ALK1	Activin-like Kinase 1
ANGPTL	Angiopoietin-like Protein
ApoA1	Apolipoprotein A1
ApoA5	Apolipoprotein A5
ApoB-100	Apolipoprotein B-100
ApoB-48	Apolipoprotein B-48
ApoC1	Apolipoprotein C1
ApoC2	Apolipoprotein C2
ApoC3	Apolipoprotein C3
ApoE	Apolipoprotein E
ApoER2	Apolipoprotein E Receptor 2
ApoJ	Apolipoprotein J (Clusterin)
APP	Amyloid Precursor Protein

ARIA	Amyloid Related Imaging Abnormalities
ASCVD	Atherosclerotic Vascular Disease
ASGR1	Asialoglycoprotein Receptor 1
ATP	Adenosine Triphosphate
Aβ	Amyloid Beta
BACE-1	Beta Secretase-1
BBB	Blood-brain-barrier
CAC	Coronary Artery Calcium
CAD	Coronary Artery Disease
CCTA	Computed Tomography Coronary Angiography
CD36	Cluster of Differentiation 36
CETP	Cholesteryl Ester Transfer Protein
CNS	Central Nervous System
CoQ10	Coenzyme Q10
CPT1A	Carnitine Palmitoyltransferase 1A
CSF	Cerebrospinal Fluid
CV	Cardiovascular
DAM	Disease-associated Microglia
DGAT	Diacylglycerol Acyltransferase
DHA	Docosahexaenoic Acid
EGF	Epidermal Growth Factor
FDA	Food and Drug Administration
FH	Familial Hypercholesterolemia
FPP	Farnesyl Pyrophosphate
FXR	Farnesoid X Receptor
GalNac	N-acetyl-galactosamine

GPIHBP1	Glycosylphosphatidylinositol Anchored High-Density Lipoprotein Binding Protein 1
GFAP	Glial Fibrillary Acidic Protein
GGPP	Geranylgeranylpyrophosphate
GLUT	Glucose Transporter
GLP-1	Glucagon-Like Peptide 1
GSK3β	Glycogen Synthase Kinase 3 Beta
GWAS	Genome Wide Association Study
HDL	High-Density Lipoprotein
HMGCR	HydroxymethylglutarylCoA Reductase
HNF1α	Hepatocyte Nuclear Factor 1 Alpha
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
IDE	Insulin-Degrading Enzyme
IDL	Intermediate-Density Lipoprotein
IDOL	Inducible Degrader of LDL Receptor
IL-1β	Interleukin-1 Beta
IL-6	Interleukin-6
Insig	Insulin-induced Gene Protein
IVUS	Intravascular Ultrasound
kD	Kilodaltons
LCAT	Lecithin Cholesterol Acyltransferase
LDL	Low-Density Lipoprotein
LDL-c	Low-Density Lipoprotein Cholesterol
LDLr	Low-Density Lipoprotein Receptor
LMF1	Lipase Maturation Factor 1
Lp(a)	Lipoprotein(a)
LPL	Lipoprotein Lipase

Lp-PLA2	Lipoprotein Phospholipase A2
LRP1	Low-Density Lipoprotein Receptor-Related Protein 1
LRP5	Low-Density Lipoprotein Receptor-Related Protein 5
LRP6	Low-Density Lipoprotein Receptor-Related Protein 6
LXR	Liver X Receptor
mAb	Monoclonal Antibody
MACE	Major Adverse Cardiovascular Events
MCI	Mild Cognitive Impairment
MCT	Monocarboxylate Transporter
MFSD2a	Major Facilitator Superfamily Domain-Containing Protein 2a
MGAT	Monoacylglycerol Acyltransferase
MHC-1	Major Histocompatibility Complex 1
MMP-9	Matrix Metalloproteinase 9
MMSE	Mini Mental State Exam
MTTP	Microsomal Triglyceride Transfer Protein
NFL	Neurofilament Light
NIRS	Near-infrared Spectroscopy
NLRC4	NLR family caspase recruitment domain-containing protein 4
NMDA	N-methyl-d-aspartate
NMR	Nuclear Magnetic Resonance
NPC1L1	Niemann-Pick C1-Like 1
OCT	Optical Coherence Tomography
PAV	Percent Atheroma Volume
PCP	Primary Care Provider
PCSK9	Proprotein Subtilisin/Kexin Type 9
PET	Positron Emission Tomography

PLTP	Phospholipid Transfer Protein
PPARα	Peroxisome Proliferator-activated Receptor Alpha
P-tau	Phosphorylated Tau
SCAP	Sterol Regulatory Element Binding Protein Cleavage Activating Protein
siRNA	Small Interfering Ribonucleic Acid
SQLE	Squalene Epoxidase
SRB1	Scavenger Receptor B1
SREBP2	Sterol Regulatory Element Binding Protein 2
RRR	Relative Risk Reduction
TFEB	Transcription Factor EB
TNF-α	Tumor Necrosis Factor Alpha
TREM2	Triggering Receptor Expressed on Myeloid Cells 2
TRL	Triglyceride-rich Lipoprotein
VLDL	Very low-density Lipoprotein
VLDLr	Very low-density Lipoprotein Receptor

CHAPTER 1-Introduction to Impairments Observed in Alzheimer's Dementia

1.1 Background and Problem

Alzheimer's Disease (AD) is a ubiquitous problem across the globe, affecting an estimated 315 million individuals worldwide encompassing the spectrum of prodromal, preclinical, and late-stage disease (Gustavsson et al, 2023). The condition, marked by progressive loss of executive function, memory impairment, and cognitive decline, has been characterized by amyloid beta (Aβ) plaques, tau hyperphosphorylation, and neurofibrillary tangles (Gustavsson et al, 2023). Despite the early recognition of glial lipid anomalies in patients with dementia by Alois Alzheimer himself (Haney et al, 2023) the current pathophysiological paradigm describes the processing of amyloid precursor protein (APP) via beta and gamma secretases into primarily Aβ-40 and Aβ-42 peptides (Shi et al, 2022). It is believed that Aβ is primarily derived from neurons in a cholesterol-dependent process (Wang et al, 2021). Teleologically, APP appears to have important functions in maintenance of neuroplasticity, and amyloid itself may have antimicrobial properties. It has also been stated to function as a "vascular plug" which confers structural integrity to the blood brain barrier (BBB) (Kent et al, 2020). Even tau proteins have been purported to have a role in microtubule function. However, when the equilibrium between production and clearance of APP, amyloid, and tau is compromised, pathology ensues. Various factors, such as hyperinsulinemia and inflammation, may steer the APP cleavage pattern from an ostensibly more benign alpha-secretase pathway toward this amyloidogenic direction (Shi et al, 2022). Subsequently, these peptides may aggregate into insoluble plaques that take residence in the brain parenchyma and vasculature, and impaired clearance can also result in tau hyperphosphorylation, aberrant seeding and folding of tau protein, and the neurofibrillary tangles that represent the hallmarks of the disease (Kent et al, 2020). This process results in widespread synaptic dysfunction, neuronal loss, and a vicious inflammatory cascade leading to progressive dementia.

Historically, AD has been a clinical diagnosis; the presence of amyloid plaques and neurofibrillary tangles is then later confirmed on post-mortem autopsy. However, there has been an ebullient interest in developing biomarkers to better identify disease in earlier stages given its lengthy prodrome. Blood based biomarkers such as glial fibrillary acidic protein (GFAP), which becomes elevated in states of neuronal damage and subsequent astrocyte activation, and neurofilament light (NFL), a marker of neuroaxonal damage, show promise (Cronje et al, 2022). Measurement of Aβ-42/40 has also correlated well with the ratio of CSF phosphorylated tau-181 (p-tau-181) to Aβ-42 and carried additional predictive value for progression to positive amyloid β on positron emission tomography (PET) (Niotis et al, 2023). Measurements of the various p-tau isoforms do show association with stages of neurodegeneration; for instance, p-tau-231 appears to increase earlier in the disease process, while p-tau-217 predicted those who would progress from mild cognitive impairment (MCI) to AD (Niotis et al, 2023). However, variables such as obesity and kidney disease may lead to confounding when using these plasma measurements, so correlation

of these markers with cerebrospinal fluid (CSF) levels and cognitive measures are necessary in larger cohorts for better validation (Niotis et al, 2023, Angioni et al, 2023). Additionally, various other imaging modalities, including PET, for amyloid and tau are being used in select cases along with other specific forms of electroencephalography (EEG). Hopefully this cadre of biomarkers along with other metabolic measures of health will result in a proactive, holistic approach to disease management in the future.

Treatments for AD, to this point, have been mostly futile in mitigating disease progression. The first Food and Drug Administration (FDA)-approved agents targeted central cholinergic transmission via acetylcholinesterase inhibition along with modulation of the n-methyl-d-aspartate (NMDA) receptor; these did little more than offer modest symptomatic relief for a small subset of individuals (Shi et al, 2022). After failure of multiple therapeutics targeting upstream processing of the amyloid processing pathway, such as beta secretase-1 (BACE-1) inhibition, the most recent innovation has come in the form of anti-amyloid monoclonal antibodies. Indeed, two agents, aducanumab and lecanemab, have now been FDA-approved at the time of this composition, although not without lingering doubts in regards to their true benefit (Shi et al, 2022). Despite their ability to reduce Aβ-40 and Aβ-42 in CSF, lingering safety concerns and the multifactorial nature of AD etiology have led many to investigate other avenues. Quite alarmingly, the most common side effect of these agents is amyloid-related imaging abnormalities (ARIA), which includes cerebral edema and microhemorrhage; this side-effect is more common in those with at least one copy of the E4 allele of the *APOE* gene (Shi et al, 2022). Given this allele is the most common genetic risk factor implicated in development of late-onset AD, a large proportion of the intended population may be at risk for adverse events. Indeed, the quest continues for well-tolerated and efficacious interventions that not only mitigate amyloid accumulation, but restore synaptic transmission and cognitive function.

Community clinicians, including primary care physicians (PCPs), often find themselves at the frontlines of AD treatment despite the aforementioned nuances of appropriate diagnosis and dearth of meaningful intervention. Indeed, a 2020 survey demonstrated that 82% of PCPs perceive themselves at the proverbial frontlines of AD care while 64% of dementia diagnoses originate from a PCP (Sideman et al, 2023). Perhaps more concerningly, gaps in knowledge represent significant barriers for PCPs in regards to AD pathogenesis and management. A seven-study analysis showed that up to 63% of respondents felt inadequately trained for management of dementia, while another four-study compilation of PCP opinions revealed that 23-66% of clinicians lacked confidence due to gaps in understanding (de Levante Raphael, 2022). Given the importance of lipid dysregulation in the pathogenesis of AD and that community clinicians routinely manage lipids in their patient population, improving knowledge of the relationships between cholesterol, atherosclerotic vascular disease (ASCVD), and AD would seem a worthwhile crusade.

1.1.1 Purpose of this Study

The purpose of this study is to explore the relationships between substrate utilization, peripheral lipid transport, and central nervous system cholesterol homeostasis. Humbly acknowledging the complexity and enigmas involved in the discussion, I will present what is currently known about the relationships between proprotein convertase subtilisin/kexin type 9 (PCSK9) and members of the low-density lipoprotein receptor (LDLr) family in both the periphery and the central nervous system (CNS). After extensive literature review to establish appropriate context, the research culminates in a more pedantic literature search and subsequent analysis of the few publications that have experimentally investigated the role of PCSK9 in the CNS. This is coupled with quantitative and qualitative data harvested from a 3-question survey administered to a sampling of community clinicians practicing in the United States. A case report employing peripheral PCSK9 inhibition as a therapeutic intervention will accompany the analysis prior to a discussion of the data ascertained.

1.1.2 Research Questions

Several salient questions will serve as a beacon on the labyrinthine expedition through the simultaneously historical and biochemical landscape.

Research Question 1: What is the relationship between PCSK9 and members of the LDLr family in the central nervous system, and is PCSK9 a bystander or active contributor to the development of Alzheimer's Disease?

Research Question 2: Is peripheral PCSK9 inhibition an efficacious strategy to mitigate the progression of Alzheimer's Disease and, if so, by what mechanisms could this confer benefit?

Research Question 3: What is the awareness of community clinicians regarding the relationship between lipids, cholesterol homeostasis, and PCSK9 in the context of AD? Do these opinions or perceptions influence prescribing patterns of cholesterol-modulating drugs?

1.1.3 Significance and Contribution to the Literature

Alzheimer's Disease is a condition that not only inevitably reduces lifespan, but mercilessly steals the life from one's years. As mentioned, the current interventions aimed at ameliorating this disease state have largely been inadequate. Viewing this multifactorial condition through a metabolic and lipid-oriented lens offers a fresh perspective that seeks to integrate the myriad of dysregulated physiological states observed in AD into one coherent model. There is currently a paucity of literature specifically examining the role of PCSK9 and the LDLr family within the CNS and their potential significance in AD pathogenesis, and this research seeks to help begin the process of bridging that chasm. If our understanding of an admittedly complicated disease state improves, then appropriate interventions can subsequently be taken to manage the pathology. This research proposes the potential utility of PCSK9 inhibition as a promising tool for not only

the well-established benefit in reducing ASCVD, but perhaps mitigating progression of neurodegeneration as well.

Additionally, the lack of perceived knowledge among PCPs regarding diagnosis and management of AD is well-documented. Community clinicians, including PCPs and cardiologists, are both involved in lipid management. However, the assessment of community clinician understanding of lipid-related factors germane to AD, including cholesterol and PCSK9, has not been previously conducted. The survey of community clinicians, the vast majority being PCPs and community cardiologists, elucidates a concerning discordance between knowledge and treatment intervention in regards to these factors. The vast majority of those surveyed had no specific awareness of the role of lipids in AD, and yet many have altered their lipid-lowering therapies due to concerns about AD.

1.1.4 Delimitations and Limitations

An important delimitation is the focus on neurodegeneration through a lipid-centric lens. AD is an intricately complicated disease state, and there is significant overlap between varying disease states that inevitably result in a similar cognitive phenotype. Rather than take an amyloid-centric view, this dissertation rather incorporates the unarguable presence of Aβ into a model prioritizing substrate utilization and lipid homeostasis. Additionally, other potential factors such as environmental toxic exposure, heavy metal deposition, and a plethora of putative contributing factors to neuronal impairment are intentionally omitted from this thesis.

This dissertation is not the culmination of AD research; rather, it attempts to accelerate the conversation regarding lipid involvement in neurodegeneration with hopes of actionable targets as we better understand the role of PCSK9's involvement in the CNS. Consequently, there are a number of limitations, including the dearth of current studies exploring these relationships and the restriction of many AD associations to post-mortem analyses given the obvious ethical limitations implicit in CNS research. Additionally, the analysis here is largely qualitative and exploratory in nature; generalizability of findings based on the limited data would be irresponsible. However, the resource limitations that have, to this point, prevented the various hypotheses presented in this project from laboratory testing will hopefully be mitigated in the future.

There are also limitations within the cohort of clinicians involved in the survey analysis portion of the research. A significant proportion of those surveyed failed to respond, so there may have been some bias in regards to respondent characteristics and data provided by these individuals. The community clinicians surveyed represented a small cohort primarily in the Western United States, and their opinions, perceptions, and baseline knowledge may not be generalizable to practitioners in other locales across the globe.

1.1.5 Conceptual Framework for the Study

There are many strong associations of various dysregulated physiologic states with AD progression. Among the strongest of these are lysosomal dysfunction, impaired amyloid beta clearance, inadequate substrate utilization, and atherosclerotic vascular disease (ASCVD); these are succinctly represented in **Figure 1.** Given the central role of lipid homeostasis as an overlapping feature of the aforementioned conditions, the exploration of this relationship will commence with a brief description of what has been observed in the brains of those either affected by AD or who would inevitably go on to succumb to the disease state.

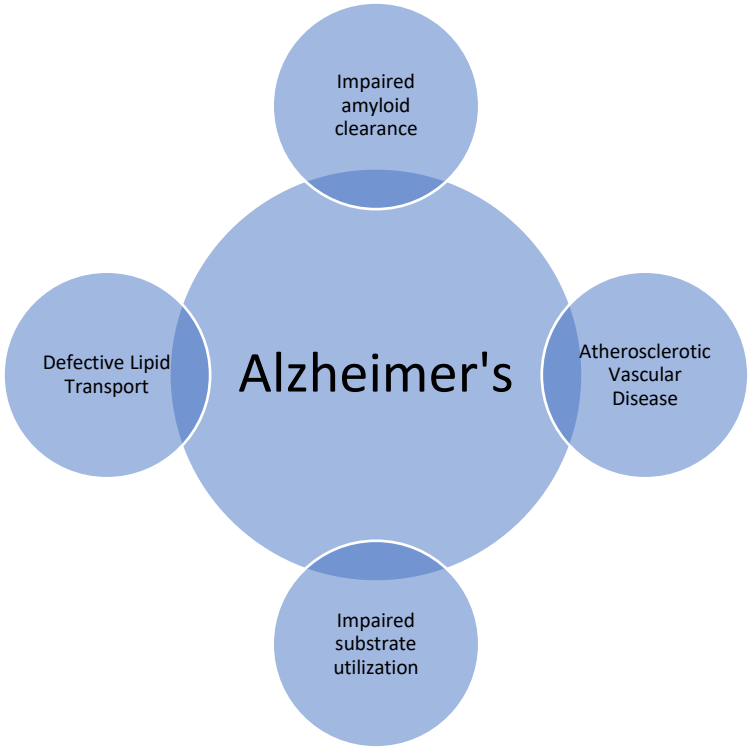

Figure 1: Core Anomalies in a Lipid-centric Model of Alzheimer's Dementia. Impairments in substrate utilization along with abnormalities in lipid trafficking contribute to atherosclerosis as well as the characteristic amyloid plaques observed in the AD brain.

1.1.6 Outline

Chapter 1 will provide an **Introduction and Background** to the AD disease state as well as further elaborate on the core defects within the conceptual framework. Special attention will be given to the Home Security System and Vascular Neighborhood, a sub-framework of ASCVD risk stratification that I personally developed to better assist clinicians in mitigating CV risk. Given the overlapping pathologic associations between ASCVD and AD, a preliminary discussion of lipid biomarkers and vascular biology of CV disease will help provide context for the literature review in Chapters 2 and 3.

Chapter 2 commences **Part 1 of the Literature Review**. A detailed review of both peripheral lipid trafficking and cholesterol homeostasis within the CNS will provide the reader with awareness of both optimal and dysregulated states in regards to lipid transport. Key players such as ApoE and members of the LDL receptor family will be discussed. With this understanding, the perturbations in lipid homeostasis observed in the AD brain will become more meaningful. The chapter then concludes with a historical exploration of lipid-lowering therapies, including a detailed discussion on the mechanisms of statins, the cornerstone of modern-day cholesterol-reducing therapeutics.

Chapter 3, which is **Part 2 of the Literature Review,** will provide an **extensive Literature Review of PCSK9**. This journey will take the reader from its discovery to drug development, sprinkling in ample helpings of vascular biology and genetics to help provide appropriate background. The relatively sparse literature germane to the role of PCSK9 in the brain will pave the way for a more detailed exploration in subsequent sections of its possible role in neurodegeneration.

Chapter 4 is the **Methods** section describing the specific criteria used in the literature search and methodology employed in the community clinician survey. The interventions employed in the case report are also explained in this section. The specific research questions used to guide the research are reiterated and the rationale for the multifaceted methodology employed is discussed in detail.

Chapter 5 is the **Contents and Results** section. The 2 specific articles meeting inclusion criteria are qualitatively analyzed in detail. The qualitative and the semi-quantitative data gleaned from the community clinician survey is thoroughly examined and reported. Themes that emerged from the research are highlighted as well.

Chapter 6 is a **Case Report** of one community clinician's approach to treatment in a patient with hyperlipidemia and concurrent AD.

Chapter 7 weaves together the literature review and provocative results from the research into a compelling **Discussion** of the research questions. The apparent interactions between LDLr family members and PCSK9 are noted, and the potential utility of PCSK9 inhibition, both peripherally and perhaps even centrally as a means to mitigate AD progression, is proposed. Knowledge gaps regarding lipid-related factors germane to AD and potential impacts on current intervention among community clinicians are highlighted. Limitations and propositions for further research and education are set forth as well.

Chapter 8 is a brief **Conclusion** that leaves the reader with hopeful optimism of a brighter future that, driven by increased knowledge, leaves the lugubrious specter of neurodegeneration in the proverbial rearview mirror.

1.2 Core Anomalies Observed in Alzheimer's Disease

1.2.1 Impaired Amyloid Beta Clearance

Under normal physiologic circumstances, the production and clearance rate of Aβ is nearly equivalent. In a study involving adults devoid of disease, the synthesis rate in CSF of Aβ was 7.6% while the clearance was estimated to be 8.3% (Yoon and Jo, 2012). However, minuscule perturbations in the clearance of Aβ can precipitate the cascade resulting in inevitable disease progression. Although they are distinct disease states, deposition in the brain parenchyma results in AD while beta amyloid accumulation in the vasculature can result in cerebral amyloid angiopathy (CAA), a common cause of intracerebral hemorrhage in the elderly (Greenberg et al, 2020). It has been reported that CAA is concurrently present in nearly 90% of those with AD (Cozza et al, 2023).

Aβ can be cleared via enzymatic and non-enzymatic mechanisms. Proteases produced by glial cells such as neprilysin, insulin-degrading enzyme (IDE), endothelin-converting enzymes, matrix metalloproteinase 9 (MMP-9), and possibly angiotensin converting enzyme (ACE) play a role in enzymatic degradation of Aβ (Yoon and Jo, 2012). Additionally, the glymphatic system, a "drainage" perivascular pathway formed by the endfeet processes of astrocytes, appears to remove metabolic wastes as well as Aβ; this is largely coordinated by the presence of aquaporin-4 (Ding et al, 2023). Non-enzymatic clearance via astrocytic uptake or microglial phagocytosis also seems to be particularly germane to central homeostatic maintenance, and receptors such as the very low-density lipoprotein receptor (VLDLr) and low-density lipoprotein receptor-related protein 1 (LRP1) also appear critical in transporting amyloid across both vasculature walls and even across the BBB for peripheral clearance (Kanekiyo and Bu, 2014). Megalin, also known as low density lipoprotein receptor-related protein 2 (LRP2) and ApoJ also may serve a role in this transcytosis of amyloid across the BBB (Raulin et al, 2022); soluble megalin levels have been shown to be decreased by 40% in the CSF of those with AD compared to those without (Spuch et al, 2015). The role of LRP1, its numerous binding partners, and its role as a ligand for myriad cellular processes both peripherally and centrally will continually resurface, as it serves relevant functions in amyloid clearance and distribution of lipids throughout the body. Purported physiologic glial-derived mechanisms for Aβ clearance will be the focus of this section given the inextricable relationship between substrate utilization, lipid trafficking, and proper functioning of both astrocytes and microglia in maintenance of the cerebral milieu. A brief summary of the routes of amyloid beta clearance are delineated in **Table 1.**

Table 1: Routes of Amyloid Clearance

Route of Clearance	Key Players	Comment
Enzymatic	IDE, neprilysin, MMP-9, endothelin-converting enzymes, ACE	Enzymes produced by glial cells
Glymphatic system	Aquaporin-4	Perivascular drainage pathway formed by astrocyte endfeet
Astrocytic	LRP1, LDLr, VLDLr	Receptor-mediated endocytosis and subsequent lysosomal degradation
Microglial phagocytosis	LPL, LRP1, TREM2, ApoE	ApoE4 impairs this process
Peripheral sink	LRP1, megalin	LRP1 and/or megalin-mediated transport across BBB for hepatic clearance

1.2.1.1 The Role of Microglia

Microglia are the resident mononuclear immune cells of the brain and represent 5 to 15% of total CNS cells in adults (Loving et al, 2020). However, when activated for prolonged periods, such as seen in the context of neurodegeneration, they can shift from an M2 anti-inflammatory phenotype to an M1 pro-inflammatory subset deemed disease-associated microglia (DAMs). These DAMs shift metabolic preference from oxidative phosphorylation to glycolysis. Microglia expressing CD11b and CD18 are capable of phagocytizing Aβ, but recent animal models suggest increased DAM proliferation is coupled with impaired clearance in models of neurodegeneration (Loving et al, 2021).

Despite the limited global utilization of fatty acids for substrate in the CNS, treatment with peroxisome proliferator activated receptor alpha (PPARα) agonists and overexpression in enzymes such as Carnitine Palmitoyltransferase 1A (CPT1A), critical in transport of long-chain fatty acids into mitochondria for oxidation, have experimentally shown benefit in preventing oxidative stress from M1 microglia polarization (Loving and Bruce, 2020). Normally, microglia are able to scavenge lipid species via LRP1, triggering receptor expressed on myeloid cells 2 (TREM2) and lipoprotein lipase (LPL) present at the cell surface; these receptors interact either directly with Aβ to facilitate subsequent endocytosis or indirectly via Aβ interaction with ApoE. Mutations in the *TREM2* gene have been associated with susceptibility to AD (Kanekiyo and Bu, 2014), and ApoE will be discussed in much greater detail in **Section 2.7**. However, while many details remain to be elucidated, the link between AD and microglial lipid regulation can be asserted with a high degree of confidence.

One intriguing microglial player lying at the nexus between substrate utilization and lipid homeostasis is lipoprotein lipase (LPL). The canonical role of lipoprotein lipase involves hydrolysis of triglyceride-rich lipoproteins (TRLs) for uptake into cells. In the periphery, adipocytes and myocytes produce LPL, which with the aid of lipase maturation factor 1 (LMF1), secretes LPL into the interstitium where it can become tethered to the capillary endothelium via glycosylphosphatidylinositol anchored high-density lipoprotein binding protein 1 (GPIHBP1) (Nelson and Cox, 2013). Cofactors such as ApoC2 and ApoA5 can then assist LPL in its hydrolytic function and subsequent uptake of free fatty acids into the cell. However, LPL also possesses phospholipase A1 activity that equips it for phospholipid hydrolysis and can also function as a chaperone to present lipoproteins to receptors such as the LDLr and LRP1 (Williams et al, 1993). In the periphery, this enhances the clearance of ApoB-containing lipoproteins, but given the dearth of TRLs in the brain, this interaction may play a different role in the CNS. Although this is an area of ongoing research, LPL may help in the presentation of amyloid beta to LRP1 for endocytic processing and subsequent clearance; TREM2 and ApoE may play roles in enhancing or abrogating the clearance depending on the context (Kim et al, 2021). Specifically, ApoE4 has demonstrated an inhibitory effect on LPL-mediated lipid uptake in microglial cell lines in the laboratory setting. Additionally, given that myelin and, by extension, myelin debris, is rich in phospholipids, it is plausible that the phospholipase A1 activity of LPL could play a role in resolution of demyelinating states. Genes associated with LPL loss of function or gain of function have resulted in decreased and increased amyloid clearance, respectively, suggesting the importance of these interactions in phagocytosis of lipid and amyloid to mitigate toxic accumulation (Loving et al, 2020). Additionally, in vitro studies measuring LPL activity show a marked polarization of microglia to an M1 phenotype marked by decreased expression of anti-inflammatory enzymes such as arginase-1, decreased capacity for fatty acid oxidation, and decreased phagocytic capacity (Loving et al, 2020).

1.2.1.2 Lysosomal Dysfunction

The lysosome resides at the hub of all physiologic processes; this ubiquitous organelle is essential in mediating autophagic housekeeping of the cellular compartment. Indeed, a litany of hydrolytic enzymes, when maintained at optimal acidic pH, properly degrade biomolecules or, via fusion with the autophagosome, cooperate to “take out the trash” inside the cell (Nelson and Cox, 2013). Many lysosomal storage diseases, such as lysosomal acid lipase deficiency and Gaucher’s disease, result in toxic substrate accumulation and generally catastrophic physiologic consequences to the affected individual. Lysosomal function has particular importance in CNS astrocytes and microglia.

The earliest defect, occurring decades prior to any cognitive decline, observed in those who progress to AD is endosomal enlargement (Van Acker et al, 2019). In regards to AD, several genome-wide association studies (GWAS) have identified alleles germane to lysosomal function

in risk of AD development, including *BIN1* and *PICALM*, which code for bridging integrator 1 and phosphatidylinositol-binding clathrin assembly protein, respectively (Ando et al, 2022). Although these proteins are a subject of ongoing research, they appear to be critical in endolysosomal trafficking of APP byproducts as well as autophagolysosomal fusion. Additionally, ApoE4, which will be discussed at length in later sections, does appear to alkalinize the pH of the lysosome, impairing the dynamic process involved in lipid transport and amyloid clearance (Krogsaeter et al, 2023).

Niemann-Pick C Disease, also known as "childhood Alzheimer's," may also be helpful in elucidating the importance of lysosomal function in preserving neurologic dysfunction. Under normal circumstances, when cholesterol is delivered to the lysosome, lysosomal acid lipase degrades the contents and the remaining free cholesterol is transported by Niemann-Pick C2 and then "handed off" to Niemann-Pick C1 on its to the plasma membrane; once there, it downregulates the expression of sterol regulatory element binding protein 2 (SREBP2) to prevent toxic accumulation of sterol subspecies (Wheeler and Sillence, 2020). However, in Niemann-Pick C disease, this transport does not occur, leading to toxic sterol accumulation via loss of the SREBP2-mediated downregulation of further lipid uptake. Subsequently, affected individuals experience the triad of ataxia, dysarthria, and dementia unless measures are taken to effectively chelate the excess sterols from within the cell (Wheeler and Sillence, 2020). While AD may not be characterized by deficient Niemann-Pick C-mediated transport mechanisms, it is intriguing that a disease characterized by early-onset dementia does somewhat parallel the early anomalies in endolysosomal function seen in those who progress to AD.

Given the importance of autophagy in the central nervous system, some have proposed Rapamycin, which indirectly inhibits the mechanistic target of rapamycin complex 1 (mTORC1), as a therapeutic option for AD prophylaxis (Kaeberlein and Galvan, 2019). Indeed, inadequate catabolism of intracellular cholesterol leads to chronic mTORC1 activation. This subsequently sequesters transcription factor EB (TFEB) in the cytoplasm, preventing its transcription and suppressing lysosomal biogenesis (Lee et al, 2023). Other avenues of modulating autophagy via PPARα, a nuclear transcription factor, may be beneficial in not only promoting lysosomal biogenesis, but regulating mitochondrial lipid processing (Wojtowicz et al, 2020). It remains to be seen whether these interventions can be effective long-term options in humans, but given that the balance between anabolism and autophagy is elegantly regulated by substrate utilization, this represents a seamless segway into the topic of glucose metabolism in the brain.

1.2.2 Impaired Substrate Utilization

Proper substrate utilization across the lifespan is a critical component in prevention of chronic disease, and fueling the human machine, so to speak, requires varying substrates that are both context and tissue-specific. Optimal metabolic flexibility includes the capacity to utilize various energy systems and substrates, but the metabolic demands of the brain are preferentially met by

glucose as a fuel source. Peripheral fuel partitioning is in large part geared towards preservation of adequate glucose to drive the perpetual neurotransmission and maintenance of the central cellular milieu required for the human existence. Glucose is processed via glycolysis into pyruvate, which can either enter the mitochondria via the pyruvate dehydrogenase complex or be shuttled to lactate in the cytosol. Pyruvate converted into acetylcoA in the mitochondria can enter the citric acid cycle and eventually drive oxidative phosphorylation, or lactate itself can be transported via monocarboxylate transporters (MCTs) to tissues as an alternative fuel source (Nelson and Cox, 2013). Although there is an underappreciated role for fatty acid oxidation in the brain and the dynamic organ is capable of utilizing ketones and lactate as alternatives in the absence of glucose, 95% of adenosine triphosphate (ATP) produced by the brain occurs via glucose metabolism (Cunnane et al, 2020). Consequently, glucose hypometabolism and subsequent dysregulation in brain bioenergetics has been consistently identified as a feature of Alzheimer's disease. Indeed, decreased activity in complexes I, IV, and V of the electron transport chain along with downregulation of mitochondrial enzymes such as alpha-ketoglutarate dehydrogenase and pyruvate dehydrogenase are characteristic of the AD brain (Pahlavani, 2023). Given that glucose homeostasis and, by extension, insulin signaling, lies at the intersection of lipid trafficking, a basic understanding of these processes, both peripherally and centrally, will help provide context for further discussion.

Glucose can be transported into cells via an insulin-dependent or non-insulin-dependent fashion. The primary insulin-dependent transporter in the periphery is GLUT4, but this is only sparsely expressed in the CNS. However, several insulin-dependent transporters, including GLUT4 and GLUT8 in hippocampal neurons, and GLUT12 in cortical astrocytes, have been identified (Kyrtata et al, 2021). However, the non-insulin-dependent transporters GLUT1, GLUT2, and GLUT3 are the predominant players in brain glucose regulation, while GLUT7 serves a secondary role in astrocytes (Raut et al, 2023). GLUT1 transports glucose from the periphery across the endothelial cells lining the blood brain barrier (BBB), and GLUT3 facilitates glucose uptake into neurons (Kyrtata et al, 2021). GLUT2 is primarily expressed in astrocytes, and astrocytes may devote this glucose to lactate production; this lactate can be shuttled to the extracellular matrix and then into the neuron by MCT1 and MCT2 transporters, respectively. This lactate, derived from glycolysis, serves as an additional fuel source for neurons, and the astrocyte functions as a critical coordinator of metabolic support for preservation of brain function (Kyrtata et al, 2021). The various CNS glucose transporters are depicted below in **Table 2.**

Table 2: Glucose Transporters in the CNS

Transporter	Insulin Dependent or Independent	Primary Location
GLUT1	Independent	BBB Endothelial Cells, Oligodendrocytes
GLUT2	Independent	Astrocytes
GLUT3	Independent	Neurons
GLUT4	Dependent	Sparse, hippocampal neurons
GLUT7	Independent	Astrocytes
GLUT8	Dependent	Sparse, hippocampal neurons
GLUT12	Dependent	Cortical Astrocytes

Diabetes represents a profound pathological disturbance in glucose homeostasis, and according to a 28-study meta-analysis, is associated with a 56% increase in development of Alzheimer's dementia (Gudala et al, 2013). Somewhat paradoxically, hyperglycemia downregulates GLUT1 transporter expression, consistent with the diabetes paradigm of cellular "starvation in the midst of plenty." However, more subtle perturbations in glucose homeostasis strongly predict cognitive decline; decreased cerebral metabolic rate of glucose in the hippocampus was present 7 years prior to development of mild cognitive impairment in a small cohort (Raut et al, 2023). This same cohort would later demonstrate glucose hypometabolism in the parietal and temporal lobes when followed over time (Raut et al, 2023). This inability to properly utilize substrate persists with disease progression. In a 1983 study of 24 patients with AD, there was a 17-24% decrease in glucose metabolism on FDG-PET scans (de Leon et al, 1983). Post-mortem brains of both humans and mice with AD show decreased GLUT1 and GLUT3 transporter expression, although it does appear that astrocytic GLUT2 expression, in an effort to compensate, does increase in the AD brain. In some studies, decreased GLUT1 expression not only correlates with decreased cognition, decreased cerebral blood flow, and increased Aβ deposition, but also a decrease in LRP1, an important player in amyloid clearance and lipid transport (Actis Dato et al, 2021).

Although the primary glucose transporters in the brain are non-insulin-dependent, hyperinsulinemia without hyperglycemia is still associated with development of memory decline; insulin receptors are present in the CNS, and insulin-resistant states decrease insulin transport into the brain. Interestingly, several human studies have demonstrated an acute improvement in declarative memory with intravenous or intranasal insulin delivery when euglycemia is preserved (Avgerinos, 2018). However, a meta-analysis of intranasal insulin in AD demonstrated no significant benefit in cognition, although this did vary according to *APOE* genotype, as non-carriers of ApoE4 did show some improvement in verbal memory (Avgerinos, 2018). This analysis was limited by small studies and varying dosing regimens; additionally, any therapeutic intervention at later stages of AD is likely hamstrung by the chronicity of the disease process. Still, given the

ubiquitous role of insulin in a diverse array of biological processes, restoration of proper insulin signaling prior to profound cognitive impairment is likely to be beneficial; various mechanistic hypotheses based on associations seen in the diseased brain are outlined below.

- Insulin is the primary hormonal activator of lipoprotein lipase (LPL). There are many factors which positively regulate LPL activity, including GPIHBP1, LMF1, ApoA5, and ApoC2. Homozygotes with complete loss-of-function in the *LPL* gene have Familial Chylomicronemia Syndrome, characterized by therapy-resistant triglyceride elevations and recurrent pancreatitis, while loss-of-function in the other alleles may result in Multifactorial Chylomicronemia Syndrome in the context of lifestyle factors such as alcohol use or insulin resistance (Feingold, 2001). ApoC3 is a potent inhibitor of LPL activity, and ApoC1 inhibits LPL to a lesser extent. Loss-of-function variants in APOC2 have been associated with cognitive decline, while loss-of-function in APOC3 seems to correlate with preservation of cognition (D Bruce et al, 2020). The *APOC1* gene on chromosome 19 has also been linked to AD as well but its function in the CNS remains to be elucidated (Fuior and Gafencu, 2019). As mentioned previously, LPL activity in microglia is highly important in amyloid clearance; whether insulin is required or supportive in this process remains to be determined.
- Glycogen synthase kinase 3 beta (GSK3β) activity is elevated in those with AD and GSK3B has been implicated in tau hyperphosphorylation (Hooper et al, 2008). Given that insulin is a negative regulator of GSK3β, which then results in glycogen synthesis, this may perhaps be relevant in mitigating the malignant cascade resulting in tau hyperphosphorylation.
- As mentioned previously, insulin-degrading enzyme is one of the enzymatic means of amyloid beta clearance. It has been proposed that chronic elevation in insulin leads to insulin "competing" with Aβ for IDE binding, thus resulting in less clearance via IDE (Yoon and Jo, 2012). Although the precise mechanism is likely more complex, the perturbations in insulin signaling and, by extension, IDE levels in those with both diabetes and AD represent an additional plausible mechanism by which impaired glucose homeostasis disrupts optimal physiologic function.
- Both hyperglycemia and loss of insulin function result in decreased LRP1 expression, and under normal physiologic circumstances insulin activates LRP1 expression (Liu et al, 2015). It stands to reason that in states of insulin resistance LRP1 activity would also be decreased, and it has been demonstrated that those with diabetes and concurrent cognitive impairment possess decreased LRP1 levels (Liu et al, 2015). LRP1 itself also promotes insulin signaling in the brain via its interactions with GLUT3 and GLUT4, and plays a critical role in not only maintaining BBB integrity but also facilitating lipid transport (Liu et al, 2015).

- A small human study of liraglutide, a glucagon-like peptide-1 (GLP-1) receptor agonist for treatment of type 2 diabetes, was used in patients with AD. Subjects did demonstrate improved blood-brain glucose transport, which also correlated positively with markers of cognition (Edison et al, 2021). Additionally, a meta-analysis of various oral antidiabetic agents demonstrated potential benefits in mitigating cognitive decline for other medications such as metformin and thiazolidenidiones; all oral agents except sulfonylureas showed benefit (Slouha et al, 2023). Interestingly, sulfonylureas, due to their mechanism of action in depolarizing the beta cell, actually lead to increased insulin levels; this speaks to the importance of minimizing glycemic excursions while simultaneously avoiding hyperinsulinemia. Additionally, glyburide, a member of the sulfonylurea drug class, inhibits ATP-binding cassette transporter A1 (ABCA1)-mediated cholesterol efflux, potentially constipating not only HDL reverse cholesterol transport, but perhaps deleteriously impacting CNS lipid trafficking (Nieland et al, 2004). Regardless, further trials of insulin-sensitizing agents are warranted, perhaps at earlier stages of cognitive impairment.
- Visceral and subcutaneous adiposity, which is inextricably linked to insulin resistance, was highly predictive of brain volume loss among a cohort of over 10,000 middle-aged individuals (Raji et al, 2023). The correlation between visceral fat and decreased cortical volume was stronger in females than males. This built upon past studies that showed an odds ratio of 3.88 for development of dementia in those with midlife obesity (Raji et al, 2023).
- An additional study of a large Korean cohort showed that those with metabolic syndrome, a constellation of characteristics unified by insulin resistance, had an elevenfold risk of developing AD (Kim et al, 2021). Each individual component of the syndrome, which includes hypertension, impaired fasting glucose, low HDL-c, elevated triglycerides, and increased waist circumference, were associated with AD development (Kim et al, 2021). Interestingly, impaired fasting glucose was the sole individual factor associated with vascular dementia in this cohort; perhaps lipid perturbations associated with insulin resistance are more germane to the AD disease process than the pathogenesis of vascular dementia.
- Arguably the most potent intervention in primary prevention of neurodegeneration is exercise, and exercise unequivocally promotes glucose disposal via insulin-dependent and independent mechanisms; exercise has been said to reduce the risk of AD by 45% (Pahlavani, 2020). An entire dissertation could be devoted to the benefits and mechanisms of both aerobic and resistance exercise in relation to vascular and cerebral health, but not paying a humble homage to the power of movement in the context of substrate utilization would be a glaring omission.

1.3 Atherosclerotic Vascular Disease-The Home Security System and the Vascular Neighborhood

Atherosclerotic vascular disease (ASCVD) is the number one cause of death across the globe. Although litany of factors, both modifiable and non-modifiable, are linked to ASCVD, this can be simplified into a relatable model known as the Home Security System and the Vascular Neighborhood. The elements of the Home Security System include 4 pillars:

1. **Normal Parameters of Glucose Metabolism**-this can be measured clinically in various ways. Hemoglobin A1c, oral glucose tolerance testing, and homeostatic model assessment for insulin resistance (HOMA-IR) as determined by measurements of fasting plasma insulin and glucose, are some of the biomarkers that may be utilized (Nelson and Cox, 2013).
2. **Normal Blood Pressure**-maintaining normal blood pressures has long been recognized as a critical aspect of cardiovascular health.
3. **Keeping Systemic Inflammation Low**-this can also be broadened to represent markers of endothelial function. Clinically, this may include a more diverse set of biomarkers. Urinary microalbumin, plasma uric acid, high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), lipoprotein phospholipase A2 (Lp-PLA2), and homocysteine are just a few of the clinically available ways that, in the proper context, may be useful in risk stratification and subsequent intervention (Al Attiq et al, 2024).
4. **Smoking Cessation** (or preferably never starting given its unequivocally deleterious effect on the endothelium).

The Vascular Neighborhood is represented by the lipid panel, as every ApoB-containing lipoprotein <90 nanometers in diameter is potentially a “criminal” that can “break in” if the Home Security System is not optimized (Ginsberg et al, 2021). Since under normal physiologic circumstances over 90% of these particles are of the low-density lipoprotein (LDL) variety, the most common way in which this may be assessed is by a calculated LDL cholesterol (LDL-c) measurement (Jang et al, 2020). LDL particles are largely the byproducts of very low-density lipoprotein (VLDL) hydrolysis and have been regarded as “vascular toxins” once their triglyceride-laden cargo has been delivered (Toth 2021); the intricacies of lipid transport will be detailed in **Chapter 2**. Additionally, European Guidelines recommend a one-time measurement of a genetically determined LDL-like particle known as lipoprotein(a) or Lp(a), as it has emerged as an independent risk factor for ASCVD; indeed, it can be particularly “felonious” if present in the neighborhood (Kronenberg et al, 2022). In rare cases the neighborhood can be so criminal-infested that even a perfect home security system is futile in prohibiting entry; a classic example of this is Homozygous Familial Hypercholesterolemia. These children have stratospheric LDL-c levels, and despite normal blood pressure, optimal glucose control, and no identifiable inflammation, have extensive premature coronary artery disease. This model is graphically depicted in **Figure 2.**

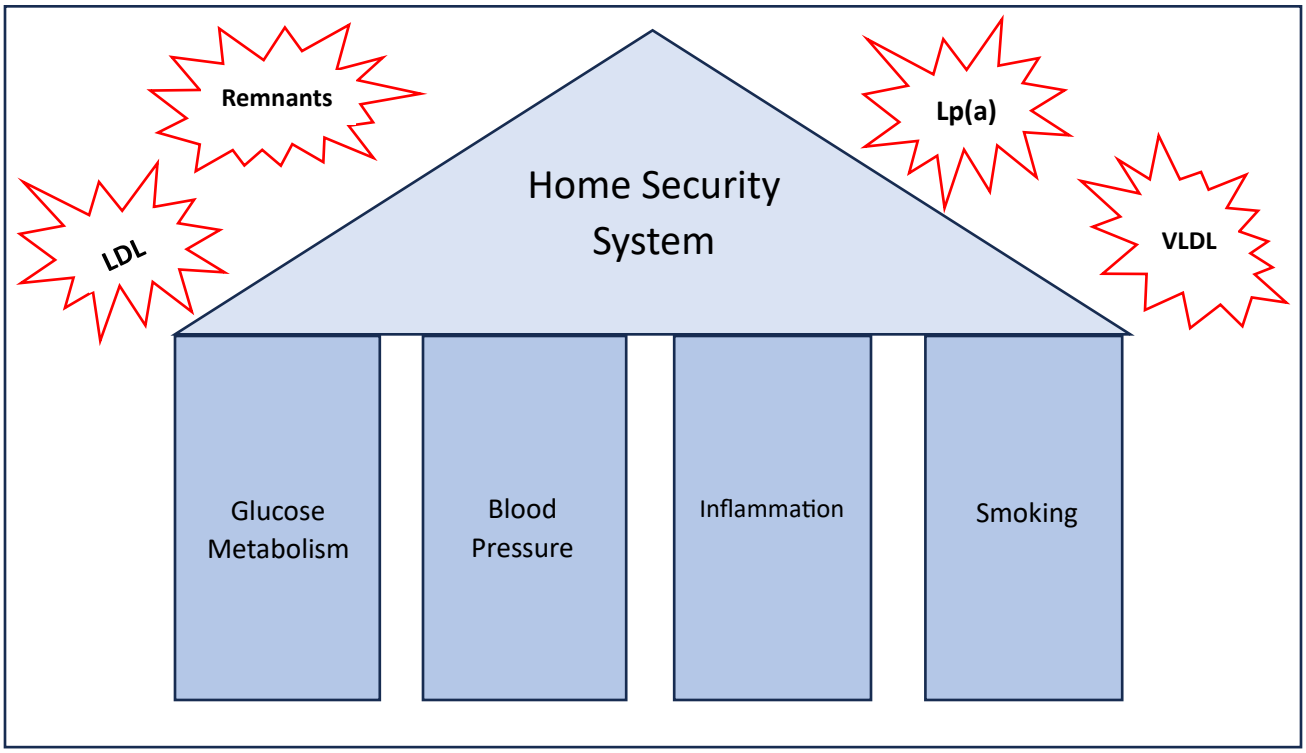

Figure 2: The Home Security System and Vascular Neighborhood. The 4 pillars of the Home Security System representative of endothelial integrity are optimization of glucose metabolism, normalizing blood pressure, keeping inflammation low, and not smoking. Every ApoB-containing lipoprotein is potentially a "criminal" that could infiltrate the neighborhood. The vast majority of these particles under normal conditions are LDL particles, although remnants and Lp(a) particles can be particularly atherogenic.

The *sine qua non* of any atherosclerotic disease state is the infiltration of an ApoB-containing lipoprotein into the subendothelial space of the arterial wall. Although this was once viewed as a passive process, carrier proteins such as caveolin-1, the bi-directional scavenger receptor B1 (SRB1), and activin-like kinase 1 (ALK1) have recently been implicated in the transcytosis of lipoproteins into the arterial intima (Jang et al, 2020). According to the response-to-retention hypothesis, the lipoprotein is subsequently modified and commences the maladaptive immune response resulting in atherosclerotic plaque formation. Native LDL must be oxidized prior to retention, while remnant lipoproteins containing ApoE may become retained independent of oxidation; this likely contributes to the increased atherogenicity per particle of triglyceride-rich remnants (Ginsberg et al, 2021). Various immune cells such as monocyte chemoattractant protein 1 (MCP-1) and scavenger receptors such as cluster of differentiation 36 (CD36) and SR-A1 further contribute to the process by which monocytes differentiate into macrophages and generate foam cells which eventually lead to atheroma formation (Jang et al, 2020). Indeed, the lipoprotein receptors which engulf sterol-rich particles within the intima are not subject to feedback

inhibition, propagating a malevolent cascade that either results in progressive stenotic lesions or vulnerable plaques prone to rupture.

1.3.1 Lipid Biomarkers

Given the role of sterol-containing particles in propagating ASCVD, the basic lipid panel has become a common clinical way to risk stratify an individual's proclivity toward pathology. This lipid panel, generally obtained in a fasted state, contains the following components:

- Total cholesterol
- HDL cholesterol (HDL-c)
- Triglycerides (measured by glycerol)
- LDL cholesterol (calculated)
- Non-HDL-cholesterol (calculated)

Using the Friedewald equation, LDL-c is calculated by subtracting the sum of HDL-c and triglycerides/5 from total cholesterol (Berberich and Hegele, 2022). This is based on the assumption that for every VLDL particle, which contains the majority of triglyceride-rich particles in a fasting state, the ratio of triglyceride to cholesteryl ester is 5:1 (Ginsberg et al, 2021). In a non-insulin resistant individual, LDL-c is often concordant with LDL particle counts. These particle counts, rather than the mass of cholesterol trafficked by the particles, have been correlated much more strongly with disease; one can view each LDL particle as a potential "criminal" that can penetrate the vascular endothelium. However, if the given mass of cholesterol in LDL particles is transported by a multitude of small, dense subspecies, the particle metric will be highly discordant compared to LDL-c, representing residual risk not captured by the basic lipid panel (Ginsberg et al, 2021). Because of this, particle metrics ascertained via nuclear magnetic resonance (NMR) spectroscopy or via a measurement of ApoB have become much more widespread. Given that one ApoB-100 molecule, which is non-transferable and has a stable molecular weight of ~550 kD, resides on each LDL particle, ApoB has become the preferred assay for many lipidologists across the globe due to its greater predictive capacity for major adverse cardiovascular events from the CARDIA consortium, the Framingham registry, and other large biobanks when compared to LDL-c (Glavinovic et al, 2022).

Along with particle metrics, there are other sophisticated assays that can measure sterol absorption and production. Desmosterol and lathosterol are markers of cholesterol synthesis, and campesterol and sitosterol are indicative of sterol absorption. Given the strong correlation between levels of CSF desmosterol and plasma desmosterol, desmosterol may be an important marker of sterol production in the CNS as well (Sato et al, 2012). This will have potential significance when discussing the nuances of cholesterol homeostasis in the brain. Additional imaging techniques, such as the coronary artery calcium (CAC) scan can be very useful supplements to blood-based biomarkers in determining whether or not an individual's vascular

abode has “been broken into,” so to speak. This non-invasive, low-dose computed tomography scan reveals whether there is calcified plaque present in the coronary tree, a late finding in the process of atherosclerosis (Budoff et al, 2023). A score of zero portends low risk of a major adverse cardiovascular event (MACE) in the immediate future, while an absolute score of 300 or greater carries equivalent risk of sustaining a CV event, even over the short-term (Budoff et al, 2023).

1.3.2 Inflammatory Markers

There are numerous biomarkers available to better quantify inflammatory risk. HS-CRP is a widely used and independent, albeit non-specific risk factor for ASCVD, although both Lp-PLA2 and IL-6 have shown complementary utility as well (Cojocaru et al, 2010). In the Multi-Ethnic Study of Atherosclerosis (MESA), hs-CRP ceased to retain significance when adjusted for IL-6; IL-6 was more strongly associated with both heart failure and ASCVD (Ferreira et al, 2024). Lp-PLA2 is an enzyme produced by macrophages that hydrolyzes oxidized phospholipids into the atherogenic precursor lysophosphatidylcholine; multiple studies have revealed Lp-PLA2 as an independent risk factor for CAD and stroke of ischemic origin (Cojocaru et al, 2010). Homocysteine has also been recognized as a biomarker of CAD in various studies. Perhaps more relevant to this discussion is the fivefold greater risk in dementia in those with homocysteine levels >15 versus those with levels <10.1 in one Italian cohort (Smith et al, 2018). Although there are many other biomarkers that can be utilized for CV risk stratification, the aforementioned biomarkers will be revisited in the Case Report discussion in **Chapter 6.**

1.3.3 Associations of ASCVD and Lipid Metrics with Alzheimer’s Dementia

In light of the Home Security System and Vascular Neighborhood model, it should perhaps come as little surprise that Alzheimer’s Disease is associated with ASCVD; a 2020 meta-analysis showed that atherosclerosis was 46% more prevalent in those with AD than the comparator group (Xie et al, 2020). Additionally, the extent of coronary artery disease was an independent risk factor for AD in individuals with the ApoE4 allele (Beeri et al, 2006) and a large sample size from the UK BioBank showed a 29% increased risk of Alzheimer’s Disease in those with established coronary heart disease in a middle-aged cohort of over 430,000 individuals (Liang et al, 2023). Myocardial infarction has also been associated with not only cognitive decline and vascular dementia, but Alzheimer’s pathology as well (Thong et al, 2022). However, despite the well-established association between LDL cholesterol and ASCVD, the relationship between traditional lipid-specific risk factors and AD is a far murkier picture. Several studies showed no relationship between LDL-c and AD, although the utilization of lipid-lowering therapies did not exclude patients from those earlier analyses, thus contributing to potential confounding. A recent 26-study meta-analysis of therapy-naïve patients, however, did show that LDL-c levels >121 in 60-70 year-olds did show a 20% increased risk of AD (Zhou et al, 2020). Additional data from a large cohort of over 450,000 individuals from the UK Biobank demonstrated a 13% increased risk of AD

in those within the highest quartile of LDL-c, while those in the highest quartile of plasma ApoB had a 32% increased risk of developing AD (Gong et al, 2022). The bulk of current evidence does point to elevated LDL-c and ApoB in midlife being associated with AD development. Interestingly, LDL/ApoB ratio was inversely associated with AD, suggesting that particle composition may be of particular importance when utilizing lipid biomarkers to stratify risk of neurodegeneration (Gong et al, 2022). Another study showed that elevated levels of another atherogenic lipoprotein, lipoprotein(a) or Lp(a), which has emerged as an independent risk factor for ASCVD, was strongly associated with AD independent of ApoE genotype (Solfrizzi et al, 2002) although this finding was not replicated in a larger cohort from the UK Biobank (Gong et al, 2022). Many of these studies have been hamstrung by background therapies and heterogeneity in patient demographics; this ultimately speaks to the intricate and tightly-regulated balance between cholesterol synthesis, transport, and efflux in the central nervous system.

CHAPTER 2-Lipid Transport in the Periphery and Central Nervous System

2.1 Cholesterol Synthesis

In order to fully appreciate the complexities of lipid dysregulation in the context of neurodegeneration, an understanding of lipid homeostasis in both the peripheral circulation and the central nervous system is necessary. In general, although ~23% of total body cholesterol resides in the brain, lipoprotein metabolism in the periphery is largely segregated from CNS lipid trafficking (Mahley 2016).

Every nucleated cell in the body is capable of synthesizing its own cholesterol, as it is a critical component of cell membranes, vital in bile acid production, and essential for steroidogenesis. This 37-step process takes place in the cytosol and commences with the condensation of 2 acetylcoA molecules into acetoacetylcoA. After the addition of a third acetylcoA molecule catalyzed by hydroxymethylglutarylcoA synthase, the committed step in this so-called mevalonate pathway proceeds via the enzyme hydroxymethlyglutarylcoA-reductase (HMGCR) (Nelson and Cox, 2013). From there, the production of various isoprenoid precursors such as geranylgeranylpyrophospate (GGPP) and other sterol intermediates takes place prior to the aromatization of the ultimate linear hydrocarbon molecule, squalene, into 2,3 epoxysqualene (Nelson and Cox, 2013). At this point, the cholesterol production pathway is bifurcated; in the peripheral circulation the majority of cholesterol production is a result of the Kandutsch-Russell pathway with a minority contribution from the Bloch pathway. The penultimate precursors in each pathway are lathosterol and desmosterol, respectively, catalyzed by the enzymatic activity of 7-dehydrocholesterol reductase (7-DHCR) and 24-dehydrocholesterol reductase (24-DHCR). Excess enzymatic activity or decreased catalysis is significant in various disease states, and both lathosterol and desmosterol can be obtained via laboratory assay as biomarkers of cholesterol production (Nelson and Cox, 2013).

The cholesterol component is tightly regulated, as cellular deficiency is incompatible with normal biologic function while cholesterol excess results in cytotoxicity. Since hydrophobic substances cannot be transported freely in aqueous plasma, lipoproteins are required for transport of lipids throughout the circulation. The purpose of these lipoproteins is to primarily deliver triglycerides to target tissues and, to a lesser extent, phospholipids and cholesterol; the sterol component confers structural properties to the lipoprotein, enhancing surface area for optimal cargo packaging (Nelson and Cox, 2013). The lipoprotein signatures indicate the origin of the particles and also function as ligands for specific receptors. This process of lipid transport involves the exogenous and endogenous pathway of ApoB-containing lipoproteins as well as the so-called reverse cholesterol transport pathway primarily mediated by ApoA-containing subspecies. This will be further elucidated below. **Table 3** categorizes selected apolipoproteins as well as their associated lipoproteins and functions.

Table 3: Selected Peripheral Apolipoproteins

Apolipoprotein	Lipoprotein Association	Function(s)
ApoA1	HDL, Prechylomicron	LCAT activator Mediates ABCA1/ABCG1 efflux
ApoA5	VLDL	Activates LPL when insulin levels are low
ApoB-48	Chylomicron	Postprandial lipid transport Ligand for LRP1
ApoB-100	VLDL, IDL, LDL, Lp(a)	Lipid transport Ligand for LDLr
ApoC1	HDL, VLDL	LCAT activator LPL inhibitor CETP inhibitor
ApoC2	HDL, chylomicrons, VLDL	LPL activator
ApoC3	HDL, chylomicrons, VLDL, LDL	LPL inhibitor Retards remnant clearance
ApoE	HDL, chylomicrons, VLDL, IDL	Triglyceride hydrolysis Regulates remnant clearance
Apo(a)	Lp(a)	Unknown Lp(a) an independent risk factor for ASCVD

2.2 Exogenous Pathway

The sterols obtained from the exogenous pathway typically represent 15-25% of what can be observed on a plasma lipid measurement (Feingold, 2021). This process begins with ingestion of triglycerides and sterols, which after initial enzymatic hydrolysis by pancreatic lipases can be transported across the brush border of the small intestine via transporters such as CD36 for fatty acids and Niemann-Pick-C1-Like-1 (NPC1L1) for sterols (Feingold, 2021). NPC1L1, the drug target of ezetimibe, functions as a gatekeeper for sterol entry into the enterocyte. Once in the enterocyte, sterols therein have three fates: esterification via acyl-coA acyltransferase (ACAT) and incorporation into a chylomicron, efflux into a pre-beta HDL particle which can then be subsequently esterified by lecithin cholesterol acyltransferase (LCAT), or in conditions of intracellular sterol excess, be effluxed via the heterodimeric adenosine triphosphate binding cassette transporter G5/G8 (ABCG5/G8) into the gut lumen for excretion into the stool (Feingold, 2001). Complete defects in ABCG5/G8, which may be described as a “failure to evict” sterols, results in an autosomal recessive condition known as beta-sitosterolemia; this is characterized by premature ASCVD and tendinous xanthomas with elevated levels of campesterol and sitosterol in the blood. However, there is much subclinical variance in ABCG5/G8 function, leading to significant interindividual variability in plasma cholesterol change after sterol ingestion.

The intestinal cholesteryl esters, fat soluble vitamins, and fatty acids are incorporated into chylomicrons; key enzymes monoacylglycerol acyltransferase (MGAT) and diacylglycerol acyltransferase (DGAT) form triglycerides while ApoB-48 is generated within the enterocyte (Feingold, 2021). Then, microsomal triglyceride transfer protein (MTTP) incorporates the triglycerides into the ApoB-48 lipoprotein before this particle, with the aid of the coat-protein complex II (COPII), is transported from the endoplasmic reticulum to the Golgi apparatus (Auclair et al, 2023). From there, the chylomicron is secreted into the lymph and delivered into the circulation via the thoracic duct. Defects in MTTP and SAR1 GTPase, a component of the COPII complex, result in abetalipoproteinemia and chylomicron retention disease, respectively; these conditions are both characterized by severe malabsorption and profound neurological deficits (Auclair et al, 2023).

The chylomicron, which has an approximate 10:1 ratio of triglyceride to cholesteryl ester, is acted upon by lipoprotein lipase and transports its cargo to adipocytes and myocytes. The action of LPL may be inhibited by the actions of angiopoietin-like proteins (ANGPTL) 3, 4, and 8 (Thorin et al, 2023). Hepatically-produced ANGPTL3 inhibits LPL and this is amplified when complexed to ANGPTL8; adipocyte-derived ANGPTL4's inhibitory action on LPL is conversely blunted when complexed to ANGPTL8. ANGPTL8 in isolation does not inhibit LPL (Thorin et al, 2023). This sophisticated system is critical in fuel partitioning in both fasted and fed states; whether or not this may pertain to LPL activity in the CNS remains to be determined, although ANGPTL4 is expressed in brain tissue (Thorin et al, 2023). After LPL hydrolysis, surface phospholipids may be shed and incorporated into HDL particles via phospholipid transfer protein (PLTP). As the chylomicron loses triglycerides, it acquires ApoE from HDL and loses its ApoA1; this lack of ApoA1 differentiates a chylomicron remnant from its parent particle. The ApoE on the chylomicron results in rapid uptake by primarily LRP1, although LDLr and other hepatic heparan sulfate proteoglycans can also serve as receptors for clearance (Feingold, 2021). Under normal physiologic conditions, chylomicrons are short-lived postprandial particles and are not present at meaningful levels in the fasted state; however, those with metabolic syndrome have 40% higher fasting ApoB-48 levels than their insulin-sensitive counterparts, which is likely to play a role in persistent loitering of atherogenic remnant lipoproteins and the approximate twofold increased risk in CV events (Yanai et al, 2023). Particles containing ApoE can be particularly atherogenic, as if they enter the subendothelial space of the arterial intima they do not require oxidative modification for subsequent retention; the inflammatory cascade that ensues then may result in atherosclerotic plaque formation (Ginsberg et al, 2021). Additionally, excessive chylomicronemia prevents clearance of VLDL particles produced in the endogenous pathway.

2.3 Endogenous Pathway

The endogenous pathway, which accounts for the majority of ApoB-100 particles captured as a static measurement on a lipid panel, involves hepatic assembly of VLDL particles containing ApoB-100; the rate of VLDL production is primarily determined by the availability of hepatic triglycerides. VLDL particles, specifically the VLDL1 subfractionation, are comprised of 70% triglycerides and deliver their triglyceride content to target tissues similarly to chylomicrons (Packard et al, 2020). LPL hydrolyzes the triglycerides and the free fatty acids are taken up into the cell, and when this hydrolysis occurs the VLDL remnant can enter a new density range, coined intermediate-density lipoprotein (IDL). This is a very brief and transient period, as roughly half of IDL particles may be cleared at the liver via its ApoE or ApoB-100 ligands. However, some of these particles are further hydrolyzed by hepatic lipase into LDL particles, which lack ApoE and circulate in plasma for 2-3 days. The majority of LDL are products of VLDL hydrolysis, although de novo synthesis can occur; plasma LDL levels are determined by the balance between production and catabolic rates. LDL particles must be cleared by the LDLr, and approximately 2/3 of total body LDL flux proceeds via hepatic LDLr (Feingold, 2021). When intracellular cholesterol levels are sufficient, sterol regulatory element binding protein cleavage-activating protein (SCAP) remains complexed to insulin-induced gene protein (Insig); however, when intracellular cholesterol levels drop, SCAP dissociates from Insig, cleaves SREBP2, and SREBP2 subsequently migrates from the endoplasmic reticulum to the Golgi apparatus to upregulate LDLr expression (Nelson and Cox, 2013). PCSK9 is a negative regulator of LDLr expression; instead of the LDLr recirculating to the plasma membrane after endocytosis of an LDL, if PCSK9 is bound to the LDL-LDLr complex, the LDLr is targeted for degradation in the lysosome along with the LDL (Feingold, 2021). Subsequently, gain-of-function variants in PCSK9 are among the autosomal dominant alleles leading to phenotypic familial hypercholesterolemia (FH) and accelerated CAD (Seidah and Prat, 2021). PCSK9 will be reviewed in great detail in chapter 3. Other lipoproteins such as ApoC3 can also retard this clearance if present on the LDL particle, and relatively minor ubiquitin ligases such as inducible degrader of the LDL receptor (IDOL), regulated by the liver x receptor (LXR), also play a role in lipoprotein clearance mechanisms (Packard et al, 2020). LXR is activated by oxysterols and promotes sterol efflux as well as lipogenesis when intracellular oxysterol concentrations rise (Duan et al, 2022).

An interesting potential link between efficient lipid trafficking and global metabolic processing is the LPL-mediated hydrolysis of VLDL, which subsequently activates PPARα (Ruby et al, 2010). This endogenous PPARα activation appears superior to medication-induced PPARα agonism achieved with agents such as Gemfibrozil, Fenofibrate, and most recently Pemafibrate. It is quite plausible that the underwhelming clinical trials of fibrates including FIELD, ACCORD, and PROMINENT are because PPARα, while critical, functions optimally when “unlocked” by proper hydrolysis of VLDL (Ruby et al, 2010); in this context, the lipoprotein is not only a carrier of triglycerides, but an orchestrator of gene expression. PPARα is a ubiquitous nuclear transcription factor and functions

as a positive regulator of fatty acid oxidation and autophagy (Ruby et al, 2010). PPARα expression is decreased in the brains of those with Alzheimer's disease and PPARα also regulates alpha-secretase activity that promotes non-amyloidogenic processing of APP (Loving and Bruce, 2020). Given the absence of VLDL particles in the brain but the apparent importance of LPL and PPARα in preserving cognition, the potential relevance of these interactions warrants further investigation.

One more lipoprotein of notable consequence is lipoprotein(a), or Lp(a). This enigmatic, hepatically produced LDL-like particle was discovered in 1963, but its teleology and route of clearance remains elusive. The *LPA* gene is located on chromosome 6 near the gene coding for plasminogen and does, in fact, share significant homology with plasminogen (Kronenberg et al, 2022). It possesses domains resembling Danish pastries denoted Kringles, and it is primarily the number of repeats on Kringle IV subsegment 2 (KIV-2) that dictates the production rate and, consequently, the plasma levels of Lp(a) (Kronenberg et al, 2022). Apo(a) is non-covalently bound to an ApoB-100 intracellularly via lysine residues on the 7^{th} and 8^{th} subsegments of Kringle IV before forming a disulfide bridge with cysteine residues on KIV-10 once extracellular (Youssef et al, 2022). Interestingly, PCSK9 is one of the determinants of Lp(a) secretion from the hepatocyte (Youssef et al, 2022); given that statins increase PCSK9 levels, this may be the reason why statins, on average, increase Lp(a) plasma levels by ~19% (Zhu et al, 2022). Of potential relevance to global ASCVD and AD stratification, ApoE4 carriers tend to have higher Lp(a) levels compared to those with E2 or E3 alleles (Moriarty et al, 2017); whether or not this is relevant when selecting treatment interventions is debatable. However, Lp(a) remains an independent risk factor for ASCVD and, in particular, calcific aortic stenosis, and remains a topic of ongoing research and target of drug development (Kronenberg et al, 2022).

2.4 Reverse Cholesterol Transport

Reverse cholesterol transport is largely mediated by the interaction between HDL subspecies and members of the ApoB family. Each HDL particle possesses between 1 and 5 copies of ApoA1 and begins its life cycle as a nascent, discoidal particle before acquiring cholesterol via the ubiquitous efflux pump ABCA1 (Feingold, 2021). This cholesterol is esterified via LCAT and as the particle matures into a larger, spherical particle it acquires phospholipids via PLTP and additional cholesterol via ABCG1 (Feingold, 2021).

HDL may deliver its cholesterol to the liver for uptake or the gonads and adrenals for steroidogenic hormone synthesis via the scavenger receptor SRB-1 (Feingold, 2001). HDL may also interact with ApoB-100 particles via the action of cholesteryl ester transfer protein (CETP) to mediate sterol delivery to the liver; the HDL particle donates its cholesteryl ester to the ApoB particle while the ApoB particle transfers triglycerides to the HDL (Nelson and Cox, 2013). It has also been proposed that HDL particles containing ApoE may be endocytosed by the LDLr as well as via a holoparticle receptor at the hepatic interface (Feingold, 2021).

HDL particles, while much smaller in diameter than their LDL counterparts, represent the majority of plasma lipoproteins despite containing only about 1/3 of plasma lipoprotein cholesterol (Nelson and Cox, 2013). However, measuring HDL-c concentrations gives no insight into the functionality of these heterogeneous particles; HDL has a complex proteome and performs a variety of physiologic actions. It was once thought that high HDL-c levels were protective against ASCVD, but there actually appears to be a J-curve; low levels of HDL-c typically seen in insulin resistant states, are associated with disease, but higher levels >80 mg/dL also appear associated with CV death and all-cause mortality (Liu et al, 2021). The low HDL-c levels that comprise an aspect of the so-called metabolic syndrome are most often due to excessive CETP activity; when triglycerides are chronically elevated HDL particles become "fat and fluffy" with triglycerides. Subsequently, these triglycerides are hydrolyzed and minuscule HDL particles are then catabolized via megalin and cubilin in the kidney for urinary elimination. High HDL states may be due to factors such as hypofunctioning CETP, decreased hepatic or endothelial lipase function, decreased SRB1 activity, or a combination of polygenic contributors. Variants causing HDL elevations may or may not be beneficial; the elusive key is determining the functionality of these particles, which often cannot be directly ascertained.

Regardless of the way in which cholesterol is returned to the liver, much of the hepatic cholesterol pool is devoted to bile acid synthesis via the action of 7-alpha hydroxylase; given the energetically demanding process of cholesterol synthesis over 90% of these bile acids are then recirculated to the liver via the ileal bile acid transporter so the sterol content may be repurposed for further use (Nelson and Cox, 2013). A schematic of peripheral lipid transport is depicted below in **Figure 3.**

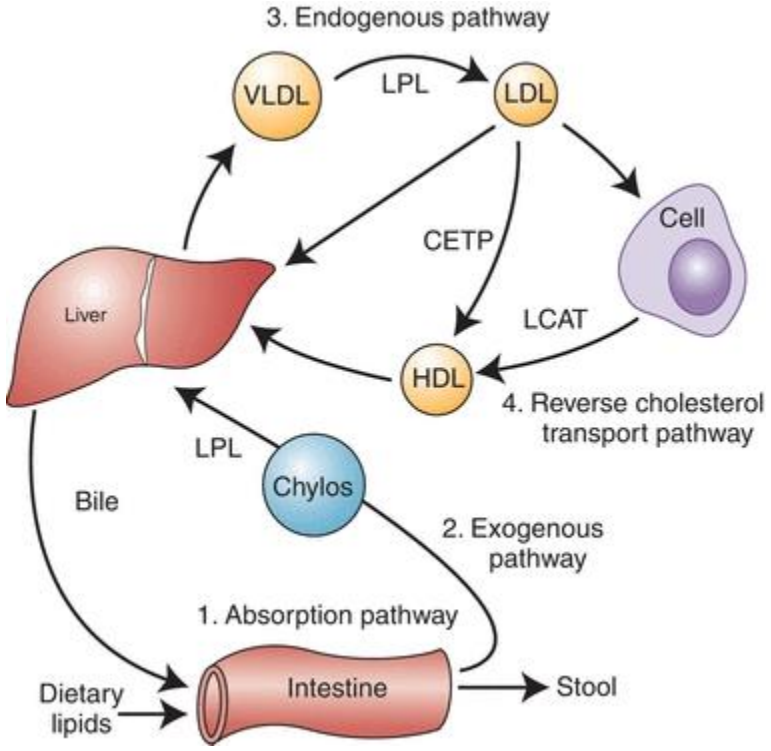

Figure 3: Peripheral Lipid Transport. Dietary sterols are absorbed at the enterocyte brush border via NPC1L1 for incorporation into ApoB-48 containing chylomicrons. LPL hydrolyzes the chylomicron triglycerides for incorporation into adipocytes and myocytes and remnants are cleared at the liver, primarily via LRP1. Excess dietary sterols may be evicted into the stool via ABCG5/G8 and hepatic cholesterol pools may be utilized for bile salt production under the enzymatic action of 7-alpha hydroxylase. The endogenous pathway involves formation of VLDL particles containing ApoB-100 prior to LPL-mediated hydrolysis of their triglyceride content. VLDL and IDL remnants may be cleared at the liver or enter a new density range of LDL; LDL particles have a lengthier plasma residence time due to their lack of ApoE and eventually are cleared by the LDLr. HDL coordinates reverse cholesterol transport via direct transport of particles to the liver or other tissues after esterification of sterols via LCAT. HDL also donates its cholesterol to ApoB-containing particles in exchange for triglyceride via CETP as a means of indirect reverse cholesterol transport. Image courtesy of: No author. (2017). Diagram of major lipoprotein metabolism pathways. Lipids & Lipoproteins. https://basicmedicalkey.com/lipids-and-lipoproteins/#Bishop-ch015-fig003.

2.5 The LDL Receptor Family

Members of the LDL receptor family are not only integral in peripheral cholesterol homeostasis, but also CNS lipid transport. A brief overview of these transmembrane receptors is listed below.

2.5.1 LDL Receptor

As alluded to previously, the hepatic LDLr, an 839 amino acid glycoprotein, is responsible for the majority of LDL flux in the peripheral circulation. The *LDLR* gene is located on chromosome 19, and loss-of-function in LDLr is the most common allele implicated in autosomal dominant FH; this is present in about 1 in 250 individuals and carries an accelerated risk of premature ASCVD (Galicia-Garcia et al, 2020). The receptor itself is characterized by 5 domains, including N and C termini, a transmembrane domain, O-linked sugars-containing domain, and the epidermal growth factor (EGF) precursor homology domain (Galicia-Garcia et al, 2020). There are 3 EGF-like domains denoted EGF-A, B, and C; EGF-A and EGF-B are separated from EGF-C by a beta propeller segment. The LDLr binds ApoE or ApoB-containing lipoproteins while in an open conformation, and then due to the acidic pH of the endosome shifts to a closed conformation; after dissociation of the particle and the LDLr this conformation returns to its original state upon recycling to the plasma membrane (Galicia-Garcia et al, 2020). Therapies that induce LDLr expression via diverse mechanisms, such as statins, PCSK9i mAb, ATP citrate lyase inhibitors, NPC1L1 inhibitors, and 7-alpha hydroxylase inhibitors have all demonstrated efficacy in reducing CV events.

LDLr is also present in most CNS cell types, interacting with ApoE given the dearth of ApoB in the brain. Overexpression of LDLr in mouse microglia does appear beneficial in mitigating M1 polarization and minimizing tau-mediated neurodegeneration (Shi et al, 2021). Additionally, LDLr overexpression enhanced the clearance of Aβ via BBB transcytosis and subsequent peripheral catabolism in another mouse model (Castellano et al, 2012). Perhaps upregulation of LDLr in the human brain may provide similar benefit if the plethora of other factors involved in cell signaling throughout the brain are not adversely affected.

2.5.2 VLDL Receptor

Despite its structural similarity to the LDLr, the VLDLr is not present in the liver; it resides on the cell surface of metabolically active tissues such as the cardiac myocyte, skeletal muscle cell, and adipocyte. Known as a remnant lipoprotein receptor, the VLDLr is incapable of binding ApoB, but does show preference for ApoE-containing triglyceride-rich particles (Takahashi et al, 2004). In the CNS, the primary ligand for VLDLr is the glycoprotein Reelin, and the interaction of Reelin with both VLDLr and ApoER2 is critical in brain development and maintenance of synaptic plasticity (Lane-Donovan and Herz, 2017). Mice lacking both ApoER2 and VLDLr display the “reeler” phenotype characterized by profound ataxia, severe developmental delays, and premature death (Lane-Donovan et al, 2015), and disruptions in Reelin signaling have been noted in the AD brain (Dlugosz and Nimpf, 2018). VLDLr may also serve a role in clearance of amyloid across the BBB along with LRP1 (Kanekiyo and Bu, 2014).

2.5.3 ApoER2

ApoER2 is also highly expressed in neurons and, along with the VLDLr, is critical in Reelin signaling. Like VLDLr, it possesses both endocytic and signal transduction properties via interaction with ApoE and other ligands (Dlugosz and Nimpf, 2018). ApoER2 levels are regulated by both IDOL and PCSK9; similar to its role in LDLr degradation, PCSK9 also targets both VLDLr and ApoER2 for lysosomal degradation (Dlugosz and Nimpf, 2018). ApoER2 also regulates selenium levels in the cell via interaction with Selenoprotein P; selenium is not only a critical co-factor in thyroid hormone regulation, but deficiency also leads to profound neurological deficits (Dlugosz and Nimpf, 2018). Additionally, ApoER2 binds specific subunits of the NMDA receptor, influencing long-term potentiation and synaptic transmission and its interaction with Reelin modulates calcium and glutamate signaling in NMDA receptors via Disabled 1 (Dab1) signaling (Lane-Donovan et al, 2015). Genetic loss-of-function variants in ApoER2 are associated with AD and mice lacking ApoER2 display premature neuronal degeneration (O'Connell and Lohoff, 2020).

2.5.4 LRP1

LRP1 is secreted as a rather large, 600 kD receptor with high affinity for ApoE; its role in hepatic clearance of triglyceride-rich remnants is well characterized. However, it is quite promiscuous, interacting with numerous ligands, including alpha-2 macroglobulin, factor VIII, and Aβ (Shinohara et al, 2017). An endoplasmic reticulum protein simply named receptor-associated protein (RAP) functions as the natural inhibitor of LRP1, thereby regulating cell surface expression. Interestingly, variants within the *LRP1* gene are associated with increased risk of atherosclerosis as well as abdominal aortic aneurysms (Strickland et al, 2014).

LRP1 is the primary member of the LDLr family in neurons, although it is also expressed in glial cells. LRP1 serves a litany of critical functions within the CNS, although variants in the *LRP1* gene are not unequivocally associated with AD. The data regarding LRP1 levels are highly conflicted within the literature; many studies demonstrate increased mRNA expression of LRP1 in the AD brain, and multiple studies show decreased LRP1 protein levels in neurons and cortical regions of those with AD (Shinohara et al, 2017). LRP1 gene expression and protein likely varies according to stage of disease progression and ApoE genotype with potentially many other factors influencing its function (Shinohara et al, 2017). Although it has been associated with increased APP processing to Aβ, mice lacking LRP1 display accelerated neurodegeneration (Lane-Donovan et al, 2014). It is also abundantly expressed in pericytes at the BBB and orchestrates Aβ transcytosis across the junction for clearance in the periphery; a soluble isoform of LRP1 has been proposed as a biomarker for this process (Shinohara et al, 2017). Increased oxidized soluble LRP1 levels were noted in one study of individuals with MCI who would progress to AD, suggesting that post-translational modification of LRP1 may dictate the proclivity of LRP1 toward mediating Aβ clearance (Shinohara et al, 2017). Interestingly, when LRP1 avidly binds ApoE4 in the CNS, this leads to sluggish recycling of LRP1 to the cell surface; instead, impaired degradation results in

TNFα sensitization. When this occurs at the BBB, matrix metalloproteinases may be upregulated, possibly increasing BBB permeability while simultaneously retarding peripheral clearance of amyloid (Kanekiyo and Bu, 2014). LRP1 is also well-known to scavenge myelin debris in the brain and also upregulate GLUT3 and GLUT4 transporters (Shinohara et al, 2017). Both insulin deficiency and hyperglycemia downregulate LRP1, and diabetic subjects with cognitive impairment have lower LRP1 levels (Shinohara et al, 2017). Interestingly, insulin induces LRP1 expression, but LRP1 itself also modulates glucose uptake (Shinohara, et al 2017). LRP1 is a key player in many biological processes; more specifics regarding interaction with its litany of ligands as well as potentially establishing biomarkers of its activity will be important in improving our overall understanding of neurodegeneration.

2.5.5 Other Members of the LDLr Family

Several other members of the LDLr family, namely megalin, low-density lipoprotein receptor-related protein 5 (LRP5) and low-density lipoprotein receptor-related protein 6 (LRP6) are also worth mentioning, as all three serve roles in both the periphery and the CNS. Megalin, which was previously mentioned in discussion of amyloid clearance and HDL catabolism, plays a pivotal role in renal tubular reabsorption of albumin and other filtered proteins, thus minimizing proteinuria (Skeby et al, 2023).

LRP5 and LRP6 are membrane co-receptors along with a protein named Frizzled for the Wnt/β-catenin signaling pathway; this nuclear transcription pathway includes proteins relevant to physiologic processes in bone homeostasis, neuronal function, glucose regulation, and cardiac fibrosis, among others (Liu et al, 2022). Briefly, when Frizzled and LRP5/6 bind to Wnt, a multi-protein Destruction complex is prevented from degrading β-catenin; consequently, a variety of nuclear transcription factors are subsequently able to activate target genes. Interestingly, increased levels of LRP5 as well as mutations in LRP6 have shown associations with both hyperlipidemia and coronary artery disease (Borrell-Pages et al, 2023, Desita et al, 2022), while decreased Wnt signaling has been observed in the AD brain (Liu et al, 2022). Recently, it was also noted that in the brains of mice with reduced LRP5 expression, neurons more readily underwent apoptosis and LRP5 was also demonstrated as an inhibitor of GSK3β, suggesting that adequate LRP5 and Wnt/β-catenin signaling serve important roles in neuroprotection (Borrell-Pages et al, 2023).

2.6 Lipid Homeostasis in the CNS

While many of the same players that participate in peripheral lipid homeostasis retain relevance, cholesterol regulation in the brain is vastly different than outside the CNS. As mentioned, 20-25% of total body cholesterol resides in the brain. The majority of this cholesterol pool is inert and comprises myelin sheaths; the half-life of brain cholesterol is 6 months to 5 years (Mahley, 2016). This stands in stark contrast to the rapid turnover of cholesterol-laden particles in the periphery,

which typically linger just a few days. However, a continual process of cholesterol production, efflux, and delivery does occur in the adult brain and is largely mediated by astrocytes. Another contrast to the peripheral circulation is that these astrocytes primarily utilize the Bloch pathway featuring desmosterol as the penultimate cholesterol precursor; the majority of peripheral cholesterol synthesis occurs via the Kandutsch-Russell pathway in which lathosterol represents the penultimate intermediate (Zhang and Liu, 2014). Neurons may utilize the Kandutsch-Russell pathway or the Bloch pathway, although neuronal cholesterol mainly arrives via an astrocytic source under normal physiologic conditions. Microglia and oligodendrocytes ostensibly favor the Kandutsch-Russell pathway of production (Zhang and Liu, 2014). ApoB-containing lipoproteins are too large to cross the BBB, so HDL-like particles containing ApoE are primarily responsible for lipid transport in the brain (Mahley, 2016). Traditional dogma has stated that ApoB does not cross the BBB and is therefore absent in the CNS; however, recent studies have suggested that ApoB may be synthesized within the brain itself. Despite the absence of LDL particles in the brain, the presence of brain ApoB has been correlated with CSF p-tau and decline in visuospatial function in individuals without AD at baseline (Picard et al, 2022). This represents an important area of ongoing investigation.

In general, astrocytes synthesize cholesterol, and then this cholesterol is effluxed to lipid-poor ApoE particles via ABCA1; these nascent HDL-like particles may also contain ApoA1 (Wang and Eckel, 2014). ApoJ, also known as clusterin, has also been identified as having a role in this transport process, although its function remains poorly understood. Further lipidation may occur via ABCG1, ABCA7, and ABCG4 and then can be taken up into various cells via receptors in the LDL receptor family such as LRP1 and LDLr. However, if these ApoE particles, which depending on ApoE genotype may have less or more affinity for lipid (ApoE2>ApoE3>ApoE4), remain poorly lipidated, they tend to aggregate abnormally (Khalil et al, 2021). This leads to a vicious cycle in which ABCA1 remains sequestered in the endosome and is unable to further lipidate the already poorly lipidated ApoE particles; this also plays a role in amyloid deposition. Additionally, microglia appear to require lipidated ApoE in order for adequate TREM2 activity; the co-expression of ApoE, LPL, LRP1, and TREM2 is important in clearance of amyloid as mentioned previously (Loving and Bruce, 2020). Typically, neurons do not produce ApoE; however, in the context of neuronal injury or impaired astrocytic delivery, neurons can themselves produce ApoE. Similar to the periphery, intracellular cholesterol content is tightly regulated and under transcriptional control of SREBP and LXR. A graphic depiction of CNS lipid transport is located in **Figure 4.**

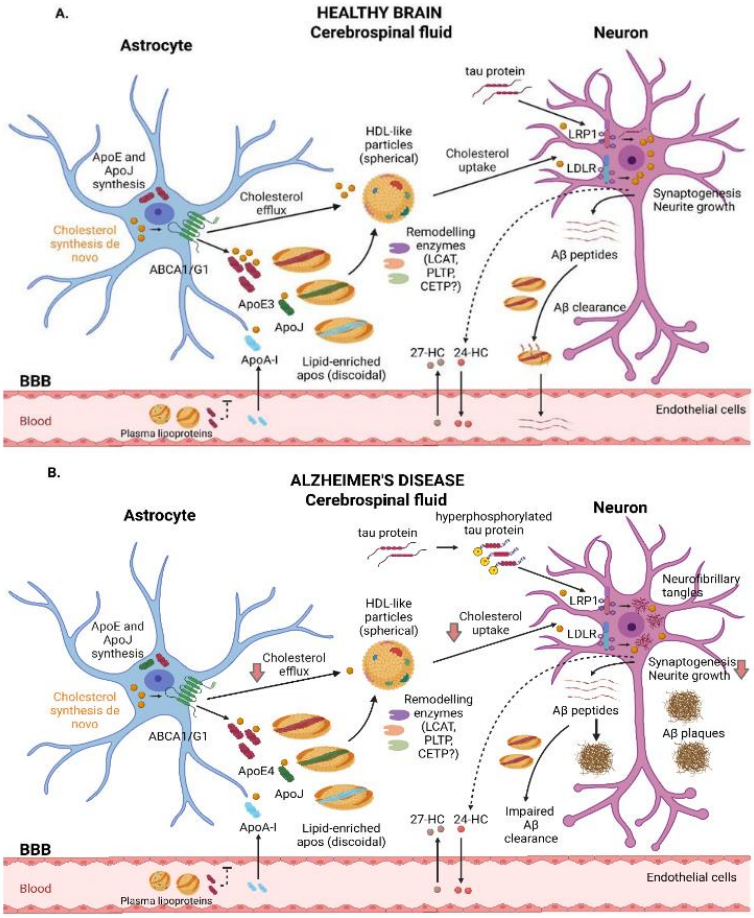

Figure 4: Lipid Transport in the Brain. Under normal physiologic conditions, astrocytes produce the bulk of cholesterol de novo via the Bloch pathway. Cholesterol is effluxed via ABC transporters into ApoE HDL-like particles and then transported to neurons for uptake via LRP1 or LDLr. Efflux, impaired lipidation, and impaired lipid uptake along with decreased amyloid clearance, both in the brain and across the BBB, are impaired in the AD brain. Image courtesy of Borras et al., 2017

2.7 ApoE

A more detailed discussion of ApoE is necessary given its importance in both peripheral lipoprotein metabolism and CNS lipid transport. The *APOE* gene is located on chromosome 19 and one copy of ApoE is inherited from each parent. Although there are rare isoforms such as ApoE1, ApoE5, ApoE7 and ApoE Christchurch, in general humans will inherit one copy of either ApoE2, ApoE3, or ApoE4 (Khalil et al, 2021). The wild type is ApoE3; approximately 75% will have an E3/E3 genotype. However, 14% will carry one copy of E4, while ~2% will be E4 homozygotes. The E4 genotype is associated with increased risk for ASCVD as well as Alzheimer's; heterozygotes carry a 3-4x risk of AD, while homozygotes may have a 12-fold risk (Yassine et al, 2017). LDL-c levels tend to be higher in ApoE4 carriers; this is because ApoE4 is "too good" at being recognized by members of the LDL receptor family such as LRP1, LDLr, and other heparan sulfate

proteoglycans in the liver. VLDL and IDL particles are rapidly cleared, but subsequently intracellular sterol content rises, downregulating SREBP2 and LDLr expression. Conversely, The E2 genotype is associated with decreased LDL-c, although E2 homozygotes in the presence of a "second hit" such as diabetes or certain medications, may develop Type III dysbetalipoproteinemia; this condition is characterized by high levels of total cholesterol and triglycerides but a normal plasma ApoB level (Berberich and Hegele, 2022). This occurs because E2 is a very poor ligand for the LDL receptor, and in the presence of accelerated production rates of ApoE particles, these are not appropriately catabolized, leading to a multitude of remnant lipoproteins, possible palmar xanthomas, and accelerated atherosclerosis. With E3 carriers, triglyceride-rich lipoproteins containing ApoE are taken up by LRP1, and the ApoE is re-secreted onto HDL particles; LRP1 prevents the ApoE from migrating to the endolysosomal complex for degradation. However, this recycling of LRP1 to the cell surface is sluggish in those with ApoE4 (Laatsch et al, 2011). These variances in the ApoE genotype are due to one amino acid substitution; ApoE3 carriers have cysteine-arginine residues on amino acids 112 and 158, respectively. ApoE4 is characterized by an arginine substitution at amino acid 112, while ApoE2 is cysteine-cysteine (Loving and Bruce, 2020). The various isoforms of ApoE are observed in **Figure 5.**

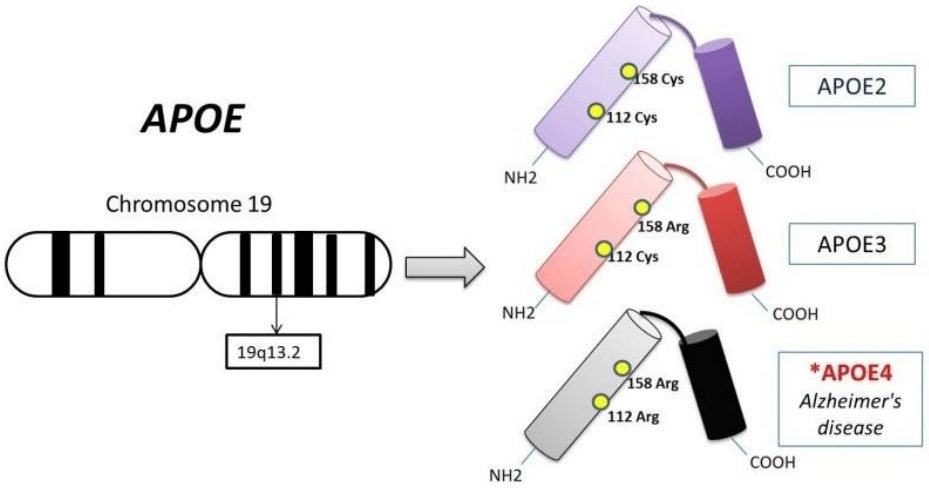

Figure 5: APOE. The APOE gene, located on the long arm of chromosome 19, encodes for the apolipoprotein with the same name. One copy is inherited from each parent, most commonly resulting in an E3/E3 genotype. However, a cysteine for arginine substitution at amino acid 158 results in E2, while an arginine for cysteine substitution at position 112 results in E4. These substitutions affect hydrolysis of triglyceride-rich lipoproteins in the periphery and lipid transport in the CNS. Image courtesy of Ortiz et al., 2015

ApoE itself is a 299 amino acid, 34 kD protein present on HDL, VLDL, and chylomicrons; it is rarely present on Lp(a) or LDL particles. ApoE resides on 60-90% of HDL particles and from 35 to 60% of VLDL (Khalil et al, 2021). It is synthesized primarily by the liver, but macrophages and glial cells

can also synthesize ApoE. Neurons, when under metabolic stress, can also synthesize ApoE. Unlike ApoB, ApoE is transferable and is often transferred from VLDL to HDL after LPL hydrolysis. Each VLDL particle contains 21 copies of ApoE. Binding affinity of ApoE varies according to type; ApoE4 binds preferentially to large VLDL, while ApoE3 more readily binds to small HDL particles (Loving and Bruce, 2020). Given there are no VLDL particles in the brain, this is one factor that results in ApoE4 carriers having poor lipidation of HDL-like particles in the CNS, which can result in defective astrocytic lipid transport. ApoE is also a ligand for ApoER2 and VLDLr, which are present in the CNS; ApoER2, in particular, is an important regulator of the n-methyl-d-aspartate (NMDA) receptor in the brain that directly impacts synaptic transmission (Dlugosz and Nimpf, 2018).

ApoE also binds LPL on microglia; when ApoE4 binds microglia, it inhibits TREM2, polarizes the microglia to an inflammatory phenotype, and impairs Aβ clearance (Loving and Bruce, 2020). ApoE4 appears to disturb the normal pH of the microglial lysosome, decreasing lysosomal acid lipase function, and leading to toxic lipid droplet accumulation (Krogsaeter et al, 2023). Perturbations in phospholipid species, particularly reduced phosphatidylethanolamine, have also been observed in ApoE4 carriers, which may further interfere with autophagic mechanisms (Lazar et al, 2022). Despite the accumulation of lipids in the microglia, genes associated with fatty acid synthesis are overexpressed in ApoE4 microglia. Additionally, the binding of ApoE4 to LRP1 is problematic; this binding inhibits the capacity of LRP1 to transport amyloid across the BBB. Instead, the binding of LRP1 to ApoE4 at the pericyte junction upregulates TNFα along with MMP-9, which has been linked to increased BBB permeability (Kanekiyo and Bu, 2014). The binding of ApoE4 to LRP1 in glia also seems to steer APP processing towards the amyloidogenic pathway and foster seeding of tau rather than appropriate clearance (Strickland and Holtzman, 2019). However, the relationship between ApoE and LRP1 is complex and may differ according to cell type and stage of disease progression, as stated previously. The ubiquity and importance of LRP1 in multiple cell types and its interaction with its binding partners, particularly in ApoE4 carriers, requires further research.

Another link between the function of ApoE in peripheral LPL hydrolysis of triglyceride-rich lipoproteins and lipid transport in the brain is the omega-3 fatty acid docosahexaenoic acid (DHA). ApoE4 carriers consistently have lower levels of DHA, and low DHA levels are associated with AD (Yassine et al, 2017). This could be due to cellular uptake of chylomicrons containing ApoE4 prior to lipolysis and subsequent delivery to target tissues; studies have demonstrated that ApoE4, while a preferred ligand for LRP1, actually is less likely to activate LPL, resulting in potential catabolism prior to complete cargo delivery (Khalil et al, 2021). This could also be why sterol absorption markers, such as campesterol, sitosterol, and cholestanol, tend to be upregulated in ApoE4 carriers; given inadequate delivery, a compensatory hyperabsorption ensues (Dayspring et al, 2015). Regardless, ApoE4 carriers often need large quantities of DHA, the preeminent omega-3 subspecies in the brain, to increase indices of omega-3 status, although it does appear that

supplementation must occur prior to onset of neurodegenerative changes to be effective (Yassine et al, 2017). Although the precise mechanisms remain to be elucidated, peripheral DHA must be transported concurrently with lysophosphatidylcholine using the major facilitator superfamily domain-containing protein 2a (MFSD2a) across the BBB. MFSD2a knockout mice display accelerated cognitive decline and neuronal defects (Yassine et al, 2017). Thus, this dynamic interplay between phospholipid transfer and fatty acid delivery from periphery to CNS is yet another example of the complex relationship between peripheral and central lipid trafficking processes.

2.8 Other Lipoproteins in the CNS

Although ApoE is the preeminent lipoprotein in the CNS, several other lipoproteins are involved in lipid transport. ApoA1, the 243 amino acid, 29 kD particle present on HDL in the periphery, may traverse across the BBB and serve a role in lipidation of HDL-like particles in the brain (Feingold 2021, Zhou et al, 2019). The brain itself does not produce ApoA1; however, pharmacologic CETP inhibition does increase production of these nascent HDL and, by extension, ApoA1 (Yu et al, 2012). Some have proposed that perhaps inhibition of CETP may help compensate for the suboptimal lipidation of ApoE4 in the brain by providing ApoA1; this is expected to be a topic of future investigation. Interestingly, CETP itself is present in the brain and there is one loss-of-function variant in the *CETP* gene, I405V, associated with exceptional longevity and preservation of cognition across the lifespan (Yu et al, 2012).

ApoJ, also known as clusterin, also serves a role in lipid transport, but elucidating its specific role has remained elusive. However, the *CLU* gene which codes for ApoJ production has been identified as the third-strongest genetic risk factor for AD (Raulin et al, 2022). Increased levels of ApoJ have been found in both the parenchyma and CSF of those with AD (Raulin et al, 2022). At this time, ApoJ has been identified as associating with lipid-poor HDL particles and its binding may be in conjunction with ApoE and ApoA1 or as an independent constituent of HDL-like subspecies. ApoJ also possesses high affinity with LRP1, VLDLr, ApoER2, and megalin; the specific result of these myriad interactions with the LDLr family remains under investigation (Raulin et al, 2022).

Other minority lipoproteins such as ApoD, ApoH, ApoA2, ApoA4, and ApoC1 have been identified in the CNS. ApoA4, which is produced in the periphery in response to fatty meals, does possess anti-eosinophilic activity, ameliorates inflammatory bowel disease, and improves glucose tolerance (Qu et al, 2019). Whether this serves any functional role in the CNS remains to be seen. ApoC1, the smallest of the lipoproteins at 6.6 kD, is located in a cluster on chromosome 19 alongside ApoE, ApoC2, and ApoC4 (Fuior and Gafencu, 2019). Certain mutations in the *APOC1* gene have also been associated with development of AD and mice either overexpressing or deficient in ApoC1 display cognitive deficits (Zhou et al, 2014). In the periphery, ApoC1 functions as an LCAT activator, LPL inhibitor, and is the only known endogenous inhibitor of CETP (Fuior and

Gafencu, 2019). Given the importance of LPL function in the microglia and the theoretical benefit of CETP inhibition for those with cognitive impairment, these may be important aspects of ApoC1 to consider as further research seeks to better understand its currently enigmatic role in the CNS.

2.9 Lipid Disturbances in the Alzheimer's Brain

There are a number of lipid anomalies seen in the Alzheimer's brain. Disturbances in both cholesterol synthesis as well as efflux have been observed in the context of neurodegeneration. Given the tight regulation of cholesterol, three different pathways may be necessary to rid the brain of excess cholesterol. These include esterification, efflux across the BBB, or conversion into 24s-hydroxycholesterol (24s-OHC). Although the vast majority of brain cholesterol is unesterified, in the context of cellular sterol excess ACAT may be upregulated to substitute a fatty acid for the hydroxyl moiety residing on carbon 3 (Varma et al, 2021). If complexed to ApoE or ApoA1, excess cholesterol may be cleared via the CSF, although the significance of this pathway is likely quite minimal. The most significant route of cholesterol elimination occurs via the enzymatic action of CYP46A1; if neuron sterol content is high, then 24s-OHC can be produced and then, given its hydrophilicity, diffuse across the BBB for eventual clearance in the liver (Gamba et al, 2021). These mechanisms will be further elucidated in the following sections.

2.9.1 Impaired Cholesterol Synthesis

One of the anomalies seen in the AD brain is impaired cholesterol synthesis, and decreased expression of 24DHCR, also known as selective Alzheimer's disease indicator 1 (Seladin-1), the enzyme catalyzing conversion of desmosterol to cholesterol, has been noted in the brains of those affected (Bai et al, 2022, Varma et al, 2021). This is theorized to result in disorganized lipid rafts, impaired cell signaling, decreased synaptogenesis, and cellular cholesterol deficiency (Bai et al, 2022). Additionally, given that desmosterol functions as a potent signaling molecule involved in LXR activation, this could represent an additional factor in impaired sterol efflux (Muse et al, 2018). LXR agonism has been a beneficial treatment in several animal models of AD, as this sterol sensor does mitigate toxic lipid droplet accumulation (Fitz 2019). LXR itself increases ABCA1 activity (Raulin et al, 2022) and given that ABCA1 is critical in proper lipidation of ApoE-containing HDL particles, a working relationship between these players is important. Interestingly, DHCR24 expression is regulated by insulin; decreased DHCR24 expression has not only been noted in AD, but also in metabolic syndrome, insulin resistance and hyperglycemia (Bai et al, 2022). These conditions of impaired substrate utilization are of clear relevance to the neurodegenerative discussion.

As stated previously, biomarkers such as lathosterol and desmosterol are markers of sterol production, and the area under the curve of receiver operating characteristics (ROC) analysis between healthy elderly adults and those with AD was 0.80 for desmosterol and desmosterol:cholesterol in a 2012 analysis (Sato et al., 2012). This value suggests that

desmosterol and desmosterol:cholesterol may be useful biomarkers for not only assessing CNS cholesterol levels, but also assessing cognition; these values similarly correlated with mild cognitive impairment. Establishing a potentially useful biomarker such as desmosterol also necessitates normative data across a large population. In an analysis of sterol absorption and production markers among a cohort of >660,000 individuals, the 20th percentile cut point for absolute level of desmosterol was 0.8 mg/L; this may represent an important cutoff when estimating an individual's sterol profile in the brain (Dayspring et al, 2015).

The initial result demonstrating the association between low desmosterol levels and cognitive decline has been replicated in the Helsinki Businessman Study; long-term follow-up of this cohort has demonstrated reduced desmosterol:cholesterol as well as lathosterol:cholesterol ratios in individuals who have gone on to develop AD. Notably, these men were not on any lipid-modulating therapies and progression to AD was more prevalent in ApoE4 carriers (Sittiwet et al, 2018). A longitudinal Japanese age-matched cohort of over 400 individuals also showed strong correlation between decreased desmosterol:cholesterol and AD; declines in desmosterol:cholesterol over time also paralleled impaired performance in Mini Mental State Exam (MMSE) scores (Sato et al., 2015). These findings were independent of gender and *APOE* genotype.

Other genes associated with cholesterol production show reduced expression in the AD brain, particularly in areas such as the hippocampus and entorhinal cortex; deficiencies in animal models include HMGCR, squalene epoxidase (SQLE) and DHCR7 (Bai et al, 2022). Additionally, decreased expression of SREBP-2, which regulates HMGCR and LDLr, has been noted in both diabetic and cognitively impaired mice (Bai et al, 2022). This decrease in HMGCR along with decreased upstream synthase activity of hydroxymethylglutaryl-coA have also been observed in human brain autopsies of the hippocampus and entorhinal cortex as well (Varma et al, 2021).

Perhaps more significantly, transcriptomic analysis of post-mortem brains from 29 individuals in the Baltimore Longitudinal Study of Aging reinforced the finding of perturbed cholesterol homeostasis in the AD brain. Brains with the greatest burden of neurofibrillary tangle had the lowest lanosterol concentrations; lanosterol is the first steroid intermediate in the post-squalene cholesterol synthetic pathway (Varma et al, 2021). Reduced gene expression of enzymes catalyzing formation of isoprenoid intermediates was also observed in the brains of those affected by AD; these prenylation proteins such as farnesyl pyrophosphate (FPP) and GGPP are important in Rho GTPase-mediated cell signaling and synaptic plasticity (Varma et al, 2021).

2.9.2 Impaired Cholesterol Elimination

The brain has several methods of mitigating a toxic rise in intracellular cholesterol, and one way in which it regulates this is esterification via the enzymatic action of ACAT1. ACAT2 is the isoform responsible for sterol esterification in the intestine and liver, while the ubiquitously expressed

ACAT1 performs this function in the CNS. Increased ACAT1 expression has been linked to increased amyloidogenic processing of APP in animal models as well as decreased autophagy in both neurons and microglia; this results in impaired lysosomal clearance of Aβ42 and tau (Shibuya et al, 2015). Increased expression of ACAT1 has also been observed in autopsies of AD brains (Varma et al, 2021), although whether this rise is a compensatory downstream effect of other perturbations to the system or represents an actionable therapeutic target remains to be determined.

The predominant means by which neurons eliminate excess sterols is via the enzymatic action of CYP46A1, which leads to generation of the hydrophilic 24s-OHC. Lower gene expression of CYP46A1 has been observed in the brains of those with diabetes as well as in the entorhinal, frontal, and occipital cortices of AD autopsies (Varma et al, 2021, Gamba et al, 2021). Not only is 24s-OHC able to traverse the BBB for peripheral clearance, it also appears to modulate the NMDA receptor in a positive manner that enhances synaptic transmission (Varma et al, 2021). Given that 24s-OHC is an oxysterol, it serves to activate LXR and the subsequent ABCA1 and ABCG1-mediated efflux of sterols into HDL-like particles in the CNS (Gamba et al, 2021). Compellingly, aging mouse models overexpressing CYP46A1 demonstrate improved memory. Additionally, Efavirenz, a non-nucleoside reverse transcription inhibitor utilized in treatment of human immunodeficiency virus, was discovered to activate CYP46A1. This resulted in reduction of Aβ and phosphorylated tau in both mouse models and in vitro; increased plasma and CSF levels of 24s-OHC were later shown in a human pilot study of low-dose Efavirenz (Lerner et al, 2022). Another study utilizing either the PCSK9 inhibitor monoclonal antibodies (mAbs) evolocumab or alirocumab in a population of 28 individuals with hyperlipidemia also showed a significant increase in 24s-OHC of 17% after just 1 month of treatment (Lutjohann et al, 2021). This was coupled with an improvement in the ratio of 24s-OHC to the neurotoxic oxysterol 27-OHC, which is capable of traversing from the periphery across the BBB to putatively interfere with synaptic function (Lutjohann et al, 2021). The authors speculated that this demonstrated a beneficial effect on the balance between CNS cholesterol synthesis and efflux. Interestingly, statins are known to decrease 24s-OHC levels; Atorvastatin at 40 mg decreases 24s-OHC by nearly 25% (Lerner et al, 2022); whether or not this is of physiologic consequence requires further investigation, although 24s-OHC measured in serum is a potentially useful biomarker of CNS lipid homeostasis (Sandebring-Matton et al, 2021).

Although there is not universal consensus on the benefits of 24s-OHC in regards to cognitive function, other oxysterol species are regarded as neurotoxic; these can be formed via enzymatic or non-enzymatic mechanisms. Increased levels of 7α-hydroxycholesterol, 7-ketocholesterol, 7β-hydroxycholesterol, and 5α, 6β-epoxycholesterol, among others, were seen in autopsies of those with AD (Varma et al, 2021). In particular, 7-ketocholesterol was associated with increased neuritic plaques and has been implicated in impaired microglial autophagy elsewhere (Anderson et al, 2020). Additionally, 27-OHC, in contrast to 24s-OHC, can diffuse from the periphery into the

CNS and increased levels have been correlated to dementia risk in several studies, including the Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER), a 2-year study of older adults (Sandebring-Matton et al, 2021). Serum levels of 27-OHC were correlated to lower cognitive scores and decreased hippocampal volume while greater reductions in this biomarker were associated with improvements in memory during the trial (Sandebring-Matton et al, 2021). Despite the promise of 27-OHC as a potential biomarker, there was rather low correlation between serum and CSF levels, and demonstrable changes on imaging were poorly defined (Sandebring-Matton et al, 2021). Still, oxysterols in the CNS appear to have mostly deleterious effects on global cognition aside from neuronally-derived 24s-OHC.

2.9.3 The Role of ABC Transporters

Proper lipid transport is essential to optimizing CNS homeostasis, and as mentioned, proper lipidation of HDL-like particles is dependent upon ATP-dependent transporters such as ABCA1, ABCA7, ABCG1, and ABCG4. Indeed, loss-of-function variants in ABCA7 enhance risk for AD and ABCA1 single-nucleotide polymorphisms have also been implicated (Raulin et al, 2022). ABCA7 is highly expressed in neurons and deficiency in ABCA7 results in impaired mitochondrial lipid homeostasis alongside downstream perturbations in synaptic transmission (Kawatani et al, 2023). Interestingly, ABCA1 and ABCA7 share greater than 50% homology; in the absence of ABCA1, it is plausible that ABCA7 may compensate; mice lacking ABCA1 do not suffer complete loss of ApoE lipidation (Raulin et al, 2022). Tangier Disease, a mutation in ABCA1 that results in near-absent plasma HDL-c levels and accumulation of macrophages in reticuloendothelial tissues such as the spleen and tonsils, is also not unequivocally associated with AD; this also suggests compensatory processes (Shahim et al, 2013). However, direct overexpression of ABCA1 or indirect activation via LXR or the retinoid x receptor have shown promise in animal studies (Raulin et al, 2022), speaking to the integral necessity of sterol efflux and lipidation to mitigate amyloid aggregation. **Table 4** summarizes some of the key lipid anomalies observed in the AD brain:

Table 4: Lipid Disturbances and Risk Factors in the Alzheimer's Brain

Category	**Observation**
Impaired cholesterol synthesis	Decreased desmosterol Decreased lanosterol Decreased lathosterol:chol ratio Reduced DHCR7 Reduced DHCR24 Reduced SQLE Reduced HMGCR Reduced FPP and GGPP
Impaired cholesterol elimination	Increased ACAT1 Decreased CYP46A1 Decreased 24s-OHC
Toxic oxysterol accumulation	Increased 27-OHC Increased 7-KC Increased 7α-OHC and 7β-OHC Increased 5α, 6β-epoxycholesterol
Decreased lipid efflux	Decreased ABCA1 Decreased ABCA7 Decreased LXR Poorly lipidated ApoE HDL-like particles Decreased ApoER2 Impaired LRP1 activity
Gene associations	APOE4 APOC1 APOJ TREM2 ABCA1 ABCA7
Other associations	Increased PCSK9 Decreased DHA Decreased p-ethanolamine Dysregulated microglial LPL

2.10 Lipid-Lowering Therapies

Once an association was made between ASCVD and cholesterol, which was later more closely tied to the LDL subfractionation, efforts were made to safely reduce plasma cholesterol levels. The first drug that targeted the cholesterol synthetic pathway was developed in the late 1950s and was named Triparanol; this agent inhibited the penultimate enzymatic step in the Bloch pathway, 24DHCR, thus reducing cholesterol synthesis (Endo, 2010). However, likely due to excessive accumulation of desmosterol, this drug was pulled from the market due to accelerated CAD and cataracts (Endo, 2010). However, continued efforts were made to find lipid-modulating agents that would demonstrate cardiovascular benefit, and the first pharmacologic tool to demonstrate statistically significant results for ASCVD mitigation was cholestyramine. This bile acid sequestrant, via its antagonism of the nuclear transcription factor FXR, upregulates 7-alpha hydroxylase activity, and utilizes the hepatic cholesterol pool to generate more bile acids. This led to a modest ~20% reduction in LDL-c and over the 7-year Lipid Research Clinics Coronary Primary Prevention Trial (LRC-CPPT), demonstrated a 19% relative risk reduction in major adverse cardiovascular events (MACE) (Pedersen 2016). However, these somewhat underwhelming results coupled with drug tolerability concerns necessitated continued research into other therapies. By the 1980s, nicotinic acid and the PPARα agonist clofibrate were commercially available in addition to cholestyramine but had not been successful in reducing MACE; however, another fibrate, gemfibrozil, did have 2 positive outcome trials. The Helsinki Heart Trial demonstrated CV benefit in men without prior CV events, demonstrating a 34% relative risk reduction (RRR) over 5 years, while the Veterans Affairs Cooperative Studies Program High-Density Lipoprotein Cholesterol Intervention Trial (VA-HIT) demonstrated a 22% RRR in a secondary prevention cohort (Pedersen 2016). These results were achieved with a drug that was actually more potent in triglyceride reduction than LDL-c; the mean LDL-c reduction in the Helsinki cohort was only 10% (Pedersen 2016).

In 1985, Brown and Goldstein discovered the LDL receptor in their efforts to better understand familial hypercholesterolemia (FH). This glycoprotein, under transcriptional regulation by SREBP2, was shown to be critical in mediating clearance of LDL from the circulation, and increasing its expression reduced plasma LDL-c levels (Duan et al, 2022). During this same era, Akira Endo, a Japanese mycologist, had been working on a class of drugs that, via inhibition of HMGCR, decreased cholesterol synthesis; these would come to be known as the statins. However, with the discovery of the LDLr, the more significant finding proved to be that reduction of intracellular cholesterol synthesis with statins resulted in increased SREBP2 expression and subsequent upregulation of the LDLr, ushering in a new therapeutic strategy (Endo 2010). Hypothesizing that increasing LDLr expression would decrease LDL-c and, over time, reduce MACE, statins were brought to market starting with Lovastatin in 1987. This hypothesis was validated in 1994 by the results of the Scandinavian Simvastatin Survival Study (4S), which demonstrated that in a secondary prevention population with phenotypic FH, the semi-synthetic,

lipophilic Simvastatin reduced MACE by 34% and all-cause mortality by 30% over 5.4 years (Pedersen 2016). This was followed by the primary prevention West of Scotland (WOSCOPS) trial of over 6000 hypercholesterolemic men aged 45-64 treated with 40 mg of the more hydrophilic, less potent Pravastatin. CHD death and MI was reduced by 31% over the follow up period, lending more credence to not only the LDL hypothesis, but the notion that by increasing clearance via the LDLr, reduction in MACE was possible (Pedersen 2016). A graphic of therapies modulating LDLr are depicted in Figure 6.

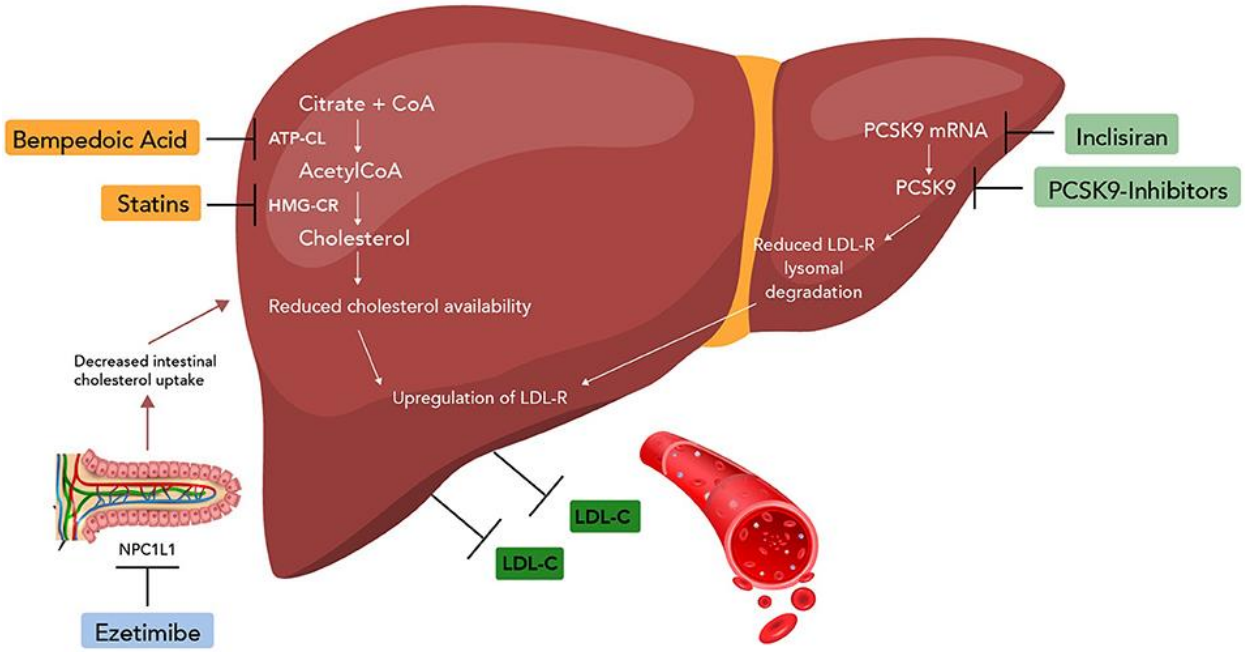

Figure 6: LDLr-modulating Therapies**.** All of the depicted drug classes upregulate LDLr expression and LDL-c clearance by various mechanisms. Ezetimibe decreases sterol absorption in the gut and at the hepatobiliary interface via inhibition of NPC1L1. Statins decrease intracellular cholesterol synthesis via inhibition of HMGCR, the committed enzymatic step in the mevalonate pathway. Bempedoic acid is a hepatoselective agent that decreases cholesterol synthesis upstream of HMGCR via ATP citrate lyase inhibition; this cytosolic enzyme splits citrate into acetylcoA and oxaloacetate prior to lipogenesis. Inclisiran halts hepatic PCSK9 production and PCSK9i mAbs bind extracellular PCSK9; both methods inhibit PCSK9-mediated degradation of LDLr in the lysosome. **Image courtesy of Bardolia et al., 2021.**

More heterogeneous cohorts were studied in further statin trials, and other statins were developed with similar degrees of success; a meta-analysis of 14 statin trials by the Cholesterol Treatment Trialists' Collaborators (CTTC) including >90,000 participants demonstrated that a 39 mg/dL reduction in LDL-c corresponded with a 22% RRR of MACE over 5 years. As time progressed, a paradigm of "lower is better" emerged in regards to LDL-c reduction, and this seemed to be validated by the Treating to New Targets (TNT) trial. In a secondary prevention

cohort, the group receiving Atorvastatin 80 mg had a significant reduction in MACE over the follow-up period compared with the group receiving the 10 mg dose; this benefit correlated well with the achieved LDL-c (Pedersen, 2016). Given that the degree of LDL-c lowering corresponded with the intensity of the statin, it became popular to employ what became known as high-intensity statins on a regular basis; this came to be known as Atorvastatin 40 mg or 80 mg or Rosuvastatin 20 mg or 40 mg, as these doses are expected to reduce LDL-c greater than or equal to 50% from baseline (Virami et al, 2023). Simvastatin 80 mg did approach the potency of these statins in LDL-c reduction but was pulled from the market due to unacceptable rates of rhabdomyolysis. Despite the success of the TNT trial, it became apparent that statin induced side effects were dose-dependent and that each subsequent doubling of statin dose only led to an additional 6% lowering of LDL-c. The myopathic symptoms of statins have been well-documented despite a variable "nocebo effect" demonstrated in the Self-Assessment Method for Statin Side-effects Or Nocebo (SAMSON) trial (Krishnamurthy et al, 2022) along with an increased risk of new-onset diabetes mellitus (NODM). Indeed, a meta-analysis of >90,000 patients demonstrated that statins do lead to an increase in risk of NODM; any dose of statin versus no statin increases risk and high-intensity versus lesser intensity also increases risk (Preiss and Sattar, 2011). The biochemical rationale for these side effects and others will be addressed in a later section. Given the relatively minuscule benefit in LDL-c reduction obtained with increased statin dosing coupled with dose-dependent risk of adverse events, there remains residual risk for CV events among not only statin-intolerant populations, but statin-treated individuals as well.

Despite the great success of statins in reducing MACE, efforts were made to develop additional lipid-lowering therapies to address this residual risk. Ezetimibe, acting on NPC1L1, typically lowers LDL-c an additional 20-25% via inhibition of sterol absorption at the enterocyte and hepatobiliary interface. When added to 40 mg of Simvastatin in the Improved Reduction of Outcomes: Vytorin Efficacy International Trial (IMPROVE-IT), Ezetimibe did, over a lengthy 7-year follow-up period, further reduce recurrent events than Simvastatin alone (Virani et al, 2023). However, it was believed that for many high-risk patients a greater degree of LDL-c lowering was necessary to "truly move them into a safer vascular neighborhood," and this notion was validated by the positive outcome trials of the PCSK9 inhibitor monoclonal antibodies (PCSK9i mAbs). In a secondary population already on statin therapy, the Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment with Alirocumab (ODYSSEY Outcomes) trial, Alirocumab reduced 5-point MACE over 2.8 years by 15% along with a statistically significant reduction in all-cause mortality (Seidah and Prat, 2021). Evolocumab also demonstrated a significant 20% RRR in MACE over 26 months in the Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects With Elevated Risk (FOURIER) trial. These monoclonal antibodies, which do not affect cholesterol synthesis, but rather prevent the lysosomal degradation of the LDLr by PCSK9, lower LDL-c an additional ~60% (Seidah and Prat, 2021). These potent agents do carry a slight increased risk of injection-site reactions, but do not interact with

skeletal muscle or result in increased myalgias versus placebo. Additionally, a meta-analysis of 38 PCSK9i mAb trials showed no increase in NODM (Monami et al, 2018); the interplay between PCSK9 and glucose homeostasis will be further explored in chapter 3. Given that statins increase PCSK9 levels in a dose-dependent manner (Macchi et al, 2019), inhibition of extracellular PCSK9 with these agents appears to complement statin therapy both biochemically and in the clinical trial setting.

Given that IMPROVE-IT, ODYSSEY, and FOURIER have shifted the paradigm away from the notion that statins, despite their utility, are not a panacea, additional innovations in the lipid-lowering space are a perpetual reality in today's pharmaceutical landscape. Indeed, bempedoic acid, a liver-specific ATP citrate lyase inhibitor that lowers LDL-c ~20% as monotherapy, recently demonstrated CV benefit in a statin-intolerant population, and other agents, including small molecule inhibitors of PCSK9, are in ongoing development (Virani et al, 2023, Bao et al, 2024). Despite the success of these non-statin therapies in clinical trials, however, various barriers to their utilization have prevented them from being routinely employed to achieve guideline-based LDL-c goals. A registry in the Northeast United States from 2015 to 2019 showed that, despite a drastic decrease in cost of PCSK9i mAbs, the rate of prescriptions for eligible patients only rose from a minuscule 0.5% to a still-paltry 3.3% (Smith et al, 2021). Alarmingly, this inertia in prescription patterns often translates into very high-risk patients not achieving LDL-c targets; the DA VINCI registry in Europe reported that a mere 18% of patients with ASCVD and guideline-directed LDL-c target <55 achieved this goal (Barrios et al, 2021). It has also been reported that >25% of those on high-intensity statin monotherapy with chronic CV disease still have an LDL-c of >100, representing significant residual risk despite the availability of additional non-statin agents (Barrios et al, 2021). **Table 5** summarizes the germane lipid-lowering therapies detailed in this section. However, despite the relationship between mitigating CV risk and the various lipid perturbations seen in neurodegeneration, it is unclear whether any of these agents can not only be safe, but effective as a tool in the quest to prevent AD. Since PCSK9 serves an intriguing role in both the peripheral circulation and in the CNS, this will be explored in great detail in **Chapter 3.**

Table 5: Selected Lipid-Lowering Therapies

Agent	Mechanism of Action	Expected LDL-c reduction	Cardiovascular Outcomes
Bile acid sequestrants (Cholestyramine)	Inhibits 7α-hydroxylase, increasing LDLr	~20%	LRC-CPPT
Fibrates (Gemfibrozil, Fenofibrate)	PPARα agonist, enhances hepatic fatty acid oxidation	~10%	Gemfibrozil: VA-HIT and Helsinki Heart Trial
Statins	Inhibits HMG-CoAr, increasing LDLr	High-intensity ≥50%	Numerous: 4S, WOSCOPS, JUPITER, many others
Sterol absorption inhibitors (Ezetimibe)	Inhibits NPC1L1 at intestinal brush border and hepatobiliary interface	~20-25%	IMPROVE-IT
PCSK9i mAbs (Alirocumab, Evolocumab)	Binds extracellular PCSK9, prevents LDLr degradation	~60%	Alirocumab: ODYSSEY Evolocumab: FOURIER
PCSK9i siRNA (Inclisiran)	Inhibits hepatically produced PCSK9	~50%	None at this time
Bempedoic acid	Inhibits ATP citrate lyase in liver, decreasing hepatic cholesterol synthesis and increasing LDLr	~20%	CLEAR-Outcomes

2.11 Statins

Prior to an exploration of PCSK9, it will provide helpful context to discuss the pleiotropic effects of statins; this will quaintly frame further discussion regarding intervention. Statins have been shown to upregulate endothelial nitric oxide synthase while antagonizing endothelin-1. Nitric oxide is a potent vasodilator, while endothelin-1 is a known vasoconstrictor, so these mechanisms of statins contribute to a beneficial vasodilatory effect (Liao and Laufs, 2005). Additionally, statins have been shown to reduce levels of the inflammatory marker hs-CRP. Various intravascular imaging trials have demonstrated an increase in the calcified content of existing plaques, which has been supposed to confer stability to the lesions. A regression in percent atheroma volume (PAV), an important measure of plaque burden, commensurate with the absolute LDL-c achieved

has also been seen observed in trials such as ASTEROID and SATURN; every 1% decrease in PAV is associated with a 20% relative risk reduction in MACE (Bhindi et al, 2019).

However, the unequivocal increase in NODM and the myalgias seen in 10-25% of patients taking statins do have a biochemical basis (Zaleski et al, 2018). Since statins inhibit cholesterol synthesis, they also decrease the production of isoprenoid intermediates produced in the mevalonate pathway which include farnesyl pyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP). In particular, GGPP has been identified as an important regulator of the Rho/ROCK1 signaling pathway resulting in GLUT4 translocation to the plasma membrane of adipocytes and myocytes (Wang et al, 2022). GLUT4 is an insulin-dependent glucose transporter and this GGPP depletion plays a role in the increased insulin resistance consistently seen in statin-treated individuals. Interestingly, supplementation with geranylgeraniol abolishes the decreased glucose uptake into insulin-dependent tissues in studies of statin-treated animals (Wang et al, 2022). Additionally, decreased insulin secretion has also been observed in statin-treated subjects; this may be due to a depletion of dolichol, which when absent leads to a reduction in the quantity of membrane insulin receptors (Brault et al, 2014). The impaired prenylation of Rap1a by GGPP could also result in impaired beta cell activity, and Rap1a in the liver may also be helpful in suppressing hepatic gluconeogenesis (Wang et al, 2022). Other recently discovered contributions of statins to perturbed glucose homeostasis include gut microbiota modification and reductions in GLP-1 (She et al, 2024).

Statins have also been said to impair mitochondrial function, and much of this may be due to decreased substrate utilization. However, other factors such as depletion of intermediates in the electron transport chain such as coenzyme Q10 (CoQ10) and heme A, may play a role in this efficiency decrement. Depletion of CoQ10 has been proposed as the source of statin-induced myalgias, but studies of supplementary CoQ10 have shown largely underwhelming results (Zaleski et al, 2018). However, depletion of CoQ10 may be more significant in the cardiac myocyte in the context of heart failure, and CoQ10 supplementation and statin cessation, respectively, have been beneficial in several heart failure studies (Langsjoen et al, 2019). Interestingly, statins are seldom tolerated in professional athletes, who typically are among the most mitochondrially efficient performers; in a study of 22 athletes with FH, only 5 of the 22 could tolerate any statin at any dose due to muscle-related side effects (Sinzinger and O'Grady, 2004). Indeed, statins have demonstrated reduced mitochondrial concentrations and oxidative capacity in skeletal muscle. In two elegant studies by Slade and colleagues, the aerobic capacity of skeletal muscle was ascertained before and after 80 mg of Atorvastatin, and this resulted in a 12% reduction in oxidative capacity from baseline (Slade et al, 2021). The second study involved a 12-week exercise protocol comparing groups on chronic low to moderate statin therapy and controls who were not taking statins. The statin-treated group had 20% lower baseline measures of muscle oxidative capacity, but were able to demonstrate similar improvements in muscle oxidative measures across the intervention; this was despite an absolute level of oxidative capacity that remained

significantly less than the control group (Slade et al, 2021). Other studies have suggested intolerance to high-intensity interval training protocols as well as a blunted exercise response to this type of intervention in statin-treated individuals (Sinzinger and O'Grady, 2004, Slade et al, 2021).

Statin trials have demonstrated mixed results concerning association with AD; some have shown an increased risk, while others have been neutral or have even been associated with decreased risk (Thong et al, 2022). It is important to recognize that no statin trial has ever been designed with development of AD as a primary endpoint, although two trials of high-intensity Atorvastatin and one of Simvastatin 20 mg were conducted in those with established AD. Disappointingly, none of these trials demonstrated any benefit in Alzheimer's Disease Assessment Scale-Cognitive scores at 12-18 months of follow-up (Wanamaker et al, 2015). Given the litany of factors that contribute to AD, some individuals may glean benefit from statins for dementia prevention due to mitigation of vascular risk factors. Those with aberrant ApoB production in the CNS, which has recently been recognized as physiologically possible, would likely derive benefit from statins as well. Others, perhaps due to impairments in insulin sensitivity, deficient substrate utilization, and decreased cholesterol synthesis in the brain may experience a net harm in risk for neurodegeneration. Notably, statins, when compared to PCSK9 inhibitors or Ezetimibe, are the only lipid-lowering therapies that cross the BBB with lipophilic agents such as Simvastatin more likely to traverse the junction than hydrophilic members of the class such as Rosuvastatin (Schwartz et al, 2018). However, real-world pharmacovigilance data from the FDA Adverse Event Reporting System has noted signals for association with neurocognitive events in both hydrophilic and lipophilic moieties. In persons over 65 years of age, signal was detected for all statins currently available in the United States, including pravastatin and rosuvastatin (Xiao et al, 2024). Since the half-life of cholesterol in the brain may be up to 5 years and even minor perturbations in sterol production could plausibly result in disturbances to the milieu, this must be taken into consideration. In particular, suppression of desmosterol, given its strong association with AD, could theoretically be a problematic aspect of statin use in regards to brain health. However, it is important to acknowledge that there may be differences in interpreting desmosterol levels for individuals who have achieved these levels on lipid-lowering therapy versus treatment naïve persons. Interestingly, individuals with genetic hypofunctioning HMGCR display decreased cortical surface area, decreased cognitive scores, and slower reaction times while those with PCSK9 loss-of-function demonstrate none of these anomalies (Rosoff et al, 2022). However, Mendelian randomization of genetically proxied PCSK9 inhibition has not shown a clear association between PCSK9 and AD; an initial study recommended pharmacovigilance with PCSK9 inhibition, while a second larger study determined a neutral cognitive profile (Williams et al, 2020, Bell et al, 2022). As mentioned, statins increase PCSK9 levels; Atorvastatin 80 mg increases PCSK9 46% while Simvastatin may increase PCSK9 up to 68% (Macchi et al, 2019), and given the importance of PCSK9 in a myriad of processes both peripherally and centrally, this also must be

taken into consideration. Consequently, an understanding of the mechanisms at play and the underlying factors contributing to risk of AD is important when discussing potential therapeutic interventions.

CHAPTER 3-Proprotein Convertase Subtilisin/Kexin Type 9

3.1 Biology of PCSK9

PCSK9 is the ninth member of the proprotein convertases, a serine protease family responsible for activation of zymogen precursors regulating diverse protein functions. This 692 amino acid protein was initially discovered in 2003 and was named neural apoptosis-regulated convertase-1 (NARC-1), as it was first observed in cerebellar neurons undergoing programmed cell death (Seidah and Prat, 2021). However, in the same year, while mapping the *PCSK9* gene location on the short arm of chromosome 1, a French family with autosomal dominant FH was noted to have a homozygous gain of function in the *PCSK9* gene (Seidah and Prat, 2021). This was notable, as at that time mutations in the *LDLR* and *APOB* genes were the only recognized non-polygenic causes of FH, often characterized by tendinous xanthomas, corneal arcus, and premature cardiovascular disease. 3 years later, the Atherosclerosis Risk in Communities (ARIC) study showed that various loss-of-function PCSK9 variants were associated with not only reduced levels of LDL-c, but dramatically less incident CVD. The R46L variant, present in 3.2% of Caucasians, was associated with a 47% decreased incidence of CVD, while African-Americans possessing the Y142X or C679x nonsense mutations in PCSK9 had a stunning 88% decreased risk of CVD (Cohen et al, 2006). Additionally, several individuals were identified with complete loss-of-function in PCSK9 and lifelong LDL-c levels of ~14-16 mg/dL; remarkably, these persons appeared to have no other perturbations to their physiology. PCSK9 subsequently became an attractive therapeutic target for pharmacologic intervention (Seidah and Prat, 2021). Current approved medications for PCSK9 include the mAbs, evolocumab and alirocumab, and a small interfering ribonucleic acid (siRNA) approach called inclisiran. The mAbs bind all extracellular PCSK9, while the siRNA approach utilizes n-acetyl-galactosamine (GalNac) to gain access to the hepatocyte via the asialoglycoprotein receptor 1 (ASGR1) unique to the liver (Macchi et al, 2019). This siRNA approach results in subsequent halting of PCSK9 protein translation; however, given that only 2/3 of PCSK9 is hepatically produced, there is logically less inhibition of PCSK9. PCSK9 is expressed in a variety of tissues, including the intestine, lung, kidney, macrophages, pancreas, and the brain (Seidah and Prat, 2021). Metaphorically, the mAb approach can be viewed as a “mopping up” of all extracellular PCSK9, while the GalNac siRNA approach is akin to “turning off the faucet” of PCSK9 production in the liver. Additional therapeutic avenues targeting PCSK9 are in development as well. The canonical role of PCSK9 in mediating LDLr degradation is graphically represented in **Figure 7**.

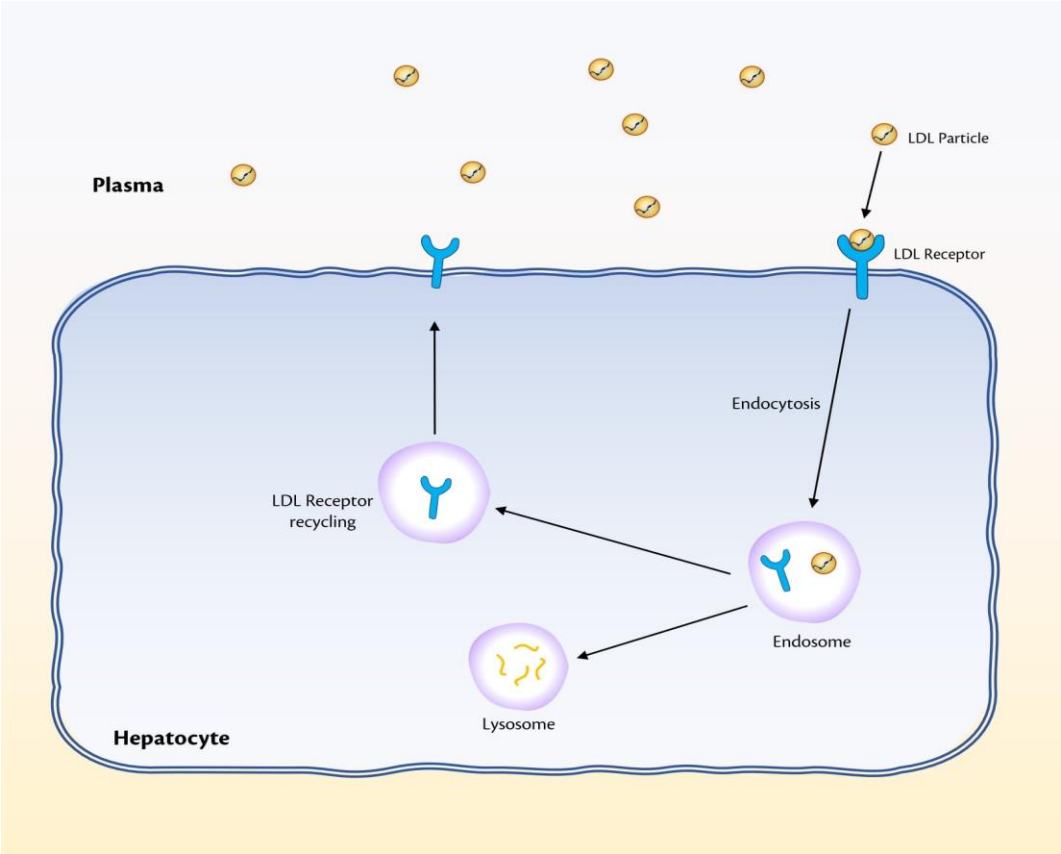

Figure 7: The Canonical Role of PCSK9. Under normal circumstances, the LDLr can internalize an LDL particle and then dissociate from the particle in the early endosome before subsequently recycling to the hepatocyte cell surface. However, when PCSK9 binds to the LDLr, the entire complex is routed to the lysosome for degradation, preventing the LDLr from continuing to clear ApoB-containing particles. **Image courtesy of Moazzeni, 2023.**

PCSK9 possesses three structural domains in addition to its 30 amino acid precursor signal peptide; interestingly, the prodomain of amino acids 31-152 remains associated with active, mature PCSK9 even after its autocatalytic processing in the endoplasmic reticulum unlike many other zymogens (Seidah and Prat, 2021). The prodomain appears to mitigate endoplasmic reticulum stress and proclivity toward apoptosis via interactions with various proteins, including Bag5 and glucose-related protein 78 (Seidah and Prat, 2021). The mature peptide is 62 kD and consists of a catalytic domain of amino acids 153-421 and a cysteine-histidine rich c-terminal domain. Both the catalytic domain and the terminal region are necessary for its canonical role in binding the EGF-A region of the LDLr and targeting it for subsequent lysosomal degradation (Seidah and Prat, 2021). PCSK9 may also be further processed by furin, resulting in a 55 kD protein with significantly less affinity for the LDLr (Macchi et al, 2019).

PCSK9 also interacts with other members of the LDL receptor family, including LRP1, VLDLr, and ApoER2 (Seidah and Prat, 2021). In particular, VLDLr and ApoER2 harbor EGF-A domains

homologous to that of the LDLr, so PCSK9 similarly targets these receptors for degradation (Ma et al, 2023). This interaction with the VLDLr likely plays a role in the decreased triglyceride uptake and adipocyte insulin resistance that results from PCSK9 overexpression in the fat cell (Shu et al, 2022). Additionally, given the importance of the VLDLr and ApoER2 in neuromodulation, the interactions between these receptors and PCSK9 in the CNS certainly warrant further investigation. The specific nature of PCSK9's interaction with LRP1 has been debated; historically, it was thought that although LRP1 is a binding partner, PCSK9 did not mediate the receptor for lysosomal degradation; however, recent work has demonstrated in vitro that PCSK9 can degrade LRP1 even in LDLr-null models (Canuel et al, 2013). Interestingly, the presence of LDLr reduced the capacity of PCSK9 to degrade LRP1; perhaps this is why, on average, extracellular PCSK9 inhibition still results in a ~31% reduction in LDL-c versus placebo in those with Homozygous FH, a condition in which virtually no LDLr activity exists (Canuel et al, 2013, Raal et al, 2015). Further exploration of this relationship not only has potential implications for peripheral clearance of remnant lipoproteins, but also regulation of lipid uptake and amyloid clearance in the CNS given the ubiquity of LRP1 in the brain.

PCSK9 interacts with both megalin and LRP5 as well, which were both mentioned in **Section 2.5.5.** Interestingly, megalin is inversely correlated with PCSK9 levels, and PCSK9 inhibition restores megalin levels and reduces proteinuria in rodent models of nephrotic syndrome (Skeby et al, 2023). Although PCSK9 does not degrade LRP5, it does function as a binding partner of LRP5. When this occurs in the blood vessel wall, this partnership amplifies foam cell formation and upregulation of toll-like receptor 4 and nuclear factor kappa-beta (NF-κβ) (Badimon et al, 2021). Whether PCSK9 interacts with neuronal LRP5 in a similarly pro-inflammatory manner is unknown.

PCSK9 also interacts with other receptors which may even be located intracellularly, including CD36, class A scavenger receptor (SRA), and lectin-like oxidized low-density lipoprotein receptor 1 (LOX-1), which likely has profound implications for vascular disease. LOX-1, in particular, is a key scavenger of oxidized LDL in the subendothelial space and PCSK9 interaction with LOX-1 appears to result in a malignant positive feedback loop which propagates foam cell formation (Ma et al, 2023). Intriguingly, soluble LOX-1 levels, when measured in plasma, correlate strongly with recurrent CV events in those who have had prior myocardial infarction; whether or not pharmacologic PCSK9 inhibition would mitigate this residual risk is unknown (Kraler et al, 2022). PCSK9 binding to platelets expressing CD36 results in an intracellular signaling cascade upregulating thromboxane A2, a significant player in prothrombotic states (Pastori et al, 2017). Indeed, plasma levels of PCSK9 directly correlate with 11-dehydrothromboxane B2, the urinary metabolite of thromboxane A2 (Pastori et al, 2017). PCSK9 bound to macrophage CD36 also leads to uptake of oxidized LDL and subsequent inflammatory signaling involving toll-like receptor 4 and NF-κβ (Punch et al, 2022). This is likely one of the factors contributing to the direct association of PCSK9 serum levels with platelet reactivity (Navarese et al, 2017). However, CD36 is also critical in mediating fatty acid uptake in the myocardium, so global loss of PCSK9 could potentially result

in heart failure or issues with substrate utilization in the context of ischemia reperfusion injury, a state in which excess triglyceride accumulation could result in toxic lipid accumulation and exacerbation (Da Dalt et al, 2020). However, in animal studies of cardiac ischemia, pre-treatment with PCSK9 inhibition actually mitigated hypoxia-induced neuroinflammatory sequelae via a reduction in reactive microglia and astrocytes (O'Connell and Lohoff, 2020). Given that PCSK9 either degrades CD36 or deleteriously modulates it in a tissue-specific manner, the increased expression of CD36 in diabetic cardiomyopathy may very well be a consequence of diacylglycerol accumulation and impaired mitochondrial oxidation of fatty acids (Shu et al, 2022). Whether or not PCSK9 overexpression is relevant in this context remains to be studied. However, given that PCSK9 is locally produced in the heart, extracellular inhibition has not demonstrated any deleterious effect on myocardial substrate utilization (Ma et al, 2023, Da Dalt et al, 2020); this has been reassuring from a pharmacologic safety standpoint. The complex and tissue-specific interaction between PCSK9 and CD36 in the CNS also warrants further exploration in the context of neurodegeneration.

In addition to targeting LDLr members for endolysosomal engulfment, PCSK9 promotes degradation of Major Histocompatibility Complex-1 (MHC-1), which may be of physiologic relevance in the context of malignancy. Increased PCSK9 mRNA has been observed in many cancers, including colorectal cancer and hepatocellular carcinoma (Seidah and Prat, 2021, Bao et al, 2024). PCSK9 also inhibits ABCA1 and ABCG1 expression in macrophages, which may lead to additional foam cell proliferation due to impaired sterol efflux (Mu et al, 2023); whether or not PCSK9 inhibits ABC transport mechanisms in the CNS is unknown. PCSK9 has recently been recognized as not only a player in LDL trafficking, but also a component of the HDL proteome; its levels positively correlate with ApoC3 (Burnap et al, 2021). Another newly discovered role for PCSK9 has been its correlation with development of cholesterol gallstone formation; inhibition of PCSK9 in vitro and in mouse models demonstrated a reduction in gall stones via upregulation of 7-alpha hydroxylase activity (Chen et al, 2024). Additionally, this study demonstrated that inhibition of PCSK9 reduced lysosomal degradation of PPARα; given the relevance of this nuclear transcription factor in fatty acid oxidation and autophagy in both the periphery and CNS, this finding may prove to be particularly salient (Chen et al, 2024). Although more investigation is warranted into its specific effects, it does appear that PCSK9 serves a role in modulation of postprandial lipemia, bile acid homeostasis, and triglyceride metabolism. In summary, the specific interactions between PCSK9 and its binding partners possess potential relevance in both the periphery and the CNS with implications for various disease states including sepsis, atherosclerosis, and Alzheimer's.

3.2 Disease Associations

Although not routinely measured in the clinical setting, there are a number of provocative observations suggesting that PCSK9 serum measurements may be additive in stratifying risk for

various disease states. In the Association of PCSK9 Serum Levels and Platelet Reactivity in Patients with Acute Coronary Syndrome Treated with Prasugrel or Ticagrelor (PCSK9-REACT) study, PCSK9 serum levels not only directly correlated with platelet reactivity in those on antiplatelet medications, but was also a valuable independent predictor of recurrent ischemic events (Navarese et al, 2017). Subjects in the highest tertile of PCSK9 had a 22% risk of recurrent MACE, while those in the lowest tertile had only a 3.4% risk (Navarese et al, 2016). Additionally, PCSK9 serum levels were independently associated with incident MACE in subjects with FH and percentage of necrotic core in coronary plaques as visualized on intravascular ultrasound (IVUS) histology (Cao et al, 2019, Cheng et al, 2016). Given that thin cap fibroatheromas and lipid-rich plaques are qualitatively unstable and prone to rupture, this association, which was independent of statin use or LDL-c levels, may be particularly salient (Cheng et al, 2016).

Other associations with serum PCSK9 include a relationship between the furin-cleaved truncated version with incident CV disease in a primary prevention setting (Kataoka et al, 2021) as well as a prognostic for MACE in women with type 2 diabetes over a lengthy 16.8-year follow-up period (Ruscica et al, 2023). PCSK9 levels also predicted all-cause mortality in males within this cohort (Ruscica et al, 2023). Interestingly, PCSK9 levels are higher in females, which may have an additional role in the increased incidence of brain atrophy and Alzheimer's Disease in this population; another study showed elevated PCSK9 levels as a predictor of short-term memory deficits only in females (Simeone et al, 2021). Indeed, females comprise over 60% of those affected by AD (Rahman et al, 2019). Autopsies of those with AD show increased levels of PCSK9 gene expression in the frontal cortex compared to age-matched controls, and CSF PCSK9 has been correlated with concurrent levels of tau as well as ApoB (Simeone et al, 2021, Picard et al, 2022). Additional associations between PCSK9 and Aβ-42 as well as both total and phosphorylated tau have been noted (Bao et al, 2024). ApoE4 carriers tend to display increased levels of PCSK9 as well, and those with alcohol use disorder, a condition characterized by neuronal degeneration, also show elevated levels of PCSK9 in both CSF and serum (Simeone et al, 2021, O'Connell and Lohoff 2020).

Genetic polymorphisms in the PCSK9 gene have not shown any deleterious effects on cognition. The C679x and Y142x variants were prospectively examined in the Reasons for Geographic and Racial Differences in Stroke (REGARDS) study over 5.6 years. In this cohort study, there were no discernible differences between those with and without PCSK9 loss-of-function variants utilizing validated assessments of global cognition from the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) and Six-Item Screener (O'Connell and Lohoff, 2020). Similarly, the R46L variant showed a neutral effect on development of AD in a French Canadian cohort, while the loss-of-function rs11583680 polymorphism was similarly benign in regards to AD in Japanese individuals (O'Connell and Lohoff, 2020). A large Mendelian randomization of over 111,000 Danish persons actually demonstrated a possible reduced risk in development of AD in PCSK9 loss-of-function variants, while several gain-of-function variants, the rs499718 and rs4927193,

were associated with increased risk of AD in females within a separate French Canadian cohort (O'Connell and Lohoff, 2020). While not conclusive, these genetic studies provide reassurance that hereditarily increased clearance of LDL via PCSK9 appears to be, at worst, a non-factor in the context of neurodegeneration.

Although not directly germane to this dissertation, PCSK9 serum levels have also been associated with predisposition to pathology or extent of disease severity in autoimmune conditions such as psoriasis, systemic lupus erythematosus, and rheumatoid arthritis (Bao et al, 2024). These conditions do confer additional CV risk due to systemic inflammatory factors, and taken as a whole, measurement of PCSK9 levels across a broader population may represent a future strategy to not only stratify risk of CV disease, but perhaps AD as well.

3.3 Other Potential PCSK9 Pleiotropy

Along with the putative role of PCSK9 in hypercoagulability, a number of studies point to PCSK9 as a contributor to pro-inflammatory states. Although inhibition of PCSK9 does not affect hs-CRP levels, which is a non-specific and widely used marker of inflammation, a number of studies have shown reductions in various inflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), and TNFα (Marfella et al 2023, Sundararaman et al, 2021). Macrophages are capable of secreting PCSK9 and upregulate these pro-inflammatory mediators in an LDLr-independent manner (Sundararaman et al, 2021). A small human study of patients with documented vulnerable atherosclerotic plaque showed significant decreases in serum interleukin-18, IL-6, TNFα, and matrix metalloproteinase-2 after being treated with PCSK9 inhibitor therapy (Basiak et al, 2022), and these findings have been replicated in other small sample sizes. Intriguingly, a small study of PCSK9i mAb in severe COVID-19 infection demonstrated a profound reduction in mortality at 30 days when treated with one 140 mg injection of evolocumab (Navarese et al, 2023). In this study of 60 patients, 50% of those in the comparator arm died, while only 12.5% of the group treated with evolocumab; this appeared to be driven by an LDL-independent 56% reduction in IL-6 (Navarese et al, 2023).

PCSK9 inhibition may have a role in improving endothelial function as well. Evolocumab was added to empagliflozin in one study of 110 patients with type 2 diabetes over 16 weeks. Subjects in the evolocumab arm experienced a significant improvement in brachial flow-mediated dilation along with reductions in plasma isoprostanes, a marker of oxidative stress (Sposito et al, 2022). A small study of evolocumab in patients with human immunodeficiency virus also showed significant improvements in coronary blood flow, which was independent of the effect on LDL-c reduction (Leucker et al, 2020), providing further support for possible endothelial benefit derived from PCSK9-targeted intervention.

While most studies of PCSK9 inhibition are polluted by background statin use, a study of PCSK9i mAb monotherapy in post-carotid endarterectomy patients supports the utilization of this

strategy in isolation and lends additional credence to the notion of pleiotropic effects. In this study of 645 patients, expression of inflammatory markers within the carotid plaque was compared between the group of 159 individuals on PCSK9i monotherapy and the 486 on other lipid-lowering agents. A composite of all-cause mortality, stroke, and myocardial infarction was also evaluated over ~2 years (Marfella et al, 2023). The results were quite remarkable; not only did those in the PCSK9i arm have significantly decreased expression of plaque inflammatory markers, including NF-κβ, TNFα, IL-1β, and nod-like receptor family pyrin domaining 3 (NLRP3) activity, there was a stunning 74% reduction in the composite 3-point MACE compared to the group receiving other lipid-lowering therapies (Marfella et al, 2023). This difference was consistent even after adjusting for LDL-c levels and occurred despite nearly identical hs-CRP serum markers (Marfella et al, 2023). Interestingly, expression of PCSK9 within plaque correlated with levels of inflammatory markers and negatively associated with sirtuin 3, a marker of plaque stability (Marfella et al, 2023). This study complements the more widely studied effects of PCSK9i mAbs on coronary plaque, which will be discussed in the subsequent section. **Table 6** summarizes the evidence of LDLr-independent mechanisms of PCSK9i.

Table 6: Potential Peripheral Pleiotropy of PCSK9 Inhibition

Mechanism	**Purported Effect**
Prevents interaction with SRA, CD36, and LOX-1	Inhibits foam cell formation
Decreases thromboxane A2	Decreases platelet aggregation and reactivity
Decreases IL-6, IL-1β, TLR4, TNFα, NF-κβ, MCP-1, NLRP3	Decreases inflammation
Inhibits MHC-1 degradation	Prevents proliferation of certain cancers
Prevents inhibition of macrophage ABCA1 and ABCG1	Promotes cholesterol efflux
Decreases 7α-hydroxylase and increases PPARα	Prevents gall stone formation
Decreases Lp(a) secretion	Reduces serum Lp(a) levels
Decreases adipocyte insulin resistance	Increases GLUT4-mediated glucose uptake in adipocyte
Reduces postprandial ApoC3 and ApoB-48	Decreases postprandial lipemic excursions
Increases brachial FMD and coronary blood flow	Improves endothelial function

3.4 Effects of PCSK9 Inhibition on Coronary Plaque

Therapeutics targeting PCSK9 have been studied for their effects on coronary plaque using various modalities, including IVUS, optical coherence tomography (OCT), near-infrared spectroscopy (NIRS), and non-invasive computed tomography coronary angiography (CCTA). All of these

studies have demonstrated beneficial effects in plaque stabilization and regression. The effect of evolocumab on coronary plaque was initially evaluated in a rather large population of 968 individuals with stable CAD in the Global Assessment of Plaque Regression With a PCSK9 Antibody as Measured by Intravascular Ultrasound (GLAGOV) trial (Nicholls et al, 2016). Over 78 weeks, those treated with the PCSK9i mAb had a 1% regression in PAV when compared to the statin monotherapy arm (Nicholls et al, 2016). This was followed by the High-Resolution Assessment of Coronary Plaques in a Global Evolocumab Randomized Study (HUYGENS) trial, a smaller study of 161 patients in the acute phase after non-ST segment elevation myocardial infarction; these patients were randomized within one week of acute coronary syndrome (Nicholls et al, 2022). In addition to IVUS, which assesses plaque burden from intima to adventitia, OCT, a much higher resolution imaging technique, was employed to better characterize high-risk features such as fibrous cap thickness, lipid arc, and macrophage index. Also, considering the utility of IVUS in quantifying plaque burden and the correlation between PAV reduction and MACE reduction, this has remained a consistent measurement across various studies. Over the year-long follow-up in HUYGENS, patients in the evolocumab arm demonstrated a 2.3% PAV regression from baseline while also increasing fibrous cap thickness, decreasing lipid arc, and decreasing macrophage index nearly twofold when compared to statin monotherapy (Nicholls et al, 2022). This supported the notion that earlier intervention with PCSK9i mAb therapy after an index MACE would lead to greater plaque regression and stabilization of high-risk features. The most recent invasive study of evolocumab was YELLOW III, which utilized IVUS, OCT and NIRS. The PAV regression over 6 months was 1.38% and a significant reduction in lipid-core burden index was noted on NIRS in the evolocumab arm alongside predictable increases in fibrous cap thickness on OCT (Gupta et al, 2023). Visually, this quantification of lipid burden within coronary plaque and subsequent reduction demonstrated by the NIRS modality resembles “melting butter” and can be appreciated visually even by non-interventionalists.

One study using the non-invasive CCTA evaluated the effect of evolocumab on coronary plaque characteristics in 170 patients with type 2 diabetes. Remarkably, a statistically significant reduction in high-risk plaque features along with prevalence of obstructive lesions was observed compared to the statin-only arm (Yu et al, 2023). Additionally, a significant reduction in pericoronary adipose tissue along with reductions in necrotic core volume were observed in the evolocumab arm (Yu et al, 2023).

Taken together, PCSK9 inhibition demonstrates favorable effects in not only stabilizing vulnerable plaque, but regressing total plaque burden, and these studies are summarized in **Table 7**. While this relationship is commensurate with LDL-c reduction, various putative pleiotropic mechanisms may also play a role in the benefits observed in these studies.

Table 7: Coronary Plaque Imaging Studies of Evolocumab

Study	Patient Population	Imaging Modality	Result
GLAGOV (Nicholls et al, 2016)	968 patients with stable CAD over 78 weeks	IVUS	-1% PAV vs control
HUYGENS (Nicholls et al, 2022)	161 patients within 1 week post-NSTEMI over 52 weeks	IVUS and OCT	IVUS: -2.38% PAV OCT: Increased fibrous cap thickness, decreased lipid arc, decreased macrophage index
YELLOW-III (Gupta et al, 2023)	137 patients with stable CAD over 6 months	IVUS, OCT, NIRS	IVUS: -1.38% PAV OCT: Increased fibrous cap thickness NIRS: Decreased LCBI
Unnamed (Yu et al, 2023)	170 patients with T2DM over 1 year	CCTA	Decreased pericoronary adipose tissue, decreased obstructive lesions, decreased high-risk plaque features

3.5 Cardiovascular Outcome Trials-FOURIER and ODYSSEY

It should perhaps come as no surprise that the benefits observed in coronary plaque stabilization with PCSK9i mAbs would, over time, translate into reductions in MACE. As alluded to in the discussion of historical lipid-lowering therapies, the FOURIER and ODYSSEY cardiovascular outcome trials of the PCSK9i mAbs evolocumab and alirocumab helped pave the way to a new era of non-statin therapeutics. These trials share many similarities, although the FOURIER trial was conducted in a population with stable CAD while inclusion criteria for ODYSSEY mandated that subjects had experienced an acute coronary syndrome between 1 and 12 months prior to randomization (Furtado and Giugliano, 2020). In both trials, subjects were at least 40 years of age, and both trials featured large sample sizes with 27,564 in FOURIER and 18,924 in ODYSSEY (Furtado and Giugliano, 2020). In both trials the population was considered well-treated at baseline, with the vast majority receiving high-intensity statin therapy along with antihypertensives and antiplatelet agents as indicated. In FOURIER and ODYSSEY the baseline LDL-c was 92 and 87 mg/dL, respectively (Furtado and Giugliano, 2020). One key difference between the studies was the variance in dosing regimens; in FOURIER subjects received Evolocumab 140 mg administered subcutaneously every 2 weeks or the equivalent monthly dose of 420 mg, while in ODYSSEY subjects received either biweekly 75 or 150 mg doses. This was

titrated based on follow-up LDL-c levels, and if patients had LDL-c levels <15 treatment with any dose of alirocumab was permanently discontinued; in fact, 7.7% of patients were switched to placebo due to this protocol stipulation (Furtado and Giugliano, 2020). The rationale for this was the concern, at the time, of very low LDL-c levels and the association with hemorrhagic stroke noted in certain past statin trials, notably the Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) of high-intensity Atorvastatin (Huisa et al, 2010). However, a subsequent meta-analysis demonstrated that while statins may be associated with hemorrhagic stroke, PCSK9 inhibition is not (Sanz-Cuesta and Saver, 2021). This speaks to the safety of enhancing efficiency of lipoprotein clearance as well as the difference between cellular cholesterol and plasma cholesterol, as the value expressed in a static plasma measurement is only a small fraction of total body cholesterol. Indeed, both short-term and long-term monitoring of patients with very low LDL-c levels from FOURIER and the open-label extension of FOURIER has demonstrated no increased risk of hemorrhagic stroke or other adverse events for those even with sustained LDL-c levels <20, and a number of those patients have been followed for more than 8 years (Gaba et al, 2023).

Both trials met their respective primary endpoints of 5-point and 4-point MACE, respectively, with a 15% relative risk reduction, and the 20% reduction in the key secondary endpoint also reached statistical significance for both evolocumab and alirocumab (Furtado and Giugliano, 2020). Although the secondary endpoint included myocardial infarction in both studies, ODYSSEY specified ischemic stroke and all-cause death in its 3-point composite secondary endpoint while FOURIER limited the analysis to CV death while including strokes of all types (Furtado and Giugliano, 2020). Subjects in FOURIER had a 27% reduction in myocardial infarction while both trials showed a particularly strong benefit in stroke reduction at 21 and 27% respectively in FOURIER and ODYSSEY (Furtado and Giugliano, 2020). Quite remarkably, these results were attained in less than 3 years for both trials, and the benefit in MACE reduction seen in FOURIER was consistent across all quartiles of baseline LDL-c. Furthermore, no significant safety signals emerged in either trial, and a separate neurocognition sub-study called EBBINGHAUS of more than 1900 patients in FOURIER reassuringly demonstrated no deleterious effects on cognition using the Cambridge Neuropsychological Test Automated Battery (CANTAB), a rigorous and well-validated battery of cognitive tests (Furtado and Giugliano, 2020). Long-term follow up of this cohort also showed no changes in cognition over up to 7.2 years (Zimmerman et al, 2024). The only significantly greater adverse event for both mAbs compared to placebo was localized injection-site reactions; there was not a significant increase in new onset diabetes, cancer, or myalgias in either trial (Furtado and Giugliano, 2020).

One of the criticisms of the initial FOURIER study was the null effect of evolocumab on CV death, and concerns remained regarding long-term safety of this new drug class. Consequently, 6635 patients were enrolled in the open-label extension of FOURIER; once the study was terminated, patients in the placebo arm were given the opportunity to add evolocumab to their regimen.

Quite intriguingly, those who had started evolocumab in the parent trial continued to demonstrate a benefit in MACE reduction versus those who had added therapy later over the initial 2 years of the open-label extension before the Kaplan-Meier curves began to parallel. Also, the exploratory endpoint of CV death was 23% lower in the arm who received evolocumab in the parent trial (O'Donoghue et al, 2022). This finding mirrored the paradigm of atherosclerotic disease mitigation; first, a reduction in atherogenic lipids is observed, followed by subsequent benefits in stabilizing and regressing coronary plaque. This would be expected to reduce CV events, which, over time, should translate into a CV mortality benefit. Additionally, a 43% reduction in CV death, myocardial infarction, and stroke was observed in subanalysis of those who achieved LDL-c levels of <10 versus those who remained >100 mg/dL with no difference in adverse events between groups (Gaba et al, 2023). Incidence of new-onset diabetes and myopathic symptoms decreased over the open-label extension follow-up period as well, and no issues related to cognition emerged (Gaba et al, 2023).

3.5.1 Nutraceuticals Impacting PCSK9

Although pharmacologic inhibition of PCSK9 has demonstrated profound efficacy in lowering LDL-c and reducing CV events, there are several common nutraceuticals that also mechanistically inhibit PCSK9. These include berberine and curcumin, and both have shown possible benefit in the context of atherogenic lipid reduction as well as neuroprotection (Bao et al, 2024). Berberine may reduce neuroinflammation and may also influence the production of Aβ-42; the precise mechanisms by which this compound confers benefit remain to be elucidated. However, it has demonstrated capacity to cross the BBB, but whether or not its ability to inhibit PCSK9 in the CNS is unknown (Cheng et al, 2022). A highly bioavailable form of curcumin actually did show an impressive reduction in amyloid and tau accumulation as measured by FDG-PET in a randomized controlled trial over 18 months in a cohort of 40 adults aged 51-84 without dementia at baseline (Small et al, 2018). This was accompanied by an impressive improvement in memory and attention in the curcumin arm when compared to age-matched controls (Small et al, 2018). Again, curcumin acts via multiple mechanisms and any potential effect of its inhibition of PCSK9 in the CNS may not be physiologically significant. Lastly, caffeine, a well-known component of popular beverages such as coffee and tea, inhibits PCSK9 via hepatic SREBP2 suppression (Lebeau et al, 2022) and greater coffee consumption has been associated with less progression to AD (Gardener et al, 2021). Teasing out potential mechanisms and minimizing other confounding variables is a tall order when determining whether or not peripheral or central PCSK9 modulation plays a role in this association.

3.6 PCSK9 and Glucose Homeostasis

Given the inextricable intertwining of lipid homeostasis and glucose metabolism, the role of PCSK9 in substrate utilization is worth a thorough exploration. PCSK9 is under tight transcriptional control by SREBPs as well as hepatocyte nuclear factor 1 alpha (HNF1α) while forkhead box

protein O3 and sirtuin 6 negatively regulate PCSK9 gene expression (Bao et al, 2024). This transcriptional regulation by HNF1α has potential relevance when discussing the role of PCSK9 in glucose homeostasis, as mutations in HNF1α are the basis for Monogenic Diabetes of the Young (MODY) Type 3 (Valkovicova et al, 2019). An initial Mendelian randomization of certain loss-of-function variants in PCSK9 and HMGCR did show an 11% and 13% increased risk of diabetes, respectively (Ference et al, 2016). However, both mechanistic studies and clinical trials of PCSK9 inhibition have been reassuring; PCSK9 loss-of-function appears to have a neutral or perhaps even beneficial effect on insulin sensitivity and glucose tolerance. PCSK9 is present in pancreatic beta cells and delta cells, but even in genetically modified mice lacking either global or pancreatic PCSK9, insulin response to glucose was unaffected in 2 recent studies (Seidah and Prat, 2021). However, variants in PCSK9 gene expression, along with other factors, may result in a tendency toward impaired insulin secretion, as other rodent studies of specific have shown a tendency for hyperglycemia in pancreatic PCSK9 knockout mice (Path et al, 2022). Regardless, PCSK9 is locally produced by the beta cell and appears to be largely unaffected by extracellular inhibition or hepatic underexpression of PCSK9 (Path et al, 2022), although depending on the degree of beta cell-specific PCSK9 loss-of-function, processing of proinsulin to insulin may become impaired. This is similar to what has been observed in the first-phase insulin response in patients with Tangier's Disease (Path et al, 2022). The complex relationship between decreased ABCA1-mediated sterol efflux and subsequent downregulation of SREBP2, which transcriptionally regulates PCSK9, would explain hyperglycemia associated with hypofunctioning PCSK9 in the beta cell.

Observation of specific loss-of-function variants in humans provide additional insights into the effects of PCSK9 on glucose metabolism. The C679x and A443T loss-of-function variants in PCSK9 demonstrated no impairments in glucose parameters, and the R46L variant only demonstrated tendency toward hyperinsulinemia if also possessing an ApoE2 copy (Path et al, 2022); despite the ostensibly protective effect of ApoE2 in cognitive impairment, it does carry a somewhat paradoxical twofold increase in risk of diabetes development (Santo Ferreira et al, 2019).

Various clinical trials have sought to investigate the role of extracellular PCSK9 inhibition on glucose tolerance, and as mentioned, a 38-study meta-analysis demonstrated a null effect on glycemia (Monami et al, 2019). Additionally, several other studies have provided additional insight into the effect of PCSK9 inhibition on beta cell function. One small cohort of 15 non-diabetic subjects underwent baseline testing of glycemic parameters, including an oral glucose tolerance test and concurrent assessment of pancreatic beta cell insulin sensitivity before and after starting evolocumab. Intriguingly, no rise in glucose was observed and there was actually an improvement in beta cell insulin sensitivity in those with higher body mass indices along with a trend towards increased insulin sensitivity in those more highly insulin resistant at baseline (Moffa et al, 2023). This effect was independent of background statin use, although beta cell sensitivity did modestly improve to a greater degree in those taking statins (Moffa et al, 2023);

perhaps this is due to the documented effect of statin-increased PCSK9 contributing to insulin resistance in the adipocyte (Shu et al, 2022).

Detailed parameters of glycemic control and postprandial lipemia in 421 subjects with pre-existing type 2 diabetes were obtained in the evolocumaB efficacy aND safety in type 2 diabetes on background statin therapy (BANTING) study (Rosenson et al, 2019). No changes were observed between groups in hemoglobin A1c, and robust reductions in non-HDL-c and ApoB were demonstrated of ~57 and ~50%, respectively. Moreover, excursions in postprandial lipemia measured after a mixed meal tolerance test showed significant reductions in chylomicron triglycerides, chylomicron cholesterol, ApoB-48, and VLDL-c along with far less insulin required to maintain glycemia in the evolocumab arm (Rosenson et al, 2019). Nearly identical benefits in postprandial lipemic excursions were observed in another similarly designed study and a 14% reduction in plasma ApoC3 was observed in addition to the decrease in ApoB-48 (Taskinen et al, 2021). Given the potential atherogenicity of remnant lipoproteins and the CV risk conferred by chronic postprandial lipemia, this study demonstrated that inhibition of extracellular PCSK9 may have additional utility in mitigating this unwanted effect present in individuals with diabetes.

In summary, certain loss-of-function variants in PCSK9, when present with a “second hit” risk factor such as an ApoE2 allele, may modestly increase risk of new-onset diabetes. However, inhibition of PCSK9 pharmacologically appears to either have a neutral or even beneficial effect on those with and without diabetes in regards to parameters of glucose metabolism.

3.7 PCSK9 Function in the Brain

Although neither PCSK9 nor PCSK9i mAbs cross the BBB, PCSK9 is expressed in the brain, particularly in the neurons of the telencephalon (Bao et al, 2024). Although there is much more to be learned in regards to its specific functions as a regulator of lipid trafficking, it does appear to modulate cellular apoptosis particularly during periods of neuronal biogenesis and differentiation (Bao et al, 2024). However, genetically modified mice lacking PCSK9 displayed no issues with cortical development, behavior, or memory (Parn et al, 2023). In fact, mixed mouse models of atherosclerosis due to PCSK9 gain-of-function and Alzheimer’s displayed a threefold increase in Aβ plaques in the hippocampus (Shabir et al, 2022). In another study, genetically modified hyperlipidemic ApoE knockout mice showed elevated expression of PCSK9 coupled with neuronal apoptosis in the hippocampus (Zhao et al, 2017). These rodent studies suggest a relationship between atherosclerosis, PCSK9, lipid trafficking, and neuronal degeneration. An in vitro investigation of human astrocytoma cells treated with PCSK9 reduced LDLr expression

Initially, it was thought that PCSK9 deviated the processing of APP toward alpha secretases via degradation of BACE-1; later studies showed that PCSK9 did not mediate BACE-1 for degradation (O’Connell and Lohoff, 2020). However, PCSK9 has been shown to degrade ApoER2 in the brain along with LDLr, thereby promoting neuronal apoptosis; this notion was reinforced by a study in

which cerebral inhibition of PCSK9 in mice mitigated sequelae of neuronal loss after ischemia was induced (Bao et al, 2024). Observing this benefit in a situation in which BBB permeability was compromised to permit PCSK9 mAb agents to infiltrate the CNS was noteworthy. Another in vitro study of CNS cells treated with PCSK9 demonstrated a 31% decrease in ApoER2 and 66% reduction in LDLr expression in astrocytes along with a 29% reduction in neuronal cholesterol (Papotti et al, 2022). PCSK9 treatment dramatically decreased lipidation of ApoE-containing HDL-like particles while simultaneously increasing neurotoxicity induced by Aβ (Papotti et al, 2022). The interaction between LRP1 and PCSK9 has also been proposed as a critical mediator of amyloid clearance across the BBB, and a study of the PCSK9i alirocumab did show increased LRP1 expression in the hippocampus along with decreased TLR4, IL-1β, IL-6, and TNF-α (Bao et al, 2024).

Given that the balance between cholesterol synthesis, lipidation of ApoE-containing HDL-like particles, transport of astrocytic cholesterol to neurons, and clearance of amyloid across the BBB appear to be linked to PCSK9, the proposition for further investigation of these relationships hardly seems unreasonable.

Chapter 4-Methods

4.1. Research Design and Rationale

The research was guided by the following research questions as described in the introduction:

Research Question 1: What is the relationship between PCSK9 and members of the LDLr family in the central nervous system, and is PCSK9 a bystander or active contributor to the development of Alzheimer's Disease?

Research Question 2: Is peripheral PCSK9 inhibition an efficacious strategy to mitigate the progression of Alzheimer's Disease and, if so, by what mechanisms could this confer benefit?

Research Question 3: What is the awareness of community clinicians regarding the relationship between lipids, cholesterol homeostasis, and PCSK9 in the context of AD? Do these opinions or perceptions influence prescribing patterns of cholesterol modulating drugs?

Given the complexity and multi-faceted nature of this research, a three-pronged qualitative approach was employed. These included a more pedantic literature source with subsequent analysis, dichotomous survey of community clinicians, and case report with analysis of a clinician approach to the patient with Alzheimer's Disease and concurrent hyperlipidemia. Qualitative research often benefits from "a wide range of interconnected interpretative practices" (Johnson et al, 2020) in hopes of addressing the questions guiding the dissertation. A solitary modality would have necessarily stunted the robustness of the discussion given the intricacy of the topic, and this triangulation approach to the data seemed best to simultaneously maximize trustworthiness and minimize bias. Additionally, some experts in the realm of qualitative research have recently argued that rigid criteria may not be the best approach to achieve optimal research quality, particularly with complicated research topics (Johnso et al, 2020). Consequently, the community clinician survey portion of the methodology aligns more with phenomenological approach rather than a more unmalleable construct. The various component parts of the data ascertained reciprocally enhance the currently incomplete understanding of the phenomenon of interest; my hope in employing this less-than-mainstream methodology was to truly exude vividness and elegance befitting of phenomenological approaches described elsewhere in the literature (Neubauer et al, 2019).

4.1.1 Specific Literature Search with Subsequent Analysis Methodology

To address research questions 1 and 2, a PubMed search employing the keywords "PCSK9" and "Alzheimer's" was conducted, yielding 52 results. Articles published prior to 2014 were excluded, leaving 46 results. The search was further specified to include "Alzheimer's models," and 11 articles remained. The desire for this literature to review was to better understand the relationship between PCSK9, PCSK9 inhibition, and its effects on lipid homeostasis and cognition in models of Alzheimer's dementia. Ergo, perusal of the remaining 11 articles sought to identify

articles in which PCSK9 inhibition was experimentally utilized. This ruled out 5 studies that employed Mendelian randomization or genome-wide association for their methodologies. In the remaining 6 publications, 2 studies met criteria for inclusion, as PCSK9 inhibition was utilized in the context of in vitro or in vivo models of Alzheimer's dementia. These articles, entitled "PCSK9 acts as a key regulator of Aβ clearance across the blood-brain barrier" by Mazura et al (2022) and "PCSK9 ablation attenuates Aβ pathology, neuroinflammation and cognitive dysfunctions in 5XFAD mice" by Vilella et al (2024) were subsequently read in their entirety and analyzed.

4.1.2. Community Clinician Survey Methodology

To foster discussion regarding research question 3, a three-question dichotomous survey was disseminated electronically to 109 community clinicians in the United States during January of 2024. These community clinicians included primary care providers (PCPs), community cardiology providers, and several clinicians practicing in endocrinology. These clinicians included physicians, nurse practitioners, and physician assistants; a breakdown of the respondents based on specialty and certification is listed below. The analysis sought to comprise a balanced representation of physicians, physician assistants, and nurse practitioners with the majority practicing in the primary care setting to best simulate the distribution of healthcare providers across the nation.

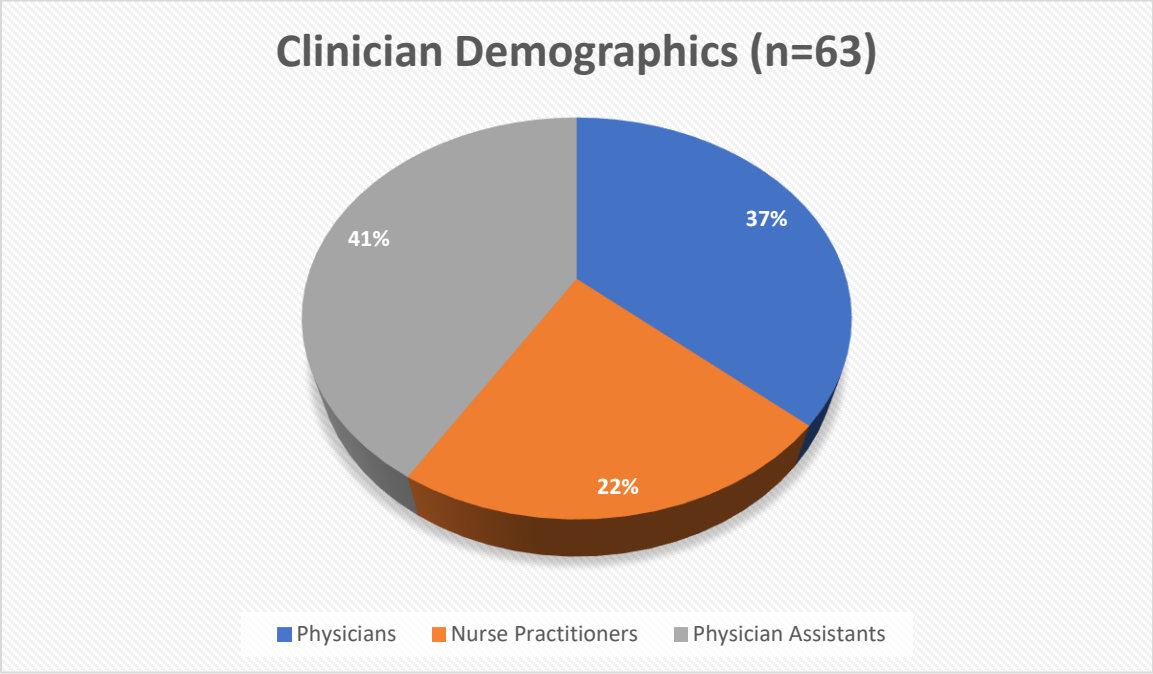

Figure 8: Community Clinician Demographics By Classification

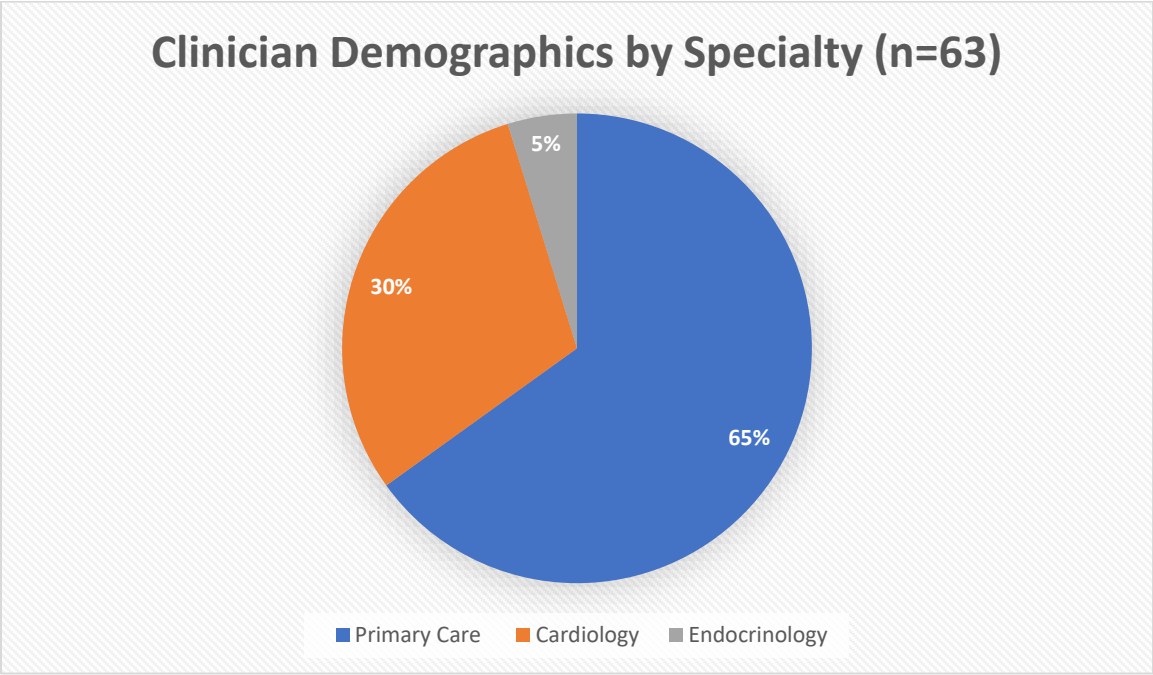

Figure 9: Community Clinician Demographics by Specialty

The sampling method was purposive in nature, as PCPs have been described as being on the frontlines of Alzheimer's Disease care while PCPs, cardiologists, and endocrinologists are all regularly involved with lipid management in their practices. Perceiving that clinicians are unlikely to respond to a lengthy questionnaire without incentivization or honoraria, the survey was intentionally kept brief and user-friendly, comprising the following questions:

Community Clinician Survey

1. Are you aware of any **SPECIFIC** relationship between cholesterol and Alzheimer's disease?
Yes or No

2. Are you aware of any potential **SPECIFIC** relationship between PCSK9 or desmosterol and Alzheimer's disease?
Yes or No

3. Has concern about Alzheimer's ever made you adjust your lipid-lowering therapy?
Yes or No

If YES, please briefly describe how your perception influenced your practice.

63 of the 109 clinicians surveyed responded to the questionnaire. Semi-quantitative descriptive data was compiled and sub-analysis based on various factors was conducted. Additional

qualitative information was gleaned from the open-ended follow-up to question 3; this was intentionally designed in hopes of capturing unique perspectives in management approaches. To maintain anonymity, each individual clinician response was categorized in the order of response and by the classification of the respondent. For instance, the first physician to respond was denoted PY1, the second physician assistant to respond was designated PA2, and the third nurse practitioner to respond was designated NP3. These data points were recorded in a Microsoft Word document in the order of reception and is located in **Appendix A**. The composite analysis of the data ascertained resulted in the emergence of two important themes that will be further discussed in the **Contents and Results** and **Discussion** sections. This purposeful sampling of these clinicians along with meticulous recording of data as it was received helped to ensure transferability and confirmability of the research. I also sought to enhance credibility by engaging with other colleagues regarding the research methodology; these colleagues were able to provide valuable peer review as well.

Due to time and resource constraints, it is unlikely that data saturation was possible in this instance. Ergo, the data accrued from this population may not be generalizable to the broader population. Admittedly, this was a rather small sample size of clinicians primarily practicing in the Western United States. However, the survey methodology was intentionally kept dichotomous to ensure reproducibility in larger cohorts for future ascertainment of data. Indeed, this represents more of a pilot approach to the issue, and full transparency of methodology lends itself to replication across broader cohorts in the future who will ideally have access to greater resources to minimize response bias.

4.1.3 Case Report Methodology

For the Case Report described in **Chapter 6**, verbal consent was obtained on 1/24/2024 from Dr. Jeffrey Boone to use images and data. Baseline blood-based biomarkers as well the WAVi imaging modality were employed for the individual patient prior to intervention. Post-treatment data was then ascertained and reported.

Reference ranges for the blood-based biomarkers are based on data from Boston Heart Diagnostics and appear below. All values falling between “Optimal” and “Increased Risk” would be designated as “Borderline.” An important caveat with these reference ranges is that, depending on the patient history and overall cardiovascular risk determined by various stratification techniques, optimal lipid values may differ from those listed below in **Table 8**.

Table 8: Reference Ranges for Selected Biomarkers

Biomarker	Reference Range
Total cholesterol	Optimal: <200 mg/dL Increased Risk: >240
LDL-c	Optimal: <100 mg/dL Increased Risk: >160
HDL-c	Optimal: 40-60 mg/dL Increased Risk: <50 (female) <40 (male)
Triglycerides	Optimal: <150 mg/dL Increased Risk: >200
ApoB	Optimal: <90 mg/dL Increased Risk: >109
HS-CRP	Optimal: <1.0 mg/L Increased Risk: >3.0 mg/L
Lp-PLA2	Optimal: <180 nmol/min/mL Increased Risk: >225
IL-6	Optimal: <2.5 pg/mL Increased Risk: >5.0
Homocysteine	Optimal: <10µmol/L Increased Risk: >14

Source: bostonheartdiagnostics.com/boston-heart-test-menu/

The WAVi® Research Platform imaging modality has been historically utilized as a non-invasive means to quantify resolution of cognitive deficits after incurring concussive injury. The instrument used in the case reports included placement of a headset with 19 electrodes according to the established International 10-20 system to obtain EEG data while reference electrodes were secured at the earlobes (Clayton et al, 2020). These electrodes were appropriately labeled based on scalp location; for example, labels with the letter "P" denoted a location in the parietal region. Event-related potential (ERP) testing using a 4 minute 2-tone audio oddball P300 protocol was used to measure amplitude and latency; these variables are said to quantify attention and speed of classification, respectively (Clayton et al, 2020). Decreases in P300 peak amplitude and increased latency have been noted in various states characterized by impaired cognition, including traumatic brain injury and dementia (Boone et al, 2022). Additionally, higher peak amplitudes and decreased latencies have been observed in those with superior cardiovascular health compared to controls with suboptimal metrics of CV health in a small cohort study (Boone et al, 2022). In the protocol employed here, a combination of 200 common tones at a frequency of 1000 hertz intermingled with 40 uncommon tones of 2777 hertz were randomly delivered over the 4-minute testing period as EEG data was extracted; each tone was 50 milliseconds in duration. The EEG was sampled at 250 hertz and bandpass filtered

between 0.5 and 3.0 hertz. From there, the WAVi software was utilized to analyze and graphically plot the extracted EEG data.

An example of WAVI imaging that topographically depicts restoration of normal brain activity is shown below in **Figure 10.**

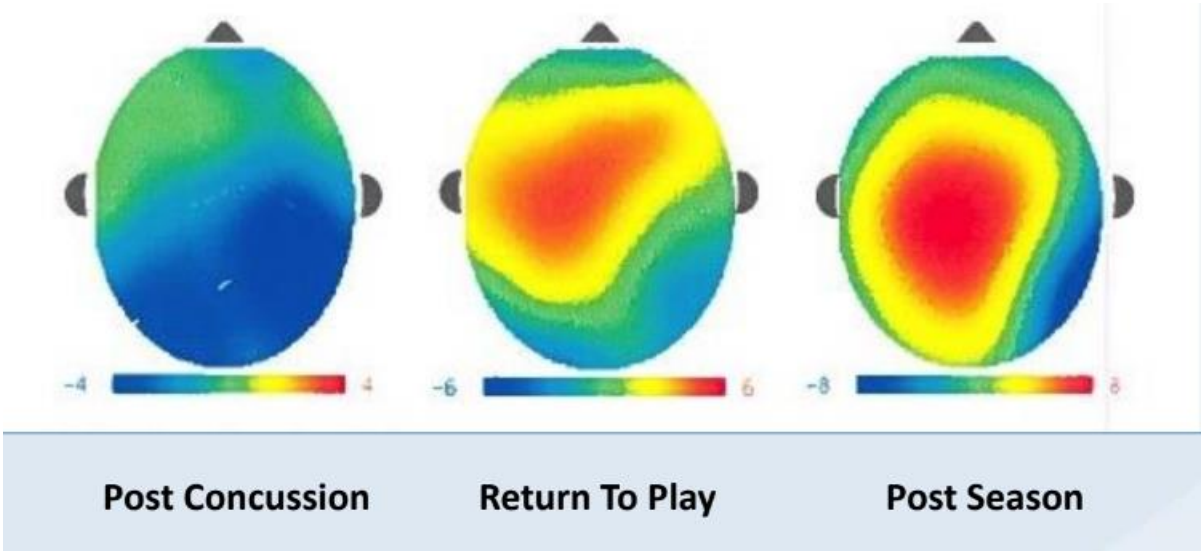

Figure 10: Example of WAVi Imaging**.** The WAVi imaging modality has historically been employed to demonstrate resolution of brain activity after concussive injury, particularly in athletes seeking a return to sport. This image represents the typical progression of an athlete who progressed through a typical return-to-play protocol after sustaining a concussion. Brighter colors are correlated with increased amplitude on the P300 assessment of event-related potentials. Image used with permission from Dr. Jeffrey Boone of Boone Heart Institute.

4.1.4 Ethical Considerations

Ethical issues were carefully considered prior to and during the research. Appropriate consent from all individuals participating in the research was obtained and no coercive maneuvers were taken to facilitate the research process. Individuals surveyed were under no obligation to respond and no honoraria or incentive was provided. However, individuals who did choose to respond to the survey were assured that their identities and responses would be kept confidential and any open-ended responses would be accurately recorded. Data was stored on a password-protected computer and appropriate credit and citations were provided when indicated.

Chapter 5- Contents and Results

5.1 Results from Specific Literature Search and Analysis

Two articles, entitled "PCSK9 acts as a key regulator of Aβ clearance across the blood-brain barrier" by Mazura et al (2022) and "PCSK9 ablation attenuates Aβ pathology, neuroinflammation and cognitive dysfunctions in 5XFAD mice" (2024) met the aforementioned inclusion criteria for evaluation. The role of the researcher in unearthing these articles was to utilize my expertise in the topic to rigorously analyze these recent contributions to the literature in efforts to provide better answers to Research Questions 1 and 2. Once again, these research questions are listed below:

Research Question 1: What is the relationship between PCSK9 and members of the LDLr family in the central nervous system, and is PCSK9 a bystander or active contributor to the development of Alzheimer's Disease?

Research Question 2: Is peripheral PCSK9 inhibition an efficacious strategy to mitigate the progression of Alzheimer's Disease and, if so, by what mechanisms could this confer benefit?

5.1.1 Review of "PCSK9 acts as a key regulator of Aβ clearance across the blood-brain barrier"

This 2022 article, published in *Cellular and Molecular Life Sciences*, sought to better elucidate the mechanisms by which Aβ is transported across the BBB and peripherally cleared. A well-established in vitro model of the BBB first identified PCSK9 as an inhibitor of LRP1-mediated Aβ transport from the CNS to the periphery. This elegant model employed physiologic concentrations of Aβ-42 and immortalized murine brain capillary endothelial cell lines; these cell lines were then engineered to produce PCSK9. After this, the clearance of amyloid across mouse brain endothelial cells lacking LRP1 but treated with recombinant PCKS9 was assessed prior to verification with endothelial cells of porcine origin.

Aβ clearance in the PCSK9-treated cells was decreased by ~42% compared to the cells not treated with PCSK9. However, when an LRP1 antibody was used, the Aβ clearance rate did not differ between cells treated or untreated with PCSK9, strongly suggesting that the LRP1-mediated "peripheral sink" Aβ transport was regulated by PCSK9. Indeed, mouse brain endothelial cells expressing PCSK9 displayed both downregulated LRP1 and LDLr upon further examination. To further confirm this role of PCSK9 on LRP1 expression, extracellular PCSK9 treatment did not influence the rate of Aβ transcytosis in murine LRP1 knockout cell lines. When porcine cells were treated in a similar manner, PCSK9 decreased LRP1-mediated Aβ transport by approximately 50% as well.

Once this physiological interaction was established, in vivo examination of PCSK9 inhibition on LRP1-mediated Aβ clearance was assessed in mouse models of severe AD. These 14-week-old female 5xFAD were treated with either a PCSK9i mAb or control. Additionally, in one group of

these mice, an LRP1 knockout model was induced via treatment with tamoxifen. The 5xFAD mice treated with the PCSK9i showed increased concentrations of LDLr and LRP1 in the periphery along with decreased serum levels of ApoE; this would indicate enhanced clearance of ApoE lipoproteins via upregulation of ApoE receptors. Conversely, cerebral LRP1 and CSF ApoE were unaffected by extracellular PCSK9i and no detectable mAb was found in the PCSK9i-treated mice, indicating that the mAb did not cross the BBB.

5xFAD mice lacking LRP1 treated with PCSK9i for 10 weeks showed no difference in clearance of both soluble and insoluble Aβ-40 and Aβ-42 compared to controls. Conversely, PCSK9i-treated mice with intact LRP1 decreased insoluble Aβ-40 and Aβ-42 by 44 and 38%, respectively, while the soluble amyloid peptides were reduced by 58 and 51%. Assessment of amyloid plaque in the prefrontal cortex and hippocampi of these animals was also performed after sacrifice. Even the highly aggregated soluble and insoluble Aβ-40 and Aβ-42 variants were reduced by 17 and 23%, respectively in the mice treated with PCSK9i and co-expressing LRP1 in endothelial cells. No difference in plaque accumulation was observed in PCSK9i-treated LRP1 knockout mice.

Assessment of hippocampal memory was also tested using a conditioned response model. In this well-established technique, an adverse external stimulus elicits a conditioned freezing response upon repeated confrontation. 5xFAD mice typically demonstrate deficits in this response by the time they reach 4-6 months of age, but those treated with PCSK9i displayed a significantly improved fear response compared to controls. However, this benefit was not demonstrated in the LRP1 knockout mice.

5.1.2 Review of "PCSK9 ablation attenuates Aβ pathology, neuroinflammation and cognitive dysfunctions in 5XFAD mice"

This 2024 article, published in *Brain, Behavior and Immunity,* also utilized in vitro and in vivo methods to assess the role of PCSK9 in the context of neuroinflammation. Acknowledging the interaction between PCSK9 and LRP1, ApoER2, and LDLr, the authors sought to examine the effect of PCSK9 ablation on 5xFAD mouse models of severe AD. Markers of inflammation, cholesterol levels, oxysterol concentrations, corticohippocampal Aβ plaque burden, reactivity of astrocytes and microglia, and cognitive performance were among the swath of parameters ascertained in this investigation.

Similar to the methodology employed in the aforementioned study by Mazura and colleagues, in vitro studies were first conducted, although a human astrocytoma cell line was analyzed. These cells were incubated with Aβ fibrils and treated with human recombinant PCSK9. Messenger RNA expression of genes germane to lipid homeostasis, inflammation, immune regulation, and microglial function obtained before and after treatment. Although there was a slight increase in MCP-1, IL-6, and IL-1β expression after Aβ incubation, PCSK9 treatment further increased the expression of these cytokines along with TNFα; PCSK9-treated cells not incubated with Aβ only

demonstrated increased mRNA of IL-6. Other genes regulating expression of the inflammasome, such as pyrin, NLRP3, and NLR family caspase recruitment domain-containing protein 4 (NLRC4) were also increased by Aβ; NLRC4 was the only gene demonstrating statistically significant elevation after PCSK9 treatment. Somewhat surprisingly, CD36 expression was unaffected by PCSK9 treatment although the interaction between PCSK9 and CD36 has been previously demonstrated in multiple cell types with varying effects on physiologic processes. However, this preliminary in vitro analysis demonstrated the synergistic amplification of inflammatory signaling between PCSK9 and Aβ.

Transgenic mice were produced by crossing 5xFAD models with mice lacking PCSK9. These were then compared to 5xFAD mice expressing PCSK9 and amyloid plaque burden was assessed. Mice lacking PCSK9 had a significant reduction in quantity and area of amyloid plaques in the corticohippocampal areas; notably, this reduction in plaque quantity was ~40% in the dentate gyrus and neocortex when assessed using a thioflavin-s staining technique. Additionally, BACE-1, the enzyme that processes APP into Aβ isoforms, was unchanged in the plaques of mice expressing or lacking PCSK9. Early studies of PCSK9 raised concern about PCSK9 inhibition potentially increasing BACE-1 expression and, by extension, amyloidogenic APP processing, but this was not observed during this analysis.

5xFAD mice lacking PCSK9 also displayed reduced microglial reactivity despite unchanged mRNA levels of genes related to microglial phagocytosis and homeostasis. This was assessed via quantification of ionized calcium-binding adapter molecule 1. Reductions in reactive astrocytes were also observed in the PCSK9 knockout mice as measured by GFAP; however, this difference only reached statistical significance in the corpus callosum and cingulate cortex. Given that both reactive microglia and astrocytes are characteristic of neuroinflammation in the AD brain, these findings, while encouraging, did not specifically elucidate the mechanisms by which this ostensible benefit was achieved.

There were no significant changes in gene expression related to lipid regulation between 5xFAD mice with and without PCSK9. These included APOE, LPL, and LRP1, although mRNA of APOE and LPL were increased in AD mice versus age-matched controls. There were also no changes in brain levels of measured oxysterols among groups, including the ostensibly neuroprotective 24s-OHC and the putatively neurotoxic 25-OHC and 27-OHC. However, a partial restoration in the levels of total brain cholesterol, which was decreased in the 5xFAD mice expressing PCSK9, was observed in the PCSK9 knockout mice. This appears to be in line with the wealth of other studies demonstrating decreased cholesterol synthesis in the AD brain; the potential benefit of CNS PCSK9 inhibition in this context is provocative.

Finally, memory and spatial learning were assessed using the Morris Water Maze. Mice lacking PCSK9 showed marked reductions in hippocampal-dependent impairments of cognition and

memory when compared to 5xFAD models expressing PCSK9. The 5xFAD PCSK9 knockout mice actually demonstrated similar performance to age-matched controls.

5.1.3 Summary and Analysis of Articles in Context of Lipid-Centric Model of AD

The first study adds compelling credence to the notion that PCSK9 plays an important role in Aβ clearance across the BBB via LRP1. Intriguingly, this benefit was demonstrated with peripheral extracellular inhibition of PCSK9 in mouse models of severe AD without any transfer of mAb across the BBB. Given that this therapeutic approach has demonstrated long-term safety with simultaneous efficacy in reducing CV events for humans, a conversation about the utility of peripheral PCSK9i for those with AD seems quite appropriate. Whether or not this same "peripheral sink" mechanism of LRP1-mediated amyloid clearance is impacted by PCSK9 in humans remains an unanswered question. Other unanswered questions are the role of PCSK9 in the CNS itself; would PCSK9i in the CNS impact LRP1 expressed in astrocytes, microglia, and neurons? And if so, does central PCSK9 mediate LRP1 degradation or influence the receptor in other ways? Additionally, the interaction between PCSK9 and LRP1 in the CNS may very well be cell-specific and context-dependent; this warrants further investigation. However, the plausibility of PCSK9i for those with established CVD and documented Aβ present in the brain would seem to be a rather low-risk, potentially high-reward intervention based on our current understanding.

The elegant and sophisticated second study demonstrated that global inhibition of PCSK9 may be an effective strategy to mitigate neuroinflammation, restore lipid homeostasis, decrease Aβ plaque burden, and attenuate impairments in cognition in animal models of AD. This suggests that the beneficial effects of PCSK9 inhibition may extend beyond the periphery to the CNS. A more detailed integration of this research will be discussed in **Chapter 7.**

Emerging data suggests that PCSK9 and its interactions with members of the LDLr family are key players in the context of neurodegeneration. The next section of the research will demonstrate whether or not community clinicians are aware of these interactions and if their current understanding of lipid-related factors in the context of AD has affected their practice patterns.

5.2 Results from Community Clinician Survey

5.2.1 Semi-Quantitative Analysis

63 out of the 109 clinicians (57.8%) responded to the 3-question survey administered in January of 2024. This included 23 physicians, 14 nurse practitioners, and 26 physician assistants. This was further subdivided into 41 primary care providers and 22 specialists. Among the primary care providers, 15 were physicians and 26 were either nurse practitioners or physician assistants, who collectively are referred to as advanced practice providers. Specialists, which included mostly community cardiology practitioners, included 8 physicians and 14 advanced practice providers. The baseline demographics and methods are detailed in the **Methods** section. The intent of

Survey Question 1 was to gauge the knowledge of clinicians regarding the association of cholesterol with Alzheimer's dementia. **Survey Question 2** aimed to assess whether or not clinicians were aware of the associations between desmosterol and PCSK9 with AD. I felt that this question would identify clinicians who possess advanced knowledge of the associations between lipid anomalies and neurodegeneration. The intent of **Survey Question 3** was to assess the impact of clinician perceptions and knowledge on their approach to treating patients. It was anticipated that many clinicians would elaborate on their approach in the free response section after answering in the affirmative or negative to this question. The specific questions in the survey as seen by the community clinicians can be found in **Section 4.1.2.**

There were 7 different combinations of answers that were possible given the dichotomous nature of the survey. These combinations are listed below:

- No, No, No
- No, No, Yes
- No, Yes, No
- Yes, No, Yes
- Yes, Yes, No
- Yes, No, No

There were zero responses of "No, Yes, No." A breakdown of the various responses is depicted below in **Figure 11.**

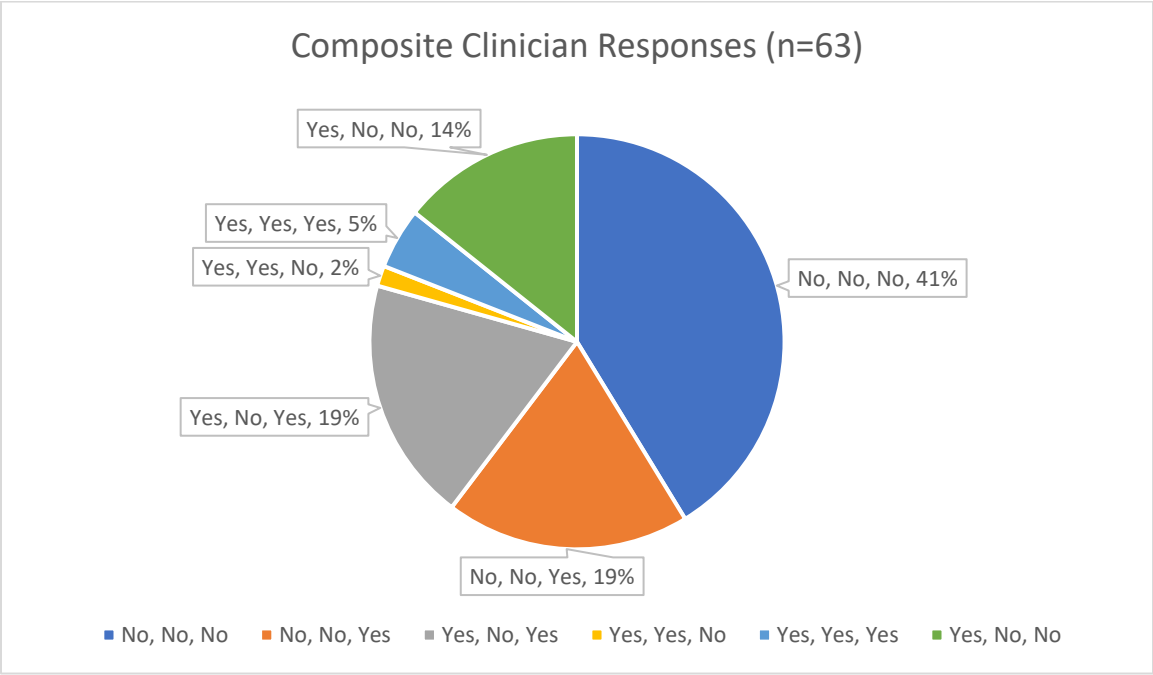

Figure 11: Composite Responses from Community Clinician Survey. The various combinations of responses from the 3-question dichotomous survey are represented above.

38 of the 63 respondents (60.3%) answered in the negative to **Survey Question 1**, and all 38 of those respondents, somewhat unsurprisingly, responded in the negative to **Survey Question 2**. The implication of these responses is that in-depth knowledge of the association between lipids and AD is unlikely if a baseline perception of the association between cholesterol and AD is not present. However, given that these clinicians obtain lipid panels and manage cholesterol metrics in their patients on a regular basis, the lack of knowledge regarding the association between cholesterol and the ubiquitous specter of neurodegenerative disease is concerning. The apparent gap in clinician knowledge regarding associations between cholesterol, lipids, and AD represents the identification of **Theme 1** gleaned from this portion of the research. Educational endeavors, both in didactic training as well as continuing medical education courses, would seem prudent given the relevance of lipid and AD management for community clinicians.

Conversely, only 25 of the 63 respondents (39.7%) answered in the affirmative to **Survey Question 1**, with even fewer (6.3%) responding in the affirmative to both questions 1 and 2. When sub-classified based on specialist versus PCP, specialists did have a higher percentage of affirmative answers (45.4%) to **Survey Question 1** when compared to PCPs. Overall, these data also highlight the lack of awareness among community clinicians regarding these associations. Logically, the majority of those who were aware of these more intricate associations adapted their practice patterns accordingly, although one respondent answered "No" to **Survey Question 3** despite answering "Yes" to the first 2 questions, representing an outlier of sorts.

Another observation that emerged was despite the implied general awareness of clinicians regarding the association between cholesterol and AD as noted by an affirmative response to **Survey Question 1,** this awareness did not necessarily result in modification of practice patterns. This occurred in 10 of the 25 (40%) who responded "Yes" to the first question. This implies that, although there is awareness of some association between cholesterol and AD, either the associations are perceived as somewhat nebulous or the clinicians' knowledge base is not robust enough to influence clinical decision making. Either way, this offers additional rationale for continuing education regarding the topic.

Curiously, 12 of the 38 clinicians (31.6%) who answered in the negative to both **Survey Questions 1 and 2** answered in the affirmative to **Survey Question 3.** This implies that, although clinicians are unaware of specific relationships between cholesterol, lipid homeostasis, and AD, they may still be adjusting their practice patterns in hopes of mitigating or ameliorating risk for neurodegeneration. Admittedly, the topic of cholesterol homeostasis in the context of neurodegeneration is complex and the relationships are less-than-straightforward. However, when practice patterns are impacted by perceptions ungrounded in empirical data or research, this represents a concerning observation noted here as **Theme 2.**

To summarize, two important themes emerged from this portion of the research.

Theme 1: There are apparent gaps in knowledge among community clinicians regarding the association of cholesterol and lipid homeostasis with Alzheimer's Dementia.

Theme 2: Despite a lack of tangible knowledge regarding specific associations between cholesterol and AD, community clinicians may be adapting their practice patterns in hopes of mitigating or ameliorating patient risk for neurodegeneration.

Figures 12-14 depict the clinician responses to each individual question of the survey.

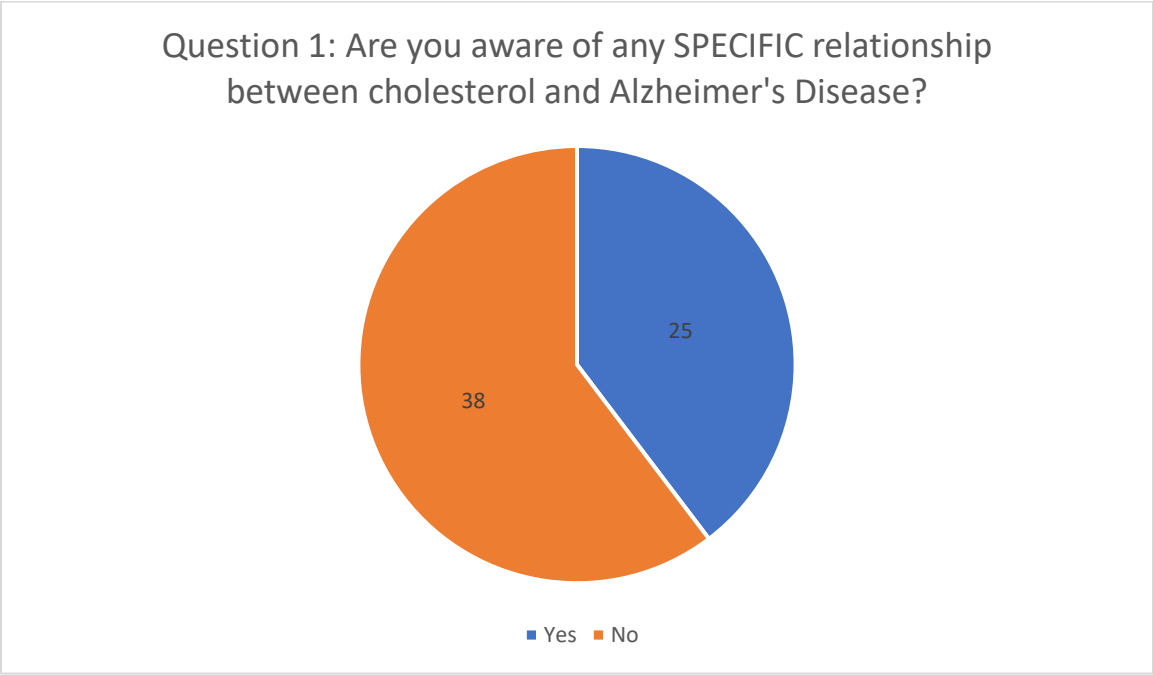

Figure 12: Clinician Responses to Survey Question 1.

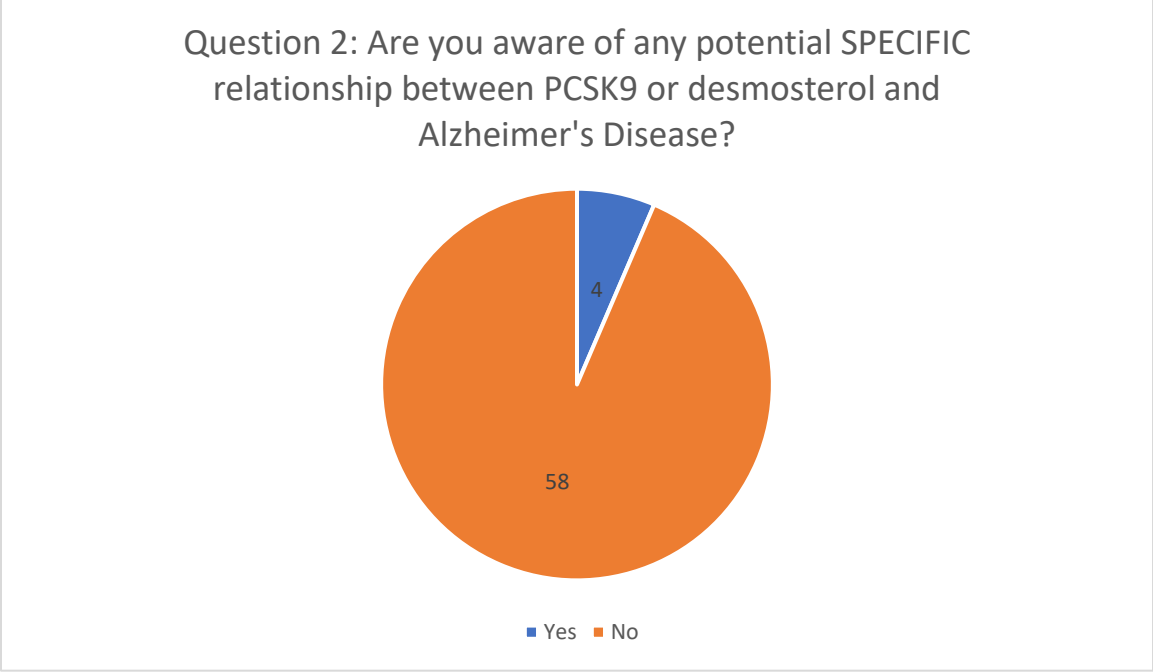

Figure 13: Clinician Responses to Survey Question 2

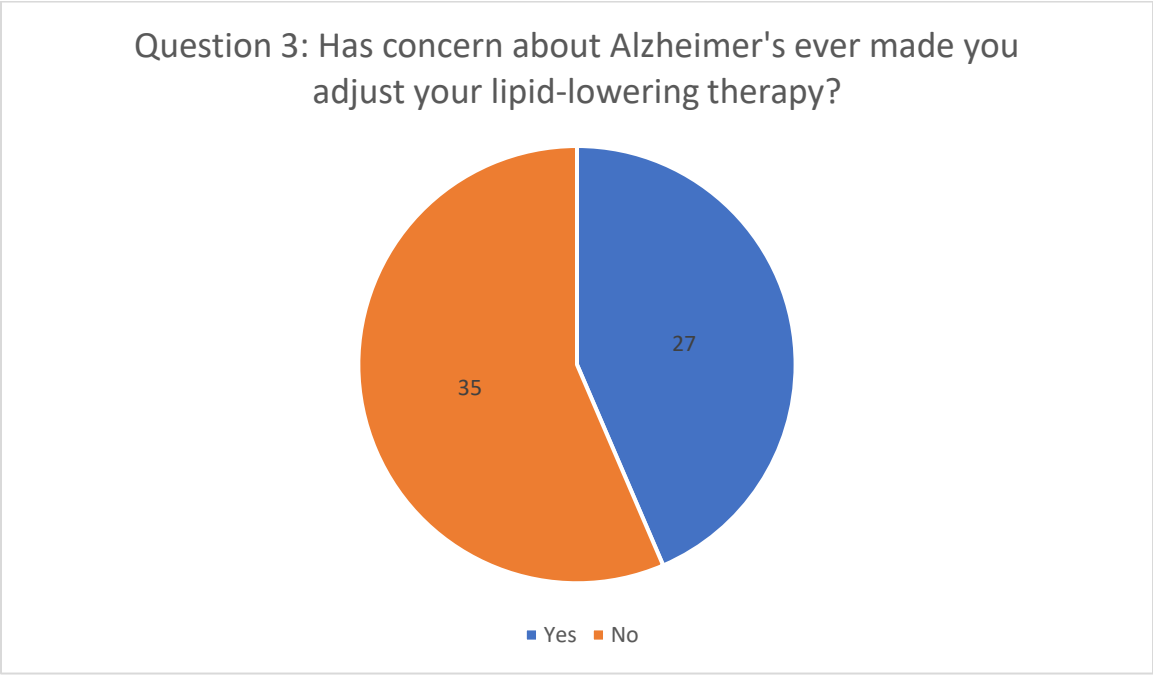

Figure 14: Clinician Responses to Survey Question 3

5.2.2 Qualitative Analysis Based on Clinician Free Response

Survey Question 3 encouraged elaboration on the part of community clinicians regarding how their perceptions regarding lipids and AD had impacted their treatment approach. This provided opportunity for further qualitative data ascertainment, particularly among the 27 (42.9%) who stated that their perceptions had impacted their clinical decision making as based on a "Yes" response to **Survey Question 3.**

A notable sub-theme emerged from this qualitative accruing of clinician perspectives. Among the clinicians who answered "Yes" to **Survey Question 3** despite answering in the negative to the first 2 questions, a common thread of patient influence surfaced repeatedly. NP-8 stated that several statins had been discontinued within the practice based on concerns of AD voiced by patients. This sentiment was echoed by several other clinicians, including PA-1, who said, "I have had patients refuse to go on statins because of the reported link to Alzheimer's." Others adjusted lipid-lowering therapy if LDL-c levels drifted below an arbitrary threshold deemed "too low" after discussing the risks and benefits with patients, and NP-4 took an approach that would be regarded as extreme by most of the medical establishment, stating the following:

"I will continue statins if a person comes to me on them, but I don't start them. I will try to focus on lifestyle changes and I will use supplements, and I will try other non-statin lipid-lowering agents first."

On one hand, the influence of patients' opinions on practice patterns suggests a patient-centered approach involving shared decision making. On the other hand, if intervention is solely based on patient biases without any foundation of scientific acumen, the risk of providing suboptimal care becomes a genuine concern. The implications of ineffective or uninformed treatment for patients when it comes to conditions such as ASCVD and AD are quite sobering, which further magnifies the need for education on these topics.

Another sub-theme that emerged from the free responses volunteered by clinicians were specific instances of patients who had experienced cognitive side effects while on statin therapy. 7 of the 63 clinicians surveyed (11.1%) mentioned one or more specific patient cases in their free responses, and multiple clinicians mentioned Atorvastatin specifically as culpable in this adverse effect. One clinician, PA-11, described an instance in which the patient complained of brain fog after starting a statin. The statin was subsequently discontinued and the patient instead was prescribed a PCSK9 inhibitor; the patient's cognitive issues resolved after several months. However, despite real-world observations of statin-associated cognitive decline within the practice, NP-1 stated "it's hard to know if the statins are causative or not." Multiple clinicians did express a preference for hydrophilic statins such as Rosuvastatin, as they cited its lesser capacity for traversing the BBB as rationale for its selection compared to more lipophilic agents. One clinician mentioned avoidance of statins if patients had evidence of cerebral amyloid angiopathy, and several other clinicians mentioned concern about statin use in the elderly. PA-16 had a specific approach to the elderly patient, stating "If patients are over age 75 I reduce statin dose to low-intensity unless there is a history of CAD with myocardial infarction."

The theme of gaps in knowledge coupled with general lack of consensus on the topic was further clarified by the free responses. Some clinicians mentioned they were aware of ApoE4 as a risk factor for AD, but not all of these clinicians were confident of its association with lipid transport or cholesterol regulation. Additionally, there was confusion regarding the differentiation between vascular dementia and AD; some clinicians correctly identified the distinction, while others implied that AD and vascular dementia were different ways to describe the same entity. One clinician asserted that the associations between cholesterol and dementia had all been debunked. This hodgepodge of opinions further reinforces the need for further education on the topic.

Aggressive risk stratification and intervention with multiple agents was a common thread among the few clinicians who answered "Yes" to all three questions. NP-7 stated that testing for ApoB, Lp(a), *APOE* genotype as well as employing diagnostic imaging such as coronary artery calcium scanning was important given the overlap between ASCVD and AD; this particular practice also readily employed multiple lipid-lowering agents utilized in combination, including PCSK9i.

PY-5, who was even aware of the associations between PCSK9, desmosterol, and AD, stated that although the associations are compelling, there remains an unmet need to study these specific

lipid-related factors in broader populations with greater specificity prior to these associations influencing practice. This effectively summarizes the need for further education and further exploration of the relationships between lipid homeostasis and the interaction between PCSK9 and its ligands proposed in this project.

Chapter 6-Case Study

6.1 Case Report

A 78 year-old male presented to the clinic for evaluation. His past medical history was significant for carotid atherosclerosis, homozygous C677T *MTHFR* gene mutation, and deep vein thrombosis. Notably, his *APOE* genotype was 3/3 and his Lp(a) levels were within normal limits. He had been diagnosed with Alzheimer's Dementia 2 years prior and sought treatment at the Boone Heart Institute. The baseline evaluation included blood-based biomarkers along with WAVi evaluation as described in the Methods section. Initial laboratory measurements are listed below in **Table 9**.

Table 9: Case Study Baseline Labs

Lab	**Result**
Total cholesterol	214
LDL-c	122
HDL-c	73
Triglycerides	201
Lp-PLA2	223
Hs-CRP	4.1
IL-6	9.6
Homocysteine	14
ApoB	91

Baseline WAVi scan showed a brain power as quantified by peak amplitude of 6.5 mV, latency of 248 mS, and reaction time of 328 mS.

The initial treatment approach from a lipid standpoint was Rosuvastatin 20 mg taken orally once per day. This was applied in conjunction with various lifestyle modification and supplementation per a typical institutional protocol. No data was available regarding other pharmacologic interventions.

After 1 year of Rosuvastatin 20 mg, repeat labs were obtained and results are listed below in **Table 10**:

Table 10: Case Study Follow-up Labs

Lab	**Result**	**Previous Result**
Total cholesterol	118	214
LDL-c	50	122
HDL-c	56	73
Triglycerides	75	201
ApoB	50	91
Lp-PLA2	186	223
HS-CRP	1.0	4.1
IL-6	12.4	9.6
Homocysteine	15	14

Repeat WAVi scan was obtained and brain power increased from 6.5 mV to 12.2 mV. Latency and reaction time increased from 248 to 280 mS and from 328 to 444, respectively. A comparison of the topographical heuristic WAVi scan results before and after intervention is depicted below in **Figure 15**.

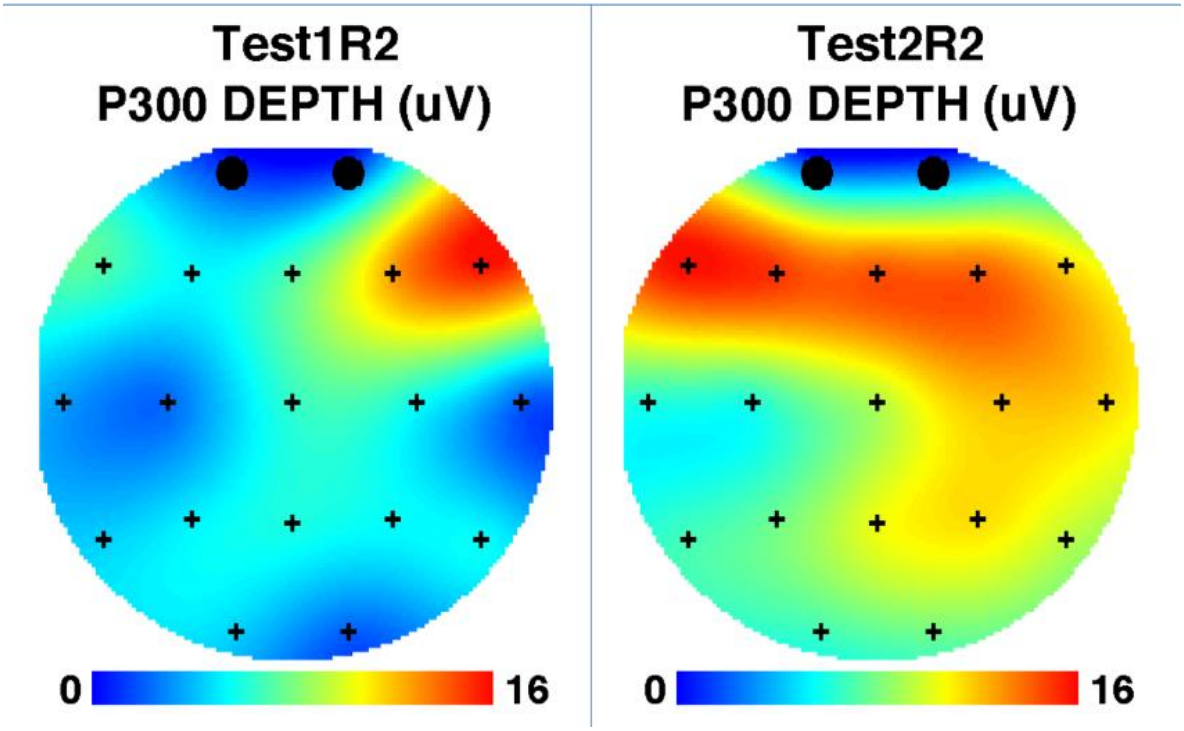

Figure 15: WAVi Scan Pre and Post Intervention. This topographical depiction of increased global brain amplitude as measured by the P300 testing of event-related potentials parallels the improvements in biomarkers seen in this patient case. Brighter colors represent increases in amplitude, which did increase from 6.5 mV to 12.2 mV after one year of initial intervention, which included Rosuvastatin 20 mg for lipid management.

The decision was made at that time to proceed with the addition of a PCSK9i mAb. This decreased the patient's LDL-c to 10 and further improved his brain power from 12.2 to mV to 16.0 mV upon repeat testing. Other lab data was not available. Remarkably, the patient reported no further cognitive decline and was stable on his regimen of statin plus PCSK9i mAb when followed for 7 years after his initial diagnosis of Alzheimer's Disease.

6.2 Case Discussion

This case report supplements the conversation regarding lipid modulation in the context of Alzheimer's Disease. This individual was selected due to having been diagnosed with Alzheimer's Disease prior to presentation at the Boone Heart Institute. Interestingly, this individual was not an ApoE4 carrier; it would have been useful to ascertain more specific data about his history, although he did have established carotid disease. Additionally, the elevated homocysteine that often accompanies those with the C677T allele of the *MTHFR* gene has been associated with development of dementia.

The initial lipid-lowering intervention was the hydrophilic Rosuvastatin, and demonstrable benefit was observed in reducing total cholesterol, LDL-c, ApoB, and triglycerides. The reduction in

triglycerides was almost assuredly due to modification in lifestyle factors, as the reduction from 201 mg/dL to 75 mg/dL would not be expected due to the selected lipid-lowering therapy alone. Given that elevated triglycerides are a harbinger of insulin resistance, this suggests that this individual's insulin sensitivity improved between baseline lab evaluation and follow-up. As glucose homeostasis and insulin sensitivity are critical in optimizing cognitive function, this was likely a contributory factor in the increased brain power observed on the WAVi scan. Pre-treatment and post-treatment HbA1c and fasting insulin/fasting glucose levels would have been helpful in further making this assertion along with specifics regarding other pharmacologic and non-pharmacologic interventions.

Along with the reduction in potentially atherogenic lipid species, the individual's hs-CRP was reduced from 4.1 to 1.0. Statins are known to reduce this non-specific measure of inflammation, but curiously the patient's IL-6 increased further from 9.6 to 12.4. IL-6 may be more specific to ASCVD, and it would have been interesting to see if the addition of PCSK9i reduced this marker, as many studies have shown a reduction in IL-6 when PCSK9i mAbs are employed. Lp-PLA2, another biomarker associated with ASCVD, was reduced by the initial intervention, but homocysteine increased slightly. Additional measurements of these markers after addition of PCSK9i were not available, although meaningful change in these biomarkers based on known mechanisms of this drug class seems unlikely.

Compellingly, addition of PCSK9i mAb resulted in an LDL-c of 10 with a very likely concordant decrease in ApoB. Additionally, the brain power as measured by WAVi further increased after one year of PCSK9i mAb. Interestingly, brain speed and reaction time decreased after one year of statin monotherapy. These reductions do characterize the normal aging process, and genetic loss-of-function in HMGCR is associated with decreased reaction time; however, one cannot draw conclusions based on this limited data. Unfortunately, follow-up latency and reaction time data after PCSK9i was not available; it would have been interesting to see if this additional intervention attenuated the loss in brain speed. However, the stability in day-to-day function and cognition of this individual 7 years after diagnosis of AD does provide hope that appropriate and nuanced interventions can mitigate what would seem to be inevitable decline.

This case report, while interesting, has many limitations. Many of the additional interventions beyond lipid modification were unavailable, and a complete dossier of data after treatment with PCSK9i was also unavailable. Additionally, despite the association of brain power assessed by WAVi with various neurodegenerative disease states, correlation with well-established markers of cognition such as the ADAS-Cog, would have been useful as well. Hopefully this non-invasive marker of brain activity can be better correlated with other imaging modalities, AD-specific biomarkers, and cognitive tests in the future to determine whether or not it can be a useful tool for prognostication and assessment of treatment efficacy.

Chapter 7-Discussion

7.1 Purpose of the Research

This dissertation sought to generate a framework that reconciles the core defects observed in AD through a lipid-centric lens. These core defects include impaired amyloid clearance, dysfunctional substrate utilization, defective lipid transport, and the constellation of overlapping factors that contribute to ASCVD. This provides a valuable perspective through which to view a complex disease and provocatively fills gaps in the current literature while identifying future directions for ongoing research. The anomalies in lipid homeostasis observed in the AD brain include dysregulated lipid transport, decreased cholesterol synthesis, accumulation of toxic oxysterols, and impaired lipid efflux capacity. In this lipid-centric model, PCSK9 and the members of the LDLr family were identified as relevant participants in the pathophysiologic process. Synthesizing this available information while continuing to explore the unknowns regarding the interplay between substrate utilization and lipid homeostasis was consequently guided by the following research questions:

Research Question 1: What is the relationship between PCSK9 and members of the LDLr family in the central nervous system, and is PCSK9 a bystander or active contributor to the development of Alzheimer's Disease?

Research Question 2: Is peripheral PCSK9 inhibition an efficacious strategy to mitigate the progression of Alzheimer's Disease and, if so, by what mechanisms could this confer benefit?

An additional aim of the research was to identify the current knowledge among community clinicians regarding the relationship between cholesterol homeostasis with AD. This was guided by the following research question:

Research Question 3: What is the awareness of community clinicians regarding the relationship between lipids, cholesterol homeostasis, and PCSK9 in the context of AD? Do these opinions or perceptions influence prescribing patterns of cholesterol modulating drugs?

From here, dual themes of insufficient knowledge and prescribing patterns ungrounded in scientific data emerged from the research. The qualitative responses of clinicians further reinforced the lack of consensus and diversity of perceptions regarding this important topic.

7.2 Discussion, Implications, and Recommendations

The 3-question survey revealed significant gaps in understanding of the relationships between lipids, cholesterol, and Alzheimer's Disease among community clinicians. Perhaps more concerning, although many reported no specific knowledge of the associations between various lipid factors and Alzheimer's, their prescription of lipid-lowering therapies was still influenced by perceptions ungrounded in tangible data. Humble admission of the topic's complexity while

establishing what is currently known regarding the topic seems an urgent endeavor to mitigate irresponsible prescribing patterns. Although the role of LDL, statins, and non-statin therapies such as PCSK9i mAbs are well-established in the context of ASCVD, there remains a paucity of high-risk patients achieving guideline-based lipid goals and a dearth of eligible patients receiving PCSK9i therapy. Educational programs must be taken to address the knowledge gaps and clinical inertia in ASCVD, and then perhaps the gaps in understanding the association of lipids with AD can subsequently be filled as well. Given the ubiquity and overlap between these conditions, increased knowledge of both ASCVD and AD can help empower clinicians to better assist their patient populations.

The research provides compelling reasons to further explore PCSK9 and its numerous ligands in the context of ASCVD, lipid homeostasis, and neurodegeneration. Harkening back to the Home Security System and Vascular Neighborhood model of ASCVD risk stratification, a therapy that confers no deleterious effect on glucose metabolism, a key pillar of the Home Security System, while allowing individuals to live in a safer Vascular Neighborhood by virtue of reducing CV events, would seem an obvious tool for ASCVD management. Consequently, PCSK9 inhibition represents a potentially attractive pharmacologic target for not only its benefits, but also for its lack of negative effects. However, this tool may have broader application beyond the peripheral circulation. Given the importance of substrate utilization in the brain, preservation of glucose homeostasis, and by extension, optimization of mitochondrial efficiency, should be prioritized. Ergo, therapies that do not adversely affect glucose metabolism would seem preferable to those that could impair this process. Therapies inhibiting PCSK9 in the periphery possess a neutral effect on glucose metabolism and have actually shown a reduction in postprandial lipemia. This reduction in potentially atherogenic remnant lipoproteins could serve a benefit in not only reducing residual CV risk, but perhaps serve benefit in maintaining the integrity of the proverbial CNS neighborhood.

PCSK9 inhibition does not affect cholesterol synthesis; it merely enhances the clearance of lipoproteins. Given that cholesterol synthesis is decreased in the brains of those with AD, further decreasing sterol production could be problematic in those with levels that are already suboptimal. Biomarkers such as plasma desmosterol may be helpful in identifying those who would benefit from peripheral PCSK9 inhibition in lieu of high-dose statin therapy; enhancing clearance via PCSK9 mitigates the lipoprotein-mediated risk that may contribute to AD while avoiding any perturbations to synthetic pathways. Additionally, PCSK9i mAbs do not traverse the BBB, whereas statins, particularly lipophilic moieties, may cross the BBB. Whether or not statins increase brain PCSK9, whether or not lipophilic statins affect brain PCSK9 to a greater degree than hydrophilic agents, and whether or not this is of physiologic significance is unknown. Real-world pharmacovigilance studies suggest that all statins, independent of hydrophilicity, may be associated with adverse neurocognitive events. However, subjective reports of these events may be confounded by a plethora of factors. Mechanistically, statins may be helpful in certain

contexts, but could perhaps be problematic if basal sterol production is already impaired. Given that LDLr overexpression has been helpful in animal models of AD, upregulation of LDLr via PCSK9 inhibition represents a means in which to achieve this without impairing cholesterol synthesis via HMGCR inhibition. Indeed, the Mendelian Randomization showing decreased cognitive scores and cortical surface area in those with HMGCR loss-of-function compared with the unaffected PCSK9 loss-of-function individuals suggests that PCSK9i may be a "cleaner" mechanism by which to increase LDLr until we better understand the myriad of interactions in the CNS that could lead to a more precise pharmacologic approach. This research suggests the current lack of precision stemming from gaps in understanding are already influencing the prescribing patterns of community clinicians, which is a sobering reality given the sheer magnitude of neurodegeneration's individual and societal impact.

Peripheral inhibition of PCSK9 appears to not only be safe, but possibly beneficial in mitigating neurodegeneration. The case report with baseline WAVi assessment before and after PCSK9i mAb intervention parallels the Mazura, et al study demonstrating improvements in cognition and accelerated amyloid clearance in animal models. These findings lend additional credence to the peripheral sink hypothesis; inhibition of PCSK9 in the endothelial cells at the BBB junction enhances transcytosis of Aβ prior to hepatic LRP1 disposal. This process is dependent upon LRP1; further studies are necessary to better understand the interaction between PCSK9 and LRP1. It would appear that PCSK9 mediates LRP1 for degradation in the pericytes at the BBB interface; follow-up investigations should explore the precise nature of this interaction between LRP1 and PCSK9 not only in the endothelial cells of the BBB, but also at the liver. Additionally, the interaction between LRP1 and PCSK9 in the CNS warrants further attention. It is not currently known whether PCSK9 mediates LRP1 for degradation in astrocytes, microglia, and neurons, or whether it impacts LRP1 function in other ways. The manner in which PCSK9 interacts with LRP1 may vary depending on cell type, ApoE genotype, and stage of disease progression. These relationships must be explored further to better understanding whether or not global PCSK9 inhibition, including in the CNS, would be an effective strategy to combat neurodegenerative changes. The mouse study of global PCSK9 ablation demonstrating decreased Aβ accumulation and improved cognition suggests that this is a compelling avenue, but more research is necessary. Similarly, a further exploration of interactions between PCSK9, megalin, and LRP5 in the CNS may be a worthwhile pursuit. PCSK9 degrades megalin in the renal tubular system, leading to albuminuria, and given the role of megalin in amyloid clearance across the BBB this relationship ought to be further explored. Additionally, LRP5 was recently shown to mitigate neuronal apoptosis; given that the partnership between LRP5 and PCSK9 in the periphery amplifies atherosclerotic progression, the consequence of any CNS relationship seems a prudent investigation as well.

On a related note, small studies of nutraceuticals such as curcumin with known inhibitory effects on PCSK9 have shown benefit in reducing Aβ and improving cognition in non-demented

individuals. Whether or not the PCSK9 inhibition, either peripherally or centrally, of curcumin has any effect on the process is unknown but represents an additional avenue of research. Any agent impacting PCSK9 in the CNS also may have greater or less utility depending on degree of Aβ aggregation and baseline cognitive status, among a host of other factors. As research progresses, testing safe and efficacious modalities in various stages of disease progression will be essential in determining when and for whom individual interventions are best employed. Other relevant innovations in this discussion include ongoing development of small molecule inhibitors of PCSK9; testing whether or not these agents possess the capability to traverse the BBB will also be of particular interest moving forward.

The interaction between other members of the LDLr family in the CNS also needs further study. Peripheral PCSK9 mediates VLDLr and ApoER2 for degradation, and investigation into the precise manner by which PCSK9 interacts with these receptors could better help identify whether or not PCSK9 is actually involved in the process of neuronal apoptosis. Indeed, the elevations of PCSK9 in AD brains and other conditions characterized by neuronal loss, such as alcohol use disorder, beg the question of whether or not PCSK9 is contributing to the disturbed cerebral milieu or is merely a marker of processes gone awry. Given its association with neuroinflammation, neutrality of PCSK9 in this process is unlikely, although the possibility of PCSK9 attempting to compensate in the context of aberrant CNS lipid trafficking cannot be fully ruled out apart from more detailed investigations. Other unanswered questions regarding the interaction of PCSK9 with CD36 in microglia and the potential link between PCSK9, LPL, TREM2, and Aβ phagocytosis ought to be considered for further experimentation as well. PCSK9 inhibition in the periphery does decrease ApoC3 to a modest degree, and given that ApoC3 is an inhibitor of LPL, whether or not this has any relevance in the action of microglial LPL activity remains to be seen. Additionally, insulin is the most potent hormonal activator of LPL; whether or not a plausible benefit of PCSK9i on adipocyte insulin resistance could indirectly modulate CNS insulin signaling is also unknown, although the physiologic significance of this interaction seems unlikely.

Correlating blood-based biomarkers with AD would also be helpful in determining individuals who could benefit from various therapies. The correlation between plasma desmosterol and AD is relatively strong but needs further validation in larger cohorts. Additionally, better correlation of plasma PCKS9 with PCSK9 levels in the CSF would be a logical next step. From there, these correlations could then be made with data gleaned from imaging techniques such as WAVi and PET scans as well as with parameters of cognition. Correlating WAVi with amyloid PET scans would also be an interesting avenue, as the ostensible resolution of impaired CNS processing seen on WAVi may or may not correlate with amyloid burden. Other biomarkers such as oxidized LRP1, 24s-OHC, GFAP, NFL, and various Aβ and tau isoforms will hopefully be more rigorously studied and correlated with imaging and measures of cognition. In the meantime, obtaining larger data sets of the better-established lipid biomarkers such as desmosterol, ApoB, and Lp(a) across diverse populations of varying ApoE genotypes could help guide research moving forward. A

summary of the research, hypotheses formulated based on the research findings, unanswered questions, and future directions is outlined in **Table 11.**

Table 11: Summary of Research and Future Directions

What the Research Indicates	Hypothesis	Unanswered Questions
PCSK9 is elevated in the brain and CSF of those with AD	PCSK9 is directly contributing to neurodegeneration via interactions with LDLr family members	Is PCSK9 promoting neurodegeneration or is it a marker/bystander? What is the precise nature of PCSK9 interaction with LDLr, LRP1, ApoER2, and VLDLr in the CNS? Do these interactions vary between cell types? Do the deleterious interactions of PCSK9 with other binding partners such as LRP5 and megalin in the periphery portend similar consequences in the CNS or at the BBB interface?
Desmosterol levels and other markers of sterol production are decreased in AD	Decreased astrocytic cholesterol production and ApoE-mediated delivery to neurons propagates cognitive decline	Are statins a useful agent in the context of neurodegeneration? Who should we be concerned about in regards to statin therapy and AD?
Peripheral PCSK9 inhibition increases plasma levels of 24s-OHC Oxysterols accumulate in the AD brain	PCSK9 inhibition may be positively regulating cholesterol efflux	Is 24s-OHC a meaningful biomarker for large population sets? If peripheral PCSK9i improves sterol efflux, what role would CNS PCSK9i have on oxysterol species?
PCSK9 inhibition improves brain power as measured on WAVi	Peripheral PCSK9i represents a safe and effective way to mitigate neurodegeneration	Is PCSK9i beneficial regardless of ApoE genotype, baseline lipid levels, or phase of disease progression?

Peripheral PCSK9 inhibition increases Aβ clearance across BBB and improves cognition in mice	Peripheral PCSK9i may enhance Aβ clearance across the BBB in a similar LRP-dependent manner in humans	Would central PCSK9 inhibition in the right context have additive value in those with established disease?
Diabetes, ASCVD, and hyperlipidemia are associated with AD	Lipid-lowering strategies that do not increase risk of NODM while reducing atherogenic lipids and ASCVD risk are preferred to mitigate risk of AD.	In what situations would statins vs PCSK9i vs other LLT be best employed in combination or monotherapy to optimize both ASCVD risk and preservation of cognition? Would this strategy vary based on stage of disease progression?
Statins increase PCSK9 in a dose-dependent manner PCSK9 does not cross the BBB but is expressed in the brain	Lipophilic statins may increase brain PCSK9 levels to a greater degree than hydrophilic moieties	Would a statin-induced increase in brain PCSK9 have any meaningful physiologic effect? Would potential increase in CNS PCSK9 expression differ depending on cell type?
Nutraceuticals that possess inhibitory properties on PCSK9 may be neuroprotective. Curcumin, which does inhibit PCSK9, reduced Aβ and improved cognition in an RCT of adults without dementia at baseline	The PCSK9 inhibition conferred by agents such as berberine and curcumin may be safe and effective ways to mitigate neurodegeneration.	Is PCSK9 inhibition the mechanism of action by which benefit is conferred with these agents? Could this have potential relevance in the development of PCSK9 small molecule inhibitors? Is the degree of PCSK9 inhibition conferred by these agents physiologically consequential? If so, is this due to peripheral inhibition, central inhibition, or both? Does the benefit apply to people with and without established AD?

7.3 Limitations

There are many limitations to this analysis, as this is a field of ongoing research; PCSK9 itself was only discovered slightly over 20 years ago. One major limitation is the dearth of available studies that have investigated these relationships. Indeed, the search criteria unearthed only 2 animal studies, and studying the effect of PCSK9 in the human CNS would be a brazen endeavor at this point given our incomplete understanding of its role. Most of the discussion in this analysis relies on paradigms of peripheral lipid trafficking and disease associations with biomarkers that need to be studied in larger cohorts. Additionally, results obtained from in vitro studies and animal experiments cannot be generalized to humans. Although this is a lipid-centric view of AD, there are other factors that may contribute to the spectrum of neurodegenerative disease; indeed, there is much overlap between AD and other conditions characterized by cognitive decline, such as Parkinson's Disease, vascular dementia, and Lewy-Body dementia.

The obvious limitation of the community clinician research was the small sample size; the results garnered from the cohort of PCPs, community cardiologists, and endocrinology practitioners may not be generalizable to the broader population. The results from the survey may have also been limited by non-response bias. However, the same sort of methodology could be applied to larger cohorts in a variety of settings to better identify the knowledge gaps regarding the topic. Additionally, the qualitative insights gained from the clinicians illustrate the diverse opinions regarding the topic of cholesterol, lipid-related factors, and AD. This further strengthens the view that a combination of further research and simultaneous education is necessary to better assist clinicians who are on the frontlines of lipid management and neurodegenerative disease intervention.

Chapter 8-Conclusion

Currently, there remains a dearth of well-established, safe, and efficacious interventions for Alzheimer's Dementia. Neurodegeneration is a complex continuum of disease processes, and I have made what I feel is a compelling case to view AD through a lipid-centric framework. This incorporates the various anomalies observed in AD, including impaired substrate utilization, dysregulated lipid homeostasis, deficient amyloid clearance, and a constellation of overlapping contributors that contribute to both ASCVD and AD. The balance between cholesterol production, transport, and efflux are critical in maintaining an optimized cerebral milieu, and PCSK9 with its litany of ligands appear to serve important roles in this process.

In conclusion, peripheral PCSK9 inhibition has demonstrated benefit in reducing atherogenic lipoproteins, stabilizing and regressing coronary plaque, and reducing major adverse cardiovascular events. It also may represent an effective strategy in mitigating AD progression via its interaction with LRP1 at the BBB and may also have other pleiotropic benefits that result in decreased neuroinflammation. Given that ASCVD, elevated LDL-c, diabetes, and decreased cholesterol synthesis have all been correlated with AD, a therapeutic class that reduces ASCVD and LDL-c while demonstrating no effect on blood glucose or cholesterol synthesis is compelling.

Significant knowledge gaps remain among community clinicians regarding the association between lipid-related factors, management of cholesterol, and AD. This research highlighted the sobering reality that, among a small cohort of community clinicians in the United States, factors such as patient perceptions regarding AD and biases not necessarily grounded in tangible scientific data are influencing prescribing patterns. These gaps in understanding among community clinicians justifies urgent attention, and filling this chasm with currently available data represents a worthwhile crusade. A good portion of this research attempted to synthesize this information, and perhaps this research itself may provide a useful resource for clinicians seeking to better understand the relationships between the litany of factors that contribute to AD.

However, the interaction between PCSK9 and members of the LDLr family warrants further investigation. Although it appears that the elevated PCSK9 levels seen in the brains of those with AD may be contributing to neurodegeneration, the precise interaction between PCSK9 and LRP1, LDLr, VLDLr, and ApoER2, among other binding partners, must be further explored. Additionally, if attributing malignant causality to a deleterious interaction between PCSK9 and one or more of these LDLr family members appears likely, quantifying the individual relative contribution to the perturbed cerebral milieu may be difficult. Studying this interplay among various ApoE genotypes, in various CNS cell types, and in varying stages of disease progression will be necessary to better understand these relationships. PCSK9 could perhaps be an actionable target not only in the periphery, but in the CNS if these interactions are better elucidated. The possibility of an intervention that weaves together the tapestry of optimizing substrate utilization, rectifying lipid homeostasis, and promoting Aβ clearance is arguably quixotic, yet undeniably exciting. Even

more compelling than this lipid-centric model of AD, however, is the thought of a future in which the vast extent of neurodegeneration becomes a distant memory. With a combination of further research and effective dissemination of the knowledge gleaned during the continued exploration, I feel this is eminently possible.

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Appendix A: Community Clinician Demographics and Responses

Respondent	Specialty	Question 1	Question 2	Question 3
NP-1	Primary Care	No	No	Yes
NP-2	Primary Care	Yes	No	Yes
NP-3	Cardiology	No	No	Yes
PA-1	Primary Care	No	No	Yes
PY-1	Cardiology	No	No	No
PY-2	Primary Care	Yes	No	Yes
NP-4	Primary Care	No	No	Yes
PA-2	Cardiology	No	No	Yes
PY-3	Primary Care	No	No	No
NP-5	Primary Care	No	No	Yes
PY-4	Primary Care	No	No	No
PY-5	Cardiology	Yes	Yes	No
NP-6	Primary Care	No	No	No
PA-3	Cardiology	No	No	No
NP-7	Endocrinology	Yes	Yes	Yes
PA-4	Primary Care	Yes	No	Yes
PY-6	Primary Care	Yes	No	No
PY-7	Cardiology	Yes	No	Yes
PY-8	Primary Care	Yes	No	Yes
NP-8	Cardiology	No	No	Yes
PA-5	Cardiology	Yes	No	Yes
PA-6	Primary Care	No	No	Yes
PA-7	Cardiology	No	No	No
PA-8	Primary Care	No	No	Yes
PY-9	Cardiology	Yes	No	No
PY-10	Primary Care	No	No	Yes
PA-9	Primary Care	No	No	No
PY-11	Cardiology	Yes	Yes	Yes
NP-9	Cardiology	Yes	No	No
NP-10	Cardiology	Yes	No	Yes
PA-10	Cardiology	No	No	No
PY-12	Primary Care	No	No	No
PA-11	Cardiology	No	No	Yes
PY-13	Primary Care	Yes	Yes	Yes
PY-14	Primary Care	No	No	No
PY-15	Primary Care	No	No	No
PA-12	Primary Care	No	No	No
PA-13	Primary Care	No	No	No

PA-14	Primary Care	No	No	No
NP-11	Primary Care	No	No	No
PA-15	Primary Care	Yes	No	No
PA-16	Primary Care	Yes	No	Yes
NP-12	Primary Care	Yes	No	Yes
PY-16	Primary Care	Yes	No	Yes
PA-17	Primary Care	No	No	No
PA-18	Primary Care	No	No	No
PA-19	Primary Care	No	No	No
PY-17	Primary Care	No	No	No
PY-18	Endocrinology	No	No	Yes
PA-20	Primary Care	Yes	No	No
PY-19	Primary Care	Yes	No	No
PA-21	Endocrinology	No	No	No
PA-22	Primary Care	No	No	No
PA-23	Primary Care	No	No	No
PA-24	Primary Care	No	No	No
PY-20	Primary Care	Yes	No	Yes
PA-25	Primary Care	Yes	No	No
NP-13	Primary Care	Yes	No	Yes
NP-14	Cardiology	No	No	No
PY-21	Primary Care	Yes	No	No
PY-22	Cardiology	Yes	No	No
PA-26	Cardiology	No	No	No
PY-23	Cardiology	No	No	Yes