Better Oral Health for a Healthy Cognition: Investigation of a New Pathway

Kimia Rohani

Faculty of Dental Medicine and Oral Health Sciences

McGill University

Montreal, Quebec, Canada

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Abstract

Background: Tooth loss has been suggested as a risk factor for cognitive decline. Several biologically plausible explanations have been put forward to explain this oral-systemic connection. However, these purported mechanisms fail to consider the role of age-related cholinergic neurons' degeneration as a potential common cause behind this association.

Objective: The overarching objective of this study was to investigate the association between cholinergic neurons' activity, and oral and cognitive health. Specifically, we aimed to first identify oral health and cognitive health clustering patterns among middle-aged to elderly Canadians, and second, to investigate the extent to which these patterns could be explained by a proxy measure of the cholinergic neurons' activity (bone mineral density).

Methods: Baseline data from the Comprehensive cohort of the Canadian Longitudinal Study of Aging (CLSA), which recruited participants aged 45 to 85, was used to fulfill the aims of this project. First, I used latent class analysis to identify oral health and cognitive health clusters. Oral health was assessed by a self-report questionnaire, whereas seven task-based instruments measured cognitive health (i.e., retrospective and prospective memory, verbal fluency, and cognitive interference inhibition). Oral health and cognitive health clusters were then used as the outcome variables in multivariate nominal logistic regression models to investigate whether bone mineral density, a proxy for cholinergic activity, can explain the odds of being classified in a certain oral/cognitive health group. In our final multivariate analysis, we adjusted for age, sex, education, total household income, ethnicity, alcohol consumption, smoking, hypertension, and diabetes.

Results: Our study sample (N=25,444: 13035 males, 12409 females) were grouped into 5 and 4 clusters based on their self-reported oral health status and performance on cognitive tasks,

respectively. In the final multivariate regression models and after adjusting for all potential covariates, most 95% confidence intervals ranged from <1.0 to around 3.0, supporting a mild association between bone mineral density and odds of membership in any of oral health or cognitive health classes, compared to the classes with the worst oral and cognitive health.

Conclusion: Middle-aged and elderly Canadians show different oral and cognitive health profiles, based on their denture wearing status and performance in memory and verbal fluency tests. Clustering of participants based on their oral health and cognitive health status could not be explained by a proxy of cholinergic activity after adjusting for sociodemographic factors, chronic conditions, and health-related behaviors.

Résume

Contexte : L’édentulisme a été suggérée comme un facteur de risque pour le déclin cognitif, avec plusieurs explications biologiques plausibles pour cette connexion orale-systémique. Cependant, ces explications ne considèrent pas le rôle de la dégénérescence des neurones cholinergiques liée à l'âge comme une cause commune potentielle.

Objectif : La cible fondamental de cette étude est investiguer le lien parmi l’activité des neurones cholinergiques et la santé buccodentaire et cognitive. Spécifiquement, nos objectifs étaient d'identifier les classes de santé bucco-dentaire et de santé cognitive parmi les Canadiens d’âge moyen et âgés, et d'étudier dans quelle mesure ces regroupements s'expliquent par une mesure indirecte de l'activité des neurones cholinergiques (densité minérale osseuse).

Méthodes : Dans une première étape, les données initiaux de la cohorte de l'Étude longitudinale canadienne sur le vieillissement (ÉLCV), volet complète, qui a recruté participants âgés de 45 à 85 ans, ont été utilisées pour identifier les classes de santé bucco-dentaire et de santé cognitive, puis pour déterminer si ce regroupement pouvait être expliqué. La santé buccodentaire déclaré par les participants a été vérifiée par un questionnaire propre, tandis que sept instruments basés sur tâches ont mesuré la santé cognitive (c.-à-d. mémoire rétrospective et prospective, fluence verbale, et inhibition de l’interférence cognitive). La densité minérale osseuse a été utilisé comme mesure indirecte de la densité minérale osseuse pour la régression logistique multivariée. Notre analyse multivariée finale a été ajusté sur l'âge, le sexe, l'éducation, le revenu total du ménage, l'origine ethnique, la consommation d'alcool, le tabagisme, l'hypertension et le diabète.

Résultats : Les participants (N=25,444: 13035 hommes, 12409 femmes) ont été regroupés en 5 et 4 groupes en fonction de leurs caractéristiques de de santé bucco-dentaire et de santé cognitive. Dans les modèles de régression finaux et après ajustement sur toutes les covariables potentielles,

la plupart des intervalles de confiance à 95% rangent de <1,0 jusqu'à environs 3,0, donc les résultats supportent une association légère entre la densité minérale osseuse et les probabilités d'appartenance à l'une des classes de santé bucco-dentaire ou de santé cognitive.

Conclusion : Les Canadiens d'âge moyen et âgés démontrent différents profils de santé bucco-dentaire et cognitive, liés à leurs conditions bucco-dentaires rapportées et l'utilisation de prothèses dentaires, et à leur performance aux examens de mémoire et fluence verbale. Le regroupement des participants en fonction de la santé bucco-dentaire et de la santé cognitive n'a pas été expliqué par la mesure indirecte de l'activité des neurones cholinergiques après ajustement pour les covariables potentielles.

1. Introduction

Oral diseases are amongst the most common health conditions, affecting more than 3.5 billion people globally[1]. These conditions incur direct and indirect costs, which makes them a prominent public health burden[2]. The effect of oral diseases is not limited to their financial burden, but they have been also extensively investigated for their association with general health. Indeed, an overwhelming number of studies have reported a link between oral health and a wide variety of health conditions such as cardiovascular diseases[3], diabetes[4], and cognitive decline[5, 6]. The majority of these chronic non-curable conditions share common risk factors. Therefore, investigating the effect of poor oral health as a potential risk factor for major chronic diseases has attracted attention from scholars in different fields.

Cognitive decline and specifically Alzheimer's disease is one of these chronic conditions with complex and unknown aetiology. Previously, it was argued that cognitive decline limits individuals' capability to maintain their oral health[7, 8]. However, recent studies suggest that oral health could be a risk factor for cognitive decline and different mechanisms have been suggested to explain the biological plausibility of this oral-systemic connection. These purported mechanisms include chronic inflammation caused by periodontitis[9], reduced sensory input resulted from ineffective mastication[10], and nutritional deficiency attributable to masticatory dysfunction[11].

Although the proposed mechanisms are biologically plausible, they overlook the role of aging and aging-related changes in cholinergic neurons' degeneration. These neurons utilize acetylcholine, are widely spread throughout the body, and are involved in different functions including learning[12], memory[13, 14], and saliva secretion[15]. On one hand, the degeneration of these neurons

results in cognitive decline and memory deficit in people with dementia and specifically Alzheimer's' disease[14, 16]. On the other hand, cholinergic system stimulation results in saliva secretion which is essential in oral homeostasis[17, 18]. Interestingly, it has been reported that older individuals with reduced salivation tend to have fewer teeth[19] .

Therefore, we hypothesize that the aging-related degeneration in cholinergic neurons is a potential common cause explaining the association between poor oral health and cognitive decline. The overall objective of this cross-sectional study is to investigate the association between Cholinergic neurons' activity and oral and cognitive health status.

2. Literature review

This chapter has six different sections. Section 2.1 summarizes the evidence on the association between oral and systemic health. Section 2.2 presents three different mechanisms described to explain the role of poor oral health as a risk factor for cognitive decline. Finally, the last four sections present a critical examination of the suggested mechanisms and introduce Cholinergic neurons' activity as a candidate to explain the association between poor oral health and cognitive decline.

2.1. Oral health and systemic health interplay through lifetime

The World Health Organization (WHO) defines oral health as "a state of being free from mouth and facial pain, oral and throat cancer, oral infections and sores, periodontal (gum) disease, tooth decay, tooth loss and other diseases and disorders that limit an individual's capacity in biting, chewing, smiling, speaking and psychosocial wellbeing"[20].

Oral diseases are among the most common health conditions worldwide, affecting 3.5 billion people globally[21]. Every year, an estimated amount of US$ 298 billion is spent on treatment of oral diseases, which accounts for 4.6% of global health expenditures. The economic burden of oral health diseases is not limited to their direct treatment costs. These diseases also have major indirect costs because they adversely impact school and work productivity. For example, global productivity loss due to the three most common oral health conditions, i.e., severe periodontitis, tooth loss, and untreated caries, has been estimated to be US$ 144.24 billion[22]. Although Canada is among the best OECD (Organization for Economic Co-operation and Development) countries in terms of overall oral health of its population, annual productivity losses caused by oral diseases

are estimated to be around US$ 1 billion. Moreover, about 39% of Canadians report time loss attributable to the oral health problems [23].

Oral health status is also associated with different systemic conditions. For example, a growing body of evidence suggests an association between poor oral health and the development of chronic conditions such as diabetes[24, 25] and cardiovascular diseases[26-28].

Dementia is another common chronic condition that has been linked to different measures of oral health status[29, 30]. According to the WHO, dementia is a syndrome, typically chronic and progressive, that causes cognitive impairment further than expected as a result of aging[31]. Symptoms of dementia include memory loss, difficult communication, and behavior changes such as repeated questioning and aggressiveness. Approximately 50 million people worldwide has been diagnosed with dementia and this number is estimated to triple by 2050 [32]. The provision of care for individuals with dementia has huge financial and emotional impact on their caregivers and has become a major public health burden[33]. To date, there is no definite treatment for dementia[34]. Therefore, an overwhelming number of studies have focused on understanding the course and modifiable risk factors of this chronic non-communicable disease.

It is worth mentioning that several diseases result in dementia, such as Alzheimer's disease, vascular dementia, Frontotemporal dementia, and dementia with Lewy bodies. Therefore, dementia is not a single condition with one specific pathology. However, researchers investigating the association between oral health and cognitive health have used "dementia" as a general term and have in fact measured "cognitive impairment" using different measures. Although some have ascertained diagnosis of Alzheimer's disease, which is the most common type of dementia, others have measured the cognitive functioning of participants in different domains using different

cognitive tasks. Therefore, in this chapter I will use "dementia" as a measure of cognitive decline although I acknowledge the difference between these two terms.

Previously, it was reported that the direction of the association between oral health and cognitive decline was from the latter to the former only; the hypothesis was that poor cognitive functioning leads to poor oral health because of the inability of individuals with impaired cognition to look after their oral health[35]. Recently, however, it has been argued that these associations may be reverse as well, meaning that poor oral health could be a risk factor for cognitive decline and dementia.

In 1994, Kondo et al. compared each of 60 Alzheimer's cases with two age and sex matched healthy controls to evaluate the effect of lifestyle on developing senile Alzheimer's disease. Their findings highlighted the importance of tooth loss as a potential risk factor for developing dementia. They measured oral health status by asking participants if they had "lost more than half of their teeth" or if they had "total denture with none of their own teeth". This relatively small study with retrospective design, which makes it subject to recall bias, was the first one suggesting that poor oral health, specifically tooth loss, could be a potential independent risk factor for dementing disorders[36].

2.2. Poor oral health as a risk factor for cognitive decline and dementia – suggested explanations:

As poor oral health was proposed as a potential risk factor for developing dementia, researchers' interest grew substantially in investigating and providing explanations for this new oral-systemic pathway. Three main suggested mechanisms for this oral-systemic connection are as follows:

i) Chronic inflammation and infection linked to periodontitis

ii) Decreased brain stimulation and blood circulation resulted from inadequate mastication

iii) Nutritional deficiency caused by impaired mastication

2.2.1. Chronic inflammation caused by periodontitis

Periodontal disease, a common inflammatory oral disease, has an estimated prevalence of 20% to 50% globally[37]. Periodontitis, an advanced form of periodontal disease, is marked by chronic inflammation of the tooth supporting tissue. This condition is characterized by a complex interaction between host immune response and local periodontal microbiome, resulting in destruction of periodontium and subsequent tooth loss[38]. This chronic inflammatory condition has frequently reported to be associated with multiple chronic systemic conditions such as cardiovascular disease, rheumatoid arthritis, and type 2 diabetes mellitus (T2DM)[39, 40]. Periodontitis has also been proposed as a potential risk factor for cognitive decline and/or Alzheimer's disease[41]. Two main plausible explanations have been suggested for the effect of periodontitis on the pathogenesis of Alzheimer's disease: the inflammation per se, and the transmission of pathogenic bacteria from the oral cavity to distant organs[42-44].

Periodontal disease promotes the local release of pro-inflammatory cytokines, including IL-1, IL-6, and TNF-α[45, 46], and the systemic release of acute phase proteins, such as C-reactive protein (CRP) [47, 48]. These pro-inflammatory mediators enter the bloodstream and induce inflammation in organs distant from oral cavity. These mediators can also enter brain tissues through the blood-brain barrier and stimulate inflammation, which can subsequently result in neurodegeneration[41].

Apart from inducing release of pro-inflammatory cytokines and initiating the systemic inflammation, periodontal pathogens can directly enter the central nervous system through neuronal pathway or circulation. These pathogens and their parts (e.g., lipopolysaccharide) can

stimulate inflammation in or directly invade brain, which can also accelerate neurodegeneration and cause cognitive dysfunction[49, 50].

Several studies with different designs have investigated the role of periodontitis as a potential risk factor for cognitive decline or dementia. Multiple cross-sectional studies have reported positive association between periodontal disease and cognitive impairment[51-55]. However, to establish that periodontitis preceded the cognitive decline, longitudinal analyses are required.

In 2007, Stein et al. used full mouth radiographs to categorize participants of the Nun Study into two groups based on the severity of alveolar bone loss. In this study, having moderate to severe periodontitis was not associated with risk of developing dementia after adjusting for age, education, and APOE genotype. However, the study findings suggest an association between lower number of teeth (0-9) and incidence of dementia in 12 years of follow up[56]. Similarly, Arrive et al. did not find any association between periodontitis and risk of dementia during nearly 10 years of follow up[57]. Results from another prospective study suggested that cognitive impairment, which was defined using Modified Mini-Mental State Examination (3MS), was not significantly associated with periodontal disease measured by probing depth, loss of attachment, gingival index score, plaque score, and bleeding on probing[58]. Furthermore, a recent study reported no difference in the level of periodontitis inflammatory markers in cognitively impaired cases compared to healthy individuals[59]. The findings of these studies were in accordance with several prospective and retrospective longitudinal studies reporting no association between different periodontal indices and risk of cognitive decline or dementia[60-62].

In contrast, Kaye et al. reported that an increase in the incidence of alveolar bone loss and pocket depth was associated with higher risk of lower Mini Mental State Examination (MMSE) score in

older men[63]. Results from another longitudinal study with a large sample size reported a 6% higher risk of developing dementia in subjects with chronic periodontitis[64]. Findings of these studies corroborate the results of similar longitudinal studies reporting a positive association between periodontitis and risk of dementia[65-68].

Although the proposed mechanisms explaining the association between periodontal health and cognitive health is biologically plausible, the studies examining this association have used different definitions and measurements for periodontal disease and cognitive dysfunction. Furthermore, these studies have multiple methodological limitations including small sample size[69], exposure and outcome misclassification, and selection bias. Some of these studies have also failed to adjust for key confounding variables (e.g., smoking history[65, 66, 70, 71], level of education[64], or socioeconomic status[68]). These factors have led systematic reviews to report heterogeneity among findings of the studies on the association between periodontitis and cognitive health [72-74]. For example, a recent review paper used Bradford Hill criteria to investigate the causal relationship between periodontal disease and cognitive decline. According to this study, it is challenging to fulfill the temporality criteria because periodontal disease and dementia are both chronic and slow-progressing conditions. Moreover, it remains unknown whether the periodontal disease initiates the process of neurodegeneration. Also, as mentioned before, due to several limitations of the studies on this association, the consistency criterion is moderately fulfilled[41].

2.2.2. Mastication inadequacy and decreased brain stimulation and brain blood circulation

As mentioned above, tooth loss has been frequently reported to increase the risk of developing dementia[75-77], and some authors have considered this oral-systemic connection to be related to the presence of prolonged periodontal disease and inflammation. Others, however, have explained

these findings through the effect of impaired mastication and reduced sensory input from stomatognathic system on the activity of certain brain regions. Several studies have shown that the act of chewing is associated with increased activity in sensorimotor areas of the brain[78, 79]. In line with findings of these studies, prosthodontic treatments such as dentures have reported to result in an increased activity in these regions[80, 81]. Some authors have also observed increased cerebral blood flow[82] and blood oxygen level in hippocampus and prefrontal cortex following a chewing task[83-85]. These areas of the brain play are involved in certain cognitive functions including learning and memory[86]. Therefore, it has been argued that inadequate chewing, caused by suboptimal dentition, results in reduced brain stimulation and blood flow, which are consequently linked to cognitive decline[87].

To support this theory, several animal studies have investigated the effect of mastication on cognitive function in mice. In these studies, diverse models of "masticatory dysfunction" were induced by extraction of posterior teeth, soft diet feeding, or using a hard material on the occlusal surface. Findings of these studies revealed that chewing dysfunction reduces neuronal activity in hippocampus and impact cognitive parameters such as memory and learning[88, 89]. Feeding the mice with soft diet has shown to negatively affect neurogenesis in hippocampal area[90]. Researchers have also observed structural and volumetric changes in several brain regions in female mice after tooth extraction[91].

Although the evidence from animal studies generally supports the role of insufficient mastication in developing cognitive dysfunction, results from epidemiological research are mixed and controversial. While most cross-sectional studies have shown a positive association between masticatory dysfunction and cognitive impairment or dementia[92-96], others demonstrated

negative[52, 97], or no significant association[98, 99] between chewing ability and cognitive functioning.

Among longitudinal studies, some reported that a lower number of teeth or reduced masticatory performance are linked to a higher risk of cognitive impairment or dementia. For example, Batty et al. reported that having ≥22 teeth compared to not having any tooth, was associated with lower risk of both cognitive decline and dementia in a 5-year follow up period[60]. Similarly, findings from two longitudinal studies that used self-reported oral health status demonstrated that tooth loss is associated with higher risk of dementia and cognitive decline[100, 101]. In contrast, some studies did not find any association between number of teeth or edentulism with consequent cognitive decline in 8-year and 20-year follow-ups[62, 95]. Most of these clinical studies used tooth loss as a proxy measure for mastication insufficiency. Nonetheless, adaptation to a reduced number of teeth in elders might cause misclassification of the exposure. In other words, through the process of aging an individual might learn how to chew more efficiently with a sub-optimal number of teeth[102]. To address these concerns with" the number of existing teeth" as the independent variable, some studies have used more direct assessments of masticatory function such as color-changeable gum and two-color mixing test. Findings of some of these cross-sectional studies indicated that there is an association between masticatory performance and cognitive health condition[11, 92, 103-105]. Nevertheless, in cross-sectional studies, researchers cannot identify whether the masticatory dysfunction proceeds the cognitive decline. Also, individuals with poor cognitive health tend to have lower bite force and cross-sectional analysis does not take this effect of cognitive dysfunction into consideration[105].

As discussed above, evidence from clinical and longitudinal studies is not sufficient to establish a causal relationship between masticatory dysfunction and cognitive impairment. There are several factors contributing to the observed heterogeneity between studies. The exposure and outcome variables are defined and measure differently in these studies. Also, dementia and cognitive decline are slow progressing and more complex in humans compared to mice.

2.2.3. Nutritional deficiency caused by impaired mastication

The third proposed explanation for the association between tooth loss and cognitive function involves nutrition deficiency as a mediating factor between tooth loss and cognitive impairment. In other words, this hypothesis suggests that inadequate masticatory performance resulted from tooth loss can lead to reduced nutrient intake[106]. Nutrition deficiency is frequently reported to be associated with cognitive decline[107]. Therefore, elders with suboptimal dentition are at higher risk for cognitive decline or dementia, because their oral health status prevents them from receiving all nutrients required for adequate cognitive performance. This hypothesis has not been fully explored yet, and there is limited evidence on the mediating effect of nutritional status[108].

Some studies have adjusted for nutritional status or risk in their multivariate analyses. For example, Kim et al investigated the association between "objective masticatory dysfunction" and the risk of cognitive impairment, adjusting for nutritional risk which was measured by Mini-Nutritional Assessment (MNA)[109]. The risk of cognitive impairment did not change significantly after this adjustment. Nevertheless, in this study "malnourished" and "at risk" individuals had lower chewing ability and were more likely to have cognitive impairment[11]. The findings of this study were in agreement with another cross-sectional study showing that low food diversity was associated with lower chewing ability[92]. In contrast, Elsig et al. reported no association between

nutritional status of the participants and the number of natural teeth[104]. A systematic review of the literature has indicated that there is an insufficient evidence on the mediating effect of nutritional deficiency on the association between mastication dysfunction and cognitive health[108].

2.3. Critical examination on the association between oral health and cognitive health

As mentioned before, an overwhelming number of studies have been conducted on the association between oral health and cognitive health. Many of these studies have based their analyses on different hypotheses to explain this oral-systemic link. Even if these hypotheses seem biologically plausible, systematic reviews have reported that these studies are far from being conclusive and provide a low level of evidence[29, 74, 77]. Moreover, these studies have overlooked the role of age-related changes as a potential common cause explaining the association between oral health and cognitive health in elders.

Throughout an individual's life course, a variety of physical and social factors, independently or through their interactions, shape one's health status including oral health status[110, 111]. Indeed, there are several lines of evidence showing the association between oral health and exposures to different factors across an individual's lifetime. In a recent paper, Thomson et al. argued that the association between oral health and cognitive health can be explained using a "life course approach". For example, in case of "tooth loss", authors described it as "a cumulative state that arises through the incremental removal of teeth as problems arise. It is conceptually and clinically distinct from edentulism, the state of having had all natural teeth removed" [112].

Life course approach has been widely adopted in understanding and explaining the role of different exposures in developing age-related and chronic conditions in elders[113]. The degeneration of cholinergic neurons, a common age-related phenomenon, is a potential candidate to explain the link between poor oral health and cognitive functioning. Cholinergic neurons use acetylcholine as

the neurotransmitter and are widely distributed in peripheral and central nervous system. In the following sections, the role of reduced cholinergic neurotransmission in oral and cognitive health status is described separately.

2.4. Cholinergic neurons and cognitive health

Cholinergic neurons are widely distributed in the central and peripheral nervous system and thus play a key role in different neural functions in the central nervous system (CNS). For example, the cholinergic neurons of basal forebrain provide cholinergic projections to neocortex and hippocampus and are involved in different cognitive functions such as learning, memory, attention, sleep-wake cycle, and response to stress[114-117]. Age-dependent degeneration of cholinergic neurons in the basal forebrain has been observed in several studies, which explains the memory deficit observed in the elders[118]. Moreover, cholinergic neurons of Nucleus basalis of Meynert in the basal forebrain go through severe degeneration in Alzheimer's disease (AD)[119, 120]. In patients with AD, administration of cholinesterase inhibitor has shown to enhance attention and executive functioning[117, 121].

2.5. Cholinergic neurons and oral health

Cholinergic neurons are of key importance in autonomic nervous system. All pre-ganglionic parasympathetic and sympathetic neurons and all post-ganglionic parasympathetic neurons are cholinergic**.** Apart from atrophic central cholinergic neurons, dysfunction of autonomic cholinergic neurons is observed in patients affected by AD and other subtypes of dementia[122-124]. In one study, a significant parasympathetic dysfunction was observed in patients with Mild Cognitive Impairment. Findings of this study suggest that autonomic nervous system dysfunction might take place earlier than clinical signs of dementia and might be due to the degeneration of cholinergic neurons[125]. Moreover, Giubilei et al. reported that in AD patients observed cardiac

autonomic dysfunction, might be caused by cholinergic dysfunction in peripheral autonomic nervous system[126].

Parasympathetic neurons innervate all three major salivary glands and stimulate salivary secretion which is crucial to maintain oral health. For example, salivary components protect teeth against demineralization and caries. Saliva also contain immunoglobin, protecting oral mucosa against infection. Decreased salivary flow has been reported to associate with higher risk of caries and periodontal disease[18].

In addition to reduced parasympathetic stimulation caused by cholinergic deficit, an age-related reduction in salivary glands' response to acetylcholine has been reported, which might also contribute to the xerostomia in elders[127]. Therefore, cholinergic deficits in the autonomic nervous system and reduced peripheral response to acetylcholine is associated with reduced saliva secretion, and consequent higher risk of tooth loss in elders.

2.6. Cholinergic neurons and regulation of bone mineral density

The autonomic nervous system plays an important role in the regulation of bone remodeling [128]. Cholinergic fibers of both arms of autonomic nervous system, including the sympathetic nervous system (SNS) and the parasympathetic nervous system (PSNS), innervate bone tissue. Moreover, cholinergic system components such as nicotinic and muscarinic receptors, have been identified in several bone cells including osteoclasts and osteoblasts[129, 130].

Cholinergic neurotransmission has been reported as a factor promoting bone-mass accrual. This effect of cholinergic system on bone remodeling is suggested to take place through up-regulation of osteoblasts and down-regulation of osteoclasts[131-133].

In animal studies, vagal dissection, which disrupts PSNS cholinergic neurotransmission, has shown to negatively affect bone mineral density[134], and stimulation of the parasympathetic

cholinergic neurons is reported to result in bone formation[131-133]. Stimulation of nicotinic receptors has shown to result in increased bone mineral density through osteoclast down-regulation, while muscarinic receptors' stimulation is reported to lead to increased osteoblast proliferation.

In accordance with findings of animal studies, several conditions that are related to decreased cholinergic activity are also present in individuals with low bone mineral density. For example, cholinergic neurons regulate the function of lacrimal and salivary glands, and thus reduced function of cholinergic system leads to dry mouth and dry eyes. Interestingly, several studies have reported concurrent manifestation of these conditions with lower bone mineral density. Moreover, administration of acetylcholine esterase inhibitors in human subject with dementia has shown to reduce risk of bone fracture [135, 136], and in epileptic patients, Vagus nerve stimulation (VNS) has resulted in bone mass gain in vertebra[137]. Since the vagus nerve (or cranial nerve X) carries main parasympathetic cholinergic fibers from CNS, this observed effect of VNS could be linked to the activity of cholinergic neurons.

3. Rationale

Oral diseases and cognitive decline are common chronic conditions affecting a large proportion of the elderly, placing a huge burden on this vulnerable population, their caregivers, and the public health systems. The observed association between these two groups of conditions has been extensively investigated to understand the extent and direction of this oral-systemic link. Better understanding of the extent of this association can inform whether public health interventions are justified and investigating the direction of the arrow connecting oral health and cognitive health could guide effective implementation of public health policies.

Recent studies suggesting poor oral health as a risk factor for cognitive decline have proposed different mechanisms to explain this oral-systemic connection. However, their results are controversial/inconclusive and reach no agreement on whether there is an association between oral health and cognitive decline. Importantly, most of them do not consider that oral and metal health have common risk factors along an individual's life span (e.g., low education levels, smoking and low physical activity level). In other words, the accumulation of factors along the life course may be responsible for age-related deterioration of both metal and oral health. In this study, I propose that life course exposure leads to age-related changes in cholinergic neurons' activity which is a potential common cause explaining association between oral health and cognitive health. Therefore, the overarching goal of this project is to investigate whether the association between oral health and cognitive health could be explained by the activity of cholinergic system. The results of this study can provide better understanding of this oral-systemic connection. More importantly, a deeper understanding of this association may prevent funding of potential

unnecessary interventions targeting poor oral health status as a definite risk factor for cognitive decline.

4. Aims and hypothesis

Hypothesis, research question(s), aims

As discussed above, age-related degeneration of central and parasympathetic cholinergic neurons is associated with cognitive disfunction and poor oral health. This may be a missing important factor behind a faster age-related cognitive decline in association with oral diseases. Therefore, we hypothesize that aging of the cholinergic system is the common cause explaining the observed association between oral health and cognitive health status.

Research questions:

Our research question had two components:

i) How do adults at risk of cognitive decline (middle-aged and elderly) group together in terms of their oral and cognitive health status?

ii) To what extent this clustering/grouping of study participants is explained by bone mineral density as a proxy measure for cholinergic neurons' function?

Specific aims:

To address our research questions the following specific aims were formulated:

1) to identify oral health classes among our study sample using measured oral health variables.

2) to identify cognitive health classes among our study sample using measured cognitive tests

3) to investigate the extent to which oral health clustering is explained by cholinergic activity indicator (i.e., bone mineral density score) after adjusting for potential confounders

4) to investigate the extent to which cognitive health clustering is explained by cholinergic activity indicator (i.e., bone mineral density score) after adjusting for potential confounders

5. Methods

5.1. Overview

This study used baseline data from the Canadian Longitudinal Study on Aging (CLSA). CLSA is a nation-wide ongoing cohort study, with 51,338 participants initially aged 45-85 years. Baseline data collection took place between 2011 and 2015, and follow-up data collections are scheduled in 3-year intervals for at least 20 years (until 2033 or decease). CLSA participants comprised of two main cohorts, namely "Comprehensive" and "Tracking". **Figure 1** presents a graphic representation of the CLSA data collection timeline. The Tracking cohort is a national representative sample with more than 21,000 participants who provided an array of information about social, medical, psychological, and lifestyle aspects of their lives through computer assisted telephone interviews (CATI) only.

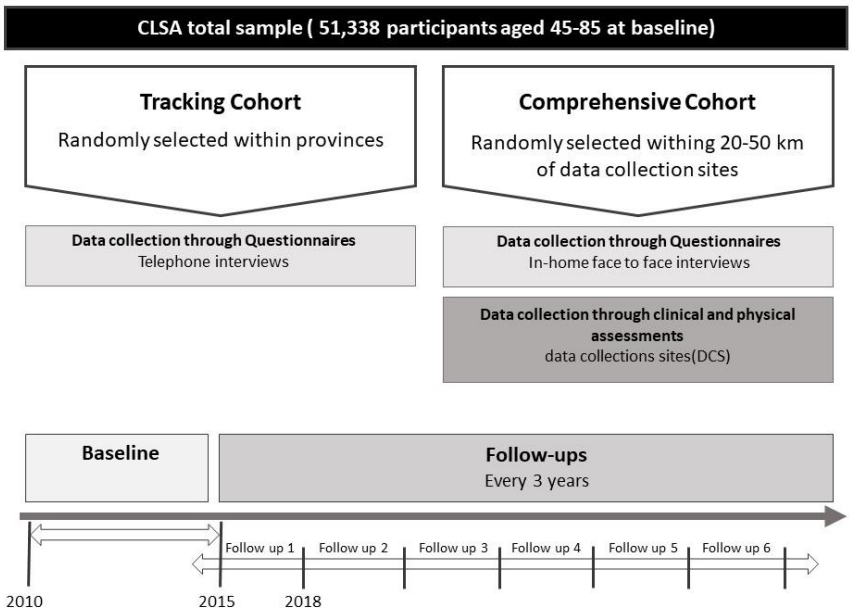

Figure 1*. Graphic representation of CLSA data collection timeline and methods adapted from Raina et al.* [(138)].

The Comprehensive cohort consists of 30,097 participants who underwent physical examinations in 11 designated data collection sites in addition to participating in telephone and in-home interviews. The cognitive performance of the participants in this cohort was assessed using a wider array of cognitive measures, compared to the Tracking cohort. Because cognitive functioning is one of the key variables in our study, we used the baseline data gathered from the Comprehensive cohort only[138].

5.2. Ethical considerations

This study was approved by the Institutional Review Board (IRB) board of McGill University (IRB study number: A07-E51-18B) and closely followed the "Sample Access Policy and Guiding Principles" specified by CLSA team [139]. Appendices 1, 2, and 3 provide the ethical approval letter, CLSA access agreement, and the source of funding for this research project, respectively.

5.3. Sample selection and recruitment

Comprehensive participants were sampled using the Provincial Healthcare Registration Databases and random digit dialing as the sampling frames. These participants were recruited from an initial age-stratified random sample of individuals living within 25- to 50-kilometer distance from the designated data collection sites (11 in 7 provinces).

Some individuals were excluded from the sampling frames, according to the following exclusion criteria [138]:

- Living in first nations reserves or in other first nations settlements;
- Living in one of three territories (YT, NT, NU) or remote regions;
- Full-time members of the Canadian Armed Forces;

- Living in long-term care facilities with 24-hour nursing services;
- Inability to communicate in English or French;
- Presence of cognitive impairment at baseline, as verified by the interviewers. Cognitive impairment can affect the reliability and validify of the data gathered through interviews. CLSA interviewers judged the cognitive function of a potential participant by assessing their ability to comprehend study aims and to provide valid and reliable data.

To recruit participants, households were randomly contacted to identify potential participants, who were asked to provide their contact information. Those who agreed to be contacted by CLSA team, were then assessed, and asked to provide informed consent prior to being considered as fully recruited participants. The participation rate and overall response rate were 45% and 10%, respectively [(138)].

5.4. Data collection for the Comprehensive cohort

Data collection for CLSA took place in different settings at baseline. For the Comprehensive cohort recruitment, "eligible postal codes" where limited to households located within 50 km distance from data collection sites (DCS). The National Coordination Center (NCC) for CLSA provided contact information of the potential participants to DCS, where the initial contact took place, the eligibility of the "pre recruit" was verified, and then the time of the baseline home-interview was set. For the Comprehensive cohort, the first step was a 90-minute face-to-face interview. Starting later, a 30-minute telephone interview, which is called "maintaining contact questionnaire", was performed for both tracking and comprehensive cohorts. As the in-home interviews were being performed, the Comprehensive cohort were asked to visit the data collection sites (DCS) for more comprehensive physical assessments.

5.5. Data management

The NCC stored participants' identifying information in a secure database, to which only limited number of authorized staff had access. The unidentified data (*"study data"*) were also password protected at the NCC. Only authenticated users had access to the workstations, which were located within a access-controlled environment. Researchers elsewhere must follow CLSA guidelines, go through data access process, and sign CLSA Access Agreement in order to receive prepared datasets.

5.6. Measures

In this thesis, the main exposure variable - cholinergic system activity - was measured using bone mineral density score. The outcomes variables were oral health clusters and cognitive health clusters. A wide variety of covariates were also included in our analyses, which are explained in section 5.6.3.

5.6.1. Exposure variable (bone mineral density)

Bone mineral density (BMD) was measured at the local data collection sites using Hologic Discovery A^{TM} Dual energy X-ray Absorptiometry (DXA), which is the "gold standard" for measuring BMD[(140)]. At baseline, BMD was measured for both hips, lateral spine, forearm, and whole body. While T- and Z-scores were available for the whole-body BMD, I opted to use the whole-body BMD raw score as a proxy measure for the activity of cholinergic system.

5.6.2. Outcome variables

a) Oral health questionnaire

The Comprehensive cohort members provided information regarding their oral health status through “the maintaining contact” telephone interviews. The oral health questionnaire was based on the Canadian Community Health Survey 2.1, incorporating subjective indicators of oral health status as proposed by Locker. These self-reported indicators have shown acceptable test-retest reliability, internal consistency levels (>0.7), and concurrent and construct validity[141].

We transformed responses for oral health questions from a Likert scale to a binary format, to improve the interpretability of oral health classes in the latent class analysis step. This was done for three oral health questions (**Table 1,** Q1, Q4 and Q5), i.e., self-rated oral health, the frequency of experiencing uncomfortable eating, and eating avoidance due to “mouth problems”. Binary transformation resulted in the following responses codes: “1” reflected favorable oral health status (original responses, Q1: excellent, very good, or good; Q4 and Q5: rarely or never) and code 2 reflected a less-favorable oral health status (Q1: fair or poor; Q4 and Q5: often, sometimes). Question 3 was an exception since wearing dentures was coded as 1 and not wearing denture was coded as 2. Finally, we had 24 variables on self-reported oral health status of the Comprehensive cohort, for whom code 1 reflected favorable oral health status and code 2 reflected less favorable oral health[142].

Table 1 summarizes the oral health questions from the baseline wave of the CLSA and their final response categories that were included in the latent class analysis step.

Table 1. *Oral health questions from Maintaining Contact Questionnaire and their response categories included in the latent class analysis step.*

	Question (Q)	**Response categories in the final dataset**
1	In general, would you say the health of your mouth is excellent, very good, good, fair, or poor?	Excellent, Very Good, Good 1 Fair, Poor 2
2	Do you have one or more of your own original teeth?	Yes 1 No 2
3	Do you wear dentures or false teeth?	Yes 1 No 2
4	In the past 12 months, how often have you found it uncomfortable to eat any food because of problems with your mouth? Would you say…	Often, Sometimes 2 Rarely, Never 1
5	In the past 12 months, how often have you avoided eating particular foods because of problems with your mouth? Would you say…	Often, Sometimes 2 Rarely, Never 1
6	In the past 12 months have you experienced any of the following?	
	6.1. Toothache	Yes 2, No 1
	6.2. Cannot chew adequately	Yes 2, No 1
	6.3. Dentures uncomfortable	Yes 2, No 1
	6.4. Dentures loose/don't fit	Yes 2, No 1
	6.5. Dentures broken	Yes 2, No 1
	6.6. Dentures missing	Yes 2, No 1
	6.7. Swelling in your mouth	Yes 2, No 1
	6.8. Dry mouth	Yes 2, No 1
	6.9. Burning mouth	Yes 2, No 1
	6.10. Jaw muscles sore	Yes 2, No 1
	6.11. Jaw joints painful	Yes 2, No 1
	6.12. Natural tooth decayed	Yes 2, No 1
	6.13. Natural tooth loose	Yes 2, No 1
	6.14. Natural tooth broken	Yes 2, No 1
	6.15. Gums around natural teeth are sore	Yes 2, No 1
	6.16. Gums around natural teeth bleed	Yes 2, No 1
	6.17. Denture-related sores	Yes 2, No 1
	6.18. Teeth or dentures dirty	Yes 2, No 1
	6.19. Bad breath	Yes 2, No 1

b) Cognitive health test battery

Trained CLSA interviewers administered the face-to-face cognitive tests and participants' responses to the tests were audio recorded[143]. Subsequently, the CLSA personnel scored the test using a standardized procedure[144].

Cognitive functioning was assessed using seven different instruments in three different domains, namely memory, executive functioning, and psychomotor speed[138]. These seven instruments are: Rey Auditory Verbal Learning Test (RAVLT), Mental Alteration Test (MAT), Prospective Memory Test (PMT), Stroop Neuropsychological Screening Test, Controlled Oral Word Association Test (COWAT), Animal Fluency Test (AF), and Choice Reaction Time (CRT). The last instrument (CRT) was not included in this study due to the roof effect, with respondents reaching an average score of 100%. Below is a detailed description of these instruments.

Rey auditory Verbal Learning Test (RAVLT)[145]: According to the CLSA protocol, this cognitive test is defined as the only measure under the memory domain of the cognitive functioning. RAVLT is a widely used cognitive measure[146] and has been administrated through in-home interviews to the Comprehensive participants. In its original form, RAVLT consists of 5 trials and it assesses learning and retention[147]. However, in CLSA this test has been administered in 2 trials only[148]. Participants were presented with a 15-item list of words and then they were asked to recall the words immediately (within 90 seconds) and after 5 minutes (within 60 seconds). For the first trial (REY I), participants scored one point for each word they recalled correctly (primary word), or for a word sounding similar to the recorded word (e.g., color and collar). For other words, the score was zero. The second trial (REY II) was scored in a similar way as REYI. However, if the participant used a variant word in test 1 and a correct word in the second one, they

did not receive a point in test 2. Also, those who were prompted by the interviewer received a score zero.

Mental Alteration Test (MAT): This cognitive test assesses mental flexibility and processing speed[148], and has been used as a measure of "executive functioning" in CLSA. MAT has shown high specificity and sensitivity in detecting cognitive impairment in HIV positive patients and elders [149, 150]. MAT involves asking the subjects to alternate between letters and numbers within 30 seconds (i.e., 1-A, 2-B, 3-C , . . .), and the final score reflects the number of correct alterations[151].

Animal Fluency Test (AF): Animal Fluency test is a semantic fluency task, which is described as another measure of executive functioning in CLSA. This task involves naming of as many animals as possible within a 60-second limit[152]. A score of 1 was given to each unique animal named. Two scoring systems were used for this cognitive task. For the first one, the participants received one point for animals from different breeds or sub-species only. However, in the second system, the participant received one point for animals named from the same breed/ sub species. For example, a participant who named "bird", "parrot", and" pheasant" would lose the bird score in the first scoring system but would score 3 in the second system. For the purpose of this study, the scores from the second system were used.

Controlled Oral Word Association Test (COWAT): COWAT is another cognitive measure that has been adopted in CLSA to assess executive functioning of the participants. This test is also a verbal fluency task (phonemic fluency). In CLSA, participants were asked to name as many words as possible with three different letters, including "F", "A", and "S" in three separate 1-minute long trials[153]. The overall score was based on the total number of unique words named across the three

trials, with each new word counting one point. Words that have the same root as the previous one mentioned, only received one point (e.g., “close”, “closer”, “closest”). Also, two words with the same meaning in two different languages received only one point and were only recorded in the participants’ preferred language.

Stroop Neuropsychological Screening Test: This task is also categorized under the executive functioning domain in CLSA and measures inhibition, attention, and mental control and speed. The Victoria version of this test, which consists of three different steps, has been applied in CLSA[154]. At each step, participants are required to name the color of printed dots or words on several cards presented to them. In the first step, they name the color of dots. In the second step, they name the color of some “non-color” words, and in the final step, they name the color of some “color” words. For example, if the word “green” is printed in yellow, they should name yellow. The third step or “interference condition” is normally done slower than the first two. The scoring of this test is based on the number of errors an individual makes and the time it takes for them to name the colors on all cards at each step. “Interference ratio” has also been used in the literature to measure the slowing from the first card to the third one[155]. This slowing, which is also known as the “color-word interference effect”[156] , is calculated by dividing the time spent on the last card by the time spent on the first one.

The Miami Prospective Memory Test (PMT): PMT is another measure to assess executive functioning of CLSA comprehensive cohort, which consists of “event-based” and “time-based” memory tests[157]. In both tasks, subjects were asked to initiate and complete a task in the next 15 to 30 minutes. In the event-based task, the participant was provided with an envelope containing 3 loonies and 5, 10, and 20 dollars bills. Then, they were required to take the five-dollar bill out of

the envelope and present it to the interviewers, and to take the ten-dollar bill for themselves when they hear the alarm sound (the event).

For the time-based prospective memory task, subjects were given another envelope containing cards with numbers 28, 14, 17, 13, and 11 printed on them. The participant then was presented with a clock set on 8 o'clock and was asked to present the card with number 17 to the interviewer at 8:15. The participant's performance was scored based on three different criteria including: 1) Intention to perform; 2) accuracy of response; and 3) need of reminders. The score for each criterion was 3 and the maximum score for each task of PMT was nine (total score=18).

5.6.3. Covariates

This thesis included data on several covariates described below. The selection of these covariates was guided by previous literature. These variables were divided into three categories: demographic and socioeconomic factors, chronic health conditions, and lifestyle factors.

a) Basic demographics and socioeconomic factors

These characteristics of the participants were obtained through face-to-face in-home interviews and included age, sex, total household outcome, education, and ethnicity. These factors have been reported to influence cognitive function[139, 158-161] and oral health status[162-166] , and to be associated with bone mineral density[167-170]. Therefore, we included these variables in our analysis as the potential confounders. These factors were recorded as the following variables:

Age and sex: The study participants reported their age (in years) and their sex as a binary variable (male /female).

Total household income: The CLSA included one question about the participant's total household income. Individuals were asked to report their total household income from all sources before taxes and deductions in the past 12 months. This question had a multiple-choice format with the following response categories: less than $20,000; $20,000 or more, but less than $50,000$; $50,000 or more, but less than $100,000; $100,000 or more, but less than $150,000; $150,000 or more.

Education: Education level of the participants was recorded in a 4-category variable including the following response categories: "less than secondary school graduation", "secondary school graduation, no post-secondary education", "some post-secondary education", and post-secondary degree/diploma".

Ethnicity: Ethnicity of the participants were recorded in 13 categories. However, due to the small number of individuals in each category, we dichotomized the participants to "white" and "non-whites". This dichotomization has been done previously in other studies using the CLSA dataset[171].

b) Chronic health conditions:

Hypertension: Blood pressure was recorder in 6 different measurements and the mean of all the measurements except the first one was recorded in the dataset. Blood pressure (in mm/Hg) was dichotomized based on a recent Canadian guideline on the definition of hypertension, according to which automated office blood pressure (AOBP) is the preferred method for blood pressure measurement and systolic blood pressure of above 135 mmHg or diastolic blood pressure of above 85 mmHg is considered hypertensive in adults[172].

Diabetes: Diabetes diagnoses was confirmed using self-reported questionnaire asking the study participants if they have been diagnosed with type I diabetes or type II diabetes, or neither. Self-reported diabetes diagnosis has been suggested as a valid measurement method in observational studies showing an acceptable agreement with the medical records[(173)].

c) Lifestyle factors:

Smoking: The questions assessing smoking behavior in the CLSA were included in the in-home interviews. Based on this information, we modeled tobacco consumption in two separate variables. First, participants were categorized into 4 categories including "current daily smokers", "current occasional smokers", "ex-smokers", and "non-smokers". Second, "cigarette-years" variable was computed, which is reflecting the average number of cigarettes smoked daily multiplied by the number of the years an individual had spent smoking daily.

Alcohol consumption: Alcohol intake was recorded as a categorical variable classifying participant as "regular drinkers", "occasional drinkers", or "non-drinkers". According to CLSA definition of this derived variable called "alcohol use", a regular drinker is someone who drinks at least once a month, an "occasional drinker" drinks less than once a month, and a "non-drinker" is someone who has not drank in the past 12 months.

5.7. Statistical analysis

The statistical analysis was carried in 3 main steps. First, we conducted descriptive statistics of our main variables. Subsequently, cluster analysis was performed to group study participants based on their cognitive and oral health status. Finally, regression analysis was conducted to address the main objective of this thesis that is to estimate the association between cholinergic system activity

and oral and cognitive health. I describe these steps in the following sections. All statistical analyses were performed using R statistical software[174].

5.7.1. Step 1: Descriptive statistics

Descriptive statistics were performed to describe the basic socio-demographic characteristics of the total study sample, and different oral and cognitive health classes. For continuous variables, mean and standard deviation and for binary and categorical variables, frequency and percentages were calculated.

5.7.2. Step 2: cluster analysis

Model-based cluster analysis, also known as latent class analysis, was used to identify latent or unobserved classes of cognitive and oral health based on the observed variables. Latent class analysis (LCA) is a technique which uses maximum likelihood estimation to categorize individuals into each latent class, i.e., to each cluster of participants. In other words, this statistical method classifies individuals into unobserved classes according to their responses to a number of observed and measured categorical variables. For example, in marketing research LCA is used to identify clusters of customers/consumers based on their preferences, previous purchase decisions, or responses to a survey. Each cluster then will be targeted with different marketing strategy.

For each participant the probability of being a member of each class is reported. In this study, we used maximum probability to assign each individual to only one class of the latent variables called "Oral health" and Cognitive health". For each latent class analysis, we ran increasingly complex models by increasing the number of latent classes/clusters in the model, and then we used Bayesian

Information Criterion (BIC) and Akaike Information Criterion (AIC) to identify the model with the best fit.

For oral health cluster analysis, 24 binary observed variables described in the methods section were used in the models. 2-, 3-, 4-, 5-, 6-, and 7-class models were fitted to the data, and AIC, BIC, and the interpretability of the classes were used to decide on the final number of classes.

For cognitive functioning, we used scores from 7 cognitive measures. Prior to running cluster analysis, Spearman correlation coefficient was used to detect collinearity between cognitive measures, and those variables with high level of correlation (correlation coefficient of more than >0.6) were removed from the model. The high correlation between REY trial I and REY trial II (r=0.68) led us to exclude the latter. This decision was based on the fact that an individual with impaired immediate recall has a higher chance of showing impaired performance on delayed recall as well. After running the correlation and removing highly correlated variables, 7 variables (i.e., REY trial I, MAT, AF, Event-based and time-based prospective memory tasks, FAS, and the interference ratio from the Stroop test) were dichotomized using the median and included in the cluster analysis. To perform all latent class analyses, we used poLCA package[175] in R statistical software[174].

5.7.3. Step 3: Multinomial logistic regression

After identifying latent classes, we ran a series of multinomial logistic regression to estimate the extent to which cholinergic system activity, measured by bone mineral density, is associated with oral health and cognitive health classifications after adjusting for potential confounders explained in the methods chapter. Please refer to **Table 2** for the list of variables which was used in the logistic regression models.

The reason for selecting multinomial logistic regression was that our outcomes (i.e., oral health and cognitive health latent classes) were not in a specific order or hierarchy, that is, the categories of the outcome variables were not ordinal. For example, oral health classes could not be ordered from poor to excellent. Therefore, ordinal logistic regression was not used.

Six different regression models were generated using different subsets of dependent variables. The worst oral health and cognitive health status classes were set as the reference outcome categories, and the odds of falling in other classes versus the reference category was estimated using different predictors in the model. **Table 2** presents 6 different fitted models for each outcome variable. These models were analyzed for each outcome variable - oral and cognitive health classes- separately and with bone mineral density score as the main independent variable. Blocks of covariates were added to the model at each step, making the model more complex as we move from model 2 to 6. The only exception was model 5, in which smoking, and alcohol consumption was substituted with chronic conditions, namely hypertension and diabetes.

Table 2. *six different regression models fitted separately for each outcome variable.*

Model number	Independent variables
Model 1	Bone mineral density
Model 2	Bone mineral density, age, sex
Model 3	Bone mineral density, age, sex, education, total household income, ethnicity
Model 4	Bone mineral density, age, sex, education, total household income, ethnicity, smoking(categorical), smoking (cigarette-years), alcohol consumption
Model 5	Bone mineral density, age, sex, education, total household income, ethnicity, diabetes, hypertension
Model 6	Bone mineral density, age, sex, education, total household income, ethnicity, smoking(categorical), smoking (cigarette-years), alcohol consumption, Hypertension, diabetes

5.7.4. Sample size considerations

Since cluster analysis does not involve hypothesis testing, no sample size estimate was performed for Aim 1. However, the interpretability of the LCA output endorses the adequateness of the sample.

For Aim 2, our sample size considerations involved determining whether the available 30,000 participants would suffice for multinominal logistic regression. An OR of at least 1.09 would be detectable as significant with $\alpha = 0.05$ and power=0.80 for the Wald test. Assumptions for this estimate included: (1) at least 3 clusters of oral health and cognitive impairment; (2) Low bone density, as a proxy for cholinergic activity, correlates with poorer oral and cognitive conditions.

5.7.5. Handling missing data

Complete case analysis was conducted and cases with missing data were excluded from the regression analysis.

6. Results

6.1. Sociodemographic characteristics

The Comprehensive cohort in CLSA comprises of 30,097 participants, among whom 15,320 (50.9%) were males and 14,777 (49.1%) were females with mean age of 63.0 (SD: 10.3). The majority of the participants had post-secondary degree (77.5%) with 9.4%, 7.4%, and 5.5% having "secondary", "some post-secondary education", and "less than secondary education", respectively. Overall, 91.1% of the subjects reported their ethnicity to be white compared to 8.1% who were considered as non-whites. **Table 3**. summarizes sociodemographic characteristics of the Comprehensive cohort (N=30,097).

Table 3. *Socio-demographic characteristics of the CLSA comprehensive cohort*

	Overall N=30,097(%)
Age(years)	
Mean (SD)	63.0 (10.3)
Median [Min, Max]	62.0 [45.0, 86.0]
Sex	
Male	15320 (50.9)
Female	14777 (49.1)
Education	
Less than secondary	1643 (5.5)
Secondary	2839 (9.4)
Some post-secondary	2238 (7.4)
Post-secondary degree	23327 (77.5)
Missing	50 (0.2)
Total household income	
< $20,000	1566 (5.2)
≥ $20,000 and < $50,000	6360 (21.1)
≥ $50,000 and< $100,000	9907 (32.9)
≥ $100,000 and< $150,000	5524 (18.4)
≥ $150,000	4799 (15.9)

Missing	1941 (6.4)
Ethnicity	
White	27412 (91.1)
Non-white	1303 (4.3)
Missing	1382 (4.6)

Table 4 and **Table 6** provide descriptive summary of oral health and cognitive health variables in each age group, respectively.

Table 4. *Descriptive statistics of oral health variables by age group in the CLSA comprehensive cohort*

	45-54 N=7,595 (%)	**55-64 N=9,856 (%)**	**65-74 N=7,362(%)**	**+75 N=5,284(%)**	**Overall N=30,097(%)**
Self-rated oral health					
Excellent	2590 (34.1)	3098 (31.4)	2272 (30.9)	1423 (26.9)	9383 (31.2)
Very good	2846 (37.5)	3815 (38.7)	2795 (38.0)	1918 (36.3)	11374 (37.8)
Good	1389 (18.3)	1942 (19.7)	1558 (21.2)	1290 (24.4)	6179 (20.5)
Fair	325 (4.3)	484 (4.9)	359 (4.9)	247 (4.7)	1415 (4.7)
Poor	82 (1.1)	144 (1.5)	96 (1.3)	65 (1.2)	387 (1.3)
Missing	363 (4.8)	373 (3.8)	282 (3.8)	341 (6.5)	1359 (4.5)
Having one or more of your original teeth					
Yes	7114 (93.7)	9125 (92.6)	6456 (87.7)	4206 (79.6)	26901 (89.4)
No	125 (1.6)	368 (3.7)	638 (8.7)	755 (14.3)	1886 (6.3)
Missing	356 (4.7)	363 (3.7)	268 (3.6)	323 (6.1)	1310 (4.4)
Denture wearing					
Yes	668 (8.8)	1866 (18.9)	2401 (32.6)	2338 (44.2)	7273 (24.2)
No	6570 (86.5)	7624 (77.4)	4692 (63.7)	2623 (49.6)	21509 (71.5)
Missing	357 (4.7)	366 (3.7)	269 (3.7)	323 (6.1)	1315 (4.4)
Uncomfortable eating					
Often	157 (2.1)	224 (2.3)	184 (2.5)	124 (2.3)	689 (2.3)
Sometimes	562 (7.4)	766 (7.8)	522 (7.1)	412 (7.8)	2262 (7.5)
Rarely	1573 (20.7)	1943 (19.7)	1348 (18.3)	891 (16.9)	5755 (19.1)
Never	4937 (65.0)	6548 (66.4)	5029 (68.3)	3526 (66.7)	20040 (66.6)
Missing	366 (4.8)	375 (3.8)	279 (3.8)	331 (6.3)	1351 (4.5)
Eating avoidance due to Oral problems					
Often	101 (1.3)	175 (1.8)	145 (2.0)	107 (2.0)	528 (1.8)
Sometimes	291 (3.8)	461 (4.7)	378 (5.1)	308 (5.8)	1438 (4.8)
Rarely	809 (10.7)	1077 (10.9)	758 (10.3)	552 (10.4)	3196 (10.6)

	45-54 N=7,595 (%)	55-64 N=9,856 (%)	65-74 N=7,362(%)	+75 N=5,284(%)	Overall N=30,097(%)
Never	6026 (79.3)	7772 (78.9)	5806 (78.9)	3980 (75.3)	23584 (78.4)
Missing	368 (4.8)	371 (3.8)	275 (3.7)	337 (6.4)	1351 (4.5)
Toothache					
Yes	1139 (15.0)	1309 (13.3)	784 (10.6)	422 (8.0)	3654 (12.1)
No	6100 (80.3)	8185 (83.0)	6310 (85.7)	4540 (85.9)	25135 (83.5)
Missing	356 (4.7)	362 (3.7)	268 (3.6)	322 (6.1)	1308 (4.3)
Chewing inadequacy					
Yes	557 (7.3)	791 (8.0)	619 (8.4)	458 (8.7)	2425 (8.1)
No	6682 (88.0)	8703 (88.3)	6475 (88.0)	4504 (85.2)	26364 (87.6)
Missing	356 (4.7)	362 (3.7)	268 (3.6)	322 (6.1)	1308 (4.3)
Uncomfortable denture					
Yes	87 (1.1)	253 (2.6)	357 (4.8)	329 (6.2)	1026 (3.4)
No	7152 (94.2)	9241 (93.8)	6737 (91.5)	4633 (87.7)	27763 (92.2)
Missing	356 (4.7)	362 (3.7)	268 (3.6)	322 (6.1)	1308 (4.3)
Loose denture					
Yes	78 (1.0)	260 (2.6)	367 (5.0)	381 (7.2)	1086 (3.6)
No	7161 (94.3)	9234 (93.7)	6727 (91.4)	4581 (86.7)	27703 (92.0)
Missing	356 (4.7)	362 (3.7)	268 (3.6)	322 (6.1)	1308 (4.3)
Broken denture					
Yes	28 (0.4)	97 (1.0)	90 (1.2)	81 (1.5)	296 (1.0)
No	7211 (94.9)	9397 (95.3)	7004 (95.1)	4881 (92.4)	28493 (94.7)
Missing	356 (4.7)	362 (3.7)	268 (3.6)	322 (6.1)	1308 (4.3)
Missing denture					
Yes	8 (0.1)	14 (0.1)	23 (0.3)	18 (0.3)	63 (0.2)
No	7231 (95.2)	9480 (96.2)	7071 (96.0)	4944 (93.6)	28726 (95.4)
Missing	356 (4.7)	362 (3.7)	268 (3.6)	322 (6.1)	1308 (4.3)
Swelling in mouth					
Yes	363 (4.8)	481 (4.9)	332 (4.5)	196 (3.7)	1372 (4.6)
No	6876 (90.5)	9013 (91.4)	6762 (91.9)	4766 (90.2)	27417 (91.1)
Missing	356 (4.7)	362 (3.7)	268 (3.6)	322 (6.1)	1308 (4.3)
Dry mouth					
Yes	809 (10.7)	1545 (15.7)	1550 (21.1)	1357 (25.7)	5261 (17.5)
No	6430 (84.7)	7949 (80.7)	5544 (75.3)	3605 (68.2)	23528 (78.2)
Missing	356 (4.7)	362 (3.7)	268 (3.6)	322 (6.1)	1308 (4.3)
Burning mouth					
Yes	89 (1.2)	142 (1.4)	135 (1.8)	77 (1.5)	443 (1.5)
No	7150 (94.1)	9352 (94.9)	6959 (94.5)	4885 (92.4)	28346 (94.2)

	45-54 N=7,595 (%)	55-64 N=9,856 (%)	65-74 N=7,362(%)	+75 N=5,284(%)	Overall N=30,097(%)
Missing	356 (4.7)	362 (3.7)	268 (3.6)	322 (6.1)	1308 (4.3)
Sore jaw muscle					
Yes	570 (7.5)	562 (5.7)	295 (4.0)	173 (3.3)	1600 (5.3)
No	6669 (87.8)	8932 (90.6)	6799 (92.4)	4789 (90.6)	27189 (90.3)
Missing	356 (4.7)	362 (3.7)	268 (3.6)	322 (6.1)	1308 (4.3)
Jaw joint pain					
Yes	562 (7.4)	609 (6.2)	347 (4.7)	206 (3.9)	1724 (5.7)
No	6677 (87.9)	8885 (90.1)	6747 (91.6)	4756 (90.0)	27065 (89.9)
Missing	356 (4.7)	362 (3.7)	268 (3.6)	322 (6.1)	1308 (4.3)
Decayed natural tooth					
Yes	994 (13.1)	1419 (14.4)	1102 (15.0)	686 (13.0)	4201 (14.0)
No	6245 (82.2)	8075 (81.9)	5992 (81.4)	4276 (80.9)	24588 (81.7)
Missing	356 (4.7)	362 (3.7)	268 (3.6)	322 (6.1)	1308 (4.3)
Loose natural tooth					
Yes	337 (4.4)	516 (5.2)	357 (4.8)	177 (3.3)	1387 (4.6)
No	6902 (90.9)	8978 (91.1)	6737 (91.5)	4785 (90.6)	27402 (91.0)
Missing	356 (4.7)	362 (3.7)	268 (3.6)	322 (6.1)	1308 (4.3)
Broken natural tooth					
Yes	779 (10.3)	1123 (11.4)	768 (10.4)	484 (9.2)	3154 (10.5)
No	6460 (85.1)	8371 (84.9)	6326 (85.9)	4478 (84.7)	25635 (85.2)
Missing	356 (4.7)	362 (3.7)	268 (3.6)	322 (6.1)	1308 (4.3)
Soreness of the gums around natural teeth					
Yes	748 (9.8)	864 (8.8)	496 (6.7)	226 (4.3)	2334 (7.8)
No	6491 (85.5)	8630 (87.6)	6598 (89.6)	4736 (89.6)	26455 (87.9)
Missing	356 (4.7)	362 (3.7)	268 (3.6)	322 (6.1)	1308 (4.3)
bleeding of gums around natural teeth					
Yes	1102 (14.5)	1226 (12.4)	638 (8.7)	263 (5.0)	3229 (10.7)
No	6137 (80.8)	8268 (83.9)	6456 (87.7)	4699 (88.9)	25560 (84.9)
Missing	356 (4.7)	362 (3.7)	268 (3.6)	322 (6.1)	1308 (4.3)
Denture related sores					
yes	66 (0.9)	164 (1.7)	244 (3.3)	193 (3.7)	667 (2.2)
no	7173 (94.4)	9330 (94.7)	6850 (93.0)	4769 (90.3)	28122 (93.4)
Missing	356 (4.7)	362 (3.7)	268 (3.6)	322 (6.1)	1308 (4.3)
Dirty denture or teeth					
Yes	266 (3.5)	312 (3.2)	165 (2.2)	87 (1.6)	830 (2.8)
No	6973 (91.8)	9182 (93.2)	6929 (94.1)	4875 (92.3)	27959 (92.9)
Missing	356 (4.7)	362 (3.7)	268 (3.6)	322 (6.1)	1308 (4.3)

	45-54 N=7,595 (%)	55-64 N=9,856 (%)	65-74 N=7,362(%)	+75 N=5,284(%)	Overall N=30,097(%)
Bad breath					
Yes	672 (8.8)	724 (7.3)	455 (6.2)	226 (4.3)	2077 (6.9)
No	6567 (86.5)	8770 (89.0)	6639 (90.2)	4736 (89.6)	26712 (88.8)
Missing	356 (4.7)	362 (3.7)	268 (3.6)	322 (6.1)	1308 (4.3)

Table 5*. Descriptive statistics of cognitive tests by age group in the CLSA comprehensive cohort*

	45-54 (N=7,595)	55-64 (N=9,856)	65-74 (N=7,362)	+75 (N=5,284)	Overall (N=30,097)
Rey Auditory Verbal Learning Test (RAVLT)-immediate recall					
Mean (SD)	6.51 (1.85)	6.16 (1.82)	5.59 (1.78)	4.67 (1.70)	5.85 (1.91)
Median [Min, Max]	6.00 [0, 14.0]	6.00 [0, 14.0]	6.00 [0, 14.0]	5.00 [0, 13.0]	6.00 [0, 14.0]
Missing	214 (2.8)	297 (3.0)	260 (3.5)	249 (4.7)	1020 (3.4)
Mental Alteration Test (MAT)					
Mean (SD)	28.9 (8.58)	27.6 (8.32)	25.5 (8.44)	22.4 (8.58)	26.5 (8.75)
Median [Min, Max]	30.0 [0, 51.0]	28.0 [0, 51.0]	26.0 [0, 51.0]	22.0 [0, 51.0]	27.0 [0, 51.0]
Missing	275 (3.6)	411 (4.2)	400 (5.4)	400 (7.6)	1486 (4.9)
Animal Fluency Test (AFT)					
Mean (SD)	23.8 (6.45)	22.5 (6.22)	20.2 (5.97)	17.6 (5.50)	21.4 (6.47)
Median [Min, Max]	24.0 [0, 52.0]	22.0[1.00,48.0]	20.0 [0, 48.0]	17.0 [0, 43.0]	21.0 [0, 52.0]
Missing	162 (2.1)	208 (2.1)	192 (2.6)	169 (3.2)	731 (2.4)
Event-based task-Miami Prospective Memory Test (MPMT)					
Mean (SD)	8.76 (0.914)	8.65 (1.10)	8.35 (1.49)	7.71 (1.98)	8.44 (1.40)
Median [Min, Max]	9.00 [0, 9.00]	9.00 [0, 9.00]	9.00 [0, 9.00]	9.00 [0, 9.00]	9.00 [0, 9.00]
Missing	37 (0.5)	79 (0.8)	69 (0.9)	59 (1.1)	244 (0.8)
Time-based task-Miami Prospective Memory Test (MPMT)					
Mean (SD)	8.82 (0.66)	8.76 (0.75)	8.63 (1.00)	8.27 (1.44)	8.66 (0.97)
Median [Min, Max]	9.00 [0, 9.00]	9.00 [0, 9.00]	9.00 [0, 9.00]	9.00 [0, 9.00]	9.00 [0, 9.00]
Missing	95 (1.3)	133 (1.3)	139 (1.9)	123 (2.3)	490 (1.6)
Controlled Oral Word Association Test (COWAT)					
Mean (SD)	41.5 (12.4)	40.3 (12.6)	37.9 (12.7)	35.6 (12.8)	39.2 (12.8)
Median [Min, Max]	41.0 [6.00, 99.0]	40.0 [3.00, 105]	37.0 [5.00, 86.0]	35.0 [4.00, 90.0]	39.0 [3.00, 105]
Missing	201 (2.6)	358 (3.6)	291 (4.0)	217 (4.1)	1067 (3.5)

	45-54 (N=7,595)	55-64 (N=9,856)	65-74 (N=7,362)	+75 (N=5,284)	Overall (N=30,097)
Stroop test-interference ratio					
Mean (SD)	1.95 (0.54)	2.10 (0.73)	2.27 (0.79)	2.42 (0.75)	2.16 (0.73)
Median [Min, Max]	1.88 [0.05, 19.5]	2.00[0.06, 32.0]	2.15 [0.04, 38.1]	2.30 [0.09, 11.3]	2.00 [0.045, 38.1]
Missing	74 (1.0)	125 (1.3)	120 (1.6)	102 (1.9)	421 .4)

6.2. Latent class analysis

As described in the methods chapter, latent class analysis was used to classify participants into unobserved clusters based on measured cognitive scores and self-reported oral health status. These oral health and cognitive health classes were then used as outcome variables. Similar to other methods of cluster analysis, LCA uses individual's responses and scores to cluster them into classes. However, in LCA the *"probability"* of being assigned to a class is obtained for individuals rather than their definite class membership. In the following sections, I describe the results of latent class analyses to identify "oral health" and "cognitive health" classes as our outcome variables.

6.2.1. Oral health

Selection of the model: After analyzing two- to seven-class models, we chose the models with the best fit indices (AIC and BIC), providing the better interpretability. AIC and BIC of these consecutively complex models are shown in **Table 6**. As we can observe, the 6-class solution showed the best model fit. However, considering the clinical interpretability of the models, 5-class model was superior to the 6-class solution. Therefore, I chose 5-class solution for oral health cluster analysis. After choosing the best model, individuals were assigned to oral health classes based on their maximum class membership probability.

Table 6. *Model fit statistics for 2- to 7-class models for oral health status*

	Model fit statistics	
	AIC	**BIC**
Number of classes		
2	314893.6	315298.5
3	305105.9	305717.4
4	300887.7	301705.7
5	298952.5	299977.1
6	283289.2	284470.8
7	296054.2	297491.9

Describing oral health classes: **Figure 2** and **Figure 3** present the likelihood of reporting "favorable oral health" for each variable/question in each class. Based on the patterns of responses to different questions, classes were named and described as below:

- *Class 1: The best oral health class*

Class 1 was the largest latent group comprising 15,922 (62.6%) of the study participants. Participants in this class had 99% probability of "having one or more of their natural teeth" and only 12% probability of having "denture or false teeth". One average, individuals in this class had 97% probability of reporting "favorable" oral health status.

- *Class 2: Denture wearers with poor oral health*

This class represents the smallest group with the poorest oral health status and included 882 individuals (3.2% of the sample). Individuals in this class had 65% probability of "having one or more of their natural teeth" and 99% probability of "wearing dentures or false teeth". Compared to others, these participants were also more likely to report uncomfortable eating (63%), uncomfortable dentures (69%), inadequate chewing (52%) and eating avoidance due to mouth problems (50%).

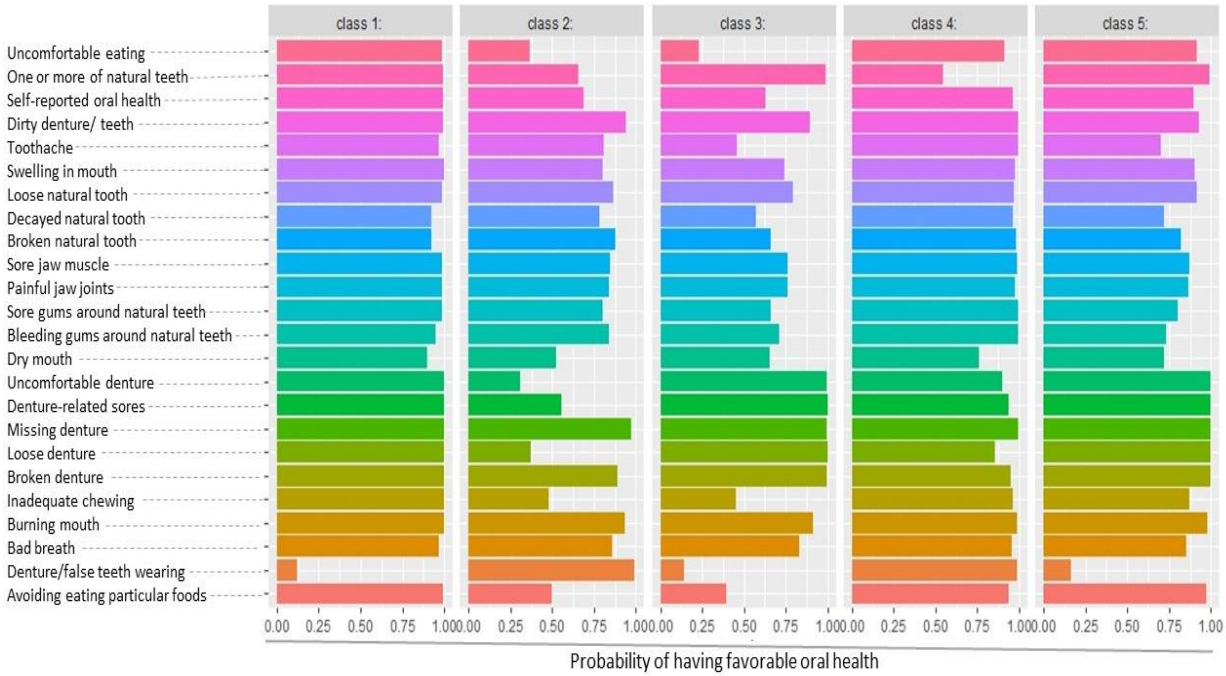

Figure 2*. Bar graphs presenting probability of reporting favorable oral health for each variable in different oral health classes.*

- *Class 3: Non-denture wearers with poor oral health*

Comprising 5.2% of the study sample (n observations= 1320), this latent class is characterized by 98% probability of having one or more of their natural teeth and 14% probability of wearing dentures/false teeth. This group was distinct from other classes in reporting the highest probability of experiencing uncomfortable eating (77%), inadequate chewing (54%), eating avoidance due to mouth problems (6%) in the past 12 months. This class members also had 23% probability of experiencing painful jaw joints and sore jaw muscles.

- *Class 4: Denture wearers with good oral health*

Class 4 represents 8.8% of the sample (n observations= 2,251). Compared to other study participants, individuals in this group had the lowest probability of "having one or more of their natural teeth" (54%). They also had the second highest probability of wearing dentures (98%). These participants were more likely to report their oral health as excellent or good (96%).

- *Class 5: Non-denture wearers with moderate oral health status*

Class 5 comprised 20.2% of the study participants (n observations=5,129). Individuals in this latent class, which was the second largest group, had 16% probability of wearing "denture/false teeth". Participants in this subgroup had moderate levels of oral disease distinguishing them from class 1 members who had higher probability of reporting favorable oral health. Participants in this group had higher probability of experiencing toothache, tooth decay in the past 12 months (30% and 28%, respectively).

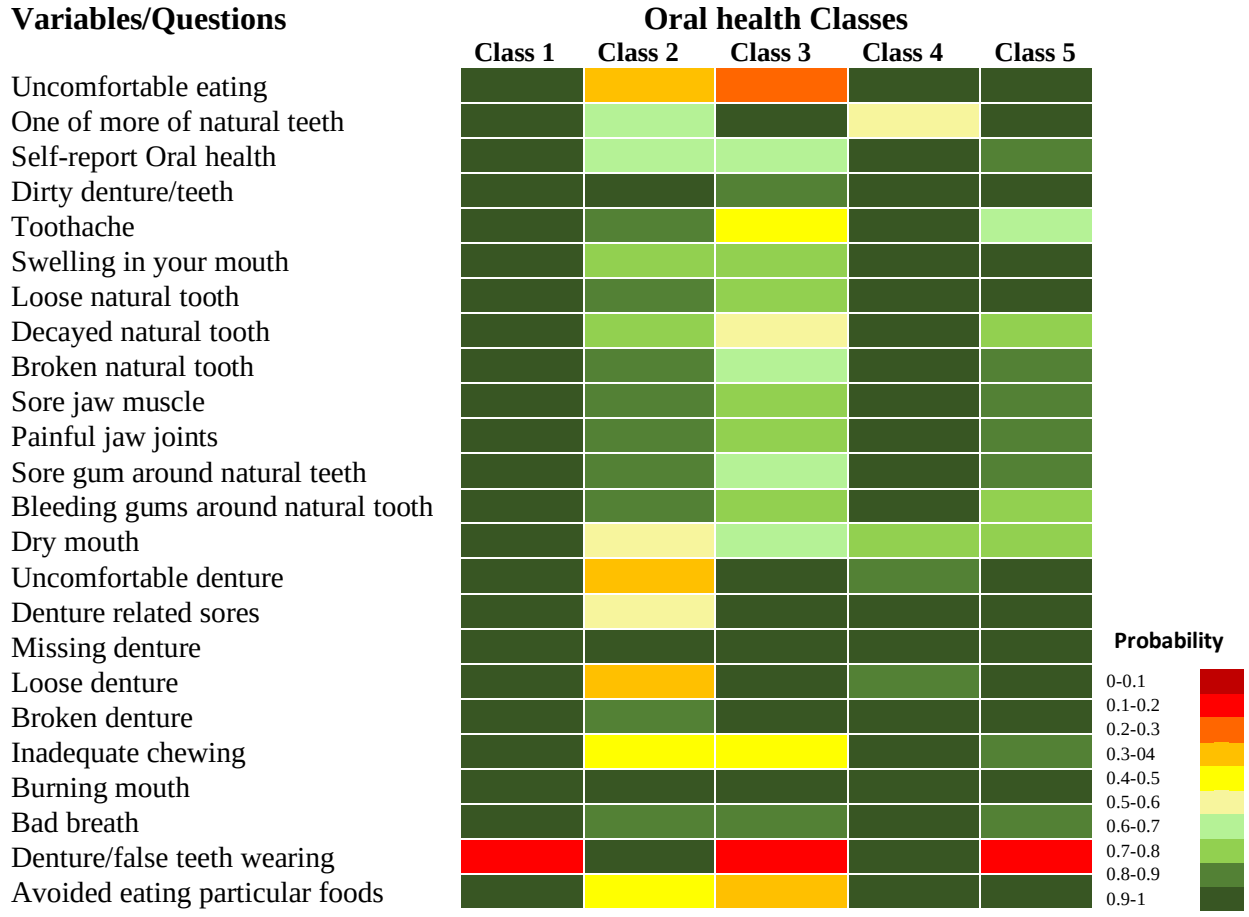

Figure 3. *Heat map visualizing the probability of individuals in each class to report favorable oral health*

Descriptive statistics for sociodemographic variables and confounders by each class: After assigning individuals to oral health latent classes and describing the classes, descriptive statistics of basic socio-demographic variables and confounders by each cluster is presented in **Table 7**.

Table 7. *Descriptive statistics of selected variables according to oral health classes*

	Class 1 N=15,922(%)	**Class 2 N=822(%)**	**Class 3 N=1,320(%)**	**Class 4 N=2,251(%)**	**Class 5 N=5,129(%)**	**Overall N=25,444(%)**
Age(years)						
Mean (SD)	62.0 (9.96)	67.8 (9.49)	60.8 (9.44)	70.6 (8.76)	60.9 (9.63)	62.6 (10.1)
Median [Min, Max]	61.0 [45.0, 86.0]	68.0 [45.0, 85.0]	60.0 [45.0, 86.0]	71.0 [45.0, 86.0]	60.0 [45.0, 86.0]	62.0 [45.0, 86.0]
Sex						
Male	7971 (50.1)	441 (53.6)	780 (59.1)	1205 (53.5)	2638 (51.4)	13035 (51.2)
Female	7951 (49.9)	381 (46.4)	540 (40.9)	1046 (46.5)	2491 (48.6)	12409 (48.8)
Education						
> secondary	527 (3.3)	131 (15.9)	71 (5.4)	370 (16.4)	177 (3.5)	1276 (5.0)
Secondary	1388 (8.7)	112 (13.6)	106 (8.0)	328 (14.6)	390 (7.6)	2324 (9.1)
Some post-secondary	1102 (6.9)	66 (8.0)	109 (8.3)	207 (9.2)	360 (7.0)	1844 (7.2)
Post-secondary	12890(81.0)	508 (61.8)	1030 (78.0)	1340 (59.5)	4195 (81.8)	19963 (78.5)
Missing	15 (0.1)	5 (0.6)	4 (0.3)	6 (0.3)	7 (0.1)	37 (0.1)
Total household income						
<$20,000	419 (2.6)	130 (15.8)	130 (9.8)	251 (11.2)	272 (5.3)	1202 (4.7)
≥$20,000-<$50,000	2658 (16.7)	317 (38.6)	317 (24.0)	899 (39.9)	1022 (19.9)	5213 (20.5)
≥$50,000-<$100,000	5421 (34.0)	227 (27.6)	429 (32.5)	677 (30.1)	1749 (34.1)	8503 (33.4)
≥$100,000-$150,000	3379 (21.2)	68 (8.3)	212 (16.1)	172 (7.6)	978 (19.1)	4809 (18.9)
≥$150,000	3161 (19.9)	25 (3.0)	149 (11.3)	79 (3.5)	816 (15.9)	4230 (16.6)
Missing	884 (5.6)	55 (6.7)	83 (6.3)	173 (7.7)	292 (5.7)	1487 (5.8)
Ethnicity						
White	14644(92.0)	736 (89.5)	1158 (87.7)	2067 (91.8)	4609 (89.9)	23214 (91.2)
Non-white	1278 (8.0)	86 (10.5)	162 (12.3)	184 (8.2)	520 (10.1)	2230 (8.8)
Diabetes						
Type I	69 (0.4)	6 (0.7)	8 (0.6)	19 (0.8)	33 (0.6)	135 (0.5)
Type II	1121 (7.0)	141 (17.2)	141 (10.7)	333 (14.8)	507 (9.9)	2243 (8.8)
Neither	14652(92.0)	664 (80.8)	1155 (87.5)	1858 (82.5)	4546 (88.6)	22875 (89.9)
Missing	80 (0.5)	11 (1.3)	16 (1.2)	41 (1.8)	43 (0.8)	191 (0.8)
Hypertension						
Yes	6449 (40.5)	432 (52.6)	532 (40.3)	1312 (58.3)	2091 (40.8)	10816 (42.5)
No	9166 (57.6)	358 (43.6)	754 (57.1)	858 (38.1)	2902 (56.6)	14038 (55.2)
Missing	307 (1.9)	32 (3.9)	34 (2.6)	81 (3.6)	136 (2.7)	590 (2.3)
Alcohol consumption						

	Class 1 N=15,922(%)	Class 2 N=822(%)	Class 3 N=1,320(%)	Class 4 N=2,251(%)	Class 5 N=5,129(%)	Overall N=25,444(%)
Regular drinker	12488(78.4)	515 (62.7)	858 (65.0)	1416 (62.9)	3781 (73.7)	19058 (74.9)
Occasional drinker	1647 (10.3)	132 (16.1)	215 (16.3)	393 (17.5)	667 (13.0)	3054 (12.0)
No drinking	1475 (9.3)	154 (18.7)	204 (15.5)	368 (16.3)	583 (11.4)	2784 (10.9)
Missing	312 (2.0)	21 (2.6)	43 (3.3)	74 (3.3)	98 (1.9)	548 (2.2)
Smoking (Categorical)						
Non-smokers	8316 (52.2)	226 (27.5)	570 (43.2)	729 (32.4)	2373 (46.3)	12214 (48.0)
Daily smokers	700 (4.4)	132 (16.1)	175 (13.3)	244 (10.8)	390 (7.6)	1641 (6.4)
Occasional smokers	241 (1.5)	10 (1.2)	15 (1.1)	27 (1.2)	101 (2.0)	394 (1.5)
Ex-smokers	5683 (35.7)	430 (52.3)	483 (36.6)	1157 (51.4)	1958 (38.2)	9711 (38.2)
Missing	982 (6.2)	24 (2.9)	77 (5.8%)	94 (4.2)	307 (6.0)	1484 (5.8)
Smoking (Cig-yrs.)						
Mean (SD)	28.7 (51.7)	82.9 (86.5)	46.8 (68.2)	74.1 (84.9)	36.5 (58.9)	37.0 (61.2)
Median [Min, Max]	0 [0, 490]	60.0 [0, 490]	6.00 [0, 384]	40.0 [0, 490]	0 [0, 392]	0 [0, 490]
Missing	1087 (6.8)	47 (5.7)	98 (7.4)	127 (5.6)	346 (6.7)	1705 (6.7)
Whole-body bone mineral density						
Mean (SD)	1.14 (0.13)	1.11 (0.14)	1.13 (0.13)	1.11 (0.14)	1.14 (0.13)	1.14 (0.13)
Median [Min, Max]	1.14 [0.71, 1.84]	1.10 [0.70, 1.75]	1.12 [0.77, 1.99]	1.10 [0.74, 1.75]	1.14 [0.74, 1.78]	1.13 [0.70, 1.99]
Missing	534 (3.4)	47 (5.7)	50 (3.8)	96 (4.3)	205 (4.0)	932 (3.7)

6.2.2. Cognitive Health

Selection of the model: We tested two- to five-class models and compared those models in terms of model fit indices and interpretability. The model with 4 latent classes showed the best model fit and lowest AIC and BIC. **Table 8** presents the goodness-of-fit indices for different LCA models. We selected the 4-class model, because it presented the best model fit and good interpretability.

Table 8. *Model fit statistics for 2- to 5-class models for cognitive health status*

	Model fit statistics	
	AIC	**BIC**
Number of classes		
2	186256.7	186363.3

3	220579.7	220768.0
4	185680.4	185901.7
5	220394.3	220713.7

Describing cognitive health classes: **Figure 4** and **Figure *5*** present and visualize the by class probability of performing higher than median in each cognitive test. Based on the cognitive performance of each group, these latent classes were described and characterized as below.

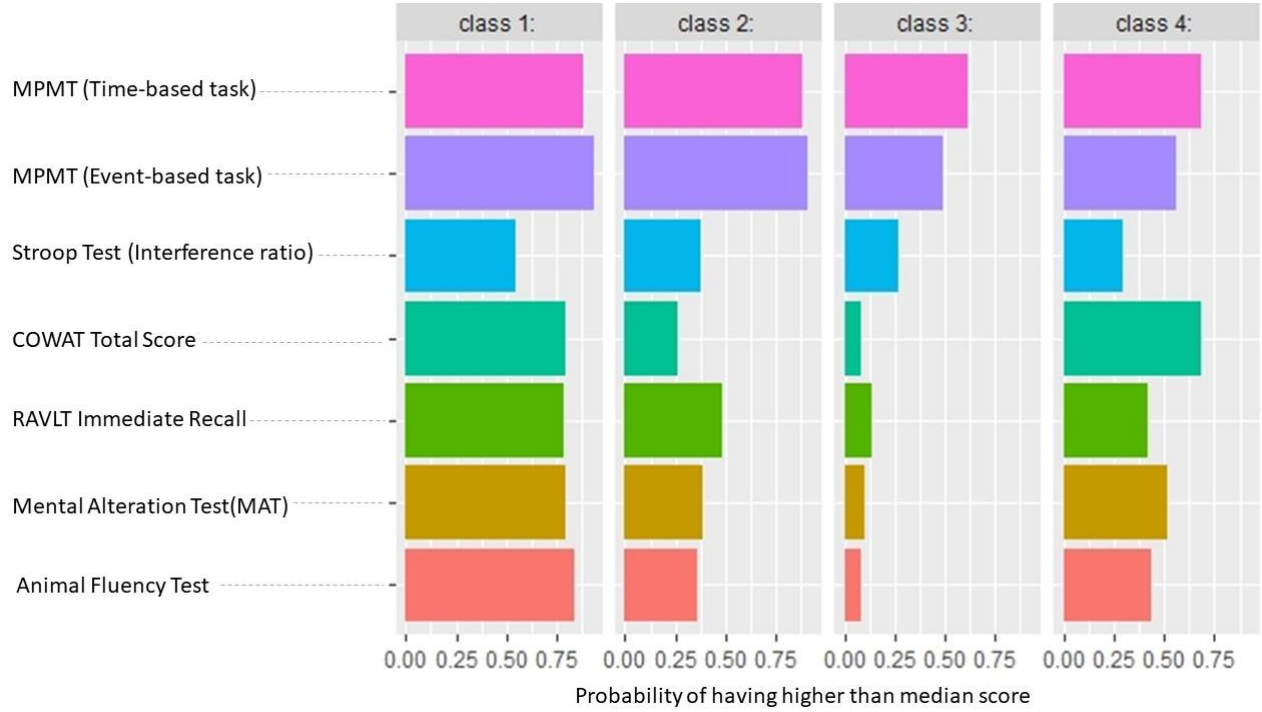

Figure 4. *Bar graphs presenting probability of performing higher than median score for each cognitive test in different cognitive health classes.*

- *Class 1: Good cognitive performance*

Class 1 represents the largest latent class, comprising 47.8% (n observation= 12,173) of the total sample. For all cognitive measures except for Stroop interference ratio, participants in this class had >75% probability of performing higher than median.

- *Class 2: Poor verbal fluency*

This group is characterized by low performance on COWAT and high performance on prospective memory test. The members of this latent class, which represents 18.8% (n observations= 4754) of the total sample, had only 0.26 probability of performing higher than median on COWAT and 90% and 88% probability of performing higher than median on event-based and time-based tasks of MPMT, respectively.

- *Class 3: Poor cognitive performance (poor verbal fluency and memory)*

Class 3 is the smallest latent class representing 7.8% (n observations= 1989) of the total sample. Individuals in this group were characterized by the lowest performance on all seven cognitive measures.

- *Class 4: Low memory performance*

This latent class was the second largest group comprising 25.5% (n observations= 6499) of the sample. Compared to other groups, this group of individuals were characterized by lower performance on memory tasks, especially prospective memory, and higher performance on COWAT (68%). Probability of performing higher than median for individuals in this group was 41%, 56%, and 68% for RAVLT, event-based and time-based task of MPMT, respectively.

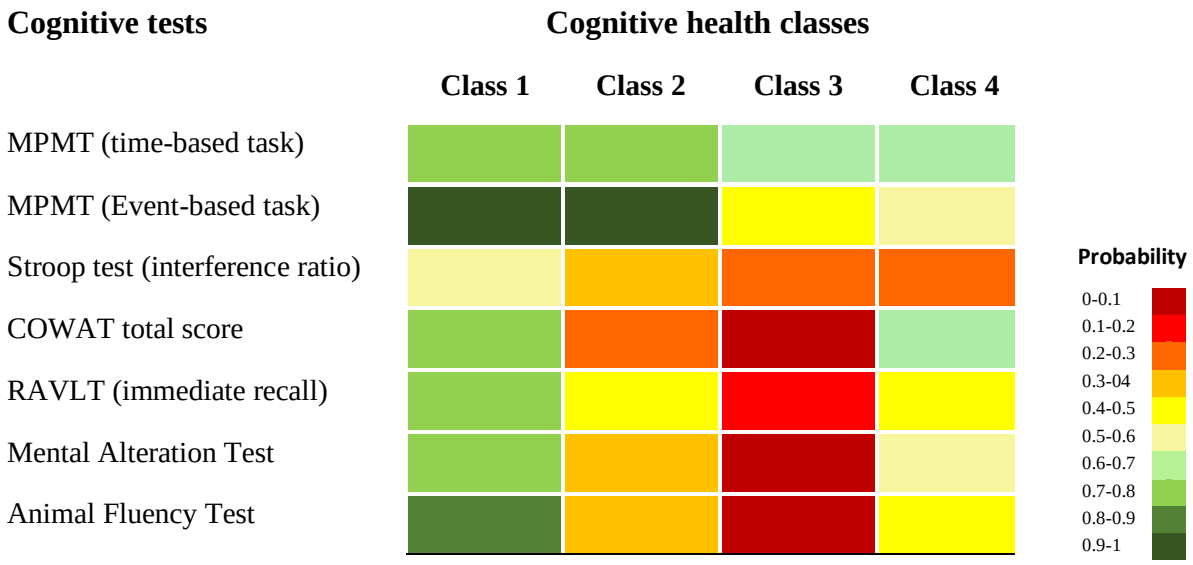

Figure 5. *Heat map visualizing the likelihood of performing higher than median in cognitive test in each class*

Descriptive statistics for selected variables in each class: After selecting the model with optimal number of cognitive health classes and describing the characteristics of each class, we assigned individuals to these latent classes and conducted descriptive statistics for sociodemographic and confounding variables by each cognitive health class (See **Table 9**).

Table 9. *Descriptive statistics of selected variables according to cognitive status class*

	Class 1 N=12,173(%)	**Class 2 N=4,783(%)**	**Class 3 N=1,989(%)**	**Class 4 N=6,499(%)**	**Overall N=25,444(%)**
Age(years)					
Mean (SD)	59.2 (8.80)	62.9 (9.57)	71.8 (9.34)	66.2 (10.1)	62.6 (10.1)
Median [Min, Max]	58.0 [45.0, 86.0]	62.0 [45.0,86.0]	74.0 [45.0, 86.0]	66.0 [45.0, 86.0]	62.0 [45.0, 86.0]
Sex					
Male	6,290 (51.7)	2,424(50.7)	980 (49.3)	3,341(51.4)	13,035 (51.2)
Female	5,883 (48.3)	2,359(49.3)	1,009(50.7)	3,158(48.6)	12,409 (48.8)
Education					
> secondary	185 (1.5)	194 (4.1)	315 (15.8)	582 (9.0)	1276 (5.0)
Secondary	805 (6.6)	453 (9.5)	260 (13.1)	806 (12.4)	2324 (9.1)
Some post-secondary	807 (6.6)	360 (7.5)	182 (9.2)	495 (7.6)	1844 (7.2)

	Class 1 N=12,173(%)	Class 2 N=4,783(%)	Class 3 N=1,989(%)	Class 4 N=6,499(%)	Overall N=25,444(%)
Post-secondary degre	10,368(85.2)	3,772(78.9)	1,224(61.5)	4,599(70.8)	19,963 (78.5)
Missing	8 (0.1)	4 (0.1)	8 (0.4)	17 (0.3)	37 (0.1)
Income					
< $20,000	327 (2.7)	210 (4.4)	232 (11.7)	433 (6.7)	1,202 (4.7)
≥ $20,000-<$50,000	1,680 (13.8)	987 (20.6)	719 (36.1)	1,827 (28.1)	5,213 (20.5)
≥$50,000-<$100,000	3,943(32.4)	1,678(35.1)	587 (29.5)	2,295(35.3)	8,503 (33.4)
≥$100,000-<$150,00	2,842 (23.3)	896 (18.7)	162 (8.1)	909 (14.0)	4,809 (18.9)
≥ $150,000	2,834 (23.3)	727 (15.2)	87 (4.4)	582 (9.0)	4,230 (16.6)
Missing	547 (4.5)	285 (6.0)	202 (10.2)	453 (7.0)	1,487 (5.8)
Ethnicity					
White	11265 (92.5)	4403 (92.1)	1771 (89.0)	5775 (88.9)	23214 (91.2)
Non-white	908 (7.5)	380 (7.9)	218 (11.0)	724 (11.1)	2230 (8.8)
Diabetes					
Type I	48 (0.4)	24 (0.5)	17 (0.9)	46 (0.7)	135 (0.5)
Type II	771 (6.3)	457 (9.6)	306(15.4)	709 (10.9)	2243 (8.8)
Neither	11305(92.9)	4262 (89.1)	1628 (81.9)	5680 (87.4)	22875 (89.9)
Missing	49 (0.4)	40 (0.8)	38 (1.9)	64 (1.0)	191 (0.8)
Hypertension					
Yes	4279 (35.2)	2130 (44.5)	1192 (59.9)	3215 (49.5)	10816 (42.5)
No	7662 (62.9)	2556 (53.4)	744 (37.4)	3076 (47.3)	14038 (55.2)
Missing	232 (1.9)	97 (2.0)	53 (2.7)	208 (3.2)	590 (2.3)
Alcohol consumption					
Regular drinker	9691 (79.6)	3571 (74.7)	1210 (60.8)	4586 (70.6)	19058 (74.9)
Occasional drinker	1176 (9.7)	597 (12.5)	347 (17.4)	934 (14.4)	3054 (12.0)
No drinking	1096 (9.0)	535 (11.2)	352 (17.7)	801 (12.3)	2784 (10.9)
Missing	210 (1.7)	80 (1.7)	80 (4.0)	178 (2.7)	548 (2.2)
Smoking (categorical)					
Non-smokers	6265 (51.5)	2203 (46.1)	896 (45.0)	2850 (43.9)	12214 (48.0)
Daily smokers	680 (5.6)	329 (6.9)	152 (7.6)	480 (7.4)	1641 (6.4)
Occasional smokers	182 (1.5)	93 (1.9)	29 (1.5)	90 (1.4)	394 (1.5)
Ex-smokers	4346 (35.7)	1868 (39.1)	809 (40.7)	2688 (41.4)	9711 (38.2)
Missing	700 (5.8)	290 (6.1)	103 (5.2)	391 (6.0)	1484 (5.8)
Smoking (Cig-yrs)					
Mean (SD)	30.0 (52.6)	38.7 (61.4)	49.3 (73.7)	45.3 (69.4)	37.0 (61.2)
Median [Min, Max]	0 [0,490]	0 [0,490]	2.00[0,392]	3.00[0,490]	0 [0,490]
Missing	799 (6.6)	333 (7.0)	117 (5.9)	456 (7.0)	1705 (6.7)

	Class 1 N=12,173(%)	Class 2 N=4,783(%)	Class 3 N=1,989(%)	Class 4 N=6,499(%)	Overall N=25,444(%)
Whole-body bone mineral density					
Mean (SD)	1.12 (0.146)	1.14 (0.132)	1.14 (0.137)	1.12 (0.139)	1.14 (0.136)
Median [Min, Max]	1.11 [0.73, 1.84]	1.14 [0.71, 1.99]	1.14 [0.70, 1.78]	1.12 [0.72, 1.93]	1.13 [0.70, 1.99]
Missing	107 (5.4)	385 (3.2)	189 (4.0)	251 (3.9)	932 .7)

6.3. Regression models

Multinomial logistic regression models were fitted to the data to estimate the extent to which whole-body bone mineral density score can explain the oral health and cognitive health classification, after adjusting for different sets of confounders. Six models were fitted to the data for each outcome variable, namely oral health status and cognitive health status. Results for the first set of regressions are presented in **Table 10**. In these models, oral health was the outcome variable and Class 2 (i.e., the poorest oral health status group) was considered as the refence group. Model 1 presents unadjusted odds ratios; one unit increase in bone mineral density score was associated with 6.54 (95% confidence interval (CI): 3.46-12.35) and 1.38 (95% CI: 0.69-2.76) times greater odds of falling into classes 5 and 4 categories, respectively compared to Class 2. After adjusting for age and sex (Model 2), one unit increase in bone mineral density was associated with 2.5 (95% CI: 1.25-5.05) times greater odds of being in class 5 rather than class 2 (in male participants and when age was kept constant). In model 3, we adjusted for all sociodemographic variables including age, sex, education, ethnicity, and total household income. In this model, the odds ratios further reduced and the odds of being a member of class 1 (i.e., the best oral health status group) was 1.84 (95% CI: 0.94,3.6) times greater the odds of being in class 2 when bone mineral density was increased for one unit and when all other covariates were kept constant and at their reference level. In all models, one unit increase in bone mineral density was associated with

higher odds of falling into other classes rather than class 2 (the poorest oral health status group). After adjusting for all confounders, one unit increase in the body bone mineral density score was associated with 1.26 (95% CI: 0.63-2.5) and 1.94 (95% CI: 1.01-3.69) times greater odds of membership in class 4 and 5 compared to odds of class 2 membership, respectively.

Table 10. *Multinomial regression models for the associations between bone mineral density scores and oral health classes*

	Model 1 *	**Model 2** #	**Model 3** Σ	**Model 4** $	**Model 5** @	**Model 6** &
Denture poor OH (class 2)	1	1	1	1	1	1
Non-denture poor OH (class 3)	2.72 [1.29,5.75]	1.35 [0.58,3.13]	1.33 [0.57,3.07]	1.23 [0.53,2.81]	1.46 [0.67,3.17]	1.31 [0.6,2.83]
Denture good OH (class 4)	1.38 [0.69,2.76]	1.45 [0.68,3.05]	1.34 [0.64,2.82]	1.23 [0.59,2.58]	1.38 [0.69,2.75]	1.26 [0.63,2.5]
Non-denture moderate OH (class 5)	6.54 [3.46,12.35]	2.5 [1.25,5.05]	2.25 [1.11,4.54]	1.81 [0.9,3.66]	2.49 [1.3,4.75]	1.94 [1.2,3.7]
Best OH (class 1)	5.81 [3.16,10.66]	2.2 [1.13, 4.27]	1.84 [0.94,3.6]	1.41 [0.72,2.76]	2.16 [1.17,3.99]	1.58 [0.85,2.92]

Class 2 is the reference group
*crude association between bone mineral density score and oral health status classification
Estimation adjusted for age and sex
Σ Estimation adjusted for age, sex, education, total household income, and ethnicity
$ Estimation adjusted for age, sex, education, total household income, ethnicity, smoking (Categorical), smoking (cigarette-years)
@Estimation adjusted for age, sex, education, total household income, ethnicity, smoking (Categorical), smoking (cigarette-years), hypertension, and diabetes
& Estimation adjusted for age, sex, education, total household income, ethnicity, smoking(categorical), smoking(cigarette-years), alcohol consumption, diabetes, hypertension

In the second set of regressions, we had "cognitive health classes" as the outcome and class 3 (i.e., the group with the lowest cognitive function) was the reference group. Therefore, the odds ratios presented in **Table 11** are the odds of falling in other groups rather than class 3. Model 1 was unadjusted. In this model, one unit increase in bone mineral score was associated with 4.32 (95% CI: 0.86-6.5) and 1.6 (95% CI: 1.03-2.48) times greater odds of falling into classes 2 and 4 rather than class 3, respectively. In model 2, we adjusted for age and sex and the odds of falling in all

classes compared to class 3 reduced (in male participants and if the age was kept constant). In all models, the odds of falling into class 4 rather than class 3 was lower than the odds of falling into class 2 and 1 when bone mineral density increased for one unit and when all confounders were kept constant and at their reference level.

Table 11. *Multinomial regression models for the associations between bone mineral density scores and cognitive health classes*

	Model 1 *	**Model 2** #	**Model 3** Σ	**Model 4** $	**Model 5** @	**Model 6** &
Lowest cognitive performance (Class 3)	1	1	1	1	1	1
Low verbal Fluency (Class 2)	3.03 [1.93,4.76]	1.72 [1.05,2.83]	1.62 [0.98,2.86]	1.53 [0.92,2.5]	1.71 [1.03,2.84]	1.61 [0.97,2.66]
Poor cognitive performance (Class 4)	1.6 [1.03,2.48]	1.2 [0.75,1.91]	1.19 [0.74-1.91]	1.13 [0.7,1.81]	1.26 [0.78,2.02]	1.19 [0.74,1.92]
Best cognitive performance (Class 1)	4.32 [0.86,6.5]	1.82 [1.14,2.9]	1.58 [0.98,2.55]	1.44 [0.89,2.32]	1.79 [1.11,2.88]	1.61 [1.00,2.6]

Class 3 is the reference group
*crude association between bone mineral density score and cognitive health status classification
Estimation adjusted for age and sex
Σ Estimation adjusted for age, sex, education, total household income, and ethnicity
$ Estimation adjusted for age, sex, education, total household income, ethnicity, smoking (Categorical), smoking (cigarette-years)
@Estimation adjusted for age, sex, education, total household income, ethnicity, smoking (Categorical), smoking (cigarette-years), hypertension, and diabetes
& Estimation adjusted for age, sex, education, total household income, ethnicity, smoking(categorical), smoking(cigarette-years), alcohol consumption, diabetes, hypertension

7. Discussion

The aims of this study were to first identify clusters of oral and cognitive health among the study sample, and second to estimate the extent to which the grouping of participants is explained by bone mineral density score as the proxy measure for cholinergic neurons' activity. In the following sections, I will discuss the results of my thesis in the context of current literature. I will also discuss the limitations and the strengths of this research project and its potential implications for future research and practice.

7.1. Oral health clusters

In this study, latent class analysis was used to assign individuals to clusters based on the pattern of their responses to a 24-item questionnaire. After identifying the optimum number of oral health classes, study participants were assigned to one of the 5 identified clusters.

Individuals in class 1 reported the best oral health among all classes and had the highest probability of reporting favorable oral health status. This cluster comprised the majority of the study sample, which indicates that the oral health status of the study sample is comparable to the report from a survey on a representative sample of Canadian population, in which 84% of Canadians reported their oral health as excellent or good[176]. Besides class 1, other two clusters included non-denture wearers (i.e., classes 3 and 5). Class 5 (i.e., the class with moderate oral health) had slightly higher probability of reporting toothache, decayed natural tooth, sore gums, bleeding gums, sore jaw muscle, painful jaw joint, bad breath, and dry mouth compared to class 1 with the best oral health status. This group also had higher probability to use dentures. Individuals in class 3 reported the poorest oral health among non-denture wearers. This group had the highest probability of reporting uncomfortable eating, eating avoidance due to mouth problems, toothache, and decayed natural

tooth. Given the characteristics of individuals in classes 3 and 5, their oral health problems seems to stem from their "natural teeth" rather than the prosthesis because they had low probability of reporting uncomfortable dentures or denture related sores.

Individuals in the other two classes, namely classes 2 and 4, had higher probability of "wearing dentures or false teeth" and being edentulous. Individuals in class 2 had the highest probability of reporting dry mouth, uncomfortable/loose dentures, denture related sores, and inadequate chewing among all classes. Compared to class 2, participants in class 4 were more likely to report edentulism, nevertheless, they reported better oral health compared to class 2 and even class 3 which comprised of non-denture wearers. These findings are in accordance with some studies reporting that adequate oral rehabilitation using complete dentures tends to improve patient-perceived oral health [177, 178]. This finding may also be due to the fact that patients who are receiving complete dentures and have fewer natural teeth have had a poor oral health status; in other words, they feel better compared to the past, when they may have been affected by severe caries and/or periodontal disease. Therefore, they might report better oral health after receiving prosthetic treatment. This argument is also mentioned in a study carried out by Montero et al[179].

Compared to non-denture wearers, denture-wearers were older on average, had lower education level, and reported lower total household income. These two classes were also characterized by higher proportion of patients with chronic conditions, namely hypertension and diabetes type II. In terms of health-related behaviors, non-denture wearers' groups had smaller number of regular drinkers and non-smokers. In summary, socioeconomic characteristics of denture wearers were distinct from denture wearers. These findings are in accordance with a study suggesting a strong association between tooth loss and 5-year age increase in the elderly[178]. Other studies have also

reported an association between tooth loss and lower education level, lower income, and tobacco consumption[180-183]. The association between alcohol consumption and oral health is controversial in the literature. One study for example has reported no association between alcohol consumption and poor oral health in a sample of young Finnish men[184] , while some others suggested that alcohol consumption is associated with higher number of teeth in the elderly[185, 186].

Tooth loss has shown to be associated with individuals' exposure to lower socioeconomic status and lower parental education level[187]. Although in CLSA information on parents' education does not exist[188], parental education has shown to impact children's educational attainment[189]. Some studies, however, have argued that one's own education rather than their parents' education is a stronger protecting factor for oral health[184].

7.2. Cognitive health clusters

Among cognitive health clusters, individuals in class 3 performed worst in all 7 cognitive tasks. This group also had the highest age mean, reported the lowest total household income, lowest education level, and had the highest percentage of chronic conditions, namely diabetes type II and hypertension. These findings are in accordance with the results of other studies reporting an association between cognitive function in the elderly and education attainment [190-193], income[194], hypertension[195], and diabetes type II [196, 197]. Also, the cumulative effect of exposure to lower socioeconomic status throughout an individual's lifetime on cognitive function has been supported by different studies using life course approaches[198].

In terms of alcohol and tobacco consumption, group with the lowest cognitive performance had the lowest percentage of regular drinkers and highest cigarette-years mean. These findings are in accordance with the results of some other studies reporting a protective effect of low to moderate

alcohol consumption against cognitive dysfunction and dementia in seniors[199-201]. In CLSA, a "regular drinker" is defined as someone who drink at least once a month, which makes the regular drinkers' group a relatively large group comprising those who consume mild to moderate amount of alcohol. Smoking, however, has been shown as a risk factor for cognitive decline[202, 203], although some studies have not found any association between cognitive health and tobacco consumption[204].

In contrast to the class with the worst performance, Class 1 is characterized by the best cognitive performance among all clusters. This group of participants were younger on average, had the highest level of education, reported the highest total household income, and had the lowest proportion of diabetic and hypertensive patients. Moreover, they had the highest proportion of regular drinkers and non-smokers.

Class 4 was the second oldest group, which comprised of participants reporting lower education and income level compared to classes 1 and 2. Compared to group 2, this group of participants showed worse performance on memory tasks including retrospective and prospective memory tasks. They specially performed worse on prospective memory tasks compared to classes 1 and 2. These findings could be explained by the differential effect of age on prospective and retrospective memory since class 4 individuals were older. This is in accordance with some studies reposting higher impact of aging on prospective memory tasks as they require more self-initiation[205, 206]. Nevertheless, some studies suggest that younger individuals perform worse in prospective memory test compared to their older counterparts[207]. Class 4 had higher probability of performing higher than median in verbal fluency tasks compared to class 2. The clustering pattern may just reflect the moderate correlation between the memory and verbal fluency of adults[208], possibly because

these functions are processed by different brain regions [209]. Specifically, class 4 performed better in phonemic fluency task (COWAT) compared to semantic fluency task (AFT). These findings could be explained by higher sensitivity of semantic fluency, and greater resistance of phonemic fluency to the effect of aging, as it is reported in some studies[210]. Moreover, the performance in fluency tasks could be affected by numerous factors other than those included in our analysis. For example, in another study on CLSA participants, verbal fluency tasks are shown to be associated with marriage status, dietary intake of certain nutrients, and immigration status [211]. Therefore, the observed difference among these groups could be due to characteristics not included in this study.

7.3. Cholinergic neurons' activity explaining oral and cognitive health clustering

Regression analysis was performed to investigate to what extent cholinergic neurons' activity could explain the clustering of participants based on their oral health and cognitive health status. In the following section, I discuss the results of these regression models separately and then jointly.

7.3.1. Cholinergic activity explaining oral health clustering

According to the results of regression models, one unit increase in bone mineral density was not associated with an increase in the odds of membership in classes with better oral health status after adjusting for all potential covariates, with a single exception (class 5/moderate OH). Based on that, it cannot be stated that cholinergic neurons' activity can explain the clustering of the participants based on their oral health status. However, as we move from model 1 to model 6 (**Table 10**)and adjust for more potential covariates, the odds of class membership, especially for classes with better oral health, reduces.

The odds of membership in class 4 (i.e., "denture wearers with better oral health") compared to class 2 (i.e., denture wearers with the worst oral health) remains the lowest in all models. Although participants in this group reported favorable oral health at CLSA baseline, this result might be an indicator of how these participants might had had experience of periodontal diseases and dental caries, which has led to tooth loss and required them to seek oral rehabilitation. This finding is supported by several studies investigating the reasons for tooth loss and suggesting caries and periodontal diseases as the most common causes for tooth loss[212]. Tooth loss has also shown to result from exposure to lower socioeconomic background as shown by the lower socio-economic status of denture wearers in our sample. Several life-course studies have reported that how exposure to adverse socioeconomic background across the individual's life results in having fewer number of teeth in adulthood[187, 213, 214].

Finally, regarding the point estimates of odds ratios, there is a gradient increase in the odds of class membership when we compare classes with better oral health (classes 1 and 5) with groups with higher probability of reporting oral health problems (classes 3 and 4) (see **Table 10**).

7.3.2. Cholinergic activity explaining cognitive health clustering

Similar to the results of regression models performed to explain oral health clustering, increase in bone mineral density was not associated with higher odds of membership in any of cognitive health classes, compared to the reference group (i.e., the class with the worst cognitive health). These findings are inclusive and suggest that the cholinergic neurons' activity could not explain the clustering of study sample based on their cognitive health status. Nevertheless, the point estimates of odds ratios for the group with poor memory performance (class 4) was the lowest in all models compared to the other groups (see **Table 11**).

7.3.3. Cholinergic neurons' activity explaining the association between oral health and cognitive health

Comparison between the results of regression models suggests that better cholinergic neurons' activity could not explain the associating between oral health and cognitive health. However, in both sets of regression models adjusting for SES reduced the odds ratios especially for the groups with better oral and cognitive health status. In other words, potential covariates, and SES in particular, could explain variability in the class memberships and the difference between membership in classes with better oral and cognitive health status. Although this study could not use a life course approach in the analysis, these findings may support the notion that the association between poor oral health and cognitive health might be explained by common causes such as exposure to lower SES as it has been recently mentioned by Thomson et al[(112)].

7.4. Strengths

The large sample size is probably the main asset of the present study, which adds precision to statistical analyses. This study controlled for a variety of covariates, including demographic characteristics, chronic conditions, and health-related behaviors. Moreover, latent class analysis as a novel analysis was used to better understand the clustering of a Canadian sample based on their oral and cognitive status, and to create subgroups of participants with heterogenous oral health and cognitive health profiles.

Furthermore, to our best knowledge, this is the first study to provide empirical evidence in support of explaining the association between oral health and cognitive health by focusing on potential common causes such as age-related cholinergic neurons' activity. Finally, the wide array of

variables provided by the CLSA allows for comprehensive analysis of potential confounders behind oral and systemic health interactions, observed in few studies.

7.5. Limitations

One of the main limitations of this study is the cross-sectional nature of the analyses, which limits our capability to infer causality based on our findings. However, this study provides cost-effective exploratory analyses and investigates a new hypothesis explaining the association between oral health and cognitive health.

Another main limitation of this study is the selection of bone mineral density as the proxy measure for cholinergic neurons' activity. Bone mineral density is a complex phenomenon and depends on a variety of factors such as medication[215, 216], genetics[217], and diet[218]. Therefore, it is challenging to isolate the effect of cholinergic neurons' activity using bone mineral density score, and the results of our analyses should be interpreted with caution. Nevertheless, the exploratory nature of this study justifies use of this measure based on the literature that is reviewed in the second chapter.

Regarding outcome variables, dichotomization of the cognitive tests scores based on the median resulted in loss of information related to the cognitive performance of the participants. Also, these tests are affected by many factors such as language, ethnicity, age, sex, and education[155] , which might limit the combined used of these scores. Binary measures of cognitive performance, however, were used to improve the interpretability of the classes in latent class analysis step. Moreover, cognitive scores in different tests are of different natures and dichotomization of these measures enabled me to use all these observed measures in our latent class analysis.

Another limitation of this study is that the confidence intervals in the regression models are wide, which indicates that our estimates are not highly precise, even with a large sample. This lack of precision could be due to measurement errors, or the complexity of the associations studied and the large number of the confounder that were not included in our models.

In our final regression models, we used complete case analysis for handling of the missing data. However, to ensure proper handling of the missing data, sensitivity analysis and multiple imputation will be performed in the next steps and prior to longitudinal analysis. Since present conclusions are conservative (e.g., multivariate regression analysis does not support rejection of H_0), it is unlikely that imputation would impact conclusions. Also, ***Table 12*** presents the difference between the Comprehensive cohort of CLSA and our final sample with complete cases in terms of basic demographic factors.

Another potential limitation of this study is the use of self-reported oral health variables instead of clinical examination, which might limit our ability to infer about clinical oral health of the study participants. However, these self-reported oral health questionnaires have been used in large cohort studies to improve feasibility, and they have shown acceptable reliability and validity[(141)].

Finally, it is important to mention that taking cholinergic or anticholinergic medications could be a confounder variable in the association between bone mineral density and oral and cognitive health. At the time of our analyses, the data on medication was not available, hence we could not adjust for the effect of medication in our analyses.

7.6. Generalizability

The Comprehensive cohort of CLSA is not a national representative sample because the participants are recruited based on their distance from data collection sites (DCS)[219]. This sample of middle-aged to older adults Canadians have shown to report higher education level, higher total household income compared to the general population[138]. Researchers in Calgary have also compared the baseline data from CLSA comprehensive cohort with data from 2016 Census and 2011 National Household Survey. The findings of this study suggest that CLSA subsample in Calgary is different from general Canadian population in basic sociodemographic factors, underrepresenting ethnic diversity, and those with lower education and income[220].Therefore, interpretations based on the results of studies using CLSA Comprehensive sample should take these factors into account.

7.7. Potential implications for future research

This study provides useful information about the clustering of a large sample of Canadians regarding their oral and cognitive health, by showing that middle-aged and older adults tend to form distinct clusters in terms of their self-reported oral health and cognitive performance. Future studies may approach how oral and cognitive health association happen in different subgroups based on the clustering patterns described in this thesis – for example, mastication inadequacy with decreased brain stimulation may be a major issue for denture wearers with poor oral health, but less relevant for other clusters. Regarding cognitive clusters, this study suggests that some individuals may perform poorly in some or all the tests and may have their cognition declining further as a consequence of exposure potential risk factors, including oral diseases. Analyses of cluster-specific patterns may be especially important for the design of future longitudinal studies,

which can lead to more powerful assumptions regarding whether oral diseases may lead to faster cognitive decline.

Moreover, this study investigated a novel hypothesis explaining the association between oral health and cognitive health. More valid measures of cholinergic activity may be used in future studies, although the associations found suggest that other aspects may be more powerful determinants of how much oral and cognitive health are associated. Those aspects probably include the wide array of environmental and socioeconomic factors experienced by participants, which may be tackled better by life course-based approaches.

8. Conclusion

In this study, a large sample of middle-aged to older adult Canadians could be classified in 5 and 4 classes based on their oral health and cognitive health status, respectively. These two sets of groupings could not be explained using bone mineral density as the cholinergic neurons' activity proxy measure. Therefore, we did not find evidence to explain the association between oral health and cognitive health using the cholinergic neurons' activity as the common cause. However, this oral-systemic association might be explained using exposure to adverse socioeconomic background throughout individuals' lifetime. More longitudinal analyses with measures across the individual life and using more specific measure of cholinergic activities are warranted.

9. References

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10. Appendix

Table 12*. Comparison of the basic demographics of the Comprehensive cohort in CLSA with our study sample*

	Final Sample (N=25,444)	**Comprehensive Cohort (N=30,097)**
Age(years)		
Mean (SD)	62.6 (10.1)	63.0 (10.3)
Median [Min, Max]	62.0 [45.0, 86.0]	62.0 [45.0, 86.0]
Sex		
male	13035 (51.2%)	15320 (50.9%)
female	12409 (48.8%)	14777 (49.1%)
Education		
less than secondary	1276 (5.0%)	1643 (5.5%)
secondary	2324 (9.1%)	2839 (9.4%)
some post-secondary	1844 (7.2%)	2238 (7.4%)
post-secondary degree	19963 (78.5%)	23327 (77.5%)
Missing	37 (0.1%)	50 (0.2%)
Total household income		
< $20,000	1202 (4.7%)	1566 (5.2%)
≥ $20,000 and < $50,000	5213 (20.5%)	6360 (21.1%)
≥ $50,000 and< $100,000	8503 (33.4%)	9907 (32.9%)
≥ $100,000 and< $150,000	4809 (18.9%)	5524 (18.4%)
≥ $150,000	4230 (16.6%)	4799 (15.9%)
Missing	1487 (5.8%)	1941 (6.4%)
Ethnicity		
white	23214 (91.2%)	27412 (91.1%)
non-white	2230 (8.8%)	1303 (4.3%)

McGill

Faculty of Medicine
3655 Promenade Sir William Osler #633
Montreal, QC H3G 1Y6

Faculté de médecine
3655, Promenade Sir William Osler #633
Montréal, QC H3G 1Y6

Fax/Télécopieur: (514) 398-3870
Tél/Tel: (514) 398-3124

CERTIFICATION OF ETHICAL ACCEPTABILITY FOR RESEARCH INVOLVING HUMAN SUBJECTS

The Faculty of Medicine Institutional Review Board (IRB) is a registered University IRB working under the published guidelines of the Tri-Council Policy Statement, in compliance with the Plan d'action ministériel en éthique de la recherche et en intégrité scientifique (MSSS, 1998), and the Food and Drugs Act (17 June 2001); and acts in accordance with the U.S. Code of Federal Regulations that govern research on human subjects. The IRB working procedures are consistent with internationally accepted principles of Good Clinical Practices.

At a Board meeting on 27 August 2018, the Faculty of Medicine Institutional Review Board, consisting of:

Frances Aboud, PhD	John Breitner, MD
Joséane Chrétien, MJur	Patricia Dobkin, PhD
Frank Elgar, PhD	Carolyn Ells, PhD
Catherine Lecompte	Kathleen Montpetit, MSc
Roberta Palmour, PhD	Alexandra Pasca, LL.M.
Daniel Saumier, PhD	Blossom Shaffer, MBA
Maida Sewitch, PhD	Lingqiao Song, LL.M.

Examined the research project **A07-E51-18B** titled: *Better oral health for a healthy cognition: investigation of a new pathway*

As proposed by: Dr. Raphael F. de Souza (Applicant) to _____ (Granting Agency, if any)

And consider the experimental procedures to be acceptable on ethical grounds for research involving human subjects.

27 August 2018
Date

Chair, IRB

Dean/Associate Dean Faculty

Institutional Review Board Assurance Number: FWA 00004545

McMaster University

CLSA Access Agreement

This agreement is entered into this March. 15 2019 (the **"Effective Date"**), at Hamilton, Ontario.

McMaster University, a University incorporated by special act of the Province of Ontario, Canada, with a main address at 1280 Main Street West, McMaster Innovation Park, Suite 309A, Hamilton, Ontario, Canada, L8S 4K1 (**"McMaster"**) is the host institution of the Canadian Longitudinal Study on Aging (***"CLSA"***).

AND

The Royal Institution for the Advancement of Learning/McGill University, with a main address at **845 Sherbrooke Street West, Montreal, Quebec, H3A 0G4 ("Approved User *Institution*")**

WHEREAS:

A. Dr. Parminder Raina (McMaster University) is the Lead Investigator for the CLSA funded by a grant funded by the Canadian Institutes of Health Research (CIHR) and is responsible for the academic obligations under this Agreement

B. Dr. Raphael Freitas de Souza (the "Approved User") is an Associate Professor, at the Institution, where he/she carries or wishes to carry out a study entitled "Better oral health for a healthy cognition: investigation of a new pathway", for which access to CLSA samples or data (or both) will be required.

C. The document titled "CLSA Data and Biospecimen Access Policy and Guiding Principles" attached, as Schedule A is an integral part of this agreement. All obligations contained therein are part of this agreement.

The parties hereto agree as follows:

1. Definitions

"Agreement" means this CLSA *Access Agreement.*

"DSAC" means the CLSA's Data and Sample Access Committee.

"Study" means the **CLSA Data and/or Biospecimen Request Application** described in Schedule B attached hereto.

"Transferred Materials," means the CLSA data and/or the biospecimens described in Schedule B attached hereto.

2. Sample and Data Security.

2.1 Security measures specified in Schedule C attached hereto will apply to all Transferred Materials. The Approved User and Institution undertakes to respect these security measures during the Study and afterwards, during storage of Transferred Materials where necessary.

2.2 The Approved User and Institution shall agree to the audit of their research facility by McMaster to ensure the security and confidentiality of Transferred Materials. These audits may be conducted with reasonable prior notice. Any discrepancies between the security measures specified in Schedule C and what is found at the Approved User and Institution's research facility will have to be corrected within sixty (60) days of notice by McMaster. McMaster will support the costs associated with these audits.

2.3 Transferred Materials, including any copies thereof, may only be used for the Study described in Schedule B and may not be disclosed, transmitted or shipped to anyone except employees working directly with the Approved User and Institution or co-investigators including co-applicants or other personnel from other institution(s), indicated in the Study who will require direct access to the CLSA Data and who agree to be bound by the terms of this Agreement or to persons expressly designated in writing by McMaster. The Approved User shall retain control of the Transferred Material at all times.

It is the responsibility of the Approved User and Institution to inform the staff and co-investigators, including co-applicants and other personnel at other third-party institution(s) entering into contact with the Transferred Materials of the obligations contained in the CLSA Data and Biospecimen Access Policy and Guiding Principles and this Agreement. As such, co-applicants and other personnel at other third-party institution(s) must submit a signed Appendix F attached hereto. Transfer of any CLSA biospecimens outside Canada is strictly prohibited. Data access will only be provided to institutional email addresses.

3. **Return of Derived Data.** The Approved User undertakes to return to McMaster the results of the Study analyses as specified in Schedule D attached hereto within the timeframe and conditions specified therein.

4. **Fees.** The Institution shall pay to McMaster the access fees and transportation fees specified in Schedule E attached hereto within forty-five (45) days after receipt of the invoice.

5. **Representations and Warranties of the Approved User and the Institution**

5.1 The Approved User and the Institution represent and warrant that the Study has received ethical approval from the Institution's research ethics committee or, if no such committee exists within the Institution, from a recognized research ethics committee for the duration of the Study. All documents in the Approved User's possession concerning ethical approval of the Study, including any subsequent amendments/renewals that may be applicable, have been provided to McMaster.

5.2 The Approved User and the Institution represent that they have read and took note of their obligations under the CLSA Data and Biospecimen Access Policy and Guiding Principles attached hereto as Schedule A.

6. **Property of Transferred Materials.** Nothing in this Agreement will operate to transfer any property rights in the Transferred Material.

7. **Exclusive Access:** No exclusive access will be granted to any portion of the Transferred Materials. McMaster may grant access to the Transferred Materials to others and may use it for its own internal purposes.

8. **Publications:** Copies of all proposed publications using Transferred Materials from McMaster must be submitted to McMaster for review at least 15 working days prior to submission. This review will be limited to ensuring that participants cannot be identified in such publications, that results are presented in a scientifically accurate manner to prevent the stigmatization of participants and of the communities they belong to.

9. **Archives and Peer Review.** Approved User will be permitted to archive the Transferred Material for the period of time required for peer review and audit purposes but not to exceed 1 year following the termination of this Agreement. Once this period of time has elapsed, the Approved User undertakes to destroy all Transferred Materials and all copies thereof in his/her possession or his/her control. When requested by McMaster, the Approved User shall certify in writing that the Transferred Material and all copies thereof were destroyed

10. **Reporting Obligations:** Approved Users shall comply with the following reporting obligations: i) a **Final Report** is to be submitted 60 days following the end date of this agreement; and ii) **notify** McMaster without delay for: a) incidents affecting the confidentiality of participants; b) incidents affecting the security or integrity of data/samples; c) suspension or lapse of any relevant authorizations (e.g. Ethics approval), professional qualifications, funding or approvals. Significant modifications to the approved project/protocol (including additional researchers or staff who will be accessing the data) or its timeline will require the submission of the CLSA Data Access Application Amendment Form available upon request to access@clsa-elcv.ca.

11. **Undertakings and Liability**

11.1 The Institution and Approved User acknowledges that the biological samples contained in the Transferred Material may carry viruses, latent viral genomes, and other infectious agents. The Approved User undertakes to treat all such biological samples as if they are not free of contamination, and to ensure that all such biological samples are handled only by trained personnel under laboratory conditions that afford adequate biohazard containment. By accepting delivery of these biological samples, the Institution and Approved User assume full responsibility and risk for their safe and appropriate use, handling, storage, and/or disposal.

11.2 The Approved User and the Institution assume all liability or damages arising from the use, storage or disposal of the Transferred Material and further agrees to defend, indemnify and hold harmless McMaster and its agents and employees from all liabilities, damages, demands, expenses and losses arising out of the acceptance, use for any purpose, handling or storage and/or disposal of the Transferred Materials or their by-products or modified or unmodified derivatives and in respect of all matters associated with the research results arising from the use of the Transferred Materials by the Approved User, the Institution or its employees.

12. **Default of Approved User or Institution.** Failure to comply with the terms of this Agreement may result, in addition to termination of this agreement pursuant to Section 13.2, in the disqualification of the Approved User or the Institution (or both) from receiving any additional data or biological samples from McMaster. McMaster reserves the right to institute and to take appropriate proceedings at law (or in equity, where applicable) against the Approved User or the Institution (or both) in connection with breaches of this Agreement.

13. **Termination**

13.1 This Agreement will terminate two years after its Effective Date for a one-year project or two (2) years after the end date for projects longer than one (1) year, unless the parties agree in writing to renew it. Upon termination of the Agreement, the Approved Users will destroy all samples in his or her possession.

13.2 McMaster may terminate this Agreement if the Approved User or the Institution are in default of any of the provisions of this Agreement and this default has not been remedied within sixty (60) days of written notice sent by McMaster to the Approved User or the Institution in respect of this default. Upon termination of this Agreement pursuant to this Section 13.2, the Approved User will return all Transferred Material in his or her possession to McMaster or destroy them and all copies thereof in possession or control of the Approved User or the Institution according to the instructions provided by McMaster. The Approved User will provide McMaster with a certificate attesting to such destruction, executed by him/her and an authorized representative of the Institution. In the event the Approved User is found to be in breach of this Agreement and such breach has not been remedied in accordance with this Section 13.2, the Approved User will not be entitled to publish the results of any Study except with the written agreement of McMaster.

14. **No Warranties.** The Transferred Materials accessed or delivered pursuant to this agreement are understood to be experimental in nature and are provided "as is". McMaster makes no representations and extends no warranties of any kind; either expressed or implied whatsoever in respect of the Transferred Materials. There are no express or implied warranties of merchantability, utility, efficacy, safety, identity, composition, non-toxicity and accuracy or fitness for a particular purpose or that the use thereof will not infringe any patent or other proprietary rights of any third party.

15. **Notices.** Any notice to be given by either party to the other shall be sent to the following:

For McMaster: CLSA Management Contact: Dr. Parminder Raina, PhD Lead Principal Investigator Canadian Longitudinal Study on Aging McMaster University 175 Longwood Rd. S. Suite 309A Hamilton, ON L8P 0A1 Tel: 905-525-9140, ext. 22197 Email: praina@mcmaster.ca	**For Legal Matters:** Executive Director, McMaster Industry Liaison Office MIP – Rm. 305 175 Longwood Rd S, Hamilton, ON L8P 0A1 Tel: 905-525-9140, ext. 22176 Fax: 905-546-1372
If for Approved User: Dr. Raphael F. de Souza Principal Investigator McGill University, Faculty of Dentistry 2001 McGill College Ave, suite 500 Montreal, QC H3A 1G1 Tel: 514 398-4777 Email: raphael.desouza@mcgill.ca	If for Institution: Agnes Wong Grants and Agreements Officer Office of Sponsored Research 845 Sherbrooke Street West Montreal, Quebec H3A 0G4 Tel: 514-398-3102 Email: agnes.wong2@mcgill.ca

16. **General Provisions**

16.1 This Agreement and the attached Schedules represent the entire understanding between the parties related to the Transferred Materials and the Study and supersedes any previous understandings, commitments or agreements, whether written or oral. If any provision of this Agreement is wholly or

partially unenforceable for any reason, all other provisions will continue in full force and effect.

16.2 This Agreement is governed by and will be construed in accordance with the laws of the Province of Ontario, without regard to conflicts of laws principles.

16.3 The following provisions will survive termination of this Agreement: 5, 8, 9, 10, 11, 12, 14, and 15.

16.4 This Agreement shall not be amended, modified, varied, or supplemented except in writing signed by each of the parties.

16.5 No party shall use, or authorize others to use, the name, symbols, or marks of another party hereto or its staff for any endorsement purposes without prior written approval from the party whose name, symbols or marks are to be used.

16.6 Neither party may assign any of its rights or obligations under this Agreement without the prior written consent of the other party.

16.7 Nothing in this Agreement creates, implies, or evidences any partnership or joint venture between the parties, or the relationship between them of principal and agent. No party shall have the authority to act on behalf of any other party or to bind another party in any manner.

16.8 Each party represents that it is permitted to enter into this Agreement; to consent to its conditions and that each has authority to sign this Agreement. This Agreement may be executed in counterparts and may be executed and delivered by electronically by PDF and all such counterparts, facsimiles and PDF copies shall together constitute one agreement. The parties agree that PDF copies of signatures have the same effect as original signatures.

IN WITNESS WHEREOF, the parties hereto have signed this Agreement.

MCMASTER UNIVERSITY

Signature

Name: Gay Yuyitung, PhD

Title: Executive Director, MILO

Date: April 3, 2019

Signature

Name: Parminder Raina, PhD

Title: Lead Investigator, CLSA

Date: APR 2/2019

The Institution, through its authorized representative who has signed below, acknowledges that it is bound by this Agreement.

The Royal Institution for the Advancement of Learning/McGill University

Signature

Name: Rupa Narasimhadevara

Title: Associate Director, Office of Sponsored Research

Date: April 2, 2019

APPROVED USER

Signature

Name: Raphael Freitas de Souza, DDS, PhD

Title: Principal Investigator

Date: February 12th, 2019

Rupa Narasimhadevara
Associate Director, Grants & Agreements
Directrice Associée, Subventions et Ententes
Office of Sponsored Research/Bureau de la recherche
McGill University/Université McGill

Schedule A:

Canadian Longitudinal Study on Aging (CLSA)

Data and Biospecimen Access Policy and Guiding Principles

1. DEFINITIONS

1.1. Applicant: an investigator affiliated with a public research organization based in Canada or elsewhere who is applying to access data/biospecimens collected as part of the CLSA.

1.2. CLSA Bioanalysis and Biorepository Centre (BBC): the centre that stores the biological samples from CLSA participants and houses a research laboratory dedicated to undertaking detailed standardized biospecimen analysis of specialized biomarkers.

1.3. CLSA Access Agreement: an agreement developed by the CLSA and the lead investigator's institution which contractually binds the parties involved in accessing CLSA data/biospecimens. An executed Access Agreement is necessary to obtain access to data/biospecimens from the CLSA.

1.4. Lead Institution: McMaster University, where the National Coordinating Centre (NCC) is located.

1.5. Canadian Institutes of Health Research (CIHR) Advisory Committee on Ethical, Legal, and Social Issues (ELSI) for the CLSA: an independent advisory body under the governance of the Canadian Institutes of Health Research set in place specifically to address the various ELSI needs of the CLSA (hereafter "CIHR ELSI Advisory Committee").

1.6. CLSA Biomarker, Genetic and Epigenetics Centres: the centres where specified analyses for biomarkers are carried out on CLSA biospecimens to ensure standardized results.

1.7. CLSA Statistical Analysis Centre (SAC): the centre where data verification and preparation is carried out. The SAC also prepares alphanumeric datasets for users.

1.8. CLSA Scientific Management Team (SMT): the executive management body within CLSA.

1.9. Custodian: as per agreements between McMaster University (Lead Institution) and all CLSA Site institutions across Canada, McMaster University is deemed the legal custodian of the CLSA data and biospecimens, regardless of where the CLSA data and biospecimens were collected

1.10. Data and Biospecimen Access Committee (DSAC): a committee with the mandate to review data and biospecimen access applications to the CLSA. The DSAC makes recommendations for approval/rejection of access requests to the SMT.

1.11. Research: any systematic inquiry into the dimensions of adult development, health and aging using CLSA data and/or biospecimens.

1.12. Study Results: all analyses, including the results of laboratory testing, obtained from the analysis, manipulation, or testing of CLSA data and/or biospecimens.

1.13. Users: Applicants that have received the necessary approvals to access CLSA data and/or biospecimens.

2. DATA AND BIOSPECIMEN ACCESS POLICIES AND PRINCIPLES

2.1. Introduction

The Canadian Longitudinal Study on Aging (CLSA) is a scientific research program and research platform. Over the course of the conduct of the CLSA, a rich resource of data and biospecimens collected from study participants will be assembled. All participants in the CLSA have provided signed informed consent that includes the stipulation that the data and biospecimens collected from them will be treated according to strict security and confidentiality standards. In addition, CLSA participants are also informed that data and biospecimens collected from them will be made available to researchers under a set of conditions that respect the CLSA consent with particular attention to security and confidentiality of the data and biospecimens. Data

and biospecimen access in large-scale longitudinal studies is complex. Governance of access to the CLSA data must balance the interests of the CLSA, the custodian, Users and study participants.

The CLSA has implemented policies and procedures that create a fair and transparent process to access its data and biospecimens. The CLSA has developed principles to guide access to, and the use of, the CLSA data and biospecimens and these are described in this document. These principles, policies, and procedures apply to the access to all CLSA data and biospecimens for research purposes. All researchers, including CLSA investigators that are requesting access to data and/or biospecimens for research are required to follow the CLSA Data and Biospecimen Access Policies and Guiding Principles.

The CLSA includes as part of its governance structure the DSAC; the body responsible for the review of applications for access to, and use of, data and biospecimens, collected as part of the CLSA. The DSAC is composed of voting members selected from the research community (in Canada and overseas) in addition to an ex officio CLSA investigator and an ex officio observer from CIHR. The Committee functions in accordance with the CLSA policies, guidelines, and procedures for data and biospecimen access.

2.2. Guiding Principles

Access to, and use of, CLSA data and biospecimens are governed by the following principles:

- The rights, privacy and consent of participants must be protected and respected at all times (see CLSA Privacy Policy at www.clsa-elcv.ca).
- The confidentiality and the security of CLSA data and biospecimens must be safeguarded at all times.
- CLSA data and biospecimens are resources that will be used optimally to support research to benefit all Canadians.
- CLSA data and biospecimens will be made available for use in a timely and responsible manner taking into account the need to assure data validity and biospecimen integrity.
- CLSA biospecimens constitute a finite resource and procedures will be put in place to ensure that this resource is used optimally, according to the long-term research goals of CLSA, and in keeping with the informed consent.
- CLSA data and biospecimens will only be released to researchers once ethics approval for the research project has been obtained from the appropriate Research Ethics Board (REB) and the CLSA Access Agreement between the CLSA Custodian and the Applicant's institution has been executed. In addition, the biospecimens will only be released once evidence of funding to analyze the biospecimens is received.
- To meet data quality standards set by the CLSA documentation pertaining to biospecimen handling and analysis will be required. This includes standard operating procedures (SOP), lot-to-lot comparisons, quality control information and a temperature record.
- Exclusive access rights to CLSA data and biospecimens will not be granted to any Applicant for any Research.
- All Applicants will be required to follow the Access Procedures.
- Approved Applicants (Users) may be required to return derived variables and/or results to the CLSA within a timeframe specified in the CLSA Access Agreement noted above.
- Data and biospecimen management for access purposes will be cost neutral to the CLSA. The CLSA has a fixed charge for each biospecimen regardless of biospecimen type and a fixed cost for data regardless of number of participants or variables requested. These costs include administration, IT, retrieval, and shipping of consumables; the cost for shipping of biospecimens is additional and will vary depending on shipping location.

- The CLSA SMT team will have access to CLSA data and biospecimens for operational activities required for developing, managing and achieving overall success of the CLSA Platform. These are for example: to conduct methodological analyses for the purposes of enhancing the design of the CLSA; enabling the development of communication materials to promote the CLSA Platform; and, facilitating partnerships in order to support long-term sustainability of the CLSA. The CLSA SMT is the decision making body for such operational activities and the CLSA will report on these activities to CIHR annually.

2.3. Limits on the Use of CLSA Data and Biospecimens

CLSA data and biospecimens can only be used by investigators affiliated with a public sector research organization[1]. Research projects must have received REB approval prior to the release of CLSA data and/or biospecimens.

In circumstances where CLSA links participant data and biospecimens to third party data holdings (e.g. provincial healthcare databases) the release of these data will be managed taking into account the terms and conditions of the third party data holdings, and thus may be subject to certain jurisdictional limitations with respect to the transfer and use of the linked data.

An important goal of the CLSA is to make the data and biospecimens available in a timely fashion for Research after data quality control and biospecimen integrity analyses are completed. If a User wishes to use CLSA data and/or biospecimens already received for a purpose other than the original purpose, then he/she must submit a new application to the DSAC. Any other change to the original application will require an amendment to the application and CLSA Access Agreement (as appropriate). CLSA Users are not permitted to share the data or biospecimens provided to them to others other than individuals identified as Users in the CLSA Access Agreement.

2.4. Access to CLSA Biospecimens

The Canadian Longitudinal Study on Aging (CLSA) collects blood and urine samples from consenting participants and stores the biospecimens in a Biorepository at McMaster University for future use. Biospecimens collected as part of the CLSA are valuable and finite resources. The CLSA SMT has the authority and the duty to responsibly manage biospecimens and to make sure the best possible scientific value is derived from these biospecimens. To achieve this objective, CLSA SMT and the DSAC will ensure that approved applications to use this resource will be of the highest scientific quality that will result in reliable, valid, informative and novel sets of biomarkers to advance the health and well-being of Canadians.

The CLSA is a longitudinal platform and the proposed use of the biospecimens should maximize the strength of this type of platform. The CLSA also requires the Users to return all the derived biomarker variables to the CLSA platform for use by other researchers. The intake of applications to access biospecimen will be once a year. The release of biospecimens to the user will require confirmation of funding to access and analyze the biospecimens. The CLSA's Biospecimen Access Guidelines can be found on the CLSA website at: https://www.clsa-elcv.ca.

2.5. Intellectual Property

The CLSA and its Lead Institution do not claim any ownership of, or exploitation rights to, any intellectual property resulting from the Users' research conducted with CLSA data/biospecimens.[2] Indeed, given the public nature of the CLSA research platform, it aims to promote a wide and accessible distribution of knowledge developed using this resource and achieves maximum public benefit. Thus, CLSA data and

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[1]Alphanumeric data is available to all public sector investigators nationally and internationally. However, currently there is no provision to transfer biospecimens to applicants outside of Canada.

[2]Note that where the Applicant in question is an investigator from McMaster University, he/she will still be bound by the university's intellectual property policies. This is independent of the CLSA intellectual property policy.

biospecimen Users are strongly encouraged to make their results (including research tools) rapidly and widely available to the scientific community.

Regarding genetic inventions, CLSA Users are strongly encouraged to follow the "Guidelines for the Licensing of Genetic Inventions" developed by the Organization for Economic Co-operation and Development (OECD) when licensing their intellectual property (presently found at: http://www.oecd.org/dataoecd/39/38/36198812.pdf).

2.6. Financial Considerations

The CLSA is a publicly funded research project and platform; access fees will be based on a cost recovery model and will be determined by the SMT.

2.7. Access Requests

Data and Biospecimen Access Application processes and procedures can be found on the CLSA website at https://www.clsa-elcv.ca.

2.8. Dissemination of Access Requests

To ensure transparency, and to ensure that participants are able to provide informed consent and withdraw if so desired, and to promote public awareness, the CLSA will provide information to study participants, to Applicants/Users and to the public on the general nature of research projects using CLSA data and/or biospecimens. Summary results from completed studies that use CLSA data and/or biospecimens will also be available in lay language. These will be provided by the researchers and will be posted on the CLSA website and in participant newsletters.

2.9. Obligations of Approved CLSA Data and Biospecimen Users

1.1. Research Quality

Users have a responsibility to enhance the value of the CLSA data by conducting high quality ethical research and sharing their findings in a timely manner to support dissemination and uptake. Formal scientific peer and ethical review of research proposals are important aspects of assuring quality and feasibility.

Safeguards will be maintained to ensure the anonymity and confidentiality of participants' data and biospecimens. Data and/or biospecimens provided to researchers from the CLSA will not contain any information that identifies any particular participant (i.e. they will be "de-identified" and coded). It is the obligation of the Users not to attempt to identify participants, and to use the data provided in a secure location to protect the privacy and confidentiality of the CLSA participants as per the CLSA Access Agreement as well as the CLSA consent form and Tri Council policies.

Return of Derived Variables

Data

As part of the conditions of the CLSA Access Agreement (as noted in Section 2) Users may be required to return to the CLSA derived variables for inclusion in the CLSA database for use by other researchers. In addition Users may be asked to return derived variables if such variables are identified in annual progress reports or manuscripts emanating from use of the CLSA data/biospecimens. In either case, Users will be asked to provide the code/syntax along with explanatory documentation to allow other researchers to understand the derivation and potential use of these derived variables. Users returning derived variables to the CLSA will work closely with the CLSA Statistical Analysis Centre.

1.2. Biospecimens

All data arising from research using CLSA biospecimens will be returned to the CLSA with exclusive use by the researcher who obtained funding for and produced the analyses lasting for a period of one year after which the data will be made available to all researchers.

2.10. Return of participants personal results from analyses conducted by Users

As a general policy, the CLSA will not return to participants their personal results from analyses conducted by Users. Nevertheless, given the duration of CLSA and the impossibility of foreseeing the nature of research projects that will be conducted using the CLSA data and biospecimens, Users shall be aware of the possibility that the CLSA may return validated results back to CLSA participants where such information is determined to be critical for the care of the participant. The decision regarding this return, whether and what to return will be taken by the SMT in consultation with the CIHR ELSI Advisory Committee and the relevant research ethics boards. Any situation in which personal results of analyses are returned to CLSA participants will be managed by the CLSA.

2.11. Public Disclosure and Proprietary Interests

The need to protect proprietary interests (e.g. patents) or pre-publication results may result in corresponding constraints on public disclosure of research results. In such situations, and where the time period during which results must be returned to CLSA is not sufficient, the User may request an extension.

2.12. Publications arising from Data and Biospecimen Access

Copies of all proposed publications using CLSA data and/or biospecimens must be submitted to the National Coordinating Centre at McMaster University for review by the CLSA Publication Review Committee at least 15 working days prior to submission. This review will be limited to ensuring that participants cannot be identified in such publications, appropriate acknowledgement has been given (see below), and that results are presented in accordance with the objectives stated in the CLSA Access Agreement. Users should review the CLSA publication policy prior to preparing manuscripts (The CLSA publication policy can be found on the CLSA Website: https://www.clsa-elcv.ca).

2.13. CLSA Acknowledgement in Publications

Full acknowledgement of the source of CLSA data and biospecimens must be included in any publications that arise from access to, and use of, the CLSA data and biospecimens. This acknowledgement must reference the sources of funding for the CLSA and its data platform and the core CLSA team responsible for the creation and implementation of the platform. Additional acknowledgements may apply if linked data have been used. All publications must include at a minimum the following acknowledgment for sources of funding:

"This research was made possible using the data/biospecimens collected by the Canadian Longitudinal Study on Aging (CLSA) [Data set version #]. Funding for the Canadian Longitudinal Study on Aging (CLSA) is provided by the Government of Canada through the Canadian Institutes of Health Research (CIHR) under grant reference: LSA 94473 and the Canada Foundation for Innovation". The specific wording of the acknowledgements will be operationalized in the CLSA Access Agreement.

Revision and Approval History

New Version #	Revision Date	CIHR Approval Date	Summary of revisions
2.0	March 2018	N/A	Editorial changes
Version #	**Date**	**CIHR Approval Date**	**Summary of revisions**
1.3	February 2017	December 2017	Modification
Version #	**Date**	**CIHR Approval Date**	**Summary of revisions**
1.2	September 2014	December 2017	Minor editorial changes
Version #	**Date**	**CIHR Approval Date**	**Summary of revisions**
1.1	January 2014	March 2014	Minor modification
Version #	**Date**	**CIHR Approval Date**	**Summary of revisions**
1.0	June 2012	July 2012	Document development

Schedule B – Approved CLSA Data Request Application

clsa élcv Canadian Longitudinal Study on Aging / Étude longitudinale canadienne sur le vieillissement

Application ID / *N° de la demande*
(office use only / *usage interne seulement*)

180901

CLSA Data and Biospecimen Request Application

Demande d'accès aux données et aux échantillons de l'ÉLCV

Instructions for completing an application. Please read carefully. / *Consignes pour remplir une demande. À lire attentivement.*

- Please consult the CLSA website for instructions, and policies and procedures for CLSA data and biospecimen access: www.clsa-elcv.ca (Data Access). Applicants are also encouraged to review the pertinent sections of the relevant CLSA protocol(s), Data Collection Tools and Physical Assessments in advance of completing the application. Additional information on the variables in the CLSA dataset is on the **CLSA Data Preview Portal.** /
  *Veuillez consulter les consignes, les politiques et la procédure de demande d'accès aux données et aux échantillons sur le site Web de l'ÉLCV : www.clsa-elcv.ca (Accès aux données). Nous encourageons les demandeurs à consulter les sections pertinentes du protocole de l'ÉLCV (en anglais seulement), les outils de collecte de données et les tests physiques avant de remplir une demande d'accès. Des informations supplémentaires sur les variables contenues dans l'ensemble de données de l'ÉLCV sont disponibles sur le **Portail de données de l'ÉLCV**.*
- Consult us for any questions regarding your application at access@clsa-elcv.ca. /
  Veuillez nous transmettre toute question relative aux demandes d'accès aux données de l'ÉLCV en écrivant à access@clsa-elcv.ca.
- The application is composed of 3 parts. Part 1: General Project Information; Part 2: Data Checklist; Part 3: Biospecimen Checklist. Parts 1 and 2 are found on the CLSA website, Part 3 will be available at a later date. /
  La demande est séparée en trois parties : 1re partie : Renseignements généraux; 2e partie : Sélection des données; 3e partie : Sélection des échantillons biologiques. Les parties 1 et 2 sont disponibles sur le site Web de l'ÉLCV. La 3e partie sera disponible ultérieurement.
- CLSA will only release data to approved users with an institutional email address. Email addresses containing domain names such as gmail, hotmail, etc. are not acceptable. /
  Les données ne seront envoyées qu'aux adresses de courriel institutionnelles. Les adresses de messagerie contenant des noms de domaine tels que gmail, hotmail, etc. ne sont pas acceptées.
- Please ensure that you have completed **all of the sections of the application** form that are relevant to your application. Incomplete applications may result in processing delays or refusal of your application. /
  *Assurez-vous de bien remplir **toutes les sections pertinentes du formulaire de demande d'accès.** Les demandes incomplètes pourront causer un retard dans le traitement de votre demande ou entraîner un refus.*
- Please complete this application using minimum size 11 font. /
  Veuillez utiliser une taille de police d'au moins 11 points pour remplir cette demande.

Submitting an application / *Envoyer la demande*

- Email applications to access@clsa-elcv.ca, clearly indicating "***Application for DSAC – your name***" in the subject line. /
  ***Envoyez** la demande par **courriel** à access@clsa-elcv.ca en indiquant clairement, dans l'objet du courriel, le titre « **Demande pour le DSAC – votre nom** ».*

Application Part 1 of 3: General Project Information / *Partie 1 de 3 : Renseignements généraux*

A1. Applicant Information / *Renseignements sur le demandeur*

Primary Applicant / *Demandeur principal*: The primary applicant will be the contact person for the CLSA Access Agreement as well as for the data release and any relevant updates. **Note:** Anyone requiring access to data must use the email address of their affiliated institution. Data will only be released to institutional email addresses. Email addresses containing domain names such as gmail, hotmail, etc. are not acceptable. /
Le demandeur principal sera la personne-ressource pour l'Entente d'accès de l'ÉLCV, ainsi que pour la transmission des données et toute mise à jour pertinente. ***Remarque :*** *Toute personne ayant besoin d'un accès aux données doit utiliser l'adresse électronique de l'établissement auquel elle est affiliée. Les données ne seront envoyées qu'aux adresses de courriel institutionnelles. Les adresses de messagerie contenant des noms de domaine tels que gmail, hotmail, etc. ne sont pas acceptées.*

For **Graduate student** (MSc, PhD) applications, the primary applicant must be the supervisor and the student must be clearly identified. **Postdoctoral Fellows** are permitted to apply as a primary applicant, but the application must be co-signed by their supervisor (see sections A7 and A8). If requesting a Fee Waiver, the Postdoctoral Fellow must be listed as the primary applicant. /
Pour les ***demandes faites par des étudiants des cycles supérieurs*** *(M. Sc., Ph. D.), le demandeur principal doit être le superviseur et l'étudiant doit être clairement identifié. Les* ***boursiers postdoctoraux*** *peuvent soumettre une demande à titre de demandeur principal, mais celle-ci doit être cosignée par leur superviseur (voir les sections A7 et A8). Le boursier postdoctoral doit être le demandeur principal pour bénéficier d'une exonération des frais.*

Name / *Nom*	180901
Position / *Poste*	Raphael F. De Souza
Affiliation / *Organisme d'appartenance*	McGill University, Faculty of Dentistry (McGill F.D.)
Mailing Address / *Adresse de correspondance*	2001 McGill College Ave, suite 500
Phone / *Téléphone*	(514) 398-4777
Email (see note above) / *Courriel (voir la remarque ci-dessus)*	raphael.desouza@mcgill.ca

Complete this section if this is a Graduate student application /
Remplir cette section si la demande est faite par un étudiant des cycles supérieurs

Name / *Nom*	
Degree and Program of Study / *Grade et programme d'étude*	
Institution of Enrollment / *Établissement d'étude*	
Current Mailing Address / *Adresse de correspondance actuelle*	
Phone / *Téléphone*	
Email (see note above) / *Courriel (voir la remarque ci-dessus)*	

A2. Project Team / *Équipe de projet*

All Co-Applicants and Other Personnel must be listed in the table below. Please note that any changes to the project team **require an amendment.** To request an Amendment Form, please email access@clsa-elcv.ca. / *Tous les codemandeurs et les membres du personnel de soutien doivent être identifiés dans le tableau suivant. Veuillez noter que tout changement à l'équipe de projet **nécessite une modification.** Pour obtenir le formulaire de modification, écrivez à access@clsa-elcv.ca.*

Co-Applicants and Other Personnel / *Codemandeurs et membres du personnel de soutien*
Please list all co-applicants including students and any other personnel who will be involved in the project (e.g.: advisor, statistician, research assistant, etc.). **Note:** Anyone requiring access to data must use the email address of their affiliated institution. Data will only be released to institutional email addresses. Email addresses containing domain names such as gmail, hotmail, etc. are not acceptable. /
*Veuillez inscrire tous les codemandeurs, y compris les étudiants et tout autre membre du personnel de soutien qui seront impliqués dans le projet (p. ex. conseiller de recherche, statisticien, auxiliaire de recherche, etc.). **Remarque :** Toute personne ayant besoin d'un accès aux données doit utiliser l'adresse électronique de l'établissement auquel elle est affiliée. Les données ne seront envoyées qu'aux adresses de courriel institutionnelles. Les adresses de messagerie contenant des noms de domaine tels que gmail, hotmail, etc. ne sont pas acceptées.*

Name / *Nom*	Position, Affiliation and email address (see note above) / *Poste, organisme d'appartenance et courriel (voir la remarque ci-dessus)*	Role on Project / *Rôle au sein du projet*	Requires Access to Data (Yes or No). / *Doit avoir accès aux données (Oui ou Non).*
Belinda Nicolau	McGill F.D. belinda.nicolau@mcgill.ca	Co-Principal Investigator	☒ Yes / Oui ☐ No / Non
Faleh Tamimi	McGill F.D. faleh.tamimimarino@mcgill.ca	Co-investigator	☒ Yes / Oui ☐ No / Non
Linda Booij	Dept. of Psychology, Concordia University, linda.booij@umontreal.ca	Co-investigator	☒ Yes / Oui ☐ No / Non
Jocelyne Feine	McGill F.D. jocelyne.feine@mcgill.ca	Co-investigator	☒ Yes / Oui ☐ No / Non
Ricardo Alchini	McGill F.D. ricardo.alchini@mail.mcgill.ca	Co-investigator (DMD student)	☒ Yes / Oui ☐ No / Non
Sreenath Madathil	McGill F.D. sreenath.arekunnathmadathil@mail.mcgill.ca	Co-investigator	☒ Yes / Oui ☐ No / Non
			☐ Yes / Oui ☐ No / Non

Name / *Nom*	Position, Affiliation and email address (see note above) / *Poste, organisme d'appartenance et courriel (voir la remarque ci-dessus)*	Role on Project / *Rôle au sein du projet*	Requires Access to Data (Yes or No). / *Doit avoir accès aux données (Oui ou Non).*
			☐ Yes / Oui ☐ No / Non
			☐ Yes / Oui ☐ No / Non
			☐ Yes / Oui ☐ No / Non
			☐ Yes / Oui ☐ No / Non
			☐ Yes / Oui ☐ No / Non
			☐ Yes / Oui ☐ No / Non
			☐ Yes / Oui ☐ No / Non
			☐ Yes / Oui ☐ No / Non
			☐ Yes / Oui ☐ No / Non
			☐ Yes / Oui ☐ No / Non
			☐ Yes / Oui ☐ No / Non
			☐ Yes / Oui ☐ No / Non

A3. Project Timeline / *Échéancier du projet*

What is the anticipated time frame for this proposed project? In planning for your project, please consider in your time frame at least six (6) months from the application submission deadline to the time you receive your dataset. /
Quel est l'échéancier prévu du projet proposé? Lors de la planification de votre projet, veuillez prévoir au moins six (6) mois à compter de la date limite de soumission de votre candidature pour recevoir votre ensemble de données.

Anticipated start date / *Date prévue de début* : 15/03/2019
(DD/MM/YYYY) / (JJ/MM/AAAA)

Proposed project duration / *Durée proposée du projet* : 2 year
(e.g. 6 months/*mois*, 1 year/*an*)

A4. Project Description / *Description du projet*

Project Description / *Description du projet*
Please adhere to word count and page limits. / *Veuillez respecter le nombre de mots et la limite de pages.*

Project Title / *Titre du projet* :

Better oral health for a healthy cognition: investigation of a new pathway

Lay Summary / *Résumé non scientifique*
Please provide a lay language summary of your project (**maximum 150 words**) suitable for posting on the CLSA website if your application is approved. Please ensure that the lay summary provides a stand-alone, informative description of your project. /
*Veuillez fournir un résumé non scientifique de votre projet (**150 mots maximum**) pouvant être publié sur le site Web de l'ÉLCV si votre demande est approuvée. Assurez-vous de fournir un résumé détaillé et complet de votre projet.*

Previous studies shows that good oral health is key for general health. They suggest that gum disease or many lost teeth raise the risk of early dementia, Alzheimer's and other neurological diseases. We believe that a certain aging-related factor is vital for this association: the degeneration of nerves that secrete a substance called acetylcholine. They are notably prone to degenerate with aging, control the production of saliva, and are involved in the onset and progress of dementia. Thus, we will check whether adults (age: 45 years or +) with dry mouth and other oral problems also have poorer memory/mental performance. We will also check if the action of acetylcholine-producing neurons can explain this association. The info will help dentists to realize their role in preventing dementia. We may also identify new ways to prevent or to slow dementia.

Word Count / *Nombre de mots* 138

Keywords / *Mots clés*
Please provide 3-5 keywords describing your project. / *Veuillez fournir 3 à 5 mots clés décrivant votre projet.*

Cognition; Cholinergic System; Oral Health

Detailed description / *Description détaillée*

Please provide a description of the proposed project. The proposal should be informative and specific and **no more than 3 pages (single spaced, minimum font size 11). No additional pages or appendices allowed. Non-compliant applications will be returned.** The proposal **MUST** contain the following sections: /

*Veuillez fournir une description du projet proposé. La proposition devrait être informative et précise **sans dépasser 3 pages (simple interligne, taille de police d'au moins 11 points). Les pages supplémentaires et les annexes ne sont pas permises. Les demandes non conformes seront renvoyées au demandeur.** La proposition **DOIT** contenir les sections suivantes :*

(a) Background and study relevance. / *Contexte et pertinence de l'étude.*

(b) Study objectives and/or hypotheses. / *Objectifs et/ou hypothèses de l'étude.*

(c) The study design and methodology including an overview of the variables and/or biospecimens requested for the project. In no more than half a page, describe the inclusion and exclusion criteria for participants to be included in your study (e.g., age, sex, etc.). /
Modèle d'étude et méthodologie comprenant un survol de la liste de variables et/ou échantillons demandés. Sans dépasser une demi-page, décrivez les critères d'inclusion et d'exclusion des participants qui seront inclus dans votre étude (p. ex. âge, sexe, etc.).

(d) Brief description of the data analysis proposed (this section should include justification for the sample size requested). /
Brève description de l'analyse de données proposée (cette section devrait inclure la justification de la taille d'échantillon demandée).

Please note that the complete CLSA alphanumeric dataset contains over 4,000 variables collected from more than 51,000 participants. If your application is approved, you will be receiving the prepared, **raw** datasets in .csv format. Depending on your choice of statistical software and proposed analyses, automatic data imports may not succeed and you may need to instruct your software how to read the file. This may require the use of advanced scripting and/or macros in some cases. The CLSA encourages you to include someone experienced in working with such complex datasets on your project team. /

*Veuillez noter que l'ensemble complet de données alphanumériques de l'ÉLCV contient plus de 4 000 variables recueillies auprès de plus de 51 000 participants. Si votre demande est approuvée, vous recevrez les ensembles de données **brutes** préparés au format .csv. En fonction de votre choix de logiciel statistique et des analyses proposées, il est possible que l'importation automatique des données échoue et que vous deviez indiquer à votre logiciel comment lire le fichier. Cela peut nécessiter l'utilisation de scripts avancés et/ou de macros dans certains cas. L'ÉLCV vous encourage à inclure dans votre équipe une personne qui a de l'expérience avec les ensembles de données complexes.*

Please note that a detailed list of data is included in Part 2 of the application and is available on the CLSA website. Biospecimens (Part 3 of the application) will be available at a later date. /

Notez qu'une liste complète des données figure à la 2e partie de la demande. Elle est également disponible sur le site Web de l'ÉLCV. Les échantillons biologiques (3e partie de la demande) seront disponibles ultérieurement.

(A) BACKGROUND AND STUDY RELEVANCE

Background: Dementing disorders are a major burden for the elderly and for healthcare systems. The estimated number of people living with dementia worldwide was 35.6 million in 2010 and is expected to double every 20 years, with 115.4 million in 2050 [1]. Likewise, oral diseases are a major public and individual financial burden with systemic implications and deleterious impact on quality of life [2]. Interestingly, oral health status has been associated with cognitive health in ageing [3]. Reasons include the increased serum levels of inflammatory mediators led by oral infectious diseases, which foster neurodegeneration, as found in mild cognitive impairment and Alzheimer's disease [4]. Oral inflammation and transient bacteremia have implications in the development of several systemic diseases. Other possible mechanisms involve malnutrition and lower afference, both linked to tooth loss [5]. Those oral health ailments tend to accrue over the lifetime and, thus, are widely prevalent in the elderly [2].

Aging is also marked by the atrophy and degeneration of brain cholinergic neurons, which have a key role in dementing disorders [6]. These changes align with aging-related atrophy of cholinergic neurons in the autonomic nervous system. Atrophy is more severe in the parasympathetic nervous system (PSNS) than in the sympathetic system. Aging is also notoriously linked to a lower peripheral response to acetylcholine, as observed in the heart, blood vessels (including cerebral vessels) and salivary glands, leading to xerostomia ("dry mouth") [7].

Previous explanations for the oral health-cognition relationship overlook the role of the aging cholinergic system. Although poor dentition status may lead to a higher risk of cognitive impairment, specific mechanisms are unknown and the link is probably bidirectional. The higher incidence of both oral and cognitive diseases with aging suggests that age-related changes trigger both groups of pathologies. These aspects led us to the following question: how are oral health diseases associated with cognitive impairment and are they both consequences of atrophy and neurodegeneration of the cholinergic system?

In order to obtain more insight into the mechanisms and the developmental time course in which both groups of disorders may develop, a first step is to clarify how oral health, cognitive function and cholinergic activity are associated.

Relevance: This proposal tackles two groups of widely prevalent, preventable conditions amongst North American elders. The likelihood of clustering among these conditions, plus their possible link with cholinergic activity, makes oral health an important target to define risk groups for dementing disorders. Results will inform public health interventions for primary care and prevention of dementia; e.g., adding cholinergic activity indicators and salivation-related items to the anamnesis of certain patient groups. We are testing a novel hypothesis that may clarify mechanisms leading to age-related oral and cognitive disease. This study will also ground plans for longitudinal analyses, e.g., how certain oral health conditions may predict dementia onset.

(B) STUDY OBJECTIVES

(I) Identify clusters of oral health status and cognitive functioning among a community sample of middle-aged and elderly individuals

(II) Estimate the extent to which indicators of cholinergic activities are associated with these clusters across different age groups.

(C) STUDY DESIGN AND METHODOLOGY

We will use data from the CLSA Comprehensive component, which includes the 30,000 participants submitted to physical and complementary exams at 11 data collection sites. The remaining 20,000 participants, or CLSA Tracking component, provided data by computer-administered telephone interviews. This subgroup will serve to ensure generalizability, as long as their responses do not depend on geographical distance from research centers. We will use CLSA Tracking data to confirm the representativeness of the CLSA Comprehensive.

Participants: Participant age ranged from 45 to 85 years during recruitment. Such a low minimum age limit intends to reveal mid-life experiences that can impact health-related and social events in later life [8]. Moreover, some cognitive

decline can be observed during mid-life [9]. Planned sample sizes for each age group were: 45-54y=9,000; 55-64y=9,000; 65-74y=6,000; 75-85y=6,000 (Comprehensive); and 45-54y=6,000; 55-64y=6,000; 65-74y=4,000; 75-85y=4,000 (Tracking). Mean age (SD) for both CLSA components is nearly 63 (10) years; 51% are women. To obtain reliable self-reported data for the cohort and informed consent, CLSA excluded respondents with severe cognitive impairment at baseline. Cognitive impairment was detected at initial contact by identifying individuals who were unable to understand the purpose of the study and provide reliable data.

Definition of the Variables:
Oral health: We will request oral health-related data collected during the "Maintaining Contact" stage. Both CLSA components used the same questionnaire to collect oral health data based on Locker et al. [10], i.e. by considering 7 major criteria: (1) self-reported oral health status (1 question, 5-point Likert scale); (2) presence of natural teeth (1 yes/no question) and (3) eventual use of dentures (1 yes/no question); (4) comfort during eating (1 question, 4-point Likert scale); (5) avoidance of specific foods (1 question, 4-point Likert scale); (6) occurrence of specific symptoms (20 yes/no questions, including xerostomia, toothache, gingival bleeding and halitosis); and (7) oral hygiene (1 question, daily frequency of oral hygiene). Adequate test-retest reliability and internal consistency were observed for this questionnaire, as well as good concurrent and construct validity [10]. All these variables will be considered as binary variables coded as good (0) or bad oral health (1).
Cognitive status: We will use results from 2 neuropsychological tests of cognitive status, i.e. Rey Auditory Verbal Learning Test (RAVLT) for memory, and the Mental Alternation Test (MAT) for executive functioning.
- RAVLT is a 15-item test that assesses short-term and long-term memory, more specifically learning and retention based on a list of words. In brief, participants listen to 15 words (1 per second) and are asked to recall as many as possible, without giving importance to the order of words. The CLSA used an adaptation of this test based on 2 trials: immediate and delayed recall; the latter respected 30 minutes before word recall. RAVLT is widely used for quantifying immediate memory performance [11], has high sensitivity in detecting early cognitive decline, and presents good reliability [12, 13]. Mean numbers of recalled words (SD) for the CLSA at baseline were: immediate: 5.9 (2.4); delayed: 4.4 (2.6). Values are lower and present much wider variation compared to the cognitively impaired elders studied by Tierney et al.[14], implying that many have low memory performance.
- MAT is a two-part test to evaluate the executive functioning component of cognition. Part A involves asking to count from 1 to 20 and say the alphabet as fast as possible; this part also confirms the feasibility of part B (a failure precludes full application of MAT). In Part B, respondents must alternate quickly between number and letters for 30 seconds. A score from 0 to 51 results from quantifying correct alternations. MAT has good sensitivity and specificity to detect cognitive impairment as detected by the Mini-Mental State Examination (MMSE) [15]. Mean values for the CLSA (SD) are 26.0 (9.6) alternations.
- Both continuous measures will be dichotomized based on clinically relevant cut points (RAVLT: mean - 1.5 SD [16]; MAT: score of 15 for abnormal performance [17]) into impaired (1) and normal (0).
Cholinergic indicators: We will analyse a set of variables linked to the PSNS activity. These will serve as indicators of the cholinergic activity and include xerostomia, intraocular pressure measured by an Ocular Response Analyzer device and bone mineral density determined by dual energy X-ray absorptiometry (DXA). Interestingly, PSNS activity promotes bone mass accrual; lower bone mineral density has been associated with dry mouth and eyes, as well as with other PSNS dysfunction-related pathologies [18]. Lower bone density is also associated with a higher risk for tooth loss [19]. Xerostomia is measured by asking if respondents have experienced "dry mouth" (i.e. xerostomia) for the last 12 months. Intraocular pressure and DXA provide continuous measures that will be categorized into binary variables based on clinically relevant cut points (intraocular pressure: 21 mm Hg in at least one eye [20]; DXA: T score below 1.0 [21]) into low and high.
Other variables: Analyses will consider data on diverse variables that may act as confounders in the association between cholinergic activity and clusters of oral and cognitive health measures (refer to "1.4. Data Analysis" for details). These variables include symptoms of mood disorders, i.e. depressed mood (CES-D10 scale) and non-specific distress (Kessler Psychological Distress Scale). The occurrence of excessive daytime sleepiness and

movement during sleep will also be considered, due to their possible association with dementing disorders, as well as physical activity. In addition, analyses will include answers regarding age, sex, tobacco and alcohol consumption habits, nutrition, hypertension (according to the CLSA algorithm) and diabetes, due to their likely patterns of association with several oral health parameters and cognitive health.
Many medications may interfere with salivation and lead to a report of xerostomia. Hence, we will dichotomize medications according to the possible occurrence of this adverse effect according to Villa et al.[22]. Examples include drugs with known anticholinergic action, diuretics, anticonvulsants, and psychotropic drugs.

(D) DATA ANALYSIS
Preliminarily, we will check the distribution of each variable for outliers and obvious errors. Besides descriptive statistics, we will conduct a sensitivity analysis to confirm that our data is representative of the main cohort.

Aim 1: "to identify clusters of oral health status and cognitive functioning among a community sample of middle-aged and elderly individuals", we will perform a hierarchical cluster analysis using complete linkage and Jaccard distance metric to identify clusters of binary cognitive and oral health variables. Dendrograms and heat maps will be reported along with the description of identified clusters.

Aim 2: "to estimate the extent to which indicators of cholinergic activities are associated with these clusters across different age groups". We will use multinomial logistic regression and report ORs and 95% CI. Our main outcome variable will be the clusters from Aim 1. The cluster showing minimal oral health and cognitive impairment will be our reference for the outcome. Our main explanatory variable is cholinergic activity as described above. We will select potential confounding factors based on directed acyclic graphs (DAGs).

We consider that there is a chance that the cluster may not be clinically interpretable. Under such situation, we will model the joint probability of having poorer cognition and oral symptoms through multivariate generalized linear regression models [23] adjusted for potential confounders. We will also perform the analysis stratified by 10-year age groups to evaluate the effect measure modification by age.

Reference List / *Liste des références*

Please include a list of the most relevant references (must fit within the allotted space on this page only). Please complete this section using a minimum font size of 11. /
Veuillez présenter une liste des références les plus pertinentes (doit entrer dans l'espace alloué sur cette page seulement). Veuillez utiliser une taille de police d'au moins 11 points pour remplir cette section.

1. Prince M, et al. The global prevalence of dementia: a systematic review and metaanalysis. Alzheimers Dement. 2013;9(1):63-75 e2.
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23. Glonek GFV, Mccullagh P. Multivariate Logistic-Models. J Roy Stat Soc B Met. 1995;57(3):533-46.

A5. Scientific Review / *Évaluation scientifique du projet*

Evidence of peer reviewed funding will be considered evidence of scientific review. If there are no plans to submit an application for financial support for this project please provide evidence of peer review (e.g. internal departmental review, thesis protocol defense, etc.) if available. If no evidence of scientific peer review is provided with this application then the project will undergo scientific review by the DSAC. /
Les documents attestant l'attribution du financement seront considérés comme une preuve d'évaluation par les pairs. Si vous ne planifiez pas demander de l'aide financière pour ce projet, veuillez fournir la preuve qu'une évaluation par les pairs a été réalisée (p. ex. évaluation départementale, défense du protocole de thèse, etc.) si disponible. Si aucune preuve d'évaluation scientifique par les pairs n'est soumise avec la demande, le DSAC procédera à l'évaluation scientifique du projet.

Peer Reviewed Funding / *Financement évalué par les pairs*

☒ Yes / *Oui* ☐ No / *Non* ☐ Requested / *Demandé*

Please provide name of funding agency. /
Veuillez fournir le nom de l'organisme de financement.

Drummond Foundation

A6. Ethics Approval / *Approbation éthique*

Has this project received ethics approval? / *Ce projet a-t-il reçu une approbation éthique?*

☒ Yes **(please attach letter)** / *Oui* ***(veuillez inclure la lettre)*** ☐ No* / *Non**

*If the project has been submitted for ethics review please provide the **expected date of response.** /
*Si le projet a été soumis à l'éthique, veuillez fournir la **date approximative de la réponse.***

Expected date of response / *Date approximative de la réponse* : 05/09/2018
(DD/MM/YYYY) / (JJ/MM/AAAA)

Please note that ethics approval is NOT required at the time of this application, but **no data or biospecimens will be released until proof of ethics approval has been received by the CLSA.** /
*Notez que l'approbation éthique n'est PAS requise à cette étape de la demande, mais **aucune donnée ou aucun échantillon ne seront transmis avant que l'ÉLCV ait reçu une preuve d'approbation éthique.***

A7. Request for a Fee Waiver for Alphanumeric Data

(subject to approval) /

Demande d'exonération des frais pour les données alphanumériques (sous réserve d'approbation)

Graduate students: In order to be eligible for the Fee Waiver, **the CLSA dataset must be for the sole use of the graduate student's thesis** (see section A1).
Postdoctoral Fellows: In order to be eligible for the Fee Waiver, **the CLSA dataset must be for the sole use of the postdoctoral research project**, the Fellow must be the primary applicant and the supervisor must sign section A8. /
Étudiants des cycles supérieurs : *Pour être admissible à l'exonération des frais,* ***l'ensemble de données de l'ÉLCV doit être utilisé uniquement pour la thèse de l'étudiant au cycle supérieur*** *(voir la section A1).*
Boursiers postdoctoraux : *pour être admissible à l'exonération des frais,* ***l'ensemble de données de l'ÉLCV doit être utilisé uniquement pour le projet de recherche du boursier postdoctoral,*** *le boursier doit être le demandeur principal et le superviseur doit signer la section A8 de la demande.*

☐ Fee Waiver for Graduate student (MSc or PhD) for thesis only /
Exonération pour un étudiant des cycles supérieurs (M. Sc. ou Ph. D.) pour la thèse seulement

☐ Fee Waiver for Postdoctoral Fellow (limit 1 waiver for postdoctoral studies) /
Exonération pour un boursier postdoctoral (limite d'une exonération pour les études postdoctorales)

A8. Primary Applicant Signature / *Signature du demandeur principal*

I certify that the information included in this application is accurate /
J'atteste que l'information fournie est exacte

➢ **Primary Applicant's Name and Signature / *Nom et signature du demandeur principal* :**

Name / *Nom* : Raphael Freitas de Souza

Signature / *Signature* : Digital signature on file

Please note that the primary applicant MUST be the supervisor for graduate student applications. /
Notez que le demandeur principal DOIT être le superviseur lors des demandes pour les étudiants des cycles supérieurs.

Date / *Date* : 12/02/2019
(DD/MM/YYYY) / (JJ/MM/AAAA)

➢ **Co-Signature** (required for postdoctoral fellow applications) /
Cosignature *(obligatoire pour les demandes de boursiers postdoctoraux)*

Postdoctoral Supervisor's Name and Signature / *Nom et signature du superviseur postdoctoral* :

Name / *Nom* : _____

Signature / *Signature* : _____

Date / *Date* : _____
(DD/MM/YYYY) / (JJ/MM/AAAA)

clsa élcv Canadian Longitudinal Study on Aging / Étude longitudinale canadienne sur le vieillissement

180901

Application ID / *N° de la demande*
(office use only / *usage interne seulement*)

CLSA Application: Data Checklist
Application Part 2

Demande de l'ÉLCV : Sélection des données
Partie 2 de la demande

Instructions for completing the Data Checklist / *Consignes pour remplir le formulaire de Sélection des données*

Please mark with an "X" the checkbox for the components containing the variables in the CLSA Baseline dataset that you are requesting.

In the **Comments** section, include other relevant information concerning your request, including rationale for the request of Additional Data (e.g. Images).

Inscrivez un « X » dans la case à côté des modules contenant les variables de l'ensemble de données de l'ÉLCV que vous demandez.

Ajoutez toute autre information pertinente à votre demande à la section ***Commentaires****, y compris une justification de la demande de données supplémentaires (par exemple, les images).*

Primary Applicant Information / *Informations sur le demandeur principal*

Name / *Nom*	Raphael F. de Sousa
Project title / *Titre du projet*	Better oral health for a healthy cognition: investigation of a new pathway

SECTION A: QUESTIONNAIRES / *QUESTIONNAIRES*

Interview module ***Module de l'entrevue***	**Tracking** (Telephone Interview) ***Évaluation de surveillance*** *(Entrevue téléphonique)*	**Comprehensive** (Face-to-face Interview - In-home or DCS visit) ***Évaluation globale*** *(Entrevue en personne - à domicile ou à un site)*
Age / *Âge* (AGE)	☒	☒
Sex / *Sexe* (SEX)	☒	☒
Socio-Demographic Characteristics / *Caractéristiques socio-démographiques* (SDC)		
Country of birth / *Pays de naissance*	☒	☒
Province of residence / *Province de résidence*	☒	☒
Urban / Rural Classification / *Classement des zones urbaines / rurales*[1]	☒	☒
Census Subdivision (Codes and Names) / *Subdivisions de recensement (Codes et noms)* [1,2]	☐	☐
Forward Sortation Areas / *Zones de tri d'acheminement* [1,2]	☐	☐
Ethnicity / *Ethnicité*	☒	☒
Culture / *Culture*	☒	☒
Language / *Langue*	☒	☒
Religion / *Religion*	☐	☐
Marital status / *État matrimonial*	☒	☒
Sexual orientation / *Orientation sexuelle*	☐	☐
Home Ownership / *Propriétaires* (OWN)	☐	☐
Education / *Éducation* (ED)	☒	☒
Veteran Identifiers / *Anciens combattants* (VET)	☐	☐
Height and Weight / *Taille et poids* (HWT)	☒	See Section B: Physical Assessments / *Voir Section B : évaluations physiques* (WGT, HGT)
Smoking / *Consommation de tabac* (SMK)	☒	☒
Alcohol Use / *Consommation d'alcool* (ALC)	☒	☒

Interview module ***Module de l'entrevue***	**Tracking** (Telephone Interview) ***Évaluation de surveillance*** *(Entrevue téléphonique)*	**Comprehensive** (Face-to-face Interview - In-home or DCS visit) ***Évaluation globale*** *(Entrevue en personne - à domicile ou à un site)*
General Health / *État général de santé* (GEN)	☒	☒
General Health – open text question / *État général de santé – question ouverte* (qualitative)	☐	☐
Nutrition: Short Diet Questionnaire / *Nutrition : Questionnaire court sur le régime alimentaire* (NUT)	Not applicable / *Ne s'applique pas*	☒
Women's Health / *Santé des femmes* (WHO)	☐	☐
Vision / *Vision* (VIS)	☐	☐
Hearing / *Audition* (HRG)	☐	☐
Self-reported Chronic Conditions / *Problèmes de santé chroniques autodéclarés* (CCT/CCC)		
Osteoarthritis or arthritis / *Arthrose ou arthrite*		
Knee / *Genou*	☒	☒
Hip / *Hanche*	☒	☒
Hand(s) / *Main(s)*	☒	☒
Rheumatoid arthritis / *Polyarthrite rhumatoïde*	☒	☒
Other arthritis / *Autres forms d'arthrite*	☒	☒
Respiratory / *Respiratoire*		
Asthma / *Asthme*	☐	☐
COPD / *MPOC*	☒	☒
Cardiovascular / *Cardiovasculaire*		
High blood pressure / *Hypertension*	☐	☐
Diabetes / *Diabète*	☐	☐
Heart disease / *Maladie cardiaque*	☐	☐
Peripheral Vascular Disease / *Maladie vasculaire périphérique*	☐	☐
Stroke / *AVC*	☐	☐
Neurological / *Neurologique*	☐	☐
Gastrointestinal / *Gastrointestinal*	☐	☐

Interview module ***Module de l'entrevue***	**Tracking** (Telephone Interview) ***Évaluation de surveillance*** *(Entrevue téléphonique)*	**Comprehensive** (Face-to-face Interview - In-home or DCS visit) ***Évaluation globale*** *(Entrevue en personne - à domicile ou à un site)*
Vision / *Vision*	☒	☒
Cancer / *Cancer*	☐	☐
Mental Health / *Santé mentale*	☒	☒
Other Conditions / *Autres maladies*	☐	☐
Infections / *Infections*	☒	☒
Medications / *Médicaments* (MEDI)	Not applicable / *Ne s'applique pas*	Not yet available / *Pas encore disponible*
Functional Status / *Capacités fonctionnelles* (FUL)	☒	☒ N = 137[3]
Life Space Index / *Évaluation de l'aire de mobilité* (LSI)	Not applicable / *Ne s'applique pas*	☐
Sleep / *Sommeil* (SLE)	Not applicable / *Ne s'applique pas*	☒
Basic Activities of Daily Living / *Activités de base de la vie quotidienne* (ADL)	☒	☒
Instrumental Activities of Daily Living / *Activités instrumentals de la vie quotidienne* (IAL)	☒	☒
Cognition – metadata & scores / ***Cognition – métadonnées et cotation*** **(COG)**[4]		
REYI / *REYI*	☒	☒
Animal Fluency Test / *Test de fluence (animaux)*	☒	☒
Mental Alternation Test / *Test d'alternance mentale*	☒	☒
REYII / *REYII*	☒	☒
Prospective Memory Test / ***Test de mémoire prospective***		
Time-Based / *En fonction du temps* (TMT)	Not applicable / *Ne s'applique pas*	☒
Event-Based / *En fonction d'un événement* (PMT)	Not applicable / *Ne s'applique pas*	☒
Stroop – Victoria Version / *Stroop – version de Victoria* (STP)	Not applicable / *Ne s'applique pas*	☒
Controlled Oral Word Association / *Test oral contrôlé d'association de mots* (FAS)	Not applicable / *Ne s'applique pas*	☒
Choice Reaction Time / *Test de temps de réaction* (CRT)	Not applicable / *Ne s'applique pas*	☒
Depression / *Dépression* (DEP)	☒	☒

Interview module ***Module de l'entrevue***	**Tracking** (Telephone Interview) ***Évaluation de surveillance*** *(Entrevue téléphonique)*	**Comprehensive** (Face-to-face Interview - In-home or DCS visit) ***Évaluation globale*** *(Entrevue en personne - à domicile ou à un site)*
Satisfaction with Life / *Satisfaction à l'égard de la vie* (SLS)	☐	☐
Posttraumatic Stress Disorder / *Trouble de stress post-traumatique* (PSD)	☐	☐
Social Networks / *Réseaux sociaux* (SN)	☒	☒
Social Support – Availability / *Soutien social – Disponibilité* (SSA)	☒	☒
Social Participation / *Participation sociale* (SPA)	☒	☒
Care Receiving 1/ Formal Care / *Soins reçus 1 / Soins à domicile* (CR1)	☒	☒
Care Receiving 2/ Informal Care / *Soins reçus 2 / Autres types de soins* (CR2)	☒	☒
Care Giving / *Prestation de soins* (CAG)	☒	☒
Injuries / *Blessures* (INJ)	☐	☐
Falls and Consumer Products / *Chutes et produits de consommation* (FAL)	☐	☐
Retirement Status / *Retraite* (RET)	☒	☒
Pre-Retirement Labour Force Participation / *Participation à la population active avant la retraite* (LFP)	☐	☐
Pre-Retirement Labour Force Participation – open text question / *Participation à la population active avant la retraite – question ouverte*[5]	☐	☐
Labour Force / *Population active* (LBF)	☐	☐
Labour Force – open text question / *Population active – question ouverte*[5]	☐	☐
Retirement Planning / *Planification de la retraite* (RPL)	☐	☐
Income / *Revenu* (INC)	☒	☒
Disease Algorithms[6] and Disease Symptoms / ***Algorithme[6] et symptômes de maladies***		
Diabetes / *Diabète* (DIA)[7]	Not applicable / *Ne s'applique pas*	☒
Stroke/Cerebrovascular Event / *AVC* (STR)[7]	Not applicable / *Ne s'applique pas*	☒
Traumatic Brain Injury / *Traumatisme crânien* (TBI)[7]	Not applicable / *Ne s'applique pas*	☒
Hypo and Hyperthyroidism / *Hypo-et hyperthyroïdie* (HYP)[7]	Not applicable / *Ne s'applique pas*	☒
Hypertension / *Hypertension* (HBP)[7]	Not applicable / *Ne s'applique pas*	☒

Interview module ***Module de l'entrevue***	**Tracking** (Telephone Interview) ***Évaluation de surveillance*** *(Entrevue téléphonique)*	**Comprehensive** (Face-to-face Interview - In-home or DCS visit) ***Évaluation globale*** *(Entrevue en personne - à domicile ou à un site)*
Ischemic Heart Disease / *Cardiopathie ischémique* (IHD)[7]	Not applicable / *Ne s'applique pas*	☒
WHO Rose Questionnaire / *Questionnaire Rose de* l'OMS (ROS)	Not applicable / *Ne s'applique pas*	☒
Osteoarthritis of the Hand / *Arthrose de la main* (OSA)	Not applicable / *Ne s'applique pas*	☒
Osteoarthritis of the Hip / *Arthrose de la hanche* (OSH)	Not applicable / *Ne s'applique pas*	☒
Osteoarthritis of the Knee / *Arthrose du genou* (OSK)	Not applicable / *Ne s'applique pas*	☒
Musculoskeletal: Other / *Musculosquelettique : autre* (OAR)	Not applicable / *Ne s'applique pas*	☒
Osteoporosis / *Ostéoporose* (OST)[7]	Not applicable / *Ne s'applique pas*	☒
Neuro-psychiatric / *Neuropsychiatrique* (DPR)[7]	Not applicable / *Ne s'applique pas*	☒
Parkinsonism / *Parkinsonisme* (PKD)[7]	Refer to Maintaining Contact Interview / *Voir l'entrevue de mi-parcours*	☒
Chronic Airflow Obstruction / *Obstruction chronique des voies respiratoires* (CAO)[7]	Not applicable / *Ne s'applique pas*	☒

Maintaining Contact Interview / *Entrevue de mi-parcours*

Interview module ***Module de l'entrevue***	**Tracking** (Telephone Interview) ***Évaluation de surveillance*** *(Entrevue téléphonique)*	**Comprehensive** (Face-to-face Interview - In-home or DCS visit) ***Évaluation globale*** *(Entrevue en personne - à domicile ou à un site)*
Falls / *Chutes* (FAL)	☐	☐
Pain and Discomfort / *Douleurs et malaises* (HUP)	☐	☐
Oral Health / *Santé bucco-dentaire* (ORH)	☒	☒
Snoring / *Ronflement* (SNO)	Not applicable / *Ne s'applique pas*	☐
Parkinsonism / *Parkinsonisme* (PKD)	☒	See Parkinsonism module above / *Voir le module sur le Parkinsonisme* (PKD) *ci-dessus*
Health Care Utilization / *Utilisation des soins de santé* (HCU)	☐	☐
Medication Use / *Consommation de médicaments* (MED)	☒	Not applicable / *Ne s'applique pas*
Dietary Supplement Use / *Usage de suppléments alimentaires* (DSU)[7]	☒	☒
Nutritional Risk / *Risque nutritionnel* (NUR)	☒	☒
Physical Activities / *Activités physiques* (PA2)	☒	☒
Psychological Distress / *Détresse psychologique* (K10)	Not applicable / *Ne s'applique pas*	☒
Personality Traits / *Traits de caractère* (PER)	Not applicable / *Ne s'applique pas*	☒
Social Inequality / *Inégalité sociale* (SEQ)	☐	☐
Online Social Networking / *Réseautage social en ligne* (INT)	☐	☐
Transportation, Mobility, Migration / *Transport, mobilité, migration* (TRA)	☐	☐
Built Environments / *Environnements construits* (ENV)	☐	☐
Wealth / *Patrimoine* (WEA)	☐	☐

[1] Determined using the Postal Code Conversion File (PCCF) from Statistics Canada. / *Déterminé à l'aide du Fichier de conversion des codes postaux (FCCP) de Statistique Canada.*

[2] Adequate justification must be provided within the project description (Application Part 1) to justify the request of these data. / *Lorsque vous demandez ces données, veuillez inclure une justification adéquate dans la description du projet (Partie 1 de la demande).*

[3] For 135 participants, the baseline DCS visit was completed by phone for various reasons including time constraints for recruitment fulfillment toward the end of Baseline data collection and some cases where the participants were unable to physically present at the DCS but wished to remain part of CLSA. For 2 participants, the Baseline DCS site visit was completed in person at the participant's residence as a pilot for the DCS at home. /

Pour 135 participants, la visite initiale au Site de collecte de données a été réalisée par téléphone pour diverses raisons, y compris des contraintes de temps liées au recrutement vers la fin de la collecte des données de départ ou des participants ne pouvant pas se présenter physiquement au site, mais souhaitant faire partie de l'ÉLCV. Pour 2 participants, la visite initiale au Site de collecte de données a été faite en personne à leur résidence dans le cadre de l'étude pilote de l'entrevue du site réalisée à domicile.

[4] Raw data available through special request. For more information and for details on how to request these data, please contact access@clsa-elcv.ca /
Les données brutes sont disponibles sur demande spéciale. Pour en savoir plus sur ces données et comment en faire la demande, veuillez écrire à access@clsa-elcv.ca

[5] Open text data for occupation and industry are available. These data are not coded. Any coding of the released open text data has to be a coordinated effort between the Approved User and the CLSA. Please contact us for details access@clsa-elcv.ca /
Des données ouvertes pour la profession et l'industrie sont disponibles. Ces données ne sont pas codées. Toute codification de données ouvertes diffusées doit être coordonnée entre l'utilisateur autorisé et l'ÉLCV. Pour plus d'information, contactez-nous à access@clsa-elcv.ca.

[6] The disease ascertainment algorithms are being prepared but are not yet available; however, some of the data contributing to the algorithms are available. /
Les algorithmes diagnostiques ont été préparés, mais ne sont pas encore disponibles; toutefois, certaines données contribuant aux algorithmes ont été préparées et sont disponibles.

[7] Open text data under review – in preparation for future release. (Please see Variables under review) /
Les données ouvertes sont en cours d'examen – en préparation pour une diffusion ultérieure. (Voir les variables en cours d'examen)

Included in all datasets / *Inclus dans tous les ensembles de données*

- Sampling weights / *Poids d'échantillonnage*

Not included in datasets / *Exclus des ensembles de données*

- Identifiable information collected (e.g. name, contact information, date of birth, health insurance number, and full postal code) /
  Informations recueillies permettant l'identification (p. ex. nom, coordonnées, date de naissance, numéro d'assurance maladie et code postal complet)

Comments / *Commentaires*

We would need data for the Medications used by the "Comprehensive" component, if it is possible by the release of data.

SECTION B: PHYSICAL ASSESSMENT VARIABLES[1]

(DCS Visit – Comprehensive Assessment) /

VARIABLES TIRÉES D'ÉVALUATIONS PHYSIQUES[1]

(Visite à un site – Évaluation globale)

Physical Assessment *Évaluation physique*[1]	**Subcategory** *Sous-catégorie*	**Data** *Données*
Contraindications Questionnaire / *Questionnaire sur les contre-indications*	Full Questionnaire / *Questionnaire complet*	☐
Weight and Height / *Poids et taille*	Weight / *Poids* (WGT)	☒
	Height / *Taille* (HGT)	☒
	Body Mass Index / *Indice de masse corporelle* (HWT)	☒
Hip and Waist Circumference / *Circonférence taille et hanche* (WHC)		☒
Pulse Rate & Blood Pressure / *Fréquence du pouls et pression sanguine*	Pulse rate / *Fréquence du pouls* (BP)	☒
	Blood Pressure / *Pression sanguine* (BP)	☒
Carotid Intima Media Thickness / *Épaisseur de l'intima media carotidienne* (CI)[2,3]	Carotid Intima / *Intima de la carotide*	☒
Spirometry / *Spirométrie* (SPR)[3]		☐
Electrocardiogram / *Électrocardiogramme* (ECG)[3]		☒
Bone Density by DEXA / *Densité osseuse avec DEXA* (DXA)[4]	Whole Body / *Corps entier*	☒
	Body Parts / *Parties du corps*	☒
	Dual Hip / *Deux hanches*	☒
	Forearm / *Avant-bras*	☒
Bio-Impedance by DEXA / *Bio-impédance avec DEXA* (DXA)[4]	Body Composition (Whole Body) / *Composition corporelle (Corps entier)*	☒
	Body Composition (Body Parts) / *Composition corporelle (parties du corps)*	☒
Hearing / *Audition* (HRG)		☒
4 Metre Walk / *Marche sur 4 m* (WLK)		☒
Timed Get Up and Go / *Lever-marcher chronométré* (TUG)		☒
Standing Balance / *Équilibre debout* (BAL)		☒
Chair Rise: Balance and Coordination / *Se lever d'une chaise : équilibre et coordination* (CR)		☒

Physical Assessment *Évaluation physique*[1]	**Subcategory** *Sous-catégorie*	**Data** *Données*
Visual Acuity / *Acuité visuelle* (VA)		☒
Tonometry / *Tonométrie* (TON)[4]		☒
Grip Strength / *Force de préhension* (GS)		☒

[1] For a detailed list of the physical assessment variables please consult the Physical Assessments Summary Table available in the Data and Sample Access Documents section of the CLSA website. / *Pour obtenir la liste complète des variables tirées d'évaluations physiques, veuillez consulter le Tableau sommaire à la section Documents d'accès aux données et aux échantillons du site Web de l'ÉLCV.*

[2] Alphanumeric data for Carotid Intima measures are available only for those images classified as useable. / *Les données alphanumériques des mesures de l'intima carotidienne sont uniquement disponibles pour les images classées comme étant utilisables.*

Additional Data available / *Données supplémentaires disponibles*

[3] Image data are currently available by special request for Carotid Intima Media Thickness (cIMT), IVA Lateral Spine (DXA), Retinal Scan (RS), Electrocardiogram (ECG; tracings) and Spirometry (SPR, flow curves).To request image data, please use the 'Comments' box below and explain in Part 1 of the Application, why your project requires use of images. Please note that a request to receive image data from the CLSA will incur additional costs, beyond the current data access fee; it may prolong processing time of your application, and the time to receive your image data may be longer than the 6 months to receive alphanumeric data. For more information, please contact access@clsa-elcv.ca / *Il est maintenant possible d'obtenir les données en format image sur demande spéciale pour les mesures suivantes : épaisseur de l'intima-média carotidienne (cIMT), analyse intervertébrale (IVA) de la colonne vertébrale (DXA), balayage de la rétine (RS), électrocardiogramme (ECG, tracés) et spirométrie (SPR, courbes de débit). Pour demander des données en format images, utilisez la case « Commentaires » ci-dessous et, à la Partie 1 de la demande, expliquez pourquoi ces images seront utiles à votre projet. Veuillez noter qu'une demande d'obtention d'images de l'ÉLCV entraînera des coûts supplémentaires, au-delà des frais d'accès aux données actuels. Cette demande peut prolonger le temps nécessaire au traitement de votre demande et le délai de réception de vos images peut être plus long que les six mois prévus pour les données alphanumériques. Pour en savoir plus sur ces demandes, veuillez contacter access@clsa-elcv.ca.*

[4] Raw data available through special request. For more information and for details on how to request these data, please contact access@clsa-elcv.ca / *Les données brutes sont disponibles sur demande spéciale. Pour en savoir plus sur ces données et comment en faire la demande, veuillez écrire à access@clsa-elcv.ca*

Comments / *Commentaires*

SECTION C: BIOMARKERS (Comprehensive Assessment – DCS Visit) / *BIOMARQUEURS (Évaluation globale – Visite à un site)*

Hematology Report / *Rapport hématologique*	**Data / *Données***
White blood cells / *Globules blancs* (WBC)	☒
Lymphocytes (relative number) / *Lymphocytes (nombre relatif)* (LY_PER)	☒
Monocytes (relative number) / *Monocytes (nombre relatif)* (MO_PER)	☒
Granulocytes (relative number) / *Granulocytes (nombre relatif)* (GR_PER)	☒
Lymphocytes (absolute number) / *Lymphocytes (nombre absolu)* (LY_NB)	☒
Monocytes (absolute number) / *Monocytes (nombre absolu)* (MO_NB)	☒
Granulocytes (absolute number) / *Granulocytes (nombre absolu)* (GR_NB)	☒
Red blood cells / *Globules rouges* (RBC)	☐
Hemoglobin / *Hémoglobine* (Hgb)	☐
Hematocrit / *Hématocrites* (Hct)	☐
Mean corpuscular volume / *Volume globulaire moyen* (MCV)	☐
Mean corpuscular hemoglobin / *Teneur corpusculaire moyenne en hémoglobine* (MCH)	☐
Mean corpuscular hemoglobin concentration / *Concentration corpusculaire moyenne en hémoglobine* (MCHC)	☐
Red blood cell distribution width / *Variation de la grosseur des globules rouges* (RDW)	☐
Platelets / *Plaquettes* (Plt)	☐
Mean platelet volume / *Volume plaquettaire moyen* (MPV)	☐

Chemistry Report / *Rapport de chimie*	**Data / *Données***
Albumin / *Albumine* (ALB)	☒
Alanine aminotransferase / *Alanine aminotransférase* (ALT)	☐
High Sensitivity C-reactive protein / *Protéine C réactive à haute sensibilité* (HSCRP)	☒
Creatinine / *Créatinine* (CREAT)	☒
Total Cholesterol / *Cholestérol total* (CHOL)	☐
Ferritin / *Ferritine* (FERR)	☒
Free Thyroxine / *Thyroxine libre* (FT4)	☐

Chemistry Report / *Rapport de chimie*	**Data / *Données***
High-Density Lipoprotein / *Lipoprotéine de haute densité* (HDL)	☐
Low-Density Lipoprotein / *Lipoprotéine de faible densité* (LDL)	☐
Non-High Density Lipoprotein / *Lipoprotéine à densité non-élevée* (non-HDL)	☐
Thyroid Stimulating Hormone / *Thyréostimuline* (TSH)	☒
Triglycerides / *Triglycérides* (TRIG)	☐
25 – Hydroxyvitamin D / *25 – hydroxyvitamine D* (VITD)	☒
Hemoglobin A1c / *Hémoglobine A1c* (HBA1c; N = 26,961)	☐

Comments / *Commentaires*

SECTION D: GENOMICS (Comprehensive Assessment – DCS Visit) / *GENOMIQUE (Évaluation globale – Visite à un site)*

Genomics / *Génomique* (N=9,900)	**Data / *Données***
Genotypes (Affymetrix Axiom array, 794k SNPs) / *Génotypes (génotypage Axiom d'Affymetrix, 794k SNP)*	☐
Imputation (Haplotype Reference Consortium release 1.1, 39.2M SNPs) / *Imputation (Haplotype Reference Consortium, version 1.1, 39.2M SNP)*	☐

Comments / *Commentaires*

SECTION E: LINKED DATA / DONNÉES LIÉES[1]

Linked Data / ***Données liées[1]***	**Tracking** (Telephone Interview) ***Évaluation de surveillance*** *(Entrevue téléphonique)*	**Comprehensive** (Face-to-face Interview - In-home or DCS visit) ***Évaluation globale*** *(Entrevue en personne - à domicile ou à un site)*
Nitrogen Dioxide / *Dioxyde d'azote*	☐	☐
Sulfur Dioxide / *Dioxyde de soufre*[2]	☐	☐
Ozone / *Ozone*[2]	☐	☐
Fine Particulate Matter / *Fines particules de matières*[2]	☐	☐
Proximity to Roadways / *Proximité des routes*	☐	☐
Nighttime Light / *Luminosité nocturne*[2]	☐	☐
Normalized Difference Vegetation Index (NDVI; greenness) / *Indice de végétation par différence normalisée (IVDN; verdeur)*[2]	☐	☐
Meteorological Data (weather and climate) / *Données météorologiques (météo et climat)*[2]	☐	☐
Material and Social Deprivation Indices / *Indices de défavorisation matérielle et sociale*[2]	☐	☐
Canadian Active Living Environments (Can-ALE) Data / *Données sur les milieux de vie actifs canadiens (Can-ALE)*[2]	☐	☐

[1] For a detailed list of the linked variables please consult the Linked Data Summary Table available in the Data and Sample Access Documents section of the CLSA website. / *Pour obtenir une liste détaillée des variables liées, veuillez consulter le tableau récapitulatif des données liées disponible à la section Documents d'accès aux données et aux échantillons du site Web de l'ÉLCV.*

[2] When requesting these data, please note that if your CLSA Data and Biospecimen Request Application is approved, you will be required to sign a Data Sharing and Use via Approved Third Party Agreement (available for consultation and download here: http://canue.ca/data/), and submit it to the CLSA and to CANUE. / *Lorsque vous demandez l'accès à ces données, veuillez noter que si votre demande d'accès aux données et aux échantillons biologiques de l'ÉLCV est approuvée, vous devrez signer une Entente de partage et d'utilisation des données via une tierce partie autorisée (disponible pour consultation et téléchargement ici : http://canue.ca/data/) et la soumettre à l'ÉLCV et à CANUE.*

Comments / *Commentaires*

PART 2 OF APPLICATION COMPLETE /
LA 2E PARTIE DE LA DEMANDE EST TERMINÉE

Schedule C – Specific security measures

Definitions

Information:

- Any CLSA data and samples obtained from the CLSA pursuant to this Agreement, with or without name or other identifying information, and any aggregation of responses that could directly or indirectly identify an individual person, business, or organization.

Authorized Person:

- Person who is the PI(s) on the approved project.

Identified Person:

- Authorized Person and all others listed as Co-investigators/Collaborators or staff on the approved project.

Transportable media:

- All types of transportable storage media on which data can be saved, including laptops, CD-ROMs, flash memory sticks, and removable hard disk.

Visitor:

- Person, other than an Authorized Person, who has been invited into the secure area by an Authorized Person, as permitted by the Institution's access policies.

Security Requirements

The Institution must ensure that adequate protection is in place to provide for the security of the Information.

The security requirements described below are the minimum requirements that must be met by the Institution.

Physical Access

1. The Information must be accessed only from within a secure location that allows access only to Authorized and Identified Persons. The secure location can be within a series of buildings, one entire building, an entire floor within a building, or a single room. Once the perimeter of the secure location is defined, the procedures apply to all areas within the perimeter. Where a series of buildings are involved, a secure perimeter must be defined for each building.

2. Access to the Information is limited to Authorized and Identified Persons. The manager responsible for ensuring that the Institution's requirements are met must maintain an auditable trail, listing the Identified Persons, the specific Information to be accessed, the period for which this access is granted, the purpose for the access, and where applicable, that the Person meets any special requirements for access.

3. Visitors may have access to the secure area. However, under no circumstances may visitors be provided access to the Information.

IT Storage and Transmission

4. All computers with access to the Information must employ logical access controls (passwords) at the device and network level.

5. Where the Information is held on laptops, CD-ROMs, flash memory sticks or other transportable media of any type, passwords and full encryption must be used. This applies equally to backups of the Information stored on transportable media.

6. The Information cannot be electronically transmitted, except as described in points 7 and 8. This includes the transmittal of the Information by facsimile or by e-mail.

7. Servers storing and transmitting unencrypted data, where used, must be located in a secure, controlled-access area, preferably in the same area where the Information is accessed. If located in a separate area, controls must be in place to ensure that only Identified Persons can access the server. Unless the Information is encrypted continuously while outside the secure area, conduit must be used for all cabling and all cross-connect areas must be physically secured.

8. Network firewalls and access rules must be in place to prevent access to the Information, other than to Identified Persons. Information may be stored on and transmitted over networks not meeting these requirements, provided that it is encrypted, except when in use by an Identified Person. Alternatively, the Information may be stored on a stand-alone computer with no external connections, or on a closed network. When a network transmits information that leaves a secure area (for example, when a series of buildings house employees within a single organization), the data must be encrypted whenever it is outside the secure area.

Physical Storage

9. When not in use, transportable media containing the Information must be stored in secure containers. This applies equally to backups of the Information.

10. The Information shall not be removed from the secure area (as described in point 1, above) in any format (e.g., laptops, printouts, flash memory sticks, transportable media of any type, etc.), except as described in points 7 and 8 above.

11. When not in use, printed documents containing the Information must always be stored in secure containers.

Information Copying and Retention & Record Management

12. Copies and extracts of the Information may only be made for the purposes of carrying out work as covered by this Agreement. When no longer needed, any such copies or extracts must be destroyed in a secure manner (as per points 13 and 14 below).

13. Paper documents containing the Information must be destroyed (shredded) in a secure manner before disposal. Destruction must occur within the secure area.

14. All electronic storage media used in the processing of the Information, including all back-up and transportable media must be sanitized or destroyed on completion of their use. Destruction must occur within the secure area.

15. These security requirements must be communicated regularly to all Identified Persons and be available for reference, as required.

Schedule D - Return of Derived Variables

Canadian Longitudinal Study on Aging (CLSA)
Policy on the Return of Derived Variables

In accordance with the Canadian Longitudinal Study on Aging's Data and Sample Access Policy and Guiding Principles and your signed CLSA Access Agreement, you may be asked to return to the CLSA Derived Variables that you created as part of your research.

What are Derived Variables?
Derived Variables (DVs) include new data-fields (apart from simple recoding) constructed by you whilst undertaking your research project using CLSA raw alphanumeric data, biomarker data, or a combination of both. A separate policy governs the return of biomarker data obtained from biospecimens to the CLSA.

Why might I be asked to return Derived Variables to the CLSA?
The objectives in asking for the DVs and documentation are that, in keeping with the CLSA as a research platform, CLSA can:

(i) expand and enhance the utility of the CLSA platform;
(ii) make your DVs available for use by other approved users;
(iii) make your methods for constructing DVs available to other researchers so that analyses can be replicated.

How do I report on Derived Variables?
In accordance with the CLSA's Data and Sample Access Policy and Guiding Principles and your signed CLSA Access Agreement, you will be asked to submit a Final Report at the end of your project. In this Report, you will be asked to describe any DVs you have created.

When do I return Derived Variables?
Once the CLSA has reviewed the Final Report and determined that the DVs would be of utility to the CLSA platform, the Statistical Analysis Centre (SAC) will send you a document with guidelines on the Return of Derived Variables to the CLSA by Approved Users, including details on what needs to be returned and how to transfer files to the CLSA. Researchers will be asked to return DVs within 6 months of the date of the first publication using the DV.

How will my Derived Variables be used by CLSA?
The DVs will be made available with acknowledgement of the provenance. The CLSA will not audit your DVs and is not responsible for its accuracy or validity. The CLSA will review the documentation and algorithms you provide to ensure that sufficient explanatory documentation has been provided. Derived variable fields and accompanying documentation may be made available for use by other approved users, and may be included in our DataPreview Portal (http://clsa-elcv.ca/).

Researchers who have any questions concerning the process or content for the return of DVs should contact the CLSA Statistical Analysis Centre via access@clsa-elcv.ca.

Schedule E – Fees

All fees in CDN dollars

Fees in accordance with this project and article 4 have been set at $3,000 and must be paid 30-45 days after receipt of an invoice that will be sent to the primary user.

Schedule F: Project Team

All Co-Applicants and Other Personnel listed below are indicated in the application Schedule B requiring direct access to the CLSA Data. Each of these Co-Applicants must sign Schedule F to agree to comply with the conditions outlined in Articles 2.1 and 2.3 (excerpts below) of this CLSA Access Agreement also located at (https://clsa-elcv.ca/doc/1042). All others should be listed but will not require a signature if they will not have direct access to the data.

Sample and Data Security.

Security measures specified in Schedule C attached hereto will apply to all Transferred Materials. The Approved User and institution undertakes to respect these security measures during the Study and afterwards, during storage of Transferred Materials where necessary.

Transferred Materials, including any copies thereof, may only be used for the Study described in Schedule B and may not be disclosed, transmitted or shipped to anyone except employees working directly with the Approved User or co-investigators including co-applicants or other personnel from other institution(s), indicated in the Study who will require direct access to the CLSA Data and who agree to be bound by the terms of this Agreement or to persons expressly designated in writing by McMaster. The Approved User shall retain control of the Transferred Material at all times. It is the responsibility of the Approved User's Institution to inform the staff and co-investigators, including co-applicants and other personnel at other third-party institution(s) entering into contact with the Transferred Materials of the obligations contained in the Data and Biospecimen Access Policy and Guiding Principles and this Agreement. As such, co-applicants and other personnel at other third-party institution(s) must submit a signed Appendix F attached hereto. Transfer of any CLSA biospecimens outside Canada is strictly prohibited. Data access will only be provided to institutional email addresses.

Co-Applicants and Other Personnel
Please list all co-applicants including students and any other personnel who will be involved in the project (eg: advisor, statistician, research assistant, etc.)

Name	Affiliation & institutional email address	Academic Position and Role on Project	Signature
Belinda Nicolau	McGill University, Faculty of Dentistry belinda.nicolau@mcgill.ca	Co-Principal Investigator	
Faleh Tamimi	McGill University, Faculty of Dentistry faleh.tamimimarino@mcgill.ca	Co-investigator	
Linda Booij	Concordia University, Department of Psychology linda.booij@umontreal.ca	Co-investigator	

Name	Affiliation & institutional email address	Academic Position and Role on Project	Signature
Jocelyne Feine	McGill University, Faculty of Dentistry jocelyne.feine@mcgill.ca	Co-investigator	Jocelyne Feine
Ricardo Alchini	McGill University, Faculty of Dentistry ricardo.alchini@mail.mcgill.ca	Co-investigator (DMD student)	Ricardo Alchini
Sreenath Madathil	McGill University, Faculty of Dentistry sreenath.arekunnathmadathil@mail.mcgill.ca	Co-investigator	

FONDATION DRUMMOND FOUNDATION

December 17, 2018

McGill University
Faculty of Dentistry
2001 McGill College Avenue, Suite 500
Montreal, Quebec H3A 1G1

Attn: Dr. Raphael F de Souza - Division of Oral Health and Society

Re: 2018 RFA – Proposal #5

Dear Dr. de Souza,

On behalf of the Board of Directors of The Drummond Foundation, I am pleased to inform you that you have been selected as one of this year's research award recipients. Your proposal received a very favourable recommendation from our Donations Committee as well as from our external scientific reviewers, and the Board believes that the innovative character of your research project aligns well with the Drummond Foundation's objectives.

Please find enclosed a cheque in the amount of **$22,500** payable to McGill University, Faculty of Dentistry as a grant for research at your institution to be carried out by you, entitled "*Better Oral Health for a Healthy Cognition: Investigation of a New Pathway.*" Other grant conditions are detailed in the original *Request for Applications (RFA) for Research Grants 2018-2019* which is also enclosed, as a reminder.

We would like to receive from you by 30 September 2019 an accounting of how the grant funds have been disbursed to that date.

The second payment of $2,500 (the balance of 10%) will be paid upon receipt of your final report for this research project which is due no later than 31 August 2020.

Would you please be sure to issue an acknowledgement and charitable donation receipt for the 2018 calendar year to The Drummond Foundation and send it to the undersigned at the address noted below.

We wish you great success in your work on this interesting project and look forward to reading about your results. If you have any questions or for any other communication with The Drummond Foundation, please do not hesitate to contact P. Stuart Iversen.

Yours truly,

P. Stuart Iversen
Secretary
cc: M. Bruce McNiven
Dr. Lucyna Lach
Marie Senécal-Tremblay

1350 Sherbrooke St. West, Suite 1201, Montreal, Quebec, H3G 1J1 | Tel : 514-866-2200 Fax : 514-866-3576
www.drummondfoundation.ca | office@drummondfoundation.ca