Intracranial artery calcifications Histology, risk factors and clinical relevance Esther de Brouwer

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Contents

CHAPTER 1

General Introduction

General introduction

Despite major advances in our comprehension of health and disease in humans and impressive results in the fields of cancer, cardiovascular disease, infections, and immune disorders, the understanding of neurodegenerative disease, and organ dysfunction with aging remains a major challenge. Researchers usually focus on amyloid, tau, dopamine, and cerebrovascular disease when investigating neurodegenerative disease and dementia. There is increasing evidence that small vascular lesions in the brain, such as microinfarcts, are associated with neurodegenerative disease.[1,2] In this thesis, we pose a less conventional hypothesis: namely, small calcifications in the brain are related to neurodegenerative disease and may even be a cause of brain dysfunction and cognitive decline. Although not much is known about their prevalence, histology, and mechanism, we posit that these calcifications are common and mostly arterial in nature. Here, we first summarize our limited knowledge and then present a plan how to solve some major knowledge gaps.

Intimal and medial calcifications in human arteries

Because we assume that intracranial calcifications are located predominantly in the arteries, we will first discuss the current knowledge on calcification in general. Over the years, there has been a considerable amount of research conducted on vascular calcifications in the coronary arteries and, more recently, also in other arteries such as the aorta and breast arteries.[3-5]

Arterial calcifications can be divided into two dominant types depending on their location in the vessel wall: calcifications in the medial layer (tunica media), which we combine with calcifications in the internal elastic lamina (IEL or internal elastic membrane) because of their non-atherosclerotic origin, and calcifications in the intimal layer (tunica intima) (figure 1).[6]

Intimal calcifications are the most well-known type of calcification. They are usually atherosclerotic lesions (plaques) associated with vascular smooth muscle cells (VSMCs) and macrophages,[8] and are seen as patchy clusters in the tunica intima.[9] The formation of these lesions begins by low-density lipoprotein (LDL) deposition in the arterial walls. There, LDLs undergo oxidation. Invading monocytes differentiate into macrophages that, after taking up oxidized LDLs, slowly transform into foam cells and form fatty streaks inside the intima of the endothelium. Endothelial VSMCs then move from the medial to the intimal layer and form a ring around the fatty streaks, which further enlarges the atheromatous plaque. With time, the VSMCs begin to calcify and cause a progressive narrowing of the vessel wall that can obstruct the blood flow or result in a thrombus if the wall ruptures. Depending on the location, this obstruction/thrombus can lead to myocardial infarction, peripheral artery disease, or stroke.[10] Atherosclerosis mostly affects large- to medium-sized arteries, including cranial arteries such as the carotid and vertebral arteries, and can lead directly, or indirectly by stroke, to cognitive impairment.[10]

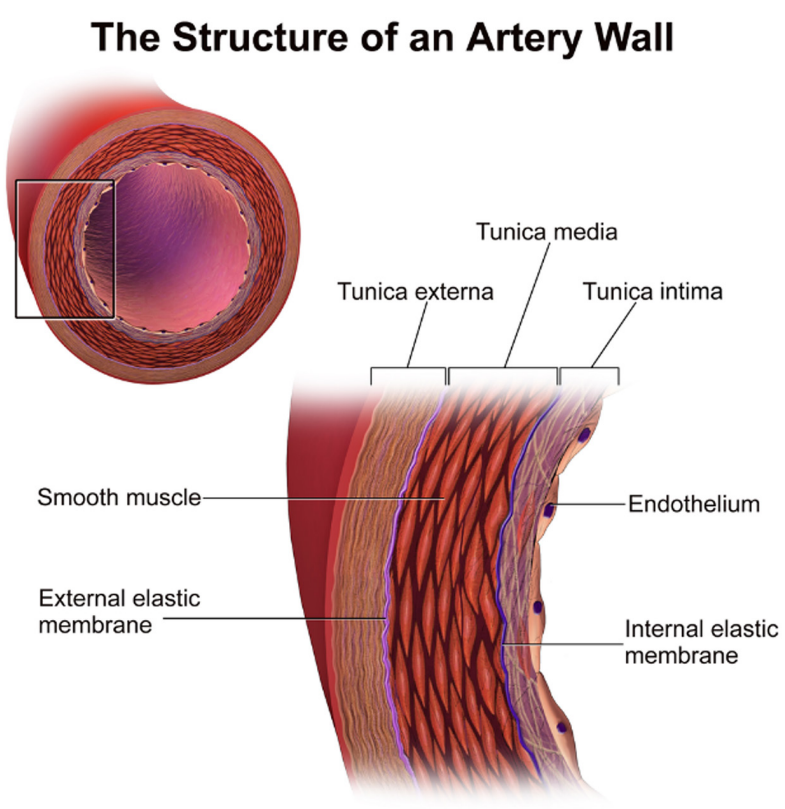
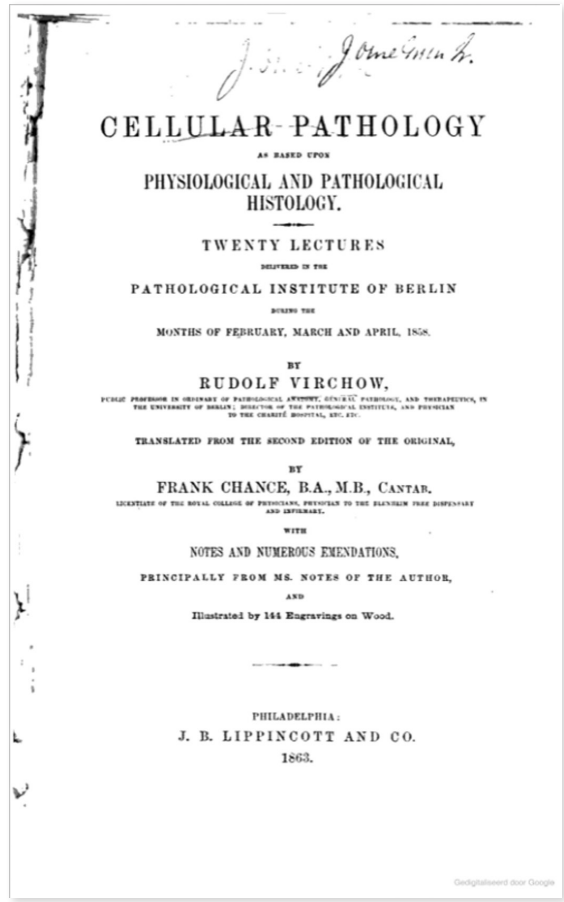


FIGURE 1. The structure of an artery wall.[7]

Medial calcifications, also known as Monckeberg sclerosis, are thought to be distinct from intimal calcifications, with different clinical consequences. They are less well known but have been recognized for a long time. Monckeberg described medial calcifications in 1903, while Virchow wrote about calcifications in 'the middle coat', a process that differs from the atheromatous process, in 1863 (figure 2).[11,12] These calcifications are located in the tunica media that comprises VSMCs and elastic tissues.[13] Calcium deposits are found both intracellularly (in VSMCs) and extracellularly alongside the IEL and nearby VSMCs. The calcium-rich deposits may thicken, form solid plates in the tunica media, and progress to involve the entire circumference like a ring. Arteries often show long tracts of continuous, linear, and circular calcifications of the tunica media and IEL. Secondary invasion of the tunica intima can also occur. Medial calcifications are mostly found in large- and medium-sized arteries but can also affect arterioles.[10] They can lead to arterial wall stiffening, which is a strong predictor of future cardiovascular events, organ failure, cognitive impairment, and all-cause mortality.[9,14]



CELLULAR PATHOLOGY

AS BASED UPON

PHYSIOLOGICAL AND PATHOLOGICAL HISTOLOGY.

TWENTY LECTURES

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FIGURE 2. Title page of Virchow's book published in 1863.[9]

Although intimal and medial calcifications are distinct entities, they often occur simultaneously in most arteries and both result in the deposition of calcium phosphate salts, mainly in the form of hydroxyapatite.[9,13] The balance between phosphate and pyrophosphate is an important factor in both types of vascular calcification. While extracellular inorganic phosphate (Pi) promotes mineralization and forms calcium phosphate salts in the presence of calcium, inorganic pyrophosphate (PPi) is the strongest known inhibitor of calcification.[15] Although the pathogenesis of arterial calcification is not yet completely understood, PPi formation is one way the body can counteract calcification. The main source of extracellular PPi is hydrolysis of extracellular adenosine-5'-triphosphate (ATP). As can be seen in figure 3, pyrophosphate is thought to regulate its own production and breakdown.[15,16]

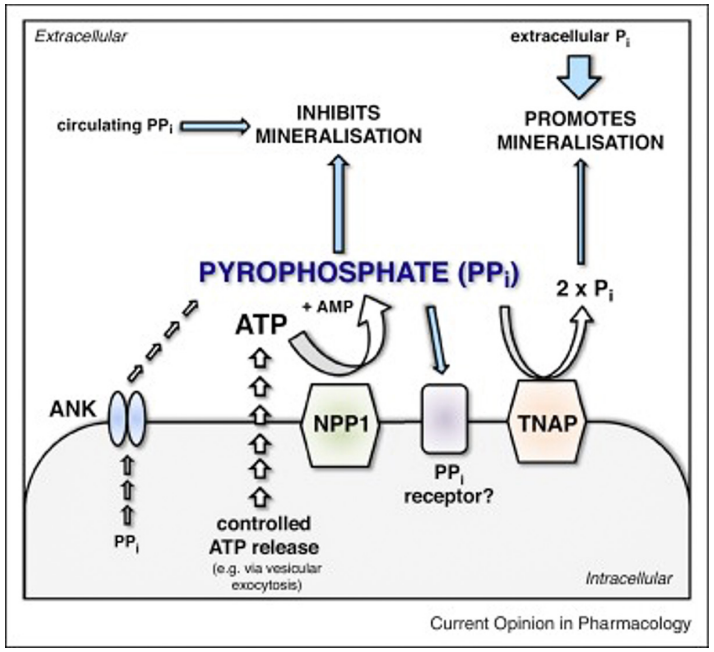

FIGURE 3. Regulation of extracellular PPi levels and mineralization.
Intracellular ATP is released from most cells via controlled mechanisms such as vesicular exocytosis. Once outside the cell, ATP is rapidly broken down by ectonucleotide pyrophosphatase phosphodiesterase 1 (NPP1) to produce adenosine monophosphate (AMP) and PPi. The progressive ankylosis protein, ANK, in the membrane can directly transport PPi, which is found at micromolar levels, from inside to outside the cell. Extracellular PPi acts to prevent mineralization by inhibiting hydroxyapatite formation and growth. It also regulates gene expression suggesting the presence of a yet unknown PPi receptor/sensor. Tissue non-specific alkaline phosphatase (TNAP) hydrolyzes PPi into two phosphate molecules, which may contribute to mineralization in association with the much higher concentrations of phosphate available in extracellular fluids. This figure is reprinted from Orriss IR, Arnett TR, Russell RG. Pyrophosphate: a key inhibitor of mineralisation. Curr Opin Pharmacol. 2016 Jun;28:57-68. doi: 10.1016/j.coph.2016.03.003. Epub 2016 Apr 7. PMID: 27061894, with permission from Elsevier.

Because intimal calcifications are atherosclerotic in origin, they are associated with the traditional risk factors for cardiovascular disease such as hypertension, smoking, adiposity, hyperlipidemia, and diabetes mellitus.[17] There is some overlap in risk factors, as medial calcifications are also associated with diabetes mellitus in addition to aging and chronic kidney disease.[9]

Intracranial artery calcifications

Histology

Intracranial calcifications can be found in larger arteries such as the carotid artery siphon and basilar artery. Calcifications in the carotid artery siphon can be found in both the intimal and medial layers of the vessel wall, although one histopathology study found calcifications in the siphon to be predominantly medial in nature.[18] Calcifications in the vertebrobasilar arteries are mostly found in the intimal layer and are related to atherosclerotic changes.[19,20] There have been very few studies conducted on smaller calcifications deeper in the brain: one histological study described small-artery calcifications in the basal ganglia,[21] while another study described calcifications in the hippocampus.[22] Three types of calcification patterns in the basal ganglia have been recognized in patients with a neurodegenerative disease such as Alzheimer's disease, frontotemporal dementia, progressive supranuclear palsy, or Parkinson's disease: calcifications in the tunica media of small- and medium-sized vessels, calcifications along capillaries, and, as opposed to other parts of the body, calcifications in the parenchyma.[21] Calcification in the hippocampus has been described as a vasculopathy with fibrosis, with a predilection for the middle hippocampal artery. This type of calcification is not associated with atherosclerosis.[22]

Computed tomography

Intracranial calcifications were already detectable using some kind of imaging almost 100 years ago. John D. Camp, for example, wrote about the value of roentgenogram in the detection of calcification in intracranial lesions in 1929.[23] Today, although magnetic resonance imaging (MRI) sequences are increasingly better able to detect intracranial calcifications, computed tomography (CT) is the imaging technique of choice for the detection and characterization of these calcifications.[24]

Intracranial calcifications, present as high-density areas (figure 4), appear to be a common finding on CT scans. However, there are no histological studies so far validating the findings of calcifications in basal ganglia and hippocampus on CT and confirming the arterial nature of these intracranial calcifications.

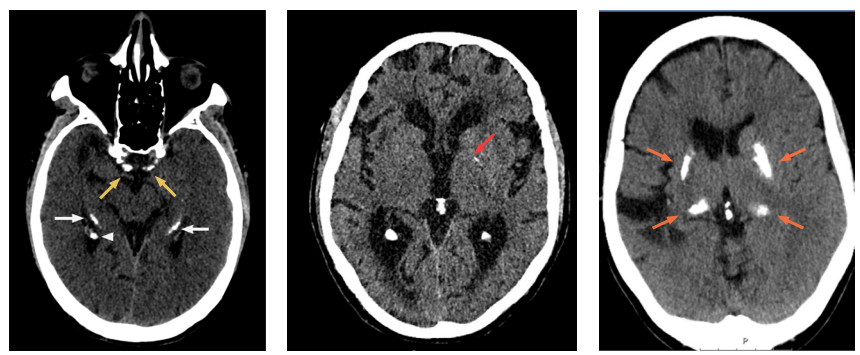

FIGURE 4. CT images of calcifications in the carotid artery siphon (yellow arrows), hippocampus (white arrows), incidental basal ganglia calcification (red arrow), and primary familial basal ganglia or Fahr disease (orange arrows). The arrowhead (in leftmost image) shows calcification in the choroid plexus.

Epidemiology

Intracranial calcifications are located most often in the carotid artery siphon and are found in almost all patients over 70 years of age.[25] Calcifications in the carotid artery siphon are strongly associated with cerebrovascular events such as stroke[26] and risk factors for these calcifications depend on the type of calcification.[27] Associations between calcifications in the carotid artery siphon and cognitive function are inconsistent. Two studies, one conducted in a hypertension clinic in Italy[28] and one in patients undergoing CT for multiple reasons in Taiwan,[29] found an association between calcifications and a lower score on the Mini Mental State Examination (MMSE), corresponding to a lower global cognitive functioning. In contrast, two Dutch studies in a population-based cohort found no association between calcification and cognitive function or dementia after adjustment for stroke or potential confounders.[30, 31]

Hippocampal calcifications have only been described in a few studies, with a prevalence of 34% found in patients with cerebrovascular disease.[32] These calcifications were associated with age, hypertension, diabetes mellitus, and hyperlipidemia.[32] A pathology study described hippocampal calcifications spreading from tail to body and occasionally to the head, resulting in patchy neuronal loss, and hypothesized that these calcifications contribute to cognitive deterioration due to hippocampal atrophy.[32] Since the hippocampus is an important area for learning and memory,[33] one can imagine that calcifications in the hippocampus can play a role in memory problems. An association between the presence of hippocampal calcification and a lower MMSE score was, in fact, found in a case-controlled study involving 67 patients visiting a memory clinic.[34]

Calcifications have also been reported in the vertebrobasilar arterial system and are more common in the vertebral arteries than in the basilar artery (prevalence: 2-31% vs 0.9-13%).[35] Vertebral and basilar calcifications are related to atherosclerotic changes and are associated with age and traditional risk factors such as hypertension, diabetes, smoking, and obesity (in men).[36] These calcifications are also associated with stroke and recurrent stroke. [37-39] A possible relation with cognitive function has not yet been explored.

Basal ganglia calcifications (BGCs) are a common finding on CT scans with a prevalence ranging from 1.3% in patients with no recorded diagnosis of any disease (mean age 46 years) to 38% in a population of elderly individuals (mean age 73 years).[40,41] Although BGCs are often considered "innocent", [40,41] a study in patients older than 85 years showed an association between BGCs and psychotic symptoms.[40] Patients with Fahr disease, which is characterized by extensive BCGs, can also suffer from movement disorders, psychiatric symptoms, and cognitive impairment.[42] Although the basal ganglia are primarily known for their role in the control of voluntary movement, there is increasing evidence that they also play a role in cognitive functioning. For example, patients with Parkinson's disease often have executive dysfunction, including working memory impairment.[43,44] To our knowledge, no studies have yet been conducted on the specific association between BGCs and cognitive function.

Fahr disease as a model for basal ganglia calcifications

Fahr disease, also known as primary familial brain calcification among others, is a rare genetic neurodegenerative disorder.[42] It is a clinically heterogenous disease and, as mentioned above, patients can suffer from movement disorders, psychiatric symptoms, and cognitive disorders.[42,45] Besides the basal ganglia, calcifications can also occur in other locations in the brain, including the cerebellum, thalamus, and cortex.[45] Case reports of patients with Fahr disease have shown calcifications in capillaries and in the tunica media of arterioles and small- and medium-sized arteries.[46,47] Mutations in six genes are known to cause Fahr disease. Mutations in *SLC20A2*, *XPR1*, *PDGFB*, and *PDGFRB* are autosomal dominantly inherited,[48] whereas *MYORG* and the recently discovered *Jam2* have an autosomal recessive inheritance pattern.[49-51]

We think that Fahr disease can serve as a model for neurodegenerative diseases with BCGs. As for many neurodegenerative diseases, there is currently no proven therapy for Fahr disease. By studying the existing knowledge on this disease, we expect to understand more about the symptoms of calcification as well as potential therapeutic options. The insights we gain from studying patients with Fahr disease may possibly be translated to elderly individuals with BGC or other intracranial calcifications.

Cognitive disorders are diverse and different underlying causes and risk factors have been identified, e.g., Alzheimer pathology (including tau and amyloid), Lewy bodies, vascular disease and its known risk factors, and excessive alcohol usage.

The underlying vascular pathology thought to be responsible for cognitive decline is heterogeneous and complex and includes chronic hypoperfusion, atherosclerosis, and increased blood–brain-barrier permeability, leading to micro- and macro-vascular damage such as cortical or lacunar infarcts, hemorrhages, and white matter disease.[52] Vascular pathology is a recognized cause or contributing factor of cognitive decline in different types of dementia. Since atherosclerosis is one of the underlying factors in vascular pathology responsible for cognitive decline, the effect of treating vascular risk factors (hypertension, hyperlipidemia) has been studied. Interestingly, in contrast to what one would expect, statins do not affect dementia occurrence or progression.[53] In addition, no significant effects have been shown so far for antihypertensive drugs.[54]

Intracranial calcifications are considered arterial in nature and are found in major arteries as well as in small arteries deeper in the brain. Since they are a manifestation of vascular disease, one can assume that intracranial calcifications are related to cognitive function. To date, little is known about this relationship and it should be further investigated.

Aim and outline of this thesis

This thesis focuses on intracranial calcifications and addresses some of the major knowledge gaps on this topic.

In Part 1 (**Chapters 2 and 3**), we compare CT findings of patients with intracranial calcifications in the basal ganglia (**Chapter 2**) and hippocampus (**Chapter 3**) with histology preparations (used as the standard). By zooming in using the microscope, we investigate whether the calcifications are indeed vascular in nature.

Part 2 (**Chapter 4**) includes a review on the mechanisms of intracranial calcification in Fahr disease. Using Fahr disease as the model disease, we expect to gain insight into the underlying mechanisms and clinical implications of intracranial calcifications.

In Part 3 (**Chapters 5–8**), we focus on risk factors for intracranial calcification and investigate the potential relevance of such calcification for cognitive dysfunction. In **Chapter 5**, we examine the prevalence of and risk factors for BGC occurrence in a large study population of patients suspected of acute ischemic stroke. In **Chapters 6, 7,** and **8**, we assess whether BGCs (**Chapter 6**), hippocampal calcifications (**Chapter 7**), and calcifications in the carotid artery siphon and basilar artery (**Chapter 8**) are associated with cognitive function in a large study population of patients visiting a memory clinic. Risk factors for BGCs, hippocampal calcifications, and calcifications in the carotid siphon and basilar artery are also investigated.

In the last part of this thesis (**Chapter 9**), we discuss the main findings and implications of the studies presented and give recommendations for future research.

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PART 1

Intracranial calcifications on computed tomography: what do we see?

CHAPTER 2

Histology and computed tomography of incidental calcifications in the human basal ganglia

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Abstract

Incidental basal ganglia calcifications are a common finding on computed tomography (CT). We investigated the histological characteristics of these calcifications and their association with CT findings, using post-mortem basal ganglia tissue from 22 patients. Eight patients had basal ganglia calcifications on histology and six patients had calcifications on CT, varying from mild to severe. Four patients had calcifications identified by both histology and CT, and two patients had calcifications detected by CT but not by histology, possibly because of insufficient tissue available. Calcifications were found mainly in the tunica media of arterioles located in the globus pallidus, which suggests that incidental CT calcifications are vascular in nature. However, tunica media calcifications, and thereby incidental basal ganglia calcifications, are probably not related to atherosclerosis.

Histology and computed tomography of incidental calcifications in the human basal ganglia

Introduction

The first radiological description of basal ganglia calcifications dates from 1924.[1] Nowadays, calcifications in the basal ganglia are most often detected incidentally during computed tomography (CT) scanning of the brain and have a prevalence of 0.32–38%.[2, 3, 4] These calcifications are usually considered innocent, although they may be associated with diabetes and psychotic symptoms.[4, 5] Patients with Fahr disease, who have severe basal ganglia calcification, suffer from movement disorders, cognitive disorders, and psychiatric symptoms.[6] This suggests that basal ganglia calcifications may not be so harmless. In case reports of patients with Fahr disease, the calcifications occurred in capillaries and the tunica media of arterioles and small- and medium-calibre arteries, with large arteries and veins sometimes showing complete calcification of the vessel wall.[7, 8] To our knowledge, there is one neuropathological study describing the histological nature of incidental basal ganglia calcifications in patients who did not have Fahr disease but who had a neurodegenerative disease, such as Alzheimer's disease, frontotemporal dementia, progressive supranuclear palsy, or Parkinson's disease.[9] The study described three patterns of calcification: deposits within the tunica media, deposits in the parenchyma, and deposits along capillaries. Calcifications in the internal elastic lamina and tunica media are usually non-atherosclerotic in origin, in contrast to calcifications of the tunica intima, and are associated with diabetes mellitus and chronic kidney disease.[10]

We investigated the histological nature of incidental basal ganglia calcifications and whether histological findings are associated with CT findings.

Methods

Local ethical committee approval was obtained for research on retained tissues after written informed consent was given by the patients during life or their next of kin after death (Medical Ethics Committee of the University Medical Center Utrecht 11-531/C). Between 1-1-2013 and 31-12-2018, we identified 22 adult patients for whom there were unenhanced CT scans of the brain (at maximum 1 year before autopsy) and brain autopsy findings available. Histological findings were compared to a consensus CT calcification score.

CT scans

The 22 unenhanced CT scans of the brain were anonymized. They were acquired on Philips Brilliance 64-slice to 256-slice CT scanners (Philips Healthcare, Best, The Netherlands) and reconstructed in thin slices (max 1 millimeter). Basal ganglia calcifications were scored as absent, mild (one

dot), moderate (multiple dots or a single artery), or severe (confluent) (figure 1).[4] Three experienced radiologists (P.A.d.J., J.W.D. and F.A.A.M.H.) blinded to the histological report scored all the scans together in a consensus meeting, using the Philips IntelliSpace Portal 7.0 (Philips Healthcare, Best, The Netherlands) in the brain window setting (centre 40 Hounsfield Units, width 80 Hounsfield Units) and axial, coronal, and sagittal views.

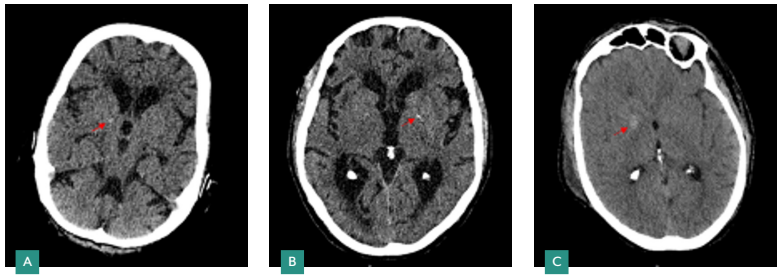

FIGURE 1. A: CT scans with mild calcification in the right basal ganglia **B:** Moderate calcification in the left basal ganglia **C:** Severe calcification in the right basal ganglia

Histology

The basal ganglia were sampled routinely and usually divided into two formalin-fixed, paraffin-embedded blocks covering the entire central grey matter up to the insular cortex. Sections were stained with hematoxylin and eosin (figure 2A). An experienced neuropathologist (WVH), who did not know the CT findings, scored the calcifications with a newly developed method as follows: mild calcification was defined as granular to fine lamellar, more often diffuse and fuchsia to purple staining; moderate calcification was defined as linear, incomplete (semi/non circular) and deep purple staining; and severe calcification was defined as linear, circular, and deep purple to dark blue staining (figure 2B-D). Beside severity, the extent of calcification was assessed as being discrete/limited (sporadic), moderate (the minority), and extensive (the majority). The anatomical distribution of calcifications, i.e. globus pallidus interna, globus pallidus externa, and putamen, was assessed.

Results

The 22 subjects (11 males) were 22–92 years old (median 69 years). On CT, six patients had calcifications, with calcification being severe in one patient, moderate in one patient, and mild in four patients (table 1). On histology, eight patients had basal ganglia calcifications located in the tunica media of arterioles (table 1). The severity of the calcifications was mild in three patients, moderate in three patients, and severe in two patients. The extent of the calcifications was assessed as discrete/limited in two patients, moderate in three patients, and extensive in three patients. The calcifications were localized in the internal globus pallidus in all patients and additionally in the external globus pallidus in six patients. Calcification of the putamen was not seen.

Comparison of the histological and CT findings showed that histologically proven calcifications were also seen on CT in four patients, whereas mild calcifications detected on CT were not detected histologically in two patients.

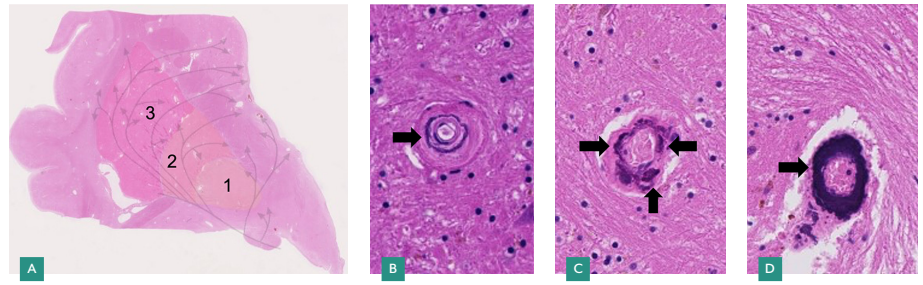

FIGURE 2. A: Histological sections of the basal ganglia with hematoxylin staining, 1 globus pallidus interna, 2 globus pallidus externa and 3 putamen **B:** Mild calcification **C:** Moderate calcification **D:** Severe calcification

On closer inspection, the deposits in the vessel wall seemed to arise along the internal elastic lamina as granular to linear deposits in an early stage, merging with more peripheral calcifications in or along the media in a later stage, ultimately forming a single (semi-)circular deposit (figure 2D). This distribution pattern started in the ventral striatopallidum and fanned out posterolaterally into the external half of the globus pallidus, seemingly following the vascular tree downstream (figure 2A).

Discussion

We investigated the histological nature of incidental basal ganglia calcifications and their association with CT findings. On histology, in an early stage calcification was detected as granular to linear deposits in the internal elastic lamina, merging with more peripheral calcifications in or along the media in a later stage, ultimately forming a single (semi-)circular deposit. This is consistent with the type 1 calcifications described by Fujita[9] in patients with neurodegenerative disease and also consistent with findings in patients with Fahr disease.[7, 8]

This study adds new information about calcifications in patients without Fahr disease, in a group patients who underwent brain autopsy after their death.

CT scanning is the most common method to detect calcifications in the basal ganglia. However, comparison of CT and histological findings is problematic, because CT may not be sensitive enough to detect small calcifications. This is probably why CT scanning did not detect histologically proven calcifications in four patients. A limitation of histology is that sampling may miss areas with calcifications that are seen on CT, which may explain why two patients had calcifications seen on CT but not confirmed by histology. Nevertheless, in four patients the basal ganglia calcifications seen on CT correlated with histological findings, namely, calcifications in small and medium-sized vessels, probably following the vascular tree downstream. As the calcifications were located in the internal elastic lamina and the tunica media, we conclude that incidental CT calcifications are vascular in nature, but probably not atherosclerotic. The relevance of incidental basal ganglia calcifications needs to be investigated and correlated with motor, cognitive, and psychiatric symptoms, such as those seen in Fahr disease.

	CT scan	Histology								
Patient number	Severity	Severity	Extent	Globus pallidus interna	Globus pallidus externa	Putamen	Age		Gender	Cause of death
1	+++	++	+++	+	+	-	56		M	Traumatic neurological injury
2	+	+++	+++	+	+	-	72		M	Pontine infarction with cerebral herniation
3	-	-	-	-	-	-	22		F	Cerebral metastatic disease
4	-	-	-	-	-	-	68		F	Pseudomembranous colitis
5	++	++	++	+	+	-	82		M	Cerebral hemorrhage with cerebral herniation
6	-	-	-	-	-	-	92		F	Subarachnoid hemorrhage
7	+	-	-	-	-	-	62		F	Ruptured aneurysm medial cerebral artery
8	+	+++	+++	+	+	-	70		F	Poor cardiopulmonary situation with bilateral pneumonia
9	+	-	-	-	-	-	71		M	Cerebral venous sinus thrombosis
10	-	+	+	+	-	-	61		F	New Onset Refractory status Epilepticus
11	-	-	-	-	-	-	67		M	Unknown
12	-	+	++	+	+	-	69		M	Iatrogenic rupture external iliac artery
13	-	-	-	-	-	-	56		M	Glioblastoma multiforma
14	-	++	++	+	+	-	87		F	Hypovolemic shock
15	-	-	-	-	-	-	80		F	Glioblastoma multiforma
16	-	-	-	-	-	-	53		F	Cerebral venous sinus thrombosis
17	-	-	-	-	-	-	67		M	Pulmonary sepsis
18	-	-	-	-	-	-	63		F	Thrombombolism
19	-	+	+	+	-	-	70		F	Cerebellar ischemia
20	-	-	-	-	-	-	70		M	Brainstem ischemia
21	-	-	-	-	-	-	30		M	Liver failure
22	-	-	-	-	-	-	69		M	Liver failure

TABLE 1. Incidental basal ganglia calcifications detected by computed tomography and histology
The severity of calcifications of the basal ganglia detected by CT and histology was scored as absent (-) mild (+), moderate (++) or severe (+++). The histological extent of basal ganglia calcifications was scored as absent (-), in discrete/limited (+), moderate (++) and extensive (+++).
Globus pallidus interna, globus pallidus externa and putamen: No calcification (-), presence of calcification (+). **M:** male; **F:** female.

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CHAPTER 3

Histological validation of calcifications in the human hippocampus as seen on computed tomography

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Abstract

Background: Calcifications within the hippocampus were recently described for the first time on computed tomography (CT). These calcifications appeared in patients older than 50 years, the prevalence increases with age and they may be associated with cognitive decline. The aim of this study was to determine the histological basis (the presence, severity and location) of these CT-detected hippocampal calcifications of post-mortem brains.

Methods: CT scans of seven post-mortem brains were scored for the presence and severity (mild, moderate, severe) of hippocampal calcification. After this, samples from nine hippocampi (bilateral in two brains, unilateral in five brains) were stained with hematoxylin and eosin (HE) to indicate the cytoarchitecture, with Elastica van Gieson to analyze the elastic connective tissue of the vessel walls and with von Kossa for detection of calcium.

Results: In four brains (six hippocampi), calcifications were both found on CT and in corresponding histology. In three brains (three hippocampi), calcifications were absent on CT and corresponding histology. In histology, mild calcifications were located in the tail and severe calcifications involved the tail, body and sometimes the head of the hippocampus. The calcifications co-localized with precapillaries, capillaries and arteries of the molecular and granular layers of the dentate gyrus and the Cornu Ammonis 1.

Conclusions: In this study, calcifications of the hippocampus as seen on CT scans were histologically located in vascular structures of the tail, body and head of the hippocampus.

Histological validation of calcifications in the human hippocampus as seen on computed tomography

Introduction

Cerebrovascular disease is a major cause of morbidity and mortality worldwide. The main clinical diseases associated with intracranial vascular problems are stroke and dementia.[1] One of the phenomena commonly present in the intracranial vessels of cerebrovascular patients is the accumulation of calcium in the arterial wall. Large calcifications may cause occlusions and restrict blood flow to certain parts of the brain and calcifications may also stiffen arteries.[2,3] Calcifications of different brain structures can be demonstrated with computed tomography (CT). The most common calcifications of the intracranial carotid artery[3,4], the choroid plexus[5] and basal ganglia structures, like the globus pallidus and dentate nucleus[6,7], have been observed on CT in human. Calcification of the choroid plexus, which is responsible for the production of cerebrospinal fluid, increases in frequency at increasing age.[5] The calcification can involve the temporal horn, the floor of the body of the lateral ventricle, the roof of the third ventricle and the foramen of Monro.[5] These regions border the hippocampus, and calcifications of the hippocampus were therefore often misinterpreted as calcification of the choroid plexus.[8,9] The evolution of CT scan techniques allowed for better quality of images and thinner CT slices, which can be read more easily in various viewing planes. On these scans, a difference between choroid plexus calcifications and adjacent hippocampal calcifications can be made. Limited research has been performed on calcifications of the hippocampus. Chew et al.[9] were the first to describe hippocampal calcification on CT. They found that hippocampal calcification appears particularly in patients older than 50 years and that the prevalence increases with age.[9] A recent study by Kockelkoren et al.[8] investigated in a case-control study the presence of hippocampal calcification and the relationship with cognitive decline. In memory clinic patients calcifications of the hippocampus were more prevalent and associated with a lower cognitive functioning.[8] One histopathological study by Wegiel et al.[10] investigated calcifications located in the hippocampus in Alzheimer's disease, Down syndrome, and control aging patients. They described the calcifications to be a manifestation of vascular disease, which they called vascular fibrosis and calcifications.[10] Currently, no evidence is available that the calcifications as observed on CT scans[8,9] are indeed corresponding to vascular fibrosis and calcifications as found in the histopathological study.[10] For this reason, the aim of this study was to determine the histological basis of these CT-detected hippocampal calcifications in human post-mortem brains.

Materials and methods

Patients

For this study, we examined a total of seven brains. One brain was derived from a body, which was included in a donation program from the Department of Anatomy. Six brains were from patients who had an autopsy at the Department of Pathology. Local ethical committee approval was obtained for research on retained tissues after written informed consent given by the patients during life or their next of kin after death (Medical Ethics Committee of the University Medical Centre Utrecht 11-531/C). All brains were CT scanned post-mortem. Table 1 shows the available information of the seven patients about age, gender and relevant medical history.

Patient number	Gender	Age (years)	Medical history	Number of hippocampi analyzed
1	F	95	• Body donation to the Department of Anatomy without available medical history	2 hippocampi: head, body and tail
2	F	87	• Cerebrovascular Accident • Atriual fibrillation	2 hippocampi: head, body and tail
3	F	62	• Subarachnoid hemorrhage, intracranial aneurysm	1 hippocampi left
4	F	81	• Atrial fibrillation • Asthma • Hypertension • Sepsis and ischemia in leg	1 hippocampi right
5	M	60	• Stem cell transplantation • Human immunodeficiency virus • Acute myeloid leukemia • Sepsis and pulmonary infection • Secondary inflammation of pituitary	1 hippocampi left
6	M	71	• Sinus thrombosis • Heart attack • Polycythemia vera	1 hippocampi right
7	F	75	• Hemorrhage in the thalamus and pons • Atherosclerosis • Atrial fibrillation • Hypertension	1 hippocampi right

TABLE 1. Patient information and relevant clinical history of the seven patients

CT examinations

The brain CT scans were made with a Philips Brilliance 64-slice or 256-slice CT scanner (Philips Healthcare, Best, The Netherlands). The brain from the patient who donated her body to the Department of Anatomy was removed from the skull before scanning. The brains from the patients from the Department of Pathology were scanned surrounded by the skull. Non-contrasted thin slice reconstructions (0.8–1.0 mm) were analyzed for hippocampal calcifications in different reconstructions, axial, coronal, and sagittal in the brain window setting (Center: 40 Hounsfield Units, Width: 80 Hounsfield Units) using the Philips IntelliSpace Portal 7.0 (Philips Healthcare, Best, The Netherlands). Calcifications were bilaterally scored on severity as absent, mild (one dot), moderate (multiple dots) or severe (confluent) (figure 1) as described first by Kockelkoren et al.[8] Calcifications on the CT scans are seen as a group of white voxels with a density similar to bone (figure 1). The hippocampi were scored by an experienced observer. His agreement in comparison with other observers was previously investigated (kappa 0.80).[8] The observer was blinded to the histological results.

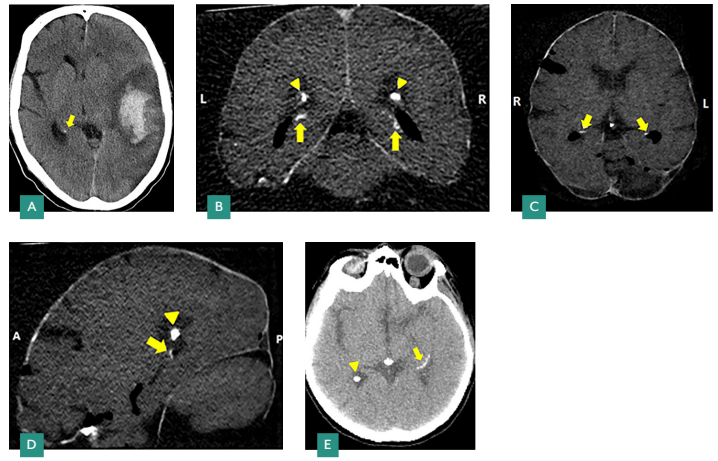

FIGURE 1. Hippocampal calcifications on CT scans of patient numbers 4 (mild, A), 1 (moderate, B-D) and 3 (severe, E). **(A)** Axial reconstructed image with mild hippocampal calcification (one dot). **(B)** Coronal reconstructed image shows (moderate) bilateral hippocampal calcification (multiple dots), indicated by arrows. Choroid plexus calcification is indicated by arrowheads. **(C)** Axial reconstructed image with moderate bilateral hippocampal calcification marked with arrows. **(D)** Sagittal reconstructed image with moderate hippocampal calcification marked with an arrow and choroid plexus calcification marked with an arrowhead. **(E)** Axial reconstructed image shows severe hippocampal calcification (confluent) indicated by an arrow and calcification of the choroid plexus is indicated with an arrowhead.

Microscopy—Histological study of nine hippocampi of seven patients

In two patients (patient number 1 and 2, corresponding with tables 1 and 2), both hippocampi (hippocampus number 1 to 4, corresponding with table 2) were evaluated by histology, in five patients (patient 3 to 7, corresponding with table 2) one hippocampus was evaluated. The tail, body and head of the hippocampus were sampled in four of the nine hippocampi (hippocampus number 1 to 4). In the other five hippocampi, only the body was evaluated (due to the standard procedure of the Department of Pathology, in which the diagnosis of the disease the patient suffered from is the most important purpose. For each patient 22 standard pieces of the brain were cut out and three pieces with own content, these three extra pieces for diagnosis of the disease were more important than pieces for research purposes. This is why not always the head, body and tail of the hippocampus were analyzed. Samples of the brains were put into cassettes. All cassettes were dehydrated in a graded series of ethanol (up to 95%) and embedded in paraffin. Subsequently, the paraffin embed-

ded samples were cut into slices of 6 µm and put on glass microscope slides for pathological study. All sections were stained with hematoxylin and eosin (HE) for characterization of the cytoarchitecture.[10] In addition to this, the hippocampal sections were stained with von Kossa method for detection of calcium[10] and Elastica van Gieson stain to identify elastic connective tissue in vessel walls.[11] An experienced neuropathologist analyzed all the specimens. The pathologist was blinded to the results of the CT scan. The calcifications in histology were quantified by the amount of vessels that were positive for calcium. Less than five vessels affected with calcium was considered mild, more than five non-confluent calcified vessels affected was considered moderate and more than five confluent calcified vessels that often had large calcium beads was considered severe.

Patient number	Hippocampus number	CT scan	Histology
		Severity	Severity
1	1	++	+++
	2	++	+
2	3	+++	+++
	4	+++	+++
3	5	+++	+++
4	6	+	+
5	7	-	-
6	8	-	-
7	9	-	-

TABLE 2. Overview of hippocampal calcifications on CT scan and in histological study.
The patients number corresponds with the patients presented in table 1.
Severity: whether the calcification is severe +++, moderate ++, mild + or absent -.
CT scoring: absent, mild (one dot), moderate (multiple dots) or severe (confluent). 8
Histology scoring: absent, mild (less than five vessels affected with calcium), moderate (more than five non-confluent calcified vessels) or severe (more than five confluent calcified vessels that often had large calcium beads).

Macroscopy of the brain of one patient

In one patient (patient number 1, as described in tables 1 and 2), a highly detailed analysis of the whole brain and both hippocampi was performed, to find more detailed information about the location of the calcifications in the vessel structures. This particular brain was obtained from the Department of Anatomy and had been fixed in 3% buffered formaldehyde. The brain was cut into coronal slices of 10 mm thickness, 25 samples were taken and put into cassettes. Figure 2 shows a coronal slice of the posterior part of the brain. The hippocampus as seen in figure 2B was removed into a cassette and used for histological study. The important samples are bilateral hippocampus anterior (head), middle (body), and posterior (tail), beside the other samples for general microscopy.

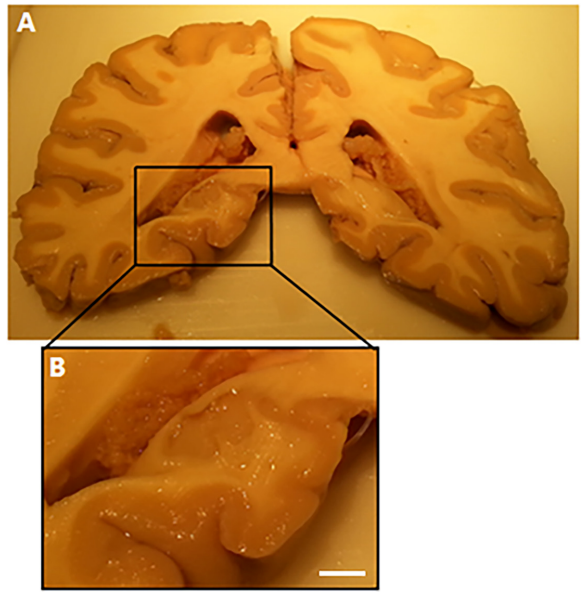

FIGURE 2. Coronal brain slice after dissection of the brain (of patient number 1).
(A) Coronal slice with the posterior part of the hippocampus (tail). **(B)** Close up of the posterior part of the hippocampus (scale bar = 10 mm). Choroid plexus is visible in the lateral ventricle. The close up shows the sample for further histological staining.

Results

Computed tomography

Calcification of the hippocampus was found bilaterally in six of the nine hippocampi on CT scan. Three hippocampi had severe calcifications, two had moderate calcifications, one had only mild calcification and three had no calcifications. Figure 1 shows, as example, the routine CT images in different planes with hippocampal calcification.

Correlation of CT findings with histology

In all six hippocampi with calcifications and all three hippocampi without calcifications on CT, these results from CT were confirmed by histology. Of the six calcified hippocampi three were considered as severe calcifications, two as moderate and one as mild. The correlation between calcifications on CT and the histopathological findings is shown in table 2. Validated with histological staining, four subjects had severe calcifications, two had mild calcifications, and three subjects had no calcifications.

Vascular localization of hippocampal calcification

We analyzed one brain with two hippocampi (patient number 1, as described in tables 1 and 2) in more detail than the other specimens. To indicate the exact location of the calcifications in the vessel structures.

In the anterior part, the head of the hippocampus, no calcification was noticed, neither in the left nor in the right hippocampus. The middle part, the body of the left hippocampus, showed mild calcification localized in the precapillaries and capillaries in the molecular layer of the dentate gyrus (DG) and Cornu Ammonis 1 (CA1). The posterior part, the tail of the left hippocampus, showed severe calcification of precapillaries, capillaries and the arteries (figure 3). These calcification beads, shown in figure 3C, were larger in comparison to the mild calcification observed in the left hippocam-

pal body. Also, an increase of number of calcifications was noticed. In this severe stage, calcifications were localized in the granular layer of the DG and the molecular layer of the DG and CA1 and spread out over the border of CA1 into the molecular layer of the subiculum (figure 3B). Figure 3D shows a calcified artery in the molecular layer of the CA1. The Elastica van Gieson stain clearly showed the structure of an artery, with calcification in the tunica adventitia and the tunica media, as seen in figure 3E. In some slices, it was difficult to distinguish the structure of the vessels in this severe stage of calcification, because sometimes no vessel walls were observed, only the calcium deposits. The right hippocampus contained no calcification in the body. The posterior part, the tail of the right hippocampus, showed mild calcification in precapillaries and capillaries (figure 4).

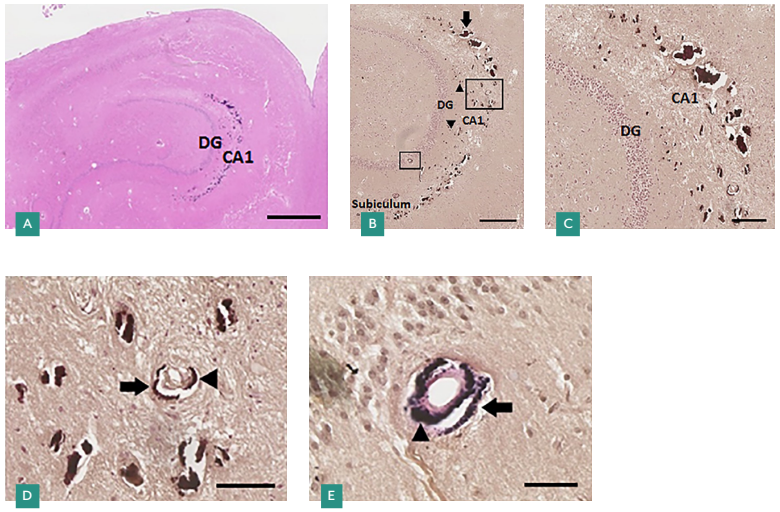

FIGURE 3. Severe calcifications in the tail of the left hippocampus (hippocampus number 1) of patient number 1 (as described in Tables 1 and 2).
(A) HE stain overview of the left hippocampal tail. Scale bar = 1 mm, 4x magnification. **(B)** Von Kossa-positive deposits in precapillaries and capillaries (arrowheads) in the molecular layer of the DG and CA1. Calcifications of the bigger vessels, mostly arteria (arrow), in the molecular layer of CA1 are clearly observed. Two arteria which are calcified are surrounded, one in the molecular layer of the CA1 and one in the granular layer of the DG. The calcifications spread out into the molecular layer of the subiculum. Scale bar = 600 µm, 10x magnification. **(C)** Zoomed in on the big calcifications of precapillaries, capillaries and bigger vessels in the molecular layer of the DG and CA1. Epithelial cells of the vascular wall are not seen, because of the big calcification deposits. Scale bar = 400 µm, 20x magnification. **(D)** Enlarged image of a von Kossa-positive calcified artery in the molecular layer of the CA1. Calcification of the tunica adventitia (arrow) and the tunica media (arrowhead) are identified. Scale bar = 200 µm, 40x magnification. **(E)** Calcified artery in the granular layer of the DG shown with Elastica van Gieson stain. Calcification of the tunica adventitia (arrow) and the tunica media (arrowhead) are identified. Scale bar = 100 µm, 40x magnification.

Onset of calcification

In the two brains (patient number 1 and 2, as described in tables 1 and 2) of which the tail, body and head of both hippocampi were analyzed, a striking pattern was seen. In one brain, both hippocampi were severely calcified in the tail, moderately calcified in the body and mildly calcified in the head. In the other brain, the tail of one hippocampus was affected by severe calcification and the body of that hippocampus showed mild calcification. In the other hippocampus only the tail was mildly calcified. This calcification pattern indicates that the calcification first appears in the tail, followed by the body, and subsequently when severe also occurs in the head of the hippocampus. This remains speculative and needs further confirmation.

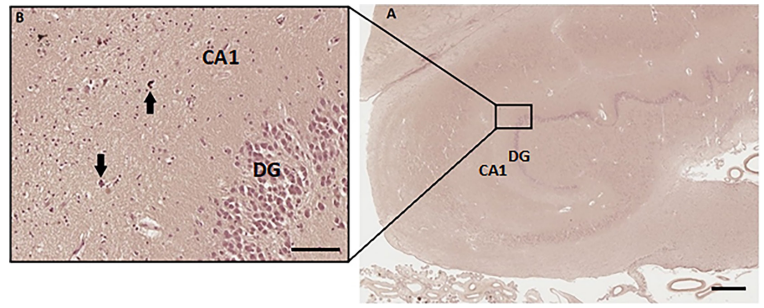

FIGURE 4. Calcification of precapillaries and capillaries in the right hippocampal tail (hippocampus number 2) of patient number 1 (as described in Tables 1 and 2).
(A) Overview of the right hippocampal tail. Scale bar = 1 mm, 4x magnification. **(B)** Zoomed picture of von Kossa-positive stain, calcifications are located in the precapillaries and capillaries (arrows) in the right hippocampal tail. Scale bar = 100 µm, 40x magnification.

Choroid plexus calcification

To demonstrate the difference between hippocampal calcification and calcification of the choroid plexus, a picture of choroid plexus calcification is included (figure 5). Small vessels, precapillaries and capillaries were calcified in the choroid plexus. The large arteries and veins were less calcified.

Discussion

The main finding of this study is that hippocampal calcifications as observed on CT images are confirmed with histology to be located in the hippocampal vasculature. Mild calcifications were found posterior in the tail of the hippocampus and extended into the body, toward the head of the hippocampus in severe stages. Whether this is a pattern that repeatedly will occur in different patients, should be further investigated and confirmed. The calcifications started in the precapillaries and capillaries of the molecular layer of the DG and CA1. Severe calcification expands in the molecular layer of the DG and CA1, the granular layer of the DG and over the border of CA1 to the molecular layer of the subiculum, as observed in four severe calcified hippocampi. The vascular calcifications seemed to be located in the adventitial and medial layer of the vessel wall. Our study is the first study that confirmed the vascular origin of the hippocampal calcifications that are detectable on CT. Limited research has been done on calcifications of the hippocampus. A histopathological study from

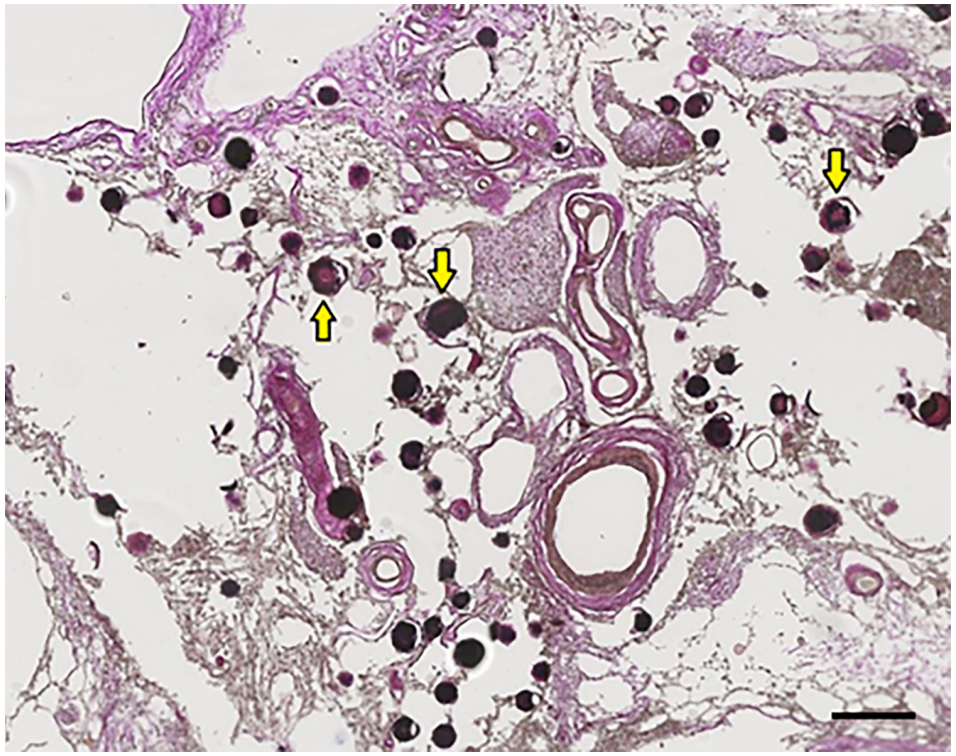

FIGURE 5. Calcification of the choroid plexus on the left side of patient number 1.
The dark red/black dots, some are indicated with yellow arrows, are calcifications of precapillaries and capillaries in the choroid plexus on the left side of the brain. Scale bar = 1 mm, 20x magnification.

2002 described vascular fibrosis and calcification in the hippocampus in subjects with Alzheimer's disease (in 59% of the patients), Down syndrome patients (in 4%) and control aging patients (in 40%).[10] In that study, just as in ours, calcifications were found in precapillaries and capillaries of the molecular layer of the DG and expanded to the granular layer and polymorphic layer of the DG, and to the molecular layer of the CA1. These calcifications found in the study of Wegiel et al.[10] apparently start in the hippocampal tail and spread in severe stages to the body and in some cases to the head of the hippocampus. A finding that we have seen as well in two brains of which the tail, body and head of the four hippocampi were analyzed. This pattern remains speculative and needs further confirmation. In the study of Wegiel et al.[10] was found that the calcifications caused changes of the endothelial cells in the wall and lumen of vessels. In later stadia, the vascular wall became thicker and the calcified beads increased, these calcium deposits degraded the vessels and occlusion of blood circulation was noticed. Which indicates that an early diagnosis of these calcifications, with for example CT, will be valuable. Also in our study we observed degraded vessel walls and big calcium deposits, which might be implicated in the pathophysiology of vascular degradation. Our study demonstrated, through comparison with histological staining, that the calcifications found on CT images are indeed located in the hippocampal vasculature. The hippocampal calcifications are located in the molecular layer of the DG and CA1 and in the granular layer of the DG as observed in all analyzed hippocampi positive

for hippocampal calcifications. Interestingly, these locations correspond with the location of vascular fibrosis and calcifications described in the histopathological study of Wegiel et al.[10] Calcifications can be detected by neuroimaging, and are seen as high density areas on CT scans, while these calcification areas are considerable less visible on magnetic resonance imaging (MRI).[12] Indicating that CT will provide a better diagnosis for hippocampal calcifications than MRI. A second study from 2012[9] was the first study to describe hippocampal calcifications on CT images. Due to thin slice CT images, a distinction between choroid plexus calcifications and hippocampal calcifications could be made. In total, 300 randomly selected CT scans were analyzed, of which intrahippocampal calcification was demonstrated in 47 patients, all these patients were older than 50 years of age. The authors concluded that intrahippocampal calcification appeared with increasing age.[9] With our study we demonstrated with histology that the CT detected calcifications were indeed located in the hippocampus. The effect of hippocampal calcifications on cognitive functioning was investigated in a recent pilot study from 2016.[8] Kockelkoren et al.[8] examined memory clinic patients and controls. In this study, it was found that hippocampal calcifications were three times as prevalent in patients of the memory clinic compared to control patients. Furthermore, memory clinic patients with hippocampal calcifications showed lower cognitive functioning measured with Mini Mental State Exam. In two other smaller studies no significant difference in the presence of hippocampal calcifications between controls and a group of Alzheimer's disease patients was found.[10, 13] More research should be performed to possibly confirm the correlation of hippocampal calcifications and dementia. In this study of Kockelkoren and colleagues it was hypothesized that the hippocampal calcification as seen on CT scan could be caused by vascular fibrosis and calcification, because it seems like it is located in the same region of the hippocampus.[8] Until now, this remained speculative, but our study provides support for this hypothesis by correlating CT findings to histology in the same brain.

In the Alzheimer's disease patients in the study of Wegiel et al.[10], neuronal cell loss was found in the CA1 region and subiculum proper, comparable to hippocampal sclerosis.[10] This was suggested to be characteristic for Alzheimer's disease patients. In normal aging patients, neuronal cell loss was rarely seen in the CA1 region of the hippocampus.[12] In our study we used material of normal aging patients. In histology we observed that the patients had some signs of aging, like amyloid-beta senile plaques, which was age-appropriate. We did not find obvious neuronal cell loss, which may be due to the fact that our subjects did not have Alzheimer's disease.[14] The posterior cerebral artery supplies the hippocampus of blood. According to the flow territories, the branches of the hippocampal arteries can be divided into two groups. Blood supply to the body and tail of the hippocampus is from the middle and the posterior hippocampal arteries. The anterior hippocampal arteries supply the head of the hippocampus and the uncus.[15] Also the choroid plexus of the lateral ventricle is supplied by branches of the posterior cerebral artery.[16] Since the calcification in the vasculature in both the hippocampus and the choroid plexus is similarly located in the precapillary and capillary vessels, and both types of calcification have the same blood supply from the posterior cerebral artery, future research could be directed to a possible relation between these calcifications. Future research may also focus on the effects of hippocampal calcifications on cognitive functioning. The main function of the hippocampus is learning and memory. Nowadays it is thought that the hippocampal sub regions, anterior (head) and posterior (tail), are involved in different aspects of memory. The exact distribution is still not known and different studies suggest different functions.[17, 18] Because the hippocampal tail is affected most early in this calcinosis process, it would be interesting to investigate the function of the posterior hippocampus in relationship with hippocampal calcifications. A potential

link with cognitive decline and dementia could be investigated not only in memory clinic patients, but also in normal aging patients. In the limited medical history of our patients, we found that some patients had neurological damage and others had cardiovascular problems, both of which may be associated with calcifications in the brain. In future research a possible association between cardiovascular diseases and hippocampal calcifications can be investigated. This study, in which we validated hippocampal calcifications as observed on CT scan with histological staining, is a crucial step for future research to investigate the underlying mechanism and consequences of these hippocampal calcifications. In the future we could detect the hippocampal vascular calcifications in early stages with thin slice CT scans. Thin slice CT scans could become a prognostic marker for cognitive decline[8] or other not yet investigated consequences of hippocampal calcifications. When finally the mechanism underlying hippocampal calcifications is known and this is related to a negative function for the patients, treatments on this mechanism could be investigated. With the prognostic marker of CT scans and applicable treatments, patients can be helped earlier. Limitations of the current study are the lack of information about cognitive ability of the patients. Nothing can be said about a possible correlation between hippocampal calcifications and cognitive functioning of these patients. Secondly, the sample size of this study was relatively small, however, it is in our opinion sufficient to validate the calcifications on CT scans with histology, the main focus of this research. In the histology of four hippocampi we saw a possible pattern of calcification severity that expanded from tail toward the head of the hippocampus, although this is a limited number, the pattern was also observed by Wegiel et al.[10] We think further studies with larger cohorts who underwent CT scanning can more firmly investigate this pattern and we did not specifically investigate this in the present study. A last limitation is the way we quantified the histological findings. There is not yet a clear standard for the quantification of hippocampal calcification in histology as it is the case for hippocampal calcifications on CT scan.[8] Therefore, we made an own quantification method for hippocampal calcification for histology. In conclusion, we showed that all hippocampal calcifications observed on thin slice CT images are confirmed by histological staining to be of vascular origin. The calcifications in mild stage were found in precapillaries and capillaries of the molecular layer of the DG and CA1. In a more severe stage also the arteries in the molecular layer of the CA1 and the granular layer of the DG were affected. Calcifications were most often found in the hippocampal tail and in a more severe stage they also became visible in the hippocampal body and sometimes in the hippocampal head. In further studies, the consequences of these hippocampal calcifications on cognitive impairment or possible other functions of the hippocampus, can be investigated

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PART 2

The pathophysiology of intracranial artery calcifications: lessons from Fahr disease

CHAPTER 4

Mechanisms of calcification in Fahr disease and exposure of potential therapeutic targets

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Abstract

Purpose of review: There is growing interest in disorders involved in ectopic mineralization. Fahr disease or idiopathic basal ganglia calcification can serve as a model for ectopic mineralization in the basal ganglia, which is fairly common in the general population. In this review, we will focus on causative gene mutations and corresponding pathophysiologic pathways in Fahr disease.

Recent findings: Patients with Fahr disease have a variability of symptoms, such as movement disorders, psychiatric signs, and cognitive impairment, but can also be asymptomatic. Fahr disease is mostly autosomal dominant inherited, and there are mutations found in 4 causative genes. Mutations in SLC20A2 and XPR1 lead to a disrupted phosphate metabolism involving brain-specific inorganic phosphate transporters. Mutations in PDGFB and PDGFRB are associated with disrupted blood-brain barrier integrity and dysfunctional pericyte maintenance. In addition, the MYORG gene has recently been discovered to be involved in the autosomal recessive inheritance of Fahr.

Summary: Knowledge about the mutations and corresponding pathways may expose therapeutic opportunities for patients with Fahr disease and vascular calcifications in the brain in general.

Mechanisms of calcification in Fahr disease and exposure of potential therapeutic targets

Fahr disease, also known as idiopathic basal ganglia calcification, is a rare autosomal dominant neurodegenerative disorder,[1, 2] characterized by bilateral calcifications in multiple basal ganglia.[3–7] Its prevalence is estimated to be 4.5 per 10.000 persons.[8] Fahr disease has several synonyms, and bilateral striopallidodentate calcinosis or idiopathic basal ganglia calcifications may be preferable, as these terms give an accurate description of the anatomic location of the calcifications.[9] A distinction is made between Fahr disease and Fahr syndrome. Fahr disease is of idiopathic or genetic origin, whereas in Fahr syndrome, the calcifications are secondary to disorders of calcium metabolism. The most common causes of Fahr syndrome are hypoparathyroidism, pseudo-hypoparathyroidism, and pseudo-pseudo-hypoparathyroidism, but other states of chronic hypocalcemia, e.g., vitamin D deficiency and renal failure, are associated with intracranial calcifications as well.[3, 4, 10]

Patients with Fahr disease may be asymptomatic or present with movement disorders such as parkinsonism, psychiatric disorders such as psychosis and depression, cognitive impairment, dementia, and a variety of other symptoms.[3, 5, 11-14] This variability in symptoms can partly be explained by the division of the basal ganglia into dorsal and ventral systems. The dorsal striatum plays a role in motor and cognitive functions, whereas the ventral striatum plays a role in motivational functions.[3]

In patients with Fahr disease, normal serum levels of calcium, phosphate, alkaline phosphatase, and parathyroid hormone are measured.[9] The age at onset is mostly between 40 and 50 years,[4] but the symptoms can manifest at any age.[15, 16] Patients who become symptomatic early in adulthood mostly develop psychiatric or cognitive disorders,[16] like psychosis,[17] whereas patients who become symptomatic later in life develop mainly movement disorders in combination with other clinical features.[16]

As Fahr disease is clinically heterogeneous, the diagnosis is based on neuroimaging in the absence of another explanation for calcification.[17] Nicolas et al.[18] developed a rating scale in which calcifications are scored from 0 (no calcification) to 5 (severe and confluent) for numerous locations in the brain (lenticular, caudate, thalamus nuclei, subcortical white matter, cortex, cerebellar hemispheres, vermis, pons, and medulla), which gives a total calcification score of 80 maximum. Calcifications are present as high-density areas on CT scans and can be more difficult to detect with conventional MRI sequences in early stages, although dedicated sequences are increasingly able to detect intracranial calcifications[19] (figure 1). The severity of calcifications on CT is correlated with an increase in age, and more calcifications were found in symptomatic patients in comparison with asymptomatic patients.[7, 20] The calcifications seem to progress in a typical pattern from mild calcification of only the lentiform nucleus and caudate nucleus to severe and confluent calcification of all basal ganglia except the midbrain.[16]

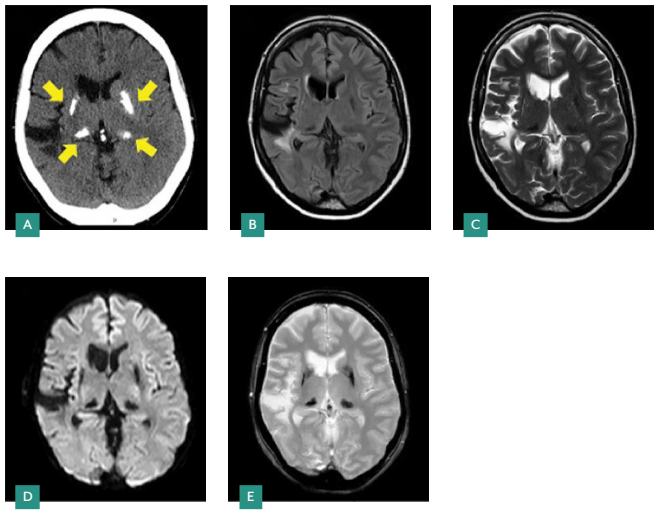

FIGURE 1. CT and MRI of a patient with Fahr disease

(A) CT of a patient with Fahr disease, with clear calcifications in the basal ganglia seen as high dense white areas (indicated by yellow arrows). **(B)** Conventional T1-weighted fluid-attenuated inversion recovery (FLAIR) MRI scan has lower sensitivity for calcifications than CT. [TR/TE 10000/140, flip angle 90]. **(C)** T2 weighted turbo spin echo (TSE) high resolution MRI scan has lower sensitivity for calcifications than CT. [TR/TE 4748/100, flip angle 90]. **(D)** Diffusion weighted imaging (DWI) MRI scan shows already better sensitivity for basal ganglia calcifications than the before mentioned MRI protocols. [TR/TE 3413/99.4, flip angle 90, b-value 1000]. **(E)** Dedicated T2 weighed fast field echo (FFE) MRI shows already better sensitivity for basal ganglia calcifications than the MRI protocols mentioned in B and C. However, CT is the most appropriate high sensitive neuroimaging method to demonstrate calcifications in the brain. [TR/TE 732/23, flip angle 18]. [All above CT and MRI scans have a slice thickness of 5 mm]. In addition, the presented MRI sequences are non-specific in the differentiation between calcium and iron as it is seen in Neurodegeneration with Brain Iron accumulations (NBIA).

On MRI, calcium deposition in Fahr disease is hard to distinguish from iron deposition in neurodegeneration with brain iron accumulations (NBIAs).[19, 21] NBIA is a neurodegenerative disease characterized by the accumulation of iron in the basal ganglia and to a lesser extend in the substantia nigra and proximity. Patients with NBIA also present movement disorders and cognitive impairment.[21] CT imaging can be used to distinguish calcium and iron depositions, as calcium has a very high density on CT and iron depositions do not.[19] It is interesting that depositions in Fahr disease also contain iron according to histopathologic studies, and calcium deposition may be found on CT in patients with NBIA.[22, 23]

There is currently no proven therapy for Fahr disease. Outside the field of neurology and neuroscience, there is growing interest in the etiology and treatment of ectopic mineralization disorders, especially in the cardiovascular system.[24-27] Much knowledge has been gained on several monogenetic disorders,[28] such as arterial calcification due to deficiency of CD73 (ACDC), and some suggestions for therapies are available.[25, 29, 30] Fahr disease may serve as a model for neurodegenerative diseases with basal ganglia calcifications. The pathophysiologic mechanisms may, to some extent,

explain such disease in the general population. Data suggest that the prevalence of symmetrical calcification of the basal ganglia observed in neuroimaging is around 1% in young patients and >20% in elderly.[31, 32] In autopsy, brain calcifications in the globus pallidus and dentate nucleus were seen in up to 70% because also microscopic calcifications are detectable in histology.[33] Calcium deposits were found in the walls of arterioles and small veins and along pericapillaries and capillaries[3, 9, 14, 34] (figure 2). We have observed similar deposits in the tail of the hippocampus in autopsy samples of patients without Fahr disease.[35]

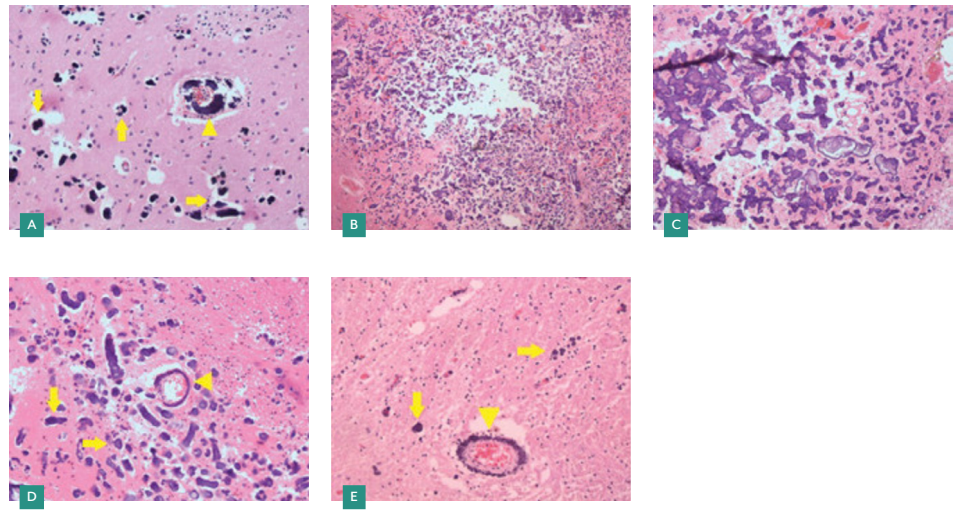

FIGURE 2. Histology of calcification of different brain structures, which belong to the basal ganglia, of a patient with Fahr disease.

(A) HE staining of calcification of the capillaries (arrows) and an artery (arrowhead) in the caudate nucleus-putamen, x20 magnification. **(B)** HE staining overview of calcifications in capillaries and arteries in the globus pallidum. The dark purple staining indicate calcium, x4 magnification. **(C)** x10 magnification of the capillaries and arteries calcified in the globus pallidum, the dark purple staining indicate calcium. **(D)** Capillaries (arrows) and arteries (arrowhead) in the putamen, pallidum and insula are calcified (HE staining), x20 magnification. **(E)** Calcified artery (arrowhead) and capillaries (arrows) in the thalamus and subthalamicus (HE staining), x20 magnification.HE = hematoxylin and eosin.

Genetics of Fahr disease

Fahr disease is autosomal dominant inherited. Since 2011, 4 causative genes have been identified. The first gene, Solute Carrier family 20 (Phosphate Transporter), Member 2 (SLC20A2), accounts for approximately 40% of the patients with Fahr disease.[14] Mutations in platelet-derived growth factor subunit B (PDGFB) and platelet-derived growth factor subunit receptor B (PDGFRB) account for 11% and 2% of the patients with Fahr disease, respectively.[6, 7] Recently, mutations in xenotropic and polytropic retrovirus receptor (XPR1) were found5 in approximately 2% of patients with Fahr disease. In half of Fahr cases, no mutations are found in one of these genes, and the cause is unknown.

In patients with a gene mutation, the penetrance is almost complete for calcium deposits.[36] However, the clinical penetrance is incomplete and may be around 70%. The penetrance can vary between

families and within families. There are no precise numbers available for the different gene mutations.[18]

In this review, we will focus on the physiologic function of these 4 genes, the pathophysiologic pathways in Fahr disease, and summarize evidence from animal models. This might give more insights into its pathophysiology and might expose therapeutic opportunities for select patients with Fahr disease and patients with vascular calcifications in the brain in general.

Solute carrier family 20 (phosphate transporter), member 2

SLC20A2 is localized to chromosome 8p11.2137 and encodes for the type III sodium-dependent inorganic phosphate (Pi) transporter 2 (PiT2).[14] PiT2 is a transmembrane Na+/Pi cotransporter that plays an important role in the maintenance of Pi homeostasis, which is essential for adenosine triphosphate synthesis.[38-40] This transporter provides the transport of Pi from the CSF into the blood.[40] SLC20A2 is expressed in many tissues. High expression levels were found in the brain, especially in the neurons, astrocytes, vascular smooth muscle cells, and vascular endothelial cells in the brain.[38, 39] PiT2 is located in the cortex, basal ganglia (especially in the globus pallidus), and substantia nigra.[41] This indicates that these brain tissues are more sensitive to an imbalance in Pi homeostasis.[14] Pi has essential functions in storage and release of metabolic energy, (bone) mineralization, nucleic acid synthesis, electrolyte transporter, and neurologic functions.[39] PiT2 contains 2 copies of the protein homology domain (PD001131), 1 in the aminoterminal (N-terminus) and 1 in the carboxy-terminal (C-terminus). These domains are highly conserved in plants and animals, which indicates that the role of these 2 domains is important in different species.[42]

Gene mutations

Wang et al.[14] were the first to describe various missense mutations in the SLC20A2 gene associated with Fahr disease. Thereafter, different other genetic studies were performed, which found mutations that mostly lead to a dysfunctional PiT2 protein.[14, 42-47] Loss-of-function mutation in the SLC20A2 gene is found in approximately 40% of patients with Fahr and causes the accumulation of Pi and formation of calcium phosphate depositions in the form of hydroxyapatite in the vascular extracellular matrix (figure 3). This occurs on tissue-specific locations where the PiT2 transporter is located.[48] Genotype-phenotype correlation studies have shown that mutations in SLC20A2 are significantly more associated with parkinsonism (in 21% of the cases in the study of Batla et al.)[48] than with mutations in the other genes associated with Fahr disease.[48] Higher levels of Pi were observed in CSF of especially SLC20A2-associated patients with Fahr disease, which suggest the possibility of Pi as biomarker for the diagnosis of this type of Fahr in patients.[49]

Analysis of the role of several SLC20A2 mutations showed the critical role in Na/Pi transport in Chinese hamster ovary (CHO) cells. When the amino acids Glu55 and Glu575, located in the transmembrane domains were exchanged by a glutamate or lysine, the phosphate transport was extremely reduced or stopped completely in CHO cells.[44] When the amino acid Ser113, located in the protein homology domain of the N-terminus, and Ser593, located in the protein homology domain of the C-terminus, were exchanged by alanine, it led to a decrease in the phosphate transport in CHO cells by 10-fold.[50] These results showed that SLC20A2 is important for phosphate transport, and mutations associated with Fahr disease will lead to an extreme reduction of phosphate import into the endothelial cells. High concentrations of phosphate remain in the vascular extracellular matrix and will cause vascular calcifications.[48]

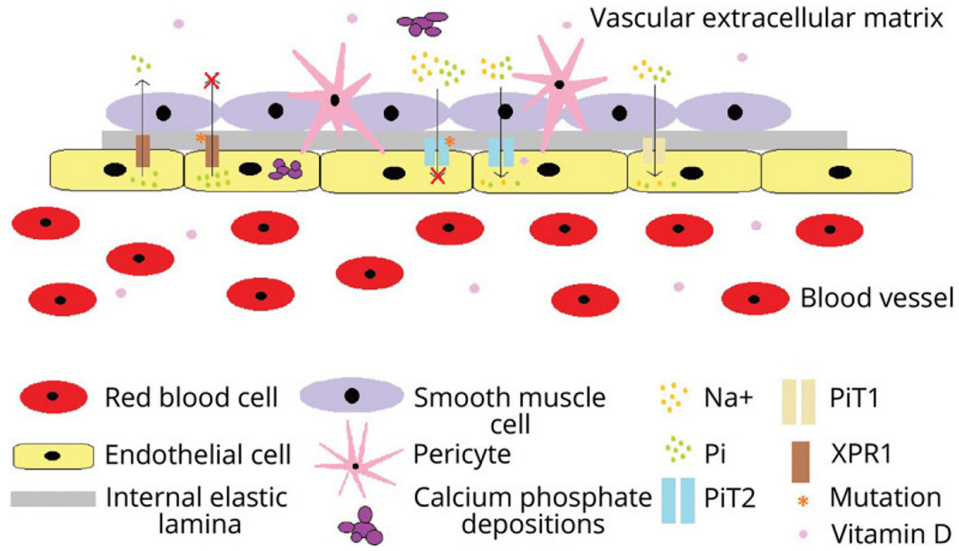

FIGURE 3. Mutations in the genes *SLC20A2* and *XPR1* are associated with a disrupted phosphate metabolism in Fahr disease patients.
On the left side of the figure, two XPR1 transporters were illustrated. The left XPR1 transporter shows the normal situation, whereby secretion of Pi from intracellular to extracellular is mediated. The right XPR1 transporter shows a mutation (orange asterisk) and because of this mutation the Pi efflux is disturbed. This leads to calcium depositions in endothelial cells. In the middle part of the figure two PiT2 transporters were illustrated. The right PiT2 transporter shows the normal situation, whereby an influx of Pi and Na+ is shown. It is suggested that vitamin D could bind to the promoter of the *SLC20A2* gene in the nucleus of endothelial cells, and increase the PiT2 expression38.The left PiT2 transporter shows a mutation (orange asterisk), which leads to a disturbed Pi influx. In some mutations vitamin D cannot bind to the promoter of *SLC20A2*, which lead to less PiT2 expression. These mutations cause calcium depositions in the vascular extracellular matrix. The right part of the figure shows the function of a PiT1 transporter, it ensures Pi influx into the endothelial cell, just like *SLC20A2*. However, the *SLC20A1* gene is not directly associated with calcifications in Fahr disease. The transporters XPR1, PiT2 and PiT1 are not only located on the vascular endothelial cells as in this figure is shown. XPR1 is also located at vascular smooth muscle cells, PiT2 and PiT1 are also located at neurons, astrocytes and vascular smooth muscle cells. For this figure it has been chosen to show the transporters only on the endothelial cells.

It has been suggested that SLC20A2 might be regulated by vitamin D because the promotor contains a predicted vitamin D receptor binding site.[38] The role of vitamin D (calcitriol) in the suppression of calcification has been studied in vitro.[38] Calcitriol could upregulate SLC20A2 mRNA expression but not SLC20A1 or XPR1 mRNA expression. High extracellular Pi concentration led to a strong decrease in SLC20A2 and SLC20A1 mRNA expression and a small decrease in XPR1 mRNA expression in calcified cells. The reduction of the Pi transporters shows that cells anticipate the situation to maintain Pi homeostasis. In vitro treatment with calcitriol resulted in reduced calcification.

To prove the hypothesis that SLC20A2 is regulated by vitamin D, the gene SLC20A2 was knocked down in SaOs2 (sarcoma osteogenic) cells, and subsequently, the cells were no longer protected for the effects of calcification by calcitriol, and the vitamin D–mediated inhibition of calcification was reduced[38] (figure 3).

Animal models

With biochemical and immunohistochemical analyses, it was found that PiT2 is broadly expressed in the mouse brain.[39] Expression was found in neurons, astrocytes, and vascular endothelial cells.[51] Inden et al.[51] investigated the locations of PiT1 and PiT2 in the mouse brain. SLC20A1 and SLC20A2 mRNA expression was found in the cortex and striatum, and the highest expression of both genes was found in the cerebellum of the mice brains.[51]

Jensen et al.[46] investigated the role of PiT2 in mice, using SLC20A2 homozygous knockout mice. These mice produced a truncated SLC20A2 mRNA, which lack 8 downstream coding exons, leading to extracellular phosphate accumulation. In SLC20A2 knockout mice, higher levels of Pi were found in the CSF compared with wildtype mice. This supports the findings that PiT2 also plays a role in Pi export from CSF and maintaining low Pi levels in the CSF.[52] Deficiency of SLC20A2 in homozygous knockout mice lead to brain calcifications in the thalamus, basal ganglia, and cortex,[46, 53] emphasizing the role of PiT2 in brain calcification. However, because Fahr concerns a heterozygous autosomal dominant disease, these results might not represent the actual pathophysiology of Fahr disease.

Xenotropic and polytropic retrovirus receptor

XPR1 is localized to chromosome 1q25.3, and it encodes for the XPR1 with Pi exporter function.[5, 54] It is suggested that mutations in XPR1 are associated with parkinsonism[5] and cognitive dysfunction (66.7%).[48] Beside calcifications in the basal ganglia and cerebellum, the cortical areas also contain heavy calcifications in patients with mutations in this gene.[48] XPR1 contains an intracellular cytoplasmic N-terminal SPX domain (named by the genes SYG1, PHO81, and XPR1), a transmembrane domain and an intracellular C-terminal domain.[54] XPR1 regulates the phosphate export, which is seen in animals, plants, and fungi.[55] It is thought to be a Pi transporter, which ensures an efflux of Pi from intracellular toward the vascular extracellular matrix[38] and thereby has an opposite function compared with PiT2. Another study into the function of XPR1, however, found it to be a G protein–coupled receptor involved in cell apoptosis via inactivation of the adenylyl cyclase pathway without Pi transporter function.[56]

Gene mutations

Legati et al.[54] first discovered that mutations in the XPR1 gene are associated with Fahr disease. After that, other studies found various mutations in the XPR1 gene resulting in a decrease in phosphate efflux and subsequently calcium depositions in endothelial cells [5, 54, 55, 57] (figure 3).

Mutations in XPR1 are associated with different neurodegenerative diseases. Xu et al.[58] found a relation between a frameshift mutation in XPR1 and schizophrenia. Fujioka et al.[59] found an alternative splicing variant of XPR1, which is related to amyotrophic lateral sclerosis and frontotemporal lobar degeneration. Manavalan et al.[60] found that altered protein expression of XPR1 in the hippocampus is linked to aging-related dementia (Alzheimer disease).

Animal models

XPR1 is expressed in different brain regions in the mouse[54]; however, deletion of the XPR1 gene in mice is embryonic lethal. It shows that this gene has an crucial function in early development, but its exact role is still unknown.[56]

Platelet-derived growth factor subunit B

PDGFB is localized to chromosome 22q13.1 and encodes a disulfide-linked dimer.[59, 61] It is a paracrine growth factor important in mesenchymal cells, neurons, smooth muscle cells, and endothelial cells and has a function in the recruitment of pericytes during angiogenesise[22, 63] (figure 4A). PDGFB signaling has different effects on these different cell types, but in every cell type, a mutation in this gene leads to calcification in the vascular extracellular matrix.[63] Endothelial cells produce and secrete PDGFB, which binds the receptor PDGF-Rβ on pericytes and vascular smooth muscle cells. Progenitor PDGFB and its receptor PDGFRβ are important for the regulation of pericyte formation and migration around blood vessels during angiogenesis.[64]

Gene mutations

Loss-of-function mutations in both genes, PDGFB and PDGFRβ, cause a vasculature lacking pericytes and leads to Fahr disease [65] (figure 4B). The pericytes play a crucial role in the blood-brain

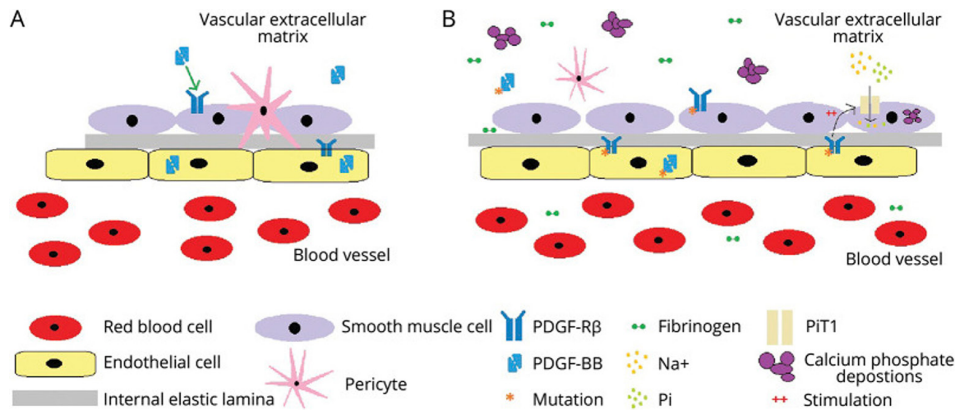

FIGURE 4. Mutations in the genes *PDGFB* and *PDGFRB* are associated with dysfunction in blood brain barrier integrity and pericyte maintenance in Fahr disease patients.
(A) This figure shows the normal situation, whereby the ligand PDGF-BB binds to his receptor PDGF-Rβ (green arrow). This ensures the blood brain barrier integrity and pericyte maintenance. (B) This figure shows a mutated situation, whereby both *PDGFB* and *PDGFRB* could be mutated (orange asterisk). The ligand cannot bind his receptor and the underlying pathway will be activated only partly or not at all. This leads to dysfunction of the blood brain barrier and pericyte recruitment. The glycoprotein fibrinogen is found in the vascular extracellular matrix, which indicates that the blood brain barrier is disrupted, plasma proteins can pass this barrier and may form locations for mineral depositions in the vascular extracellular matrix. Activating mutation in *PDGFRB* could also stimulate the expression of the Na+/Pi transporter PiT1, which leads to a phosphate influx into the smooth muscle cells, this can cause calcium phosphate depositions in this cell type.

barrier. It is therefore likely that dysfunction of the blood-brain barrier plays an essential role in Fahr disease. The finding of glycoprotein fibrinogen, which is important for the formation of blood clots, in the perivascular environment in a patient with Fahr disease also implies that dysfunction of the blood-brain barrier plays an important role in disease development.[66] In addition, in mice with a dysfunction of the blood-brain barrier caused by a deficiency of the occludin protein, calcifications of the brain were observed.[67] Moreover, in humans, calcifications occur by loss-of-function mutations in the occludin gene,[68] which supports the involvement of the blood-brain barrier in the formation of calcifications.[6]

Animal models

Homozygous PDGFB deletion in mice led to a severe lack of vascular smooth muscle cells and pericytes, which was lethal.[62, 69] As well as mutated PDGFB, mutations in PDGFRB resulted in blood-brain barrier abnormalities and a lack of pericyte recruitment, which led to an increased permeability.[6] Keller et al.[6] investigated the role of PDGFB in mice, using hypomorphic PDGFB alleles. In this study, PDGFB knockout mice developed calcifications in the brain. In early stages (2 months), matrix deposits were found in the midbrain and thalamus; these expanded in size and number in later stages (4 months). One-year old mice showed severe calcifications in the basal ganglia and thalamus. With energy-dispersive X-ray spectroscopy analysis, it was found that the calcifications consist of calcium and phosphate. This indicates that the process is comparable between PDGFB knockout mice and humans with Fahr disease.[6]

Because we know that PDGFB in the human brain is expressed in endothelial cells and neurons, the researchers investigated in which cell type PDGFB is relevant in Fahr disease. They used PDGFB-null mice with transgenic re-expression of PDGFB in endothelial cells by the rescue allele R26P. No expression of PDGFB in neurons was found, but in the endothelial cells, the expression depended on the copy number of the rescue allele. Not neuronal but only endothelial levels of PDGFB were correlated with calcification in the brain in mice. When the mouse had 2 copies of the R26P allele, no brain calcifications were seen, but when the mouse had only 1 copy of this allele, only 50% PDGFB expression was seen in the endothelial cells. They compared this with total loss of function, retention motif knockout (PDGFBret/ret) mouse in which the retention motif on the C-terminal is deleted. The dimer PDGF-BB could not bind to the receptor anymore. The researchers found that mice that did have only 1 R26P copy allele developed smaller calcifications, but on the same locations and composition as the PDGFBret/ret mice. A correlation between the severity of calcification and the degree of pericyte deficiency and blood-brain barrier dysfunction was found.[6] In PDGFBret/ret mice, a decrease in pericytes around the blood vessels was seen, and the pericytes that were present were not tightly clamped to the endothelial cells. It is suggested from these results that the retention motif of PDGFB is needed for the presentation of endothelial PDGF-BB to the adjacent pericytes.[65]

Platelet-derived growth factor subunit receptor B

The genes PDGFRA and PDGFRB encode for 2 related tyrosine kinase receptors PDGF-Rα and PDGF-Rβ. PDGFA and B are ligands that bind these receptors, respectively.[6, 7, 13] The gene PDGFRB is localized to chromosome 5q33.1.[61, 70] Expression of PDGFRB is especially found in the neurons, endothelial cells, vascular smooth muscle cells, and pericytes of the basal ganglia and dentate nucleus of the cerebellum.[19] PDGFRB has an important role in maintaining the blood-brain barrier by pericyte recruitment and angiogenesis as described above.[68, 71]

After activation, the receptor dimerizes, autophosphorylation takes place, and the downstream signaling pathway of mitogen-activated protein kinases, phosphatidylinositol-3 kinase, phospholipase Cγ, signal transducers, and activators of transcription is activated. This activation of the pathway stimulates cell proliferation, differentiation, survival, and migration and has different functions in vascular smooth muscle cells.[7, 72] When the ligand is absent, the kinase receptor is inactive.[7] The most important function of this pathway is the proliferation and migration of vascular smooth muscle cells and pericytes.

Gene mutations

Different loss-of-function mutations in PDGFRB in patients with Fahr disease were described in various studies,[7, 73, 74] which lead to permeability of pericytes around vessels and calcium deposits in the vessel wall and perivascular space.[6, 7] Patients with mutations in the PDGFRB gene had an early age at onset of disease symptoms in comparison with mutations in the other genes (SLC20A2, PDGFB, and XPR1).[48] Presumably, headache and depression are the most common features for mutations in this gene, but evidence is limited.[48]

Mutations in PDGFRB are not only involved in the formation of calcification due to loss of blood-brain barrier integrity and pericyte maintenance but also cause dysregulation of Pi transport in vascular smooth muscle cells. Different studies show that an activating mutation in PDGFRB is involved in vascular calcifications by regulating the phosphate transporter PiT1, encoded by SLC20A1.[7, 75, 76] PiT1 is located on the cell membrane and in the endoplasmic reticulum of vascular smooth muscle cells and endothelial cells.[7, 76] Activating mutation of PDGFB increases the expression of PiT1 causing a Pi influx from vascular extracellular matrix into the vascular smooth muscle cells. This results in the formation of calcium phosphate depositions in the smooth muscle cells[76] (figure 4B). It appears that PDGFRB has different functions, and mutations in this gene can lead to different types of calcifications in the brain. A recent study demonstrated that PDGFRB colocalizes with XPR1, and these genes regulate each other.[77] It is not yet known whether PDGFRB also regulates the PiT2 transporter in the brain.[6, 7]

The above-mentioned genes account for approximately 55% of the patients with Fahr disease. For the other 45% of the cases, it is unclear why these patients have Fahr disease, but until recently, no causative genes for the other 45% of the cases were found. A recent study (June 2019) found another gene, the MYORG gene, which causes Fahr with an autosomal recessive pattern of inheritance. Sixteen patients with MYORG mutations, with a median age at onset of 52 years, showed a high clinical penetrance and motor impairment, which mainly manifested in dysarthria. In addition to extensive calcifications in the basal ganglia, MYORG patients also exhibit calcifications in the brainstem.[78] The prevalence of these mutations in patients with Fahr disease and the pathophysiologic mechanism behind the calcifications remain to be established.

Recap

The above-mentioned genes encode for proteins that are located at different cell types in the brain; this may explain the different locations of calcification in the brain. Mutations in SLC20A2 and PDGFB lead to calcifications in the vascular extracellular matrix,[48, 63] mutations in XPR1 lead to calcifications in the endothelial cells,[5] and mutations in PDGFRB lead to calcifications in smooth muscle cells.[76] It is unknown why these mutations in these various genes are limited to the basal gan-

glia. The different mechanisms behind brain calcifications in patients with Fahr are unknown yet; future research is warranted to understand why calcium depositions occur in these different cell types. The wide spectrum of symptoms makes the diagnosis difficult. However, a slight separation can be made between the symptoms in comparison to the location of calcification. As mentioned earlier, patients with mutations in PDGFRB have an early age at onset, resulting mostly in headache and depression.[48] This might be explained by the effect of the mutations in this gene, loss of blood-brain barrier integrity, and pericyte maintenance. The blood-brain barrier is important for the whole brain, and it is to imagine that damage of the blood-brain barrier will express at an early age at onset. Patients who become symptomatic later in life, mostly have mutations in SLC20A2 and XPR1 and mainly develop movement disorders in combination with other clinical features.[16]

Potential treatment options

Because no treatment or therapy to reduce the progression of calcification currently exists,[3, 79] treatment focuses on symptom management with, for example, anti-Parkinson, antidepressant, and antipsychotic medication.[79] Deeper understanding of the genetics and the pathophysiologic mechanism behind this disease can provide new insight into treatment of patients with Fahr disease and of ectopic (brain) calcification beyond Fahr.

A first potential treatment for patients with Fahr disease is bisphosphonate therapy. Newer nitrogen-containing bisphosphonates, such as alendronate, predominantly inhibit osteoclasts. Although used for the treatment of osteoporosis, older non–nitrogen-containing bisphosphonates were initially shown to prevent heterotopic mineralization.[80] The bisphosphonate etidronate is a molecular homologue of the circulating calcification inhibitor inorganic pyrophosphate (PPi). The potential of etidronate for the treatment of vascular calcification is convincingly shown in patients with calcification disorders, such as generalized arterial calcifications in infancy and pseudoxanthoma elasticum.[25, 81] A clinical trial into the effect of etidronate in patients with ACDC is currently running (clinicaltrials.gov: NCT01585402).[26] Of interest, possible beneficial effects of non–nitrogen-containing bisphosphonates on arterial calcifications in the population were also demonstrated in a recent meta-analysis.[29] The authors concluded that these bisphosphonates reduce all-cause mortality in various non cardiovascular patient groups.[29] Non–nitrogen-containing bisphosphonates were also tested on patients with Fahr disease where they were shown to pass the blood-brain barrier and improve symptoms, but not visual assessment of calcification burden.[82, 83]

A second candidate therapy could be vitamin D. Keasey et al.[38] suggested that the gene SLC20A2 is regulated by vitamin D and that vitamin D reduced calcifications in the brain. They found vitamin D deficiency in patients with Fahr disease. It is suggested that in a normal situation, vitamin D can bind to a promoter region of the SLC20A2 gene and upregulate SLC20A2 mRNA. This mechanism could therefore be a possibility for a treatment for patients with Fahr disease. First, it should be considered whether patients with Fahr disease often have a vitamin D deficiency in combination with SLC20A2 mutations. If this mutation is not located in the vitamin D binding site, vitamin D therapy could be considered. Taking into account that vitamin D can pass the blood-brain barrier to a limited amount and high levels of vitamin D could be harmful, further research is certainly needed.[40, 84]

Third, some mutations found in Fahr disease resemble those in a specific subgroup of patients with cystic fibrosis who have a nonsense mutation leading to a truncated nonfunctional protein. Treatment with ataluren or PTC124 ensures that an early stop codon will not be read and that translation occurs to the normal stop codon. This results in a full-length cystic fibrosis transmembrane conductance regulator product, with normal function. A similar treatment is also used for patients with Duchenne muscular dystrophy.[85] In patients with Fahr disease, truncating mutations are also found in the SLC20A2 genee2 and the PDGFB gene.[86] Similar treatments could therefore be an option for patients with Fahr disease with nonsense mutations.

Pi/PPi imbalance is known to be involved in genetic syndromes that lead to ectopic mineralization outside the brain. Bisphosphonates, vitamin D, and drugs that target nonsense mutations are of interest for further investigation in Fahr disease and ectopic mineralization in the brain in the population. Outside neurodegenerative disease interest in the treatment of arterial calcifications is growing, and several food supplements and drugs, such as vitamins, antibodies, and proteins, are at various stages of (clinical) testing as recently reviewed; however, still novel therapeutics will be discovered and investigated. It is important that these drugs inhibit the calcification growth and avoid reacting on bones or interfering with physiologic mineralization.[87] Incomplete understanding and the complexities about Fahr disease and vascular calcification in general impede the development of specifically targeted medicines. However, those drugs could eventually play a role in the treatment of Fahr disease and ectopic brain mineralization in the population.

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PART 3

Risk factors and clinical relevance of incidental intracranial artery calcifications

CHAPTER 5

Prevalence and vascular risk factors of basal ganglia calcifications in patients at risk for cerebrovascular disease

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Abstract

Background and purpose: Risk factors for and meaning of basal ganglia calcifications outside Fahr syndrome are poorly understood. We aimed to assess the prevalence of basal ganglia calcifications and the association with vascular risk factors.

Materials and methods: 1133 patients suspected of acute ischemic stroke from the Dutch acute stroke study (DUST) who underwent thin-slice unenhanced brain CT were analyzed. Basal ganglia calcifications were scored bilaterally as absent, mild (dot), moderate (multiple dots or single artery) and severe (confluent). Uni- and multivariable logistic regression analysis was used to determine possible risk factors (age, gender, history of stroke, smoking, hypertension, diabetes mellitus, hyperlipidemia, body mass index (BMI), renal function and family history of cardiovascular disease under 60 years) for presence of basal ganglia calcifications and ordinal regression analysis for severity of basal ganglia calcifications.

Results: Mean age was 67.4 years (SD: 13.8), 56.8% were male. 337 (29.7%) patients had basal ganglia calcifications, of which 196 (58%) were mild, 103 (31%) moderate, 38 (11%) severe. In multivariable logistic regression analysis, age (OR: 1.02, 95% CI 1.01-1.03, p<0.01) and BMI (OR: 0.95, 95% CI 0.91-0.98, p 0.01) were significantly associated with the presence of basal ganglia calcifications. Ordinal regression analysis gave comparable results. Age (OR: 1.02, 95% CI 1.01-1.03, p<0.01) and BMI (OR: 0.95, 95% CI 0.92-0.99, p 0.01) were significantly associated with severity of basal ganglia calcifications.

Conclusions: In this study with patients suspected of acute ischemic stroke, basal ganglia calcifications were common and significantly associated with older age and lower BMI.

Prevalence and vascular risk factors of basal ganglia calcifications in patients at risk for cerebrovascular disease

Introduction

The risk factors for and meaning of calcifications in the basal ganglia are poorly understood and they are often regarded as a harmless incidental finding on computed tomography (CT) imaging. Evidence, mainly from genetic disorders, however suggests that they may be less harmless than they seem.

These calcifications in the basal ganglia were first described by Delacour[1] in 1850 and are relatively common. In early studies, the prevalence found with CT ranged from 0.3%[2] to 12.5%.[3] Newer studies also showed variable, but overall higher prevalence rates, possibly due to a higher sensitivity of thin slice CT scans and increased age of the subjects.[4-7] In 2016, a large CT study showed basal ganglia calcifications in 1.3% of 11941 people with no medical history and average age of 46 years.[4] The vast majority of the basal ganglia calcifications were seen in globus pallidus (98.4%).[4, 5] Research, published in 2013, among Japanese people who underwent a CT scan in two Japanese cities showed calcifications in the basal ganglia in 26.1% and 34.1% of patients above 65 years old.[6] A Swedish study of elderly, even found a prevalence of 38.7%.[7]

Not much is known about risk factors for basal ganglia calcifications, but they seem to be associated with female gender and older age.[4, 7] No association with disturbed calcium metabolism or endocrine disorders has been observed outside Fahr syndrome, a syndrome that is characterized by extensive calcifications in the basal ganglia, dentate nucleus and thalamus.[2] To our knowledge, there are no studies investigating the association between vascular risk factors and basal ganglia calcification.

The small, punctate calcifications in the basal ganglia are usually found by chance and are considered as non-pathological. However, in a study in very old patients, aged over 85 years, basal ganglia calcifications were associated with hallucinations and delusions.[8] Also in patients with Fahr syndrome neurological, psychiatric and cognitive symptoms occur.[9] The calcifications may develop as a consequence of ischemia, trauma or infections and start as microvascular and perivascular accumulations.[10, 11] In a case report describing a patient with Fahr disease the calcifications were histologically found in the tunica media of small arteries, arterioles and capillaries.[12] Other studies in patients with Fahr syndrome identified calcification in the basal ganglia both within and around the vessel walls.[13-16]

We hypothesize that vascular risk factors can lead to microvascular pathology and thereby to these vascular and perivascular calcifications in the basal ganglia. In this study, we investigated the association between vascular risk factors and basal ganglia calcifications in a well characterized population suspected of acute ischemic stroke who underwent standardized high-quality CT imaging of the brain.

Materials and methods

Population

For this study, we used the existing study population of the Dutch acute stroke (DUST) study;[17] a prospective multi-center cohort study of patients with suspected acute ischemic stroke. In this study 1393 consecutive patients were included from May 2009 until July 2013 in six university hospitals and eight non-university hospitals of the Netherlands. Inclusion criteria were age of 18 years or older and symptoms of acute ischemic stroke of less than 9 hour duration. All patients underwent unenhanced CT, CT angiography and CT perfusion examinations. Exclusion criteria were known contrast allergy or renal failure. We excluded 260 patients for whom no thin-slice unenhanced CT was available in the central archive. The medical ethics committee of the University Medical Center Utrecht, the Netherlands, as well as the local medical ethics committees of the participating centers gave approval for the DUST study. Informed consent was signed by all patients or their legal representatives. The need for consent was waived by the medical ethics committee if a patient died before consent could be obtained.

Baseline measurements

At baseline, patient characteristics were obtained by history taking, physical examination and laboratory tests, including age, gender, medical history of stroke, history of hypertension (systolic RR ≥ 140 mmHg and/or a diastolic RR ≥ 90 mmHg), diabetes mellitus, hyperlipidemia, family history of cardiovascular disease (CVD) (positive or negative for 1st grade, <60 years of age), smoking (current, former or never), body mass index (BMI) and estimated glomerular filtration rate (eGFR) (calculated using the 'modification of diet in renal disease' (MDRD) formula in µmol/L). In this study, no information about race was available. Since race is a necessary factor in calculating the eGFR, the eGFR was calculated assuming all patients were non-black, as the majority of the population in the Netherlands is Caucasian.

Technical information on image acquisition

Multidetector row CT scanners were used, with the number of detectors ranging from 40 to 320 (LightSpeed VCT, GE Healthcare; Brilliance 40, Brilliance 64, and Brilliance iCT 256, Philips Healthcare; Sensation 64, Siemens; Aquilion ONE, Toshiba Medical Systems) at 120 kV and 300-375mAs. Patients were scanned from the skull base to the vertex. Scans were reconstructed with a slice thickness ranging from 0.625 to 1 mm.

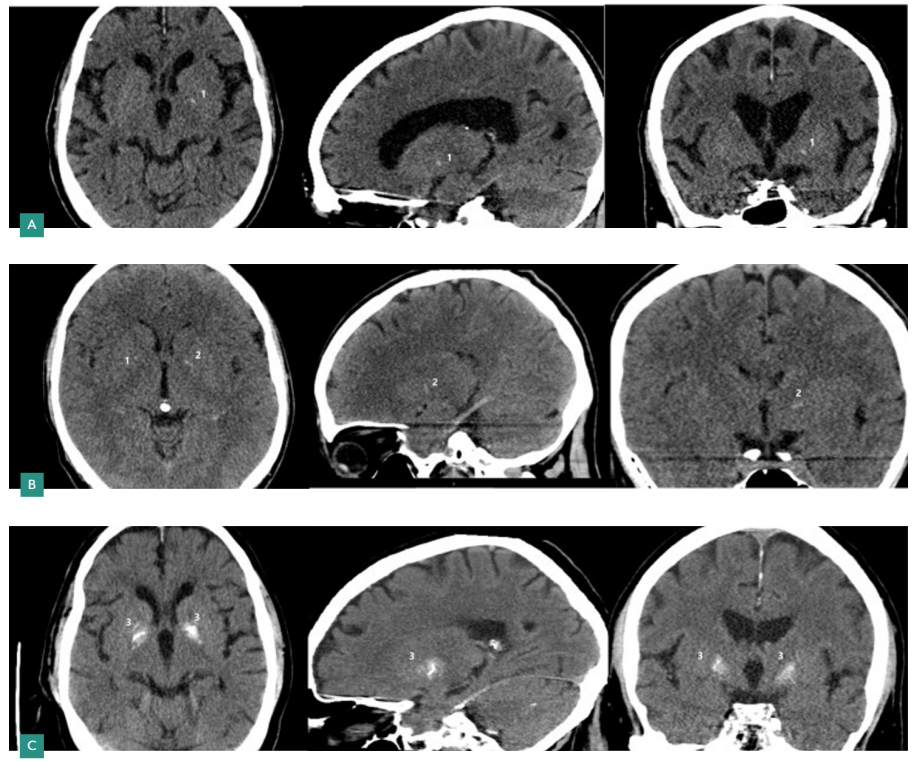

FIGURE 1A-C. CT image of basal ganglia calcifications shown in axial, sagittal and coronal plane

A. The image shows mild basal ganglia calcification (1).

B. The image shows mild (1) and moderate (2) basal ganglia calcification.

B. The image shows severe basal ganglia calcifications (3).

Calcification measurements

An experienced radiologist, a radiology resident and a medical doctor doing a PhD project on intracranial calcification with 14, 4 and 2 years of experience in reading (brain) CT scans (P.A.d.J., J.B.D.V. and R.K., respectively) rated the non-contrast enhanced thin slice reconstructions independently and blinded to the subjects characteristics. Images were analyzed in axial, coronal and sagittal plane in the brain window setting (Center: 40, Width: 100). Basal ganglia calcifications are seen in the brain CT window setting as white (clustered) dense configurations comparable to bone. Calcifications were scored bilaterally in the basal ganglia as absent, mild (dot) (figure 1A), moderate (multiple dots or single artery) (figure 1B) or severe (confluent) (figure 1C). For analyzing basal ganglia calcification severity the most severe calcification of either side was used.

Statistical analysis

Descriptive statistics were used to describe the characteristics of the study population; means with standard deviations and medians with interquartile ranges for continuous variables, depending on their distribution, and counts and percentages for categorical variables. To determine differences of baseline values between groups, chi-squared, Fishers exact, Fisher Freeman Halton and ANOVA tests were used for categorical and continuous data, respectively.

The association between presence of basal ganglia calcifications and vascular risk factors was studied with multivariable logistic regression analysis in an unadjusted model and a multivariable model adjusted for vascular risk factors: age, gender, history of stroke, hypertension, diabetes mellitus, hyperlipidemia, smoking, BMI, eGFR and family history of CVD below the age of 60.

To analyze the association between severity (absent, mild or moderate/severe) of basal ganglia calcification and vascular risk factors we used ordinal regression. In the multivariable model we adjusted for vascular risk factors: age, gender, history of stroke, hypertension, diabetes mellitus, hyperlipidemia, smoking, BMI, eGFR and family history of CVD below the age of 60.

For the logistic and ordinal regression analysis, multiple imputation was used for missing variables in all patients.

A subgroup analysis was performed for patients with an established diagnosis at discharge of TIA or ischemic stroke, excluding patients with another diagnosis than TIA or ischemic stroke.

Interobserver agreement was analyzed using Cohens Kappa.

Statistical significance was defined as $p<0.05$. Statistical analysis was performed using SPSS (IBM SPSS Statistics, Version 25.0. IBM Corp).

Results

Baseline characteristics

Characteristics of the study population are shown in table 1. The final study population consisted of 1133 patients with a mean age of 67.4 years (SD 13.8). The majority was male (56.8%). History of hypertension (52.7%) and hyperlipidemia (34.3%) were highly prevalent. In 30% of patients the BMI was unknown and family history of vascular disease was missing in 34% of patients. The other variables had less than 10% missing values.

Prevalence of basal ganglia calcifications

As shown in table 1, basal ganglia calcifications were present in 337 patients (29.7%) and was mild in 196 (17.3%), moderate in 103 (9.1%) and severe in 38 (3.4%) patients. Patients with more severe calcifications were significantly older, more frequently female and had more often hypertension. They had a significantly lower BMI and eGFR. There was no significant difference in prevalence of basal ganglia calcifications between patients with an established diagnosis of TIA or ischemic stroke at discharge and patients without a diagnosis of TIA or ischemic stroke at discharge (29.7% versus 28.8%, respectively). The interobserver agreement between P.A.d.J., J.B.D.V. and R.K. was 0.82.

Vascular risk factors of basal ganglia calcifications

Results of the uni- and multivariable logistic regression analysis after multiple imputation are presented in table 2. In the multivariable analysis, age (OR 1.02, 95% CI 1.01-1.03) and BMI (OR 0.95, 95% CI 0.91-0.98) were significantly associated with basal ganglia calcifications.

Results of the ordinal regression analysis after multiple imputation are presented in table 3. In the multivariable analysis, age (OR 1.02, 95% CI 1.01-1.03) and BMI (OR 0.95, 95% CI 0.92-0.99) were significantly associated with severity of basal ganglia calcifications. Results were comparable before and after multiple imputation (appendix tables A.1 and A.2) and for the subgroup of patients with an established diagnosis at discharge of TIA or ischemic stroke (data not tabulated).

Discussion

In this study, we assessed the prevalence of basal ganglia calcifications and the association with vascular risk factors in a large cohort of patients who visited the hospital because of suspected acute ischemic stroke. Basal ganglia calcification is a frequent finding on CT scans in these patients and presence of basal ganglia calcification is significantly associated with older age and a lower body mass index. Contrary to our hypothesis we did not observe associations with other known vascular risk factors. In this cohort we cannot investigate the symptoms and prognosis of these calcifications.

Our prevalence of basal ganglia calcification was 29.7%. This is a high prevalence in comparison with older studies who found prevalence's between 0.28%[2] and 12.5%[3] and a study in a cohort with a younger age (prevalence 1.3%).[4] However, our prevalence is comparable to several recent studies.[6,7] This is probably explained by the better sensitivity of current thin-slice CT scans. In our study the patients were on average 67 years of age and they had significant comorbidity, which could be also an explanation for the high prevalence. As this study is not population-based, therefore it is difficult to assess the true prevalence of basal ganglia calcification in the general population.

We found that basal ganglia calcifications were significantly associated with older age, which corresponds with earlier research.[6]

A lower body mass index was significantly associated with presence and severity of basal ganglia calcifications in our cohort. This was an unexpected finding, but there is more literature that shows a reverse association between BMI and calcifications in the carotid siphon,[18] aorta,[19] the aortic valve[20] and the coronary arteries.[21] The explanation for this inverse relation is not yet clear, but there are a number of theories. For example, it is hypothesized that bone demineralization in patients with a low BMI plays a role,[19] or that loss of fat and muscle mass in older people is an indicator of global deterioration in health.[20]

This is the first study to assess the association of vascular risk factors with basal ganglia calcifications. Previous post mortem studies in patients with Fahr disease described basal ganglia calcifications as non-atherosclerotic calcifications.[12-16] Since we did not find an association of hyperlipidemia, hypertension and smoking with basal ganglia calcifications, all well known risk factors for atherosclerosis, our findings support that theory in a study population outside Fahr disease. A post mortem study in a patient with Fahr disease found basal ganglia calcifications in the tunica media.[12] Tunica media calcifications are associated with diabetes mellitus and renal failure.[22] In our study we did not find an association with diabetes mellitus and renal function. However patients with renal failure were excluded in our study, so we could have missed an association between renal function and basal ganglia calcifications. Nevertheless the lack of association with diabetes was unexpected.

An important limitation of our study is the cross sectional design. This makes it impossible to conclude on a causative relation. As this study is not population-based, therefore caution is needed

	Total N=1133 100%	Absent N=796 70.3%		Mild N=196 17.3%	Moderate N=103 9.1%	Severe N=38 3.4%	P-value
Age yr (mean, SD)	67.4 (13.8)	65.9 (14.3)		70.2 (12.0)	71.3 (11.9)	73.2 (10.4)	<0.01[a]
Gender (male)	644 (56.8%)	471 (59.2%)		112 (57.1%)	45 (43.7%)	16 (42.1%)	0.01[a]
Stroke in medical history	276 (24.4%)	189 (23.7%)		47 (24.0%)	29 (28.2%)	11 (28.9%)	0.73
Hypertension	597 (52.7%)	398 (50.0%)		116 (59.2%)	63 (61.2%)	20 (52.6%)	0.05[a]
Diabetes mellitus	171 (15.1%)	109 (13.7%)		40 (20.4%)	18 (17.5%)	4 (10.5%)	0.10
Hyperlipidemia	389 (34.3%)	256 (32.3%)		81 (41.3%)	41 (39.8%)	11 (28.9%)	0.07
Smoking							0.80
Current	305 (26.9%)	218 (27.4%)		50 (25.5%)	27 (26.2%)	10 (26.3%)	
Former	346 (30.5%)	249 (31.3%)		57 (29.1%)	27 (26.2%)	13 (34.2%)	
Never smoked	403 (35.6%)	274 (34.4%)		74 (37.8%)	43 (41.7%)	12 (31.6%)	
BMI, mean (SD)	26.7 (4.6)	27.1 (4.9)		25.9 (4.2)	25.9 (3.2)	25.5 (4.8)	0.02[a]
eGFR under 60							
eGFR, mean (SD)	81.5 (30.0)	83.0 (33.0)		79.3 (21.4)	78.2 (20.1)	70.1 (19.8)	0.02[a]
Positive family history CVD	247 (33%)	183 (35%)		34 (26%)	23 (37%)	7 (25%)	0.22
Calcium antagonist	127 (11.2%)	83 (10.4%)		31 (15.8%)	8 (7.8%)	5 (13.2%)	0.13
Antihypertensive drugs	555 (49.0%)	373 (46.9%)		108 (55.1%)	52 (50.5%)	22 (57.9%)	0.14
Oral anticoagulant	153 (13.5%)	104 (13.1%)		24 (12.2%)	17 (16.5%)	8 (21.1%)	0.35
Platelet inhibitor	397 (35.0%)	266 (33.4%)		70 (35.7%)	43 (41.7%)	18 (47.4%	0.12
Diagnosis at discharge							
TIA	76 (6.7%)	47 (5.9%)		22 (11.2%)	6 (5.8%)	1 (2.6%)	0.24
iCVA	995 (87.8%)	706 (88.7%)		163 (83.2%)	90 (87.4%)	36 (94.7%)	
Other	59 (5.2%)	42 (5.3%)		10 (5.1%)	6 (5.8%)	1 (2.6%)	

with the generalization of our prevalence results to non-stroke populations and to older populations as our stroke population was relatively young compared to other stroke cohorts. Yet, we found no significant difference in outcomes between patients with and without a discharge diagnosis of TIA or ischemic stroke.

Another limitation is that we cannot comment on the clinical significance of these vascular calcifications. Finally, we had a large percentage of missing values for BMI and family history of cardiovascular disease. We used multiple imputation to impute missing data for an optimal analysis. Before and after data imputation, BMI and age were significantly associated with the presence and severity of basal ganglia calcifications.

TABLE 1. Baseline characteristics

Data analyzed with Chi-square, Fishers exact (eGFR <60), Fisher Freeman Halton test (diabetes mellitus, calcium antagonist, oral anticoagulant, diagnosis at discharge) and Oneway ANOVA (age, BMI, eGFR). Outcome of family history was defined as negative or positive for first grade family members with cardiovascular disease under 60 years of age. **N:** number, **SD**: standard deviation, **BMI:** body mass index, **eGFR:** estimated glomerular filtration rate, **CVD:** cardiovascular disease, **TIA:** transient ischemic attack, **iCVA:** ischemic cerebrovascular accident.

[a] significant value.

	Univariable analysis			Multivariable analysis		
Risk factors	OR	95% CI	P	OR	95% CI	P
Age per year older	1.03	1.02-1.04	<0.01 ª	1.02	1.01-1.03	<0.01 ª
Female gender	1.37	1.06-1.78	0.02 ª	1.22	0.93-1.60	0.15
Stroke	1.10	0.82-1.48	0.52	0.89	0.64-1.22	0.45
Hypertension	1.43	1.10-1.85	0.01 ª	1.16	0.86-1.56	0.32
Diabetes mellitus	1.41	1.00-1.99	0.05 ª	1.21	0.83-1.76	0.32
Hyperlipidemia	1.34	1.03-1.75	0.03 ª	1.26	0.92-1.73	0.15
eGFR	0.99	0.99-1.00	<0.01 ª	0.97	0.99-1.00	0.20
Smoking	1.10	0.94-1.30	0.23	1.02	0.85-1.22	0.87
BMI	0.95	0.91-0.98	<0.01 ª	0.95	0.91-0.98	0.01 ª
Family history of CVD	0.77	0.52-1.14	0.20	0.81	0.55-1.20	0.29

TABLE 2. Risk factors for basal ganglia calcification (logistic regression)
Multivariable model: adjusted for cardiovascular risk factors: age, gender, history of stroke, hypertension, diabetes mellitus type 2, hyperlipidemia, smoking (current, former, never), BMI, eGFR and family history of CVD positive or negative for first grade family members with cardiovascular disease under 60 years of age. **OR:** odds ratio, **CI:** confidence interval, **eGFR:** estimated glomerular filtration rate, **BMI:** body mass index, **CVD:** cardiovascular disease.

ª significant value.

In conclusion, basal ganglia calcifications were a frequent finding in patients suspected of having acute ischemic stroke (prevalence of 29.7%). Risk factors were older age and lower BMI, but no vascular risk factors like hypertension, hyperlipidemia, diabetes and smoking were found to be associated with basal ganglia calcifications.

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	Univariable analysis			Multivariable analysis		
Risk factors	OR	95% CI	P	OR	95% CI	P
Age	1.03	1.02-1.04	<0.01 ª	1.02	1.01-1.03	<0.01 ª
Female gender	1.44	1.12-1.85	<0.01 ª	1.25	0.96-1.64	0.10
Stroke	1.13	0.84-1.50	0.42	0.93	0.68-1.28	0.67
Hypertension	1.41	1.09-1.82	0.01 ª	1.16	0.87-1.55	0.33
Diabetes mellitus	1.34	0.96-1.88	0.09	1.13	0.79-1.64	0.50
Hyperlipidemia	1.31	1.01-1.70	0.05 ª	1.24	0.91-1.67	0.17
eGFR	0.99	0.99-1.00	<0.01 ª	1.00	0.99-1.00	0.17
Smoking						
Never	*	*	*	*	*	*
Former	0.81	0.59-1.10	0.18	0.80	0.58-1.11	0.18
Current	0.84	0.61-1.15	0.27	1.03	0.72-1.46	0.88
BMI	0.95	0.91-0.98	<0.01 ª	0.95	0.92-0.99	0.01 ª
Family history of CVD	1.35	0.94-1.95	0.11	1.29	0.89-1.86	0.18

TABLE 3. Risk factors for basal ganglia calcification according to severity (ordinal regression) Multivariable model: adjusted for cardiovascular risk factors: age, gender, history of stroke, hypertension, diabetes mellitus type 2, hyperlipidemia, smoking (current, former, never), BMI, eGFR and family history of CVD positive or negative for first grade family members with cardiovascular disease under 60 years of age. **OR:** odds ratio, **CI:** confidence interval, **eGFR:** estimated glomerular filtration rate, **BMI:** body mass index, **CVD:** cardiovascular disease.

ª significant value.

Declaration of interest

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There are no other declarations of interest.

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Appendix

Both tables below show the analysis done with the original database, so without data imputation.

	Univariable analysis			Multivariable analysis		
Risk factors	OR	95% CI	P	OR	95% CI	P
Age per year older	**1.03**	**1.02-1.04**	**<0.01**	**1.03**	**1.01-1.05**	**<0.01**
Female gender	**1.37**	**1.06-1.78**	**0.02**	1.08	0.71-1.66	0.71
Stroke	1.11	0.82-1.48	0.51	1.13	0.69-1.85	0.63
Hypertension	**1.43**	**1.11-1.86**	**0.01**	0.84	0.52-1.34	0.46
Diabetes mellitus	**1.41**	**1.00-1.99**	**0.05**	1.85	0.99-3.48	0.06
Hyperlipidemia	**1.36**	**1.04-1.77**	**0.02**	1.12	0.69-1.81	0.57
eGFR	**0.99**	**0.99-1.00**	**<0.01**	1.00	0.99-1.01	0.75
Smoking	1.09	0.93-1.29	0.29	1.00	0.76-1.31	0.99
BMI	**0.94**	**0.91-0.98**	**<0.01**	**0.95**	**0.90- 1.00**	**0.04**
Family history of CVD (1st grade, <60 years)	0.77	0.55-1.09	0.14	0.69	0.44 – 1.09	0.11

TABLE A.1 Risk factors for basal ganglia calcification (logistic regression)

Multivariable model: adjusted for cardiovascular risk characteristics: age, gender, history of stroke, hypertension, diabetes mellitus type 2, hyperlipidemia, smoking (current, former, never), BMI, eGFR and family history of CVD (one or more first grade family members with cardiovascular disease under 60 years of age).

	Univariable analysis			Multivariable analysis		
Risk factors	OR	95% CI	P	OR	95% CI	P
Age	**1.32**	**1.02-1.04**	**<0.01**	**1.02**	**1.01-1.04**	**<0.01**
Female gender	**1.44**	**1.12-1.85**	**<0.01**	1.30	0.92-1.83	0.13
Stroke	1.13	0.85-1.51	0.41	1.01	0.68-1.50	0.96
Hypertension	**1.42**	**1.10-1.83**	**0.01**	1.02	0.70-1.48	0.94
Diabetes mellitus	1.34	0.96-1.88	0.09	1.42	0.88-2.30	0.15
Hyperlipidemia	**1.32**	**1.02-1.72**	**0.04**	1.25	0.84-1.85	0.27
eGFR	**0.99**	**0.99-1.00**	**<0.01**	1.00	0.99-1.00	0.51
Smoking						
Never	*	*	*	*	*	*
Former	0.83	0.61-1.13	0.23	0.80	0.54-1.19	0.27
Current	0.85	0.62-1.17	0.33	1.05	0.68-1.61	0.82
BMI	**0.94**	**0.91-0.98**	**<0.01**	**0.94**	**0.90-0.98**	**<0.01**
Family history of CVD (1st grade, <60 years)	0.91	0.71-1.17	0.46	0.79	0.56-1.11	0.18

TABLE A.2 Risk factors for basal ganglia calcification according to severity (ordinal regression)

Severity was defined as absent, mild or moderate/severe. Multivariable model: adjusted for cardiovascular risk characteristics: age, gender, history of stroke, hypertension, diabetes mellitus type 2, hyperlipidemia, smoking, BMI, eGFR and family history of CVD (one or more first grade family members with cardiovascular disease under 60 years of age).

CHAPTER 6

Basal ganglia calcifications: No association with cognitive function

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Abstract

Background and purpose: Basal ganglia calcifications (BGC), a form of vascular calcification, are a common brain computed tomography (CT) finding. We investigated whether BGC are associated with cognitive function and examined the association between vascular risk factors and BGC.

Material and methods: Patients who visited a memory clinic of a Dutch general hospital between April 2009 and April 2015 were included. The patients underwent a standard diagnostic work up including cognitive tests (Cambridge Cognitive Examination, including the Mini Mental State Examination) and brain CT. Vascular risk factors such as hypertension, diabetes mellitus, hyperlipidemia and smoking were assessed. CTs were analyzed for presence and severity (absent, mild, moderate or severe) of BGC. Multivariable logistic regression was used to identify risk factors for BGC and linear regression for the association between BGC and cognitive function.

Results: Of the 1992 patients, 40.3% was male. The median age was 80 years and 866 patients (43.5%) had BGC. BGC was associated with female gender (odds ratio (OR) 1.27, 95% confidence interval (CI) 1.06-1.53, p 0.011), and inversely associated with hypertension (OR 0.74, 95% CI 0.60-0.89, p 0.002) and use of antihypertensive drugs (OR 0.79, 95% CI 0.64-0.98, p 0.031). No association was found between presence and severity of BGC and cognitive function or other vascular risk factors.

Conclusions: No association with cognitive function was found. Risk factors for BGC were female gender, while hypertension and antihypertensive drug use were associated with a lower risk of BGC.

Basal ganglia calcifications: No association with cognitive function

Introduction

Basal ganglia calcifications (BGC) are a common incidental computed tomography (CT) finding.[1] Histologically they are described as non-atherosclerotic vascular calcifications located in the tunica media.[2-6] Although tunica media calcifications are associated with diabetes mellitus (DM), a previous cross-sectional study in 1133 patients suspected of acute ischemic stroke found no association of BGC with traditional vascular risk factors including DM.[7-10]

BGC are mostly seen as harmless. However, some previous studies have reported associations with psychiatric symptoms.[11] Also patients with Primary Familial Brain Calcification (PFBC), who have extensive calcifications in the basal ganglia, have movement disorders, behavioural problems and/or cognitive symptoms such as executive dysfunction.[12,13]

Although the basal ganglia are primarily known for their role in control of voluntary movement, there is increasing evidence that the basal ganglia play a role in cognitive functioning.[14] Patients with Parkinson disease often have executive dysfunction, such as working memory impairment, probably as a result of hypoexcitation of the frontostriatal networks.[15] There is also a study suggesting a possible link between Alzheimer's disease and the basal ganglia. This study reported striatal atrophy in patients with Alzheimer's disease. However, the meaning and relevance of this atrophy is unknown.[16] Another study in patients with mild cognitive impairment and Alzheimer disease showed that amyloid PET detects amyloid first in the cortex and then in the striatum. A three-category staging including the striatum better predicted hippocampal volumes and subsequent cognition than a staging including only cortical amyloid.[17] To the best of our knowledge, the relationship between BGC and cognitive function has not been studied previously.

In this study, we examined the association between BGC and cognitive function. We hypothesize that BGC can impair cognitive function, mainly in the executive domain. Secondly, we explored the relationship between BGC and vascular risk factors.

Material and methods

Study population

The study population consisted of 2000 consecutive patients referred to the memory clinic of Tergooi Medical Center between April 2009 and April 2015 because of cognitive complaints. Our only exclusion criteria was absence of a CT scan of the brain, which led to a final study population of 1992 patients. This population has been described previously.[18] The study was approved by the local Medical Ethics Committee of Tergooi Medical Center.[18]

Diagnostic procedures

Patients were examined by a team consisting of a geriatrician, neurologist, neuropsychologist and a specialized nurse. They underwent a standard examination including history taking, assessment of medication use, assessment of education level, physical examination, cognitive screening and a brain CT scan. Information about vascular risk factors as hypertension (HT), DM, hyperlipidemia and smoking (never, former and current) were taken from the medical history.

Cognitive function was examined with the Cambridge Cognitive Examination (CAMCOG), which also includes the Mini Mental State Examination (MMSE). The CAMCOG consists of a memory part and non-memory part, the memory part includes orientation and memory tasks, the non-memory part includes language, attention, praxis, abstraction, calculation and perception tasks. Patients were diagnosed with Mild Cognitive Impairment (MCI), Dementia due to Alzheimer's disease (AD) or Vascular dementia (VaD). Dementia due to AD and VaD was called 'mixed dementia'. Frontotemporal dementia, Parkinson dementia and dementia with Lewy Bodies were labeled as 'other dementia'. A neurologic or psychiatric disorder was named 'other diagnosis'. If there was no evidence for cognitive impairment, or an underlying psychiatric or neurologic disorder to explain the memory complaints, patients were diagnosed with subjective cognitive impairment (SCI).

Computed tomography scan

All patients underwent a CT scan (Siemens Somatom definition AS, Erlangen, Germany) from the base of the skull to the vertex. Scans were reconstructed as oblique coronal slices of 3.0 mm, axial slices of 5.0 mm with soft tissue window and 1.5 mm axial slices in bone window.

CT scans were analyzed for BGC by one of two trained medical doctors (EdB or NG), who were blinded for the outcomes and clinical variables. BGC were scored bilateral as absent, mild, moderate or severe (figure 1) as previously described.[3] The most severe score has been used. The investigators were trained by a radiologist and before analyzing the CT-scans separately, they achieved an adequate weighted kappa (>0.7) on 100 random scans.

Statistical analysis

Descriptive statistics were used to summarize the baseline characteristics. The Chi-squared test and Mann-Whitney U test were performed to compare categorical and continuous variables, respectively, between individuals with and without BGC.

Logistic regression was used to identify vascular risk factors associated with the presence of BGC. Model 1 shows an univariable analysis, model 2 an age and gender adjusted analysis, and model 3 an analysis adjusted for age, gender, HT, DM, hyperlipidemia and smoking. Linear regression was used to determine a possible association of presence and severity of BGC with cognitive functioning. Model 1 is an univariable analysis, model 2 is adjusted for age and gender and model 3 is adjusted for age, gender and education level.

The CAMCOG total and CAMCOG memory section have a normal distribution (skewness between -1 and 1), but this was not the case for the CAMCOG non-memory section (skewness -1.089). We decided to accept this mildly skewed distribution, as with multiple transformations the skewness did not improve.

A two-sided p-value below 0.05 was considered statistically significant. Statistical analyses were performed using SPSS version 26 (IBM Corp., Armonk, New York, United States).

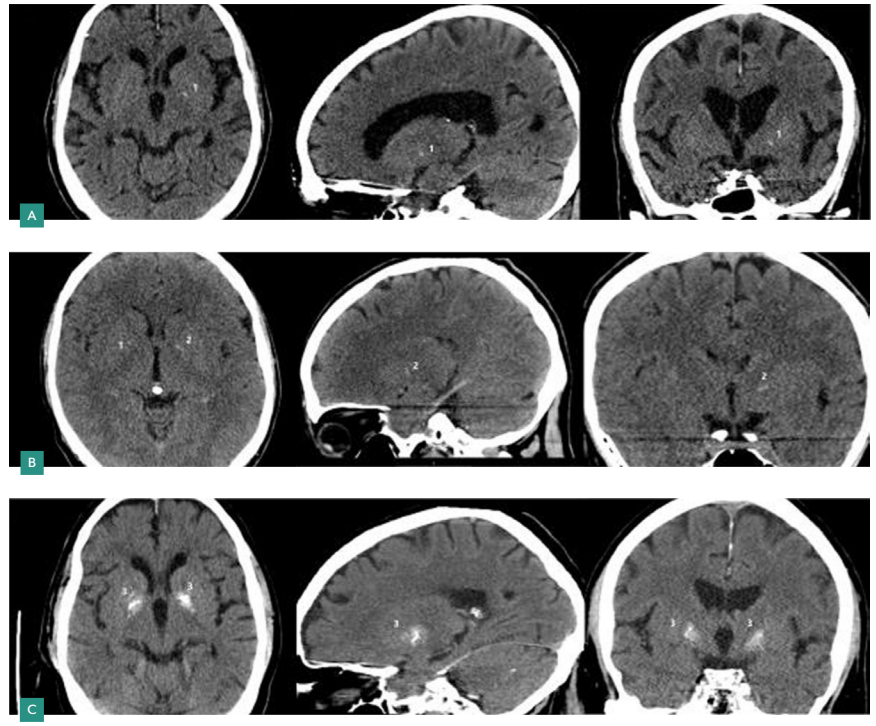

FIGURE 1A-C. CT image of basal ganglia calcifications shown in axial, sagittal and coronal plane **A.** The image shows mild BGC (1). **B.** The image shows mild (1) and moderate (2) BGC. **C.** The image shows severe BGC (3). **A-C.** This figure was published before[7]

Results

Patient characteristics

Of the 1992 patients, 866 patients (43.5%) had BGC (table 1). BGC were mild in 20.4%, moderate in 19.7% and severe in 3.3% of patients. The median age of the patients was 80 years and 803 patients (40.3%) were male. Patients with BGC were more often female (62.8% vs 57.3%, p = 0.013), and less often had HT (30.8% vs 36.4%, p = 0.009) and a medical history with TIA (4.4 vs 6.9, p = 0.016)(table 1). They were also less frequently using antihypertensive drugs (43.2% vs 50.0%, p = 0.003) (table 1). Of all variables, less than one percent of the data were missing.

Risk factors for BGC

When using logistic regression, there was a significant association of female gender with presence of BGC (model 3 odds ratio (OR) 1.27, 95%-confidence interval (CI) 1.06-1.53, p = 0.011) (table 2). A reverse association was found of BGC with HT (model 3 OR 0.74, 95%-CI 0.60-0.89, p = 0.002) (table 2). An additional analyses showed an association between presence of BGC and antihypertensive drug use (model 3 OR 0.79, 95%-CI 0.64-0.98, p =0.031). No other associations with vascular risk factors were found.

Association of BGC with cognitive function

With linear regression, we found no significant association between presence and severity of BGC and cognitive functioning (table 3 and 4). This applied to the CAMCOG, also when divided in a memory part and non-memory part, and the MMSE.

Characteristic	Total, n= 1992 (100%)	BGC absent, n= 1126 (56.5%)	BGC present, n= 866 (43.5%)	P-value
Age (yrs), median (IQR)	80 (12)	79 (11)	80 (11)	0.122
Gender, male (%)	**803 (40.3)**	**481 (42.7)**	**322 (37.2)**	0.013
Cardiovascular riskfactors, n (%) (%)				
• Hypertension	**677 (34.0)**	**410 (36.4)**	**267 (30.8)**	**0.009**
• Diabetes mellitus	317 (15.9)	174 (15.5)	143 (16.5)	0.522
• Hyperlipidemia	154 (7.7)	92 (8.2)	62 (7.2)	0.402
• Smoking, yes	227 (11.4)	125 (11.1)	102 (11.8)	0.637
Current	227 (11.4)	125 (11.1)	102 (11.8)	0.792
Former	454 (22.8)	259 (23.0)	195 (22.5)	
Never	1309 (65.7)	740 (65.7)	569 (65.7)	
Cardiovascular disorders, n (%)				
• Stroke	104 (5.2)	59 (5.2)	45 (5.2)	0.966
• TIA	**116 (5.8)**	**78 (6.9)**	**38 (4.4)**	**0.016**
• Myocardial infarction	202 (10.1)	114 (10.1)	88 (10.2)	0.978
• Peripheral arterial disease	179 (9.0)	95 (8.4)	84 (9.7)	0.329
Medication, n (%)				
• Antidiabetic drugs	267 (13.4)	150 (13.3)	117 (13.5)	0.894
• Statins	448 (22.5)	259 (23.0)	189 (21.8)	0.533
• Antihypertensive drugs	**937 (47.0)**	**563 (50.0)**	**374 (43.2)**	**0.003**
• Anticoagulant drugs	194 (9.7)	101 (9.0)	93 (10.7)	0.187

TABLE 1. Baseline characteristics by BGC presence

Age, MMSE, CAMCOG analyzed with Mann-Whitney U test, other characteristics analyzed with Chi-square test. **BGC:** basal ganglia calcification, **IQR**: Interquartile Range, yrs: years, n: number, **TIA:** Transient Ischemic Attack, **MMSE:** Mini Mental State Examination, **CAMCOG:** Cambridge Cognitive Examination, **SCI:** subjective cognitive impairment, **MCI:** Mild cognitive impairment

Characteristic	Total, n= 1992 (100%)	BGC absent, n= 1126 (56.5%)	BGC present, n= 866 (43.5%)	P-value
Cognitive tests				
• MMSE, median (range)	23 (0-30)	22 (0-30)	23 (1-30)	0.548
• CAMCOG, median (range)	76 (1-102)	76 (1-102)	76 (1-101)	0.725
Non-memory part	53 (0-67)	53 (1-67)	53 (0-67)	
Memory part	23 (0-36)	23 (0-36)	23 (1-35)	
BGC, n (%)				
• None	1126 (56.5)			
• Mild	407 (20.4)			
• Moderate	393 (19.7)			
• Severe	66 (3.3)			
Diagnosis, n (%)				
• SCI	332 (16.7)	187 (16.6)	145 (16.7)	0.936
• MCI	491 (24.6)	274 (24.3)	217 (25.1)	0.710
• Dementia	939 (47.1)	527 (46.8)	412 (47.6)	0.732
• Other	230 (11.5)	138 (12.3)	92 (10.6)	0.258
Dementia, n (%)				
• Alzheimer disease	702 (35.2)	388 (34.5)	314 (36.3)	0.404
• Vascular dementia	56 (2.8)	33 (2.9)	23 (2.7)	0.713
• Mixed dementia	128 (6.4)	74 (6.6)	54 (6.2)	0.762
• Other dementia	53 (2.7)	32 (2.8)	21 (2.4)	0.566

TABLE 1. Continued

	Univariable analysis				Age/sex adjusted analysis			Multivariable analysis		
	Model 1				Model 2			Model 3		
Risk factors	OR	95%-CI	P		OR	95%-CI	P	OR	95%-CI	P
Age	1.01	1.00-1.02	0.058		1.01	1.00-1.02	0.112	1.01	1.00-1.02	0.069
Gender	**1.26**	**1.05-1.51**	**0.013**		**1.24**	**1.03-1.48**	**0.023**	**1.27**	**1.06-1.53**	**0.011**
Hypertension	**0.78**	**0.65-0.94**	**0.009**		**0.75**	**0.62-0.90**	**0.003**	**0.74**	**0.60-0.89**	**0.002**
Diabetes mellitus	1.08	0.85-1.38	0.522		1.09	0.86-1.39	0.485	1.17	0.91-1.50	0.215
Smoking	0.98	0.92-1.04	0.502		0.99	0.94-1.04	0.572	0.99	0.94-1.04	0.635
Hyperlipidemia	0.87	0.62-1.21	0.403		0.87	0.62-1.22	0.422	V0.94	0.67-1.33	0.732

TABLE 2. Risk factors for presence of basal ganglia calcification
Data analyzed with logistic regression. Multivariable analysis adjusted for age, gender, hypertension, diabetes mellitus, smoking and hyperlipidemia. **OR** = odds ratio, **95%-CI** = 95% confidence interval.

	Univariable analysis				Age/sex adjusted			Multivariable adjusted		
	Model 1				Model 2			Model 3		
Cognitive tests	Beta	95%-CI	P		Beta	95%-CI	P	Beta	95%-CI	P
CAMCOG TOTAL	0.419	-1.07-1.91	0.582		1.080	-0.32-2.48	0.129	1.022	-0.29-2.33	0.127
Memory part	0.194	-0.49-0.88	0.579		0.515	-0.13-1.15	0.115	0.489	-0.13-1.11	0.121
Non-memory part	0.168	-0.76-1.10	0.722		0.513	-0.37-1.40	0.256	0.480	-0.35-1.31	0.256
MMSE	0.179	-0.32-0.68	0.485		0.387	-0.09-0.86	0.110	0.375	-0.08-0.83	0.105

TABLE 3. Association between presence of basal ganglia calcification and cognition
Data analyzed with linear regression. Multivariable analysis adjusted for age, gender and education level. **CAMCOG:** Cambridge Cognitive Examination, **MMSE:** Mini Mental State Examination, **95%-CI** = 95% confidence interval

	Univariable analysis				Age/sex adjusted			Multivariable adjusted		
	Model 1				Model 2			Model 3		
Cognitive tests	Beta	95%-CI	P		Beta	95%-CI	P	Beta	95%-CI	P
CAMCOG TOTAL	-0.283	-1.11-0.54	0.502		0.296	-0.48-1.07	0.453	0.370	-0.36-1.10	0.318
Memory part	-0.058	-0.44-0.32	0.765		0.222	-0.13-0.58	0.220	0.244	-0.10-0.59	0.164
Non-memory part	-0.258	-0.77-0.26	0.324		0.044	-0.45-0.54	0.862	0.095	-0.37-0.56	0.685
MMSE	-0.085	-0.36-0.19	0.550		0.095	-0.17-0.36	0.478	0.122	-0.13-0.37	0.342

TABLE 4. Association between severity of basal ganglia calcification and cognition
Data analyzed with linear regression. Multivariable analysis adjusted for age, gender and education level. **CAMCOG:** Cambridge Cognitive Examination, **MMSE:** Mini Mental State Examination, **95%-CI** = 95% confidence interval

Discussion

In this study, including 1992 patients who visited a memory clinic, we found a high prevalence (43.5%) of BGC. The majority of BGC were mild or moderate and only 3.3 % had severe BGC. We observed no association between BGC and cognitive function.

Comparison with previous literature concerning the association between BGC and cognitive function is not possible as, to our knowledge, this is the first study to assess this association. Yet, we hypothesized that BGC would be associated with cognitive impairment, especially with executive dysfunction. We therefore particularly expected to find an association with the non-memory part of the CAMCOG. However, assessment of executive function in the non-memory part of the CAMCOG is limited. This could explain why we did not observe an association, despite our large cohort. Further research with more comprehensive cognitive analysis of executive functions are needed to confirm our findings. In patients with PFBC, who have extensive calcifications in the basal ganglia, cognitive problems are common.[12] It is possible that only extensive calcifications cause cognitive problems. In our experience even the severe calcifications in our cognition cohort were less extensive than calcifications in patients with PFBC. Another explanation could be that BGC are not responsible for cognitive dysfunction and that cognitive problems in patients with PFBC are caused by other mechanisms than the calcifications in the basal ganglia.

The prevalence of BGC in this population is 43.5%. This high incidence is likely explained by the relatively high age of patients in this cohort (median age of 80 years) and in line with a prevalence of 38.7% found in another study with an older population.[19] We found an association between presence of BGC and female gender. Although this is consistent with a prior report, we don't have a clear explanation for this finding.[20]

A recent study in patients at risk for cerebrovascular disease showed no relation with vascular risk factors.[7] Our results are consistent with this study and support the previously suggested hypothesis that BGC are non-atherosclerotic and are located in the tunica media of small vessels.[2-6] We also did not find an association with DM, which is known to be associated with tunica media calcifications, even in the same study population.[8-10]

Although different studies reported about a positive association between HT and media calcification,[8, 21] we found an inverse association between BGC and HT. A possible explanation can be underrepresentation of patients with HT and severe cardiovascular disease by selective mortality, given the relatively old age in this cohort. Or one can hypothesize that antihypertensive drugs, specifically calcium channel blockers and inhibitors of the renin–angiotensin–aldosteron system, lead to less arterial calcification by their effect on vascular smooth muscle cells.[20, 22] Although we do not have information about which specific type of antihypertensive drugs patients used, the fact that we also found an inverse association with antihypertensive drugs can support this hypothesis. However, it does not explain the discrepancy with earlier studies and we have no data to substantiate this speculation.

A strength of this study is the large sample size of 1992 patients. Also the extensive standard routine workup, including a cognitive screening and a CT scan of the brain, gave little missing data. A CT scan is low burdensome and easily accessible in memory clinics, which led to exclusion of only eight patients.

This study has some limitations. First, the cross-sectional design asks for caution regarding causative relationships and direction of causality. Secondly, data about vascular risk factors are based on history taking, which could have led to an underestimation of the number of patients with DM, HT and hyperlipidemia. However, we knowingly chose not to take medication use in account, as this could have led to an overestimation of the number of patients with HT and hyperlipidemia, since the indication for antihypertensive drugs and statins is also determined by other diagnoses.

Also in a population with a median age of 80 years, selective mortality may influence associations. This may have led to underestimation of the associations found in this study.

Finally, thin slice CT reconstructions were not available for the soft tissue settings, which could have led to missing subtle calcifications and thereby to an underestimation of the prevalence of BGC. However, it is more likely that severe calcifications in particular would affect cognitive function.

Conclusions

This study showed that BGC are a frequent finding with CT in patients referred to a memory clinic because of cognitive complaints. No association with cognitive function was found. Risk factors for BGC were female gender, while HT and antihypertensive drug use were associated with a lower risk of BGC.

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CHAPTER 7

Hippocampal calcifications: risk factors and association with cognitive functioning

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Abstract

Purpose: To identify risk factors for hippocampal calcifications and to investigate the association of hippocampal calcification with cognitive function.

Method: For this retrospective study, consecutive patients visiting a memory clinic at a Dutch general hospital between April 2009 and April 2015 were identified. All individuals underwent a standard diagnostic work-up including cognitive tests and brain CT scan. The following vascular risk factors were assessed: hypertension, diabetes mellitus, hyperlipidemia and smoking. Cognitive screening consisted of the Cambridge Cognitive Examination, which includes the Mini Mental State Examination. CT scans were analyzed for presence and severity (absent, mild, moderate, severe) of hippocampal calcifications. One measure per patient, only the most severe score, was used. We used logistic regression to identify risk factors for hippocampal calcifications and linear regression for the association between hippocampal calcifications (patient level) and cognitive function.

Results: A total of 1991 patients (mean age 78 years (females 79 vs males 77), range 45;96, (females 47;96 vs males 45;95)) were included, of whom 380 (19.1%) had hippocampal calcifications. Older age (odds ratio [OR] per year 1.05, 95% confidence interval [CI] 1.03-1.06), diabetes mellitus (OR 1.50, 95% CI 1.12-2.00) and smoking (OR 1.49, 95% CI 1.05-2.10) were associated with the presence of hippocampal calcifications. No associations were found between presence and severity of hippocampal calcification and cognitive function.

Conclusion: Older age, diabetes mellitus and smoking were associated with an increased risk of hippocampal calcifications. Greater degree of hippocampal calcifications was not associated with lower cognitive function in patients with memory complaints.

Hippocampal calcifications: risk factors and association with cognitive functioning

Introduction

Dementia is a substantial health problem, with 46.8 million people having this condition worldwide.[1] Dementia mostly appears to result from a combination of factors, including Alzheimer disease (AD), vascular lesions, Lewy bodies, and inflammation, which eventually leads to atrophy of the cortex and hippocampus.[2] The hippocampus is an important area of interest in dementia research. Current research into hippocampal abnormalities in dementia focuses more on neurodegenerative causes and less on vascular causes. Hippocampal calcifications were first described in a pathology study in 2002 as a vasculopathy with fibrosis and calcification with a predilection for the middle hippocampal artery.[3] These calcifications can spread from the tail to the body of the hippocampus and occasionally to the head and may lead to patchy neuronal loss. Accordingly, it has been hypothesized that hippocampal calcifications may be a manifestation of vascular abnormalities that could contribute to hippocampal atrophy and, consequently, cognitive deterioration.[3]

Advances in radiologic imaging have provided opportunities to explore the role of hippocampal calcifications in dementia. In a study in which multiplanar brain computer tomography (CT) scans were used, hippocampal calcifications were frequently observed and appeared to increase with age, with hippocampal calcifications detected in more than 20% of individuals older than 50 years.[4] Hippocampal calcifications may be difficult to distinguish from plexus calcifications because of the proximity of these structures, and before multiplanar CT became available, hippocampal calcification may have been mistaken for choroidal calcification. The literature on the association between hippocampal calcification and cognitive impairment is limited to a small case-control study in which hippocampal calcifications were found more often in patients from a memory clinic than in matched control subjects. Moreover, individuals with hippocampal calcifications were found to have a lower score on the Mini Mental State Examination (MMSE).[5]

We hypothesized that patients with hippocampal calcifications are more likely to have vascular risk factors and that hippocampal calcifications are associated with lower scores on cognitive tests.

The aim of our study was to identify vascular risk factors for hippocampal calcifications in a large cohort of individuals who visited an outpatient memory clinic. Furthermore, we aimed to evaluate the clinical importance of hippocampal calcifications by studying the association between hippocampal calcifications and cognitive function.

Methods

Study population

Our retrospective cross-sectional study consisted of 2000 consecutive patients who visited the memory clinic of Tergooi hospital, a general hospital in the Netherlands, between April 2009 and April 2015. All 2000 patients were included in a previously published article investigating the prevalence of mixed dementia in patients with late-onset AD.[6] Herein, we report the risk factors of hippocampal calcifications and the association with cognitive function.

The inclusion criterion for this study was referral to the memory clinic of Tergooi hospital between April 2009 and April 2015 because of memory complaints. All patients underwent a standard diagnostic work-up, including a brain CT scan. The only exclusion criterion for our study was absence of a brain CT scan, which led to exclusion of nine patients. None of the CT scans had to be excluded because of motion artifacts. Of the 1991 remaining patients, 40.3 % were male and the mean patient age was 78 years (range, 45-96).

The local medical ethics committee of Tergooi Hospital approved our study and waived the requirement to obtain informed consent.

Diagnostic Procedures

All patients referred to the memory clinic underwent a standard diagnostic work-up including history taking; medical and neurological examinations; assessment of vital functions; assessment of education level according to Verhage (details in Appendix);7 cognitive screening including the Cambridge Cognitive Examination (CAMCOG) which contains the MMSE (full details in the Appendix);8, 9 electrocardiography; laboratory tests; head CT; and history taking with a relative or other acquaintance. The CAMCOG is a well validated cognitive evaluation and includes the most important cognitive domains that might be impaired in dementia. It has a high sensitivity and specificity (92% and 96%, respectively) for the detection of cognitive decline.8, 9 Vascular risk factors including hypertension, diabetes mellitus (DM), hyperlipidemia, and smoking status were assessed during history taking (yes/no). Smoking status was classified into smoking or nonsmoking (also including previous smoking). Finally, a diagnosis was established, including mild cognitive impairment, AD, or Vascular Dementia, according to the standard clinical diagnostic criteria (further details are in Appendix).10, 11, 12

CT protocol

All patients underwent brain CT. A 64-detector row CT scanner (Siemens Somatom Definition AS, Siemens Healthineers, Erlangen, Germany) was used to scan patients from the base of the skull to the vertex. The acquisition parameters were as follows: 120 kV; 260 mAs; collimation, 64 x 0.6 mm; pitch, 0.55; window center 40 HU; and window width, 80 HU. The CARE kV tool (dose optimation slider for noncontrast examinations) was used. Scans were reconstructed as oblique coronal sections of 3.0 mm, axial sections of 5 mm with soft-tissue window, and 1.5-mm axial sections in bone window.

Hippocampal Calcifications

CT scans were analyzed for hippocampal calcifications by one physician (E.J.M.d.B. or R.K.) who was blinded to clinical outcomes and all risk factors except age and gender. All CT scans were analyzed during a time span of 2 weeks. One physician (E.J.M.d.B.) has 5 years of clinical experience in geriatrics. The other physician (R.K.) has 3 years of experience in the assessment

of CT images during his doctoral degree program. These investigators were trained by a vascular radiologist (P.A.d.J., with 10 years of experience) and by a neuroradiologist (J.H., with 15 years of experience). Images were analyzed in axial and coronal planes in the brain window setting (Center, 40 HU; Width, 80 HU) with use of a previously established scoring system.5 Calcifications were scored according to presence and severity, as follows: absent, mild (one high-attenuation area)(figure 1), moderate (multiple high-attenuation areas)(figure 2) or severe (confluent)(figure 3). Both hippocampi were scored separately. In the analysis of severity, the more severe calcification score, either for right or for left hippocampi, was used. In the analysis of the presence of hippocampal calcifications, hippocampal calcifications were considered present if a calcification was scored in at least one hippocampus. In this way, we used only one measure for hippocampal calcification presence and one measure for hippocampal calcification severity per patient.

Statistical analysis

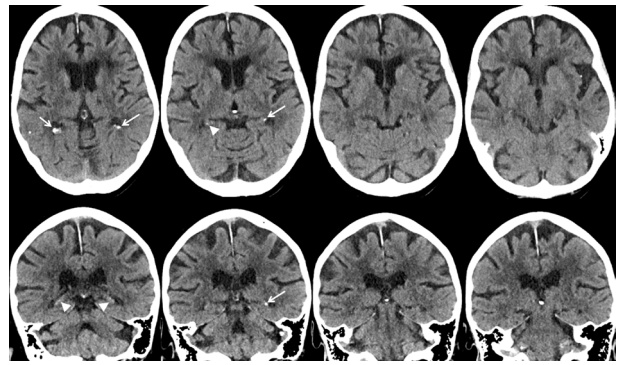

FIGURE 1. Mild hippocampal calcification on CT examination (arrowheads) of an 88-year old female. The image shows mild hippocampal calcification (arrowheads) in the axial and coronal plane. The arrows show calcification of the choroid plexus.

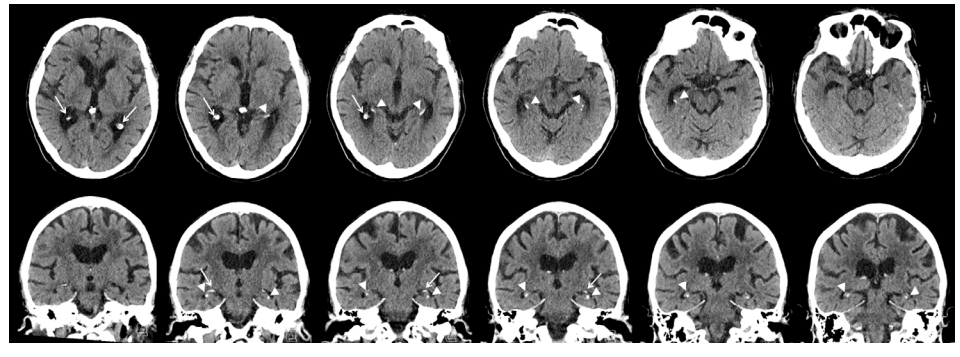

FIGURE 2. Moderate hippocampal calcification on CT examination (arrowheads) of a 74-year old female. The image shows mild hippocampal calcification (arrowheads) in the axial and coronal plane. The arrows show calcification of the choroid plexus.

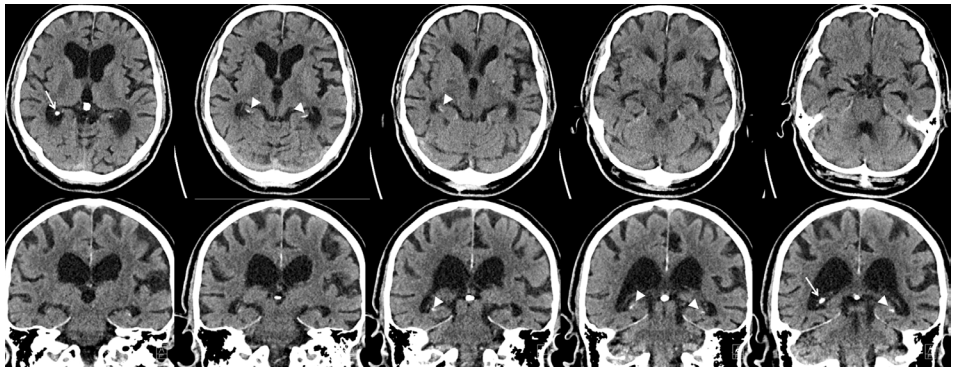

FIGURE 3. Severe hippocampal calcification on CT examination (arrowheads) of a 76-year old man. The image shows mild hippocampal calcification (arrowheads) in the axial and coronal plane. The arrows show calcification of the choroid plexus.

Descriptive statistics were used to summarize the baseline characteristics. The Chi-squared test (for trend) and Mann-Whitney U test were performed to compare categorical and continuous variables, respectively, between individuals with and without hippocampal calcification. Logistic regression was performed to identify risk factors (explanatory variables) associated with the presence of hippocampal calcifications (outcome variable), including univariable analyses (model 1), age- and sex adjusted analyses (model 2), and analyses adjusted for age, sex, hypertension, DM, hyperlipidemia, and smoking status (model 3). The Chi-squared test was used to analyze whether number of risk factors (age >80 years, DM, and smoking) was associated with severity of hippocampal calcifications. Linear regression was used to analyze whether hippocampal calcification (explanatory variable) were associated with cognitive function (outcome variable). A reflect and square root transformation (√((largest value of outcome variable + 1) – original value of outcome variable)) was used to obtain a normal distribution, checked with the Q-Q plot, of the CAMCOG and MMSE outcomes. The beta levels obtained after transformation of the outcome variables (MMSE or CAMCOG) to a normal distribution are presented in the Cognitive Outcomes section. Univariable analyses (model 1), age- and sex-adjusted analyses (model 2), and analysis adjusted for age, sex, and education level (model 3) were performed. In addition, subgroup analyses were performed for patients with mild cognitive impairment, AD, or vascular dementia. Patients with missing values on the cognitive tests were excluded from the regression analyses. Two-sided P, .05 was considered indicative of a statistically significant difference. The Cohen Kappa was used to estimate the interobserver agreement of hippocampal calcification presence (yes or no) in a sample of 50 CT scans. Statistical analyses were performed with software (SPSS, version 24; IBM, Armonk, NY).

Results

Patients

The cohort consisted of 2000 patients. Nine patients were excluded from the analysis because there was no CT scan of the brain present. This resulted in a total of 1991 patients. Of the 1991 patients, 380 (19.1%) had hippocampal calcifications (table 1). The interobserver agreement was good (kappa coefficient of 0.80 and 0.78 when weighted for severity scores).

Risk Factors for Hippocampal Calcifications

Before correcting for confounders, we found that patients with hippocampal calcification were older than those without hippocampal calcification (mean age, 81 vs 78 years, respectively; p-value <0.01) and had a lower education level (mean level, 4.20 vs 4.37, respectively; p-value 0.05). More patients with hippocampal calcification had a history of DM (20.8% vs 14.8%, p-value 0.01) (table 1). Other vascular risk factors were similarly prevalent among both groups. When we corrected for confounders, we found that older age (odds ratio (OR), 1.05; 95% confidence interval (CI): 1.03-1.06, p-value < 0.01), DM (OR, 1.50; 95% CI: 1.12-2.00, p-value < 0.01) and smoking (OR, 1.49; 95% CI: 1.05-2.10, p- value 0.02) were associated with the presence of hippocampal calcification (table 2, model 2). Education level was no longer associated with the presence of hippocampal calcifications (OR, 0.95; 95% CI: 0.88-1.02, p-value 0.17). Hypertension (OR, 0.91; 95% CI: 0.71-1.17, p-value 0.47) and hyperlipidemia (OR, 0.89; 95% CI: 0.57-1.39, p-value 0.60) were not associated with a higher risk of hippocampal calcifications. The number of risk factors (age > 80 years, DM, smoking) was associated with the severity of hippocampal calcifications ($p < 0.01$). In patients without hippocampal calcifications or with mild calcifications, 11.1% had two risk factors and 0.5% had three risk factors. In patients with moderate or severe calcifications 17.0% had two risk factors and 3.1% had three risk factors (table 3).

Cognitive Outcomes

Of the 1991 patients in our cohort, most (n = 829, 41.6%) had AD, followed by mild cognitive impairment (n = 490, 24.6%) and subjective cognitive impairment (n = 332, 16.7%) (Appendix table 1). Less than 1% of patients had missing values from the CAMCOG and MMSE.

When analysis was uncorrected for confounders, the total CAMCOG score in patients with hippocampal calcifications was lower than that in patients without calcifications (median score, 75 vs 76; p-value 0.04). After correction for confounders, we did not find differences in outcomes of the CAMCOG (beta = -0.05, p-value 0.48) or MMSE (beta = -0.08, p-value 0.09) between patients with and patients without hippocampal calcifications (table 4). There were also no differences in cognitive function when data were analyzed according to severity (p-values 0.37 for CAMCOG and 0.07 for MMSE)(table 4). Analysis according to subgroups of patients with mild cognitive impairment, AD, or vascular dementia gave the same results, with p-values ranging from 0.11 to 0.67 (Appendix tables 2, 3, 4).

Characteristic	Total, N= 1991 (100%)	HC present, n= 380 (19.1%)	HC absent, n= 1611 (80.9%)	P-value
Age (y), mean (IQR)	78 (45;96)	81 (62;95)	78 (45;96)	<0.01
No. of men (%)	802 (40.3)	164 (43.2)	638 (39.6)	0.22
Education level, mean (SD)	4.34 (1.5)	4.20 (1.5)	4.37 (1.5)	0.05
Cerebral infarct, n (%)	104 (5.2)	13 (3.4)	91 (5.6)	0.10
TIA, n (%)	116 (5.8)	25 (6.6)	91 (5.6)	0.47
Hypertension, n (%)	675 (33.9)	129 (33.9)	546 (33.9)	1.00
Diabetes mellitus, n (%)	317 (15.9)	79 (20.8)	238 (14.8)	0.01
Hyperlipidemia, n (%)	153 (7.7)	27 (7.1)	126 (7.8)	0.75
Smoking, n (%)	228 (11.5)	51 (13.4)	177 (11.0)	0.18
MMSE score, median (IQR)	23 (18;26)	22 (18;26)	23 (18;26)	0.39
CAMCOG score, median (IQR)	76 (63;85)	75 (61;84)	76 (63;86)	0.04

TABLE 1. Baseline characteristics by hippocampal calcification presence

Age, Mini Mental State Examination (MMSE) score, Cambridge Cognitive Examination (CAMCOG) score were analyzed with the Mann-Whitney U test. Other characteristics were analyzed with Chi-square test, with education analyzed with the Chi-square test for trend. **N:** number, **HC:** hippocampal calcification, **IQR:** Interquartile Range, yrs: years, **SD:** standard deviation, **TIA:** Transient Ischemic Attack

	Univariable analysis				Age/sex adjusted analysis			Multivariable analysis		
	Model 1				Model 2			Model 3		
Risk factors	OR	CI	P		OR	CI	P	OR	CI	P
Age	1.04	1.03-1.06	<0.01		1.05	1.03-1.06	<0.01	1.05	1.03-1.06	<0.01
Sex	0.86	0.69-1.08	0.20		0.78	0.62-0.98	0.03	0.81	0.64-1.02	0.07
Hypertension	1.00	0.79-1.27	0.98		0.95	0.75-1.21	0.68	0.91	0.71-1.17	0.47
Diabetes mellitus	1.51	1.14-2.01	<0.01		1.47	1.10-1.95	<0.01	1.50	1.12-2.00	<0.01
Smoking	1.25	0.90-1.75	0.18		1.50	1.06-2.11	0.02	1.49	1.05-2.10	0.02
Hyperlipidemia	0.90	0.59-1.39	0.64		0.91	0.59-1.41	0.67	0.89	0.57-1.39	0.60
Education level	0.93	0.86-1.00	0.05		0.94	0.87-1.01	0.10	0.95	0.88-1.02	0.17

TABLE 2. Risk factors for presence of hippocampal calcification

Data analyzed with logistic regression. Multivariable analysis adjusted for age, gender, hypertension, diabetes mellitus, smoking and hyperlipidemia. **OR:** Odds ratio, **CI:** confidence interval.

Discussion

The results of our cross-sectional study in patients attending a memory clinic showed that older age, DM, and smoking were associated with the presence of hippocampal calcifications. The presence and higher severity of hippocampal calcification were not associated with lower cognitive function in our cohort of memory clinic patients, which included patients with subjective cognitive impairment, mild cognitive impairment, dementia due to AD, and vascular dementia. There currently are limited data on risk factors for hippocampal calcifications. To our knowledge, only three previous articles have been published on hippocampal calcifications.[3-5] The prevalence of hippocampal calcification in our study was 19.1%, which is comparable to the 21.7% observed in an Australian university hospital study of 217 randomly selected CT scans of patients older than 50 years, although another study observed a higher prevalence of 38.8% in 67 patients visiting a memory clinic and a prevalence of 13.4% in the control group.[4,5] Both studies also used multiplanar CT scans.[4,5] All studies showed a strong association between hippocampal calcification and older age. The results of our study are consistent with this finding. One previous study assessed the association of vascular risk factors with hippocampal calcification.[4] There was no significant difference in the number of vascular risk factors in patients with hippocampal calcification or those without hippocampal calcification. However, in this previous study, the sample of 47 patients with hippocampal calcification compared with 253 patients without hippocampal calcifications was too small to draw any firm conclusions.[4] In our study, we showed an association of hippocampal calcification with DM and smoking, but not with other vascular risk factors. Therefore, hippocampal calcification could be a marker of a vascular abnormality. Wegiel et al (3) performed a pathology study and described a non-arteriosclerotic process of vasculopathy with fibrosis and calcification. The lack of association with hypertension and dyslipidemia may support nonatherosclerotic arterial calcifications, although this remains speculative until further verification with histologic assessment is performed. Our findings support that these calcifi-

Number of risk factors	Severity of hippocampal calcification			Severity of hippocampal calcification		Total
	No HC	Mild HC		Moderate HC	Severe HC	
0	600 (85.2)	61 (8.7)		32 (4.5)	11 (1.6)	704 (100)
1	840 (80.7)	117 (11.2)		59 (5.7)	25 (2.4)	1041 (100)
2	160 (69.6)	43 (18.7)		22 (9.6)	5 (2.2)	230 (100)
3	9 (64.3)	0 (0.0)		3 (21.4)	2 (14.3)	14 (100)
Total	1609	221		116	43	1989 (100)

TABLE 3. Number of risk factors and severity of hippocampal calcification

Data are numbers of patients, with percentages in parentheses. There was a significant between the groups, p-value <0.01. Data were analyzed with the Chi-squared test. Risk factors are age older than 80 years, diabetes mellitus and smoking. HC = hippocampal calcification.

	Univariable analysis				Age/sex adjusted analysis			Multivariable analysis		
	Model 1				Model 2			Model 3		
Cognitive tests	Beta	CI	P		Beta	CI	P	Beta	CI	P
	Hippocampal calcification presence				Hippocampal calcification presence					
CAMCOG	0.18	0.00-0.35	0.05		-0.00	-0.16-0.16	0.99	-0.05	-0.20-0.10	0.48
MMSE	0.05	-0.05-0.16	0.33		-0.06	-0.16-0.05	0.28	-0.08	-0.18-0.01	0.09
	Hippocampal calcification severity				Hippocampal calcification severity					
CAMCOG	0.05	-0.05-0.15	0.32		-0.02	-0.11-0.08	0.72	-0.04	-0.13-0.05	0.37
MMSE	0.00	-0.06-0.07	0.93		-0.04	-0.10-0.02	0.17	-0.05	-0.11-0.00	0.07

TABLE 4. Association between hippocampal calcification and cognition

Data were analyzed with linear regression. Multivariable analysis was adjusted for age, sex, and education level. CI = confidence interval, CAMCOG = Cambridge Cognitive Examination, MMSE = Mini Mental State Examination.

cations may be of vascular origin. Currently, if radiologists decide to report hippocampal calcifications in a radiology report, we suggest reporting the presence of hippocampal calcification as mild, moderate, or severe (vascular) calcifications in the hippocampus on the right or left side, with the remark that this is an incidental finding of currently unknown clinical relevance. Given the location of hippocampal calcifications in the hippocampal tail and the association with some vascular risk factors, we hypothesized that hippocampal calcifications could be associated with cognitive decline. In a pilot study of patients visiting a memory clinic,[5] hippocampal calcifications were three times more common in these patients than in control subjects and were associated with lower MMSE scores. In our study, however, we did not find a significantly lower score on any of the cognitive tests in patients with hippocampal calcifications, including the MMSE. A possible explanation for this discrepancy is the correction for age, sex, and education level in our study; the pilot study did not correct for these

potential confounding factors. Hippocampal calcification does not seem to be associated with cognitive decline. Further population-based cohort studies are needed to confirm our findings. There are limitations of our study that must be taken into account when interpreting the data. First, there was a referral bias because all patients were referred to a memory clinic. Although some patients did not have cognitive impairment at examination, they did have memory complaints for which they had been referred. We did not have a control group of persons without memory complaints, which might have led to an underestimation of the association between hippocampal calcification and cognition. Thin-section reconstructions of the CT scans were unavailable, which could have led to an underestimation of the presence of hippocampal calcifications if subtle calcifications were missed. However, if hippocampal calcifications had an effect on cognitive function, we would expect to find this with the more severe calcifications and not with the subtle calcifications we might have missed. Another lim-

itation is the measurement of cardiovascular risk factors. The presence of cardiovascular risk factors was based on history taking. This may have led to misclassification, especially in patients with memory complaints, because some patients classified as not having a cardiovascular risk factor actually did have the risk factor as determined with laboratory tests or the use of medication. This might have led to an underestimation of the associations that were found between the risk factors and the presence of hippocampal calcifications. In addition, the smoking classification was arbitrary, because nonsmokers may have stopped smoking recently. This could have led to an overestimation of the risk of smoking. In conclusion, older age, DM, and smoking appear to be associated with an increased risk of hippocampal calcifications on CT images, which suggests that these calcifications may be of vascular origin. The findings from our study do not support hippocampal calcifications as a marker of cognitive impairment in patients visiting a memory clinic, although further confirmation is needed.

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Appendix

Details of diagnostic procedure

Education levels were classified according to Verhage, a Dutch classification system including 7 categories; 1= did not finish primary school, 2= finished primary school, 3= did not finish secondary school, 4= finished secondary school, low level, 5= finished secondary school, medium level, 6= finished secondary school, highest level, and/or college degree, 7= university degree.[1]

Cognitive screening consisted of the Cambridge Cognitive Examination (CAMCOG) (part B of the Cambridge Examination for Mental Disorders). The CAMCOG consists of 67 items with a maximum score of 107 and can be divided into the following subscales: orientation, expressive and comprehensive language, memory (remote, recent and learning), attention, praxis, calculation, abstraction and perception.[2, 3] All items of the MMSE are incorporated into the CAMCOG.

Following the diagnostic work-up, a consensus meeting with a geriatrician, neurologist, neuropsychologist and a specialized nurse took place, during which a diagnosis was made. A diagnosis of Mild Cognitive Impairment, AD and Vascular Dementia was made according to the standard clinical diagnostic criteria at that time.[4, 5, 6] Patients were diagnosed with other forms of dementia (including Frontotemporal Dementia, Parkinson Dementia, Dementia with Lewy Bodies), or other diagnoses (i.e., a neurologic or psychiatric disorder) also according to the guidelines at that time.[7, 8, 9] If cognitive testing was normal and there was no sign of an underlying psychiatric or neurologic disorder to explain the memory complaints, patients were diagnosed with subjective cognitive impairment.

Diagnosis	Total, n= 1991 (100%)	HC present, n= 380 (19.1%)	HC absent, n= 1611 (80.9%)	P-value
SCI, n (%)	332 (16.7)	55 (14.5)	277 (17.2)	0.22
MCI, n (%)	490 (24.6)	91 (23.9)	399 (24.8)	0.79
AD, n (%)	829 (41.6)	173 (45.5)	656 (40.7)	0.09
VaD, n (%)	56 (2.8)	11 (2.9)	45 (2.8)	0.86
Other dementia, n (%)	53 (2.7)	7 (1.8)	46 (2.9)	0.37
Other diagnosis, n (%)	231 (11.6)	43 (11.3)	188 (11.7)	0.93

APPENDIX TABLE 1. Diagnosis by hippocampal calcification presence

Data analyzed with Chi-square test. SCI: Subjective Cognitive Impairment, MCI: Mild Cognitive Impairment, AD: Alzheimer disease, VaD: Vascular Dementia.

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	Univariable analysis				Age/sex adjusted analysis			Multivariable analysis		
	Model 1				Model 2			Model 3		
Cognitive tests	Beta	CI	P		Beta	CI	P	Beta	CI	P
CAMCOG	-0.02	-0.24-0.21	0.90		-0.02	-0.23-0.19	0.85	-0.07	-0.27-0.12	0.46
MMSE	-0.02	-0.17-0.13	0.80		-0.03	-0.18-0.12	0.70	-0.06	-0.20-0.09	0.43

APPENDIX TABLE 2. Association between presence of hippocampal calcification and cognition in patients with Mild Cognitive Impairment

Data analyzed with linear regression. Multivariable analysis adjusted for age, gender and education level. CI: confidence interval, CAMCOG: Cambridge Cognitive Examination, MMSE: Mini Mental State Examination.

	Univariable analysis				Age/sex adjusted analysis			Multivariable analysis		
	Model 1				Model 2			Model 3		
Cognitive tests	Beta	CI	P		Beta	CI	P	Beta	CI	P
CAMCOG	0.12	-0.08-0.32	0.25		0.10	-0.10-0.30	0.31	0.06	-0.13-0.24	0.54
MMSE	0.00	-0.12-0.12	0.98		-0.00	-0.13-0.12	0.95	-0.03	-0.14-0.09	0.67

APPENDIX TABLE 3. Association between presence of hippocampal calcification and cognition in patients with Alzheimer disease

Data analyzed with linear regression. Multivariable analysis adjusted for age, gender and education level. CI: confidence interval, CAMCOG: Cambridge Cognitive Examination, MMSE: Mini Mental State Examination.

	Univariable analysis				Age/sex adjusted analysis			Multivariable analysis		
	Model 1				Model 2			Model 3		
Cognitive tests	Beta	CI	P		Beta	CI	P	Beta	CI	P
CAMCOG	-0.70	-1.43-0.03	0.06		-0.68	-1.37-0.02	0.06	-0.50	-1.19-0.19	0.15
MMSE	-0.52	-1.04-0.00	0.05		-0.52	-1.05-0.00	0.05	-0.43	-0.96-0.10	0.11

APPENDIX TABLE 4. Association between presence of hippocampal calcification and cognition in patients with Vascular Dementia

Data analyzed with linear regression. Multivariable analysis adjusted for age, gender and education level. CI: confidence interval, CAMCOG: Cambridge Cognitive Examination, MMSE: Mini Mental State Examination.

CHAPTER 8

Intracranial artery calcifications: Risk factors and association with cardiovascular disease and cognitive function

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Abstract

Background and aims: we know little about clinical outcomes of arterial calcifications. This study investigates the risk factors of intracranial artery calcifications and its association with cardiovascular disease and cognitive function.

Methods: patients were recruited from a Dutch memory clinic, between April 2009 and April 2015. The intracranial internal carotid artery (iICA) and basilar artery were analyzed on the presence of calcifications. Calcifications in the iICA were also assessed on severity and location in the tunica intima or tunica media. Using logistic regression, risk factors of intracranial artery calcifications were analyzed, as well as the association of these calcifications with cardiovascular disease, cognitive function and type of cognitive disorder (including subjective cognitive impairment, mild cognitive impairment and dementia). Cognitive function was assessed with the Cambridge Cognitive Examination.

Results: 1992 patients were included (median age: 78.2 years, ±40% male). The majority of patients had calcifications in the iICA (±95%). Basilar artery calcifications were less prevalent (±8%). Risk factors for cerebral intracranial calcifications were age ($p < 0.001$), diabetes mellitus (medial iICA, $p = 0.004$), hypertension (intimal iICA, $p < 0.001$) and basilar artery, $p = 0.019$) and smoking (intimal iICA, $p = 0.008$). iICA calcifications were associated with stroke and intimal calcifications also with myocardial infarction. Intracranial artery calcifications were not associated with cognitive function or type of cognitive disorder.

Conclusion: the majority of memory clinic patients had intracranial artery calcifications. Cardiovascular risk factors are differentially related to medial or intimal iICA calcifications. iICA calcifications were associated with myocardial infarction and stroke, but not with cognitive outcomes.

Intracranial artery calcifications: Risk factors and association with cardiovascular disease and cognitive function

Introduction

Arterial calcifications are often seen on imaging, but the exact implications are mostly unclear. Arterial calcifications can be situated in the intimal layer and the medial layer, which consists of the tunica media and the internal elastic lamina.[1] There seem to be differences in the risk factors of intimal and medial calcifications. A study with patients suspected of stroke showed that risk factors for intimal calcifications were older age, smoking, hypertension and a positive family history for vascular diseases, whereas risk factors for medial calcifications were besides older age and a positive family history, also diabetes mellitus and previous vascular disease.[2] In addition to the differences in risk factors there are also differences in consequences of intimal and medial calcifications. Calcifications in the intimal layer are atherosclerotic lesions and are associated with stenosis of the artery.[1] In contrast, an autopsy study found that calcifications in the medial layer of the intracranial internal carotid artery (iICA) were histologically non-atherosclerotic.[3] Calcifications in this layer give stiffening of the artery with an increase of pulse wave velocity and pulse pressure, instead of stenosis resulting in chronic damage of the tissue the artery supplies.[1, 4]

Studies only investigated the clinical consequences of arterial calcifications, without taking the differences between intimal and medial calcifications into account. Most studies focused on coronary artery calcifications (CAC). It has been shown that CAC is associated with coronary artery disease, but also cerebrovascular disease.[5] There are some studies that focused on intracranial artery calcifications. They found an association between these calcifications and cerebrovascular disease.[5, 6] There are no studies about intracranial artery calcifications in association with vascular disease outside of cerebrovascular disease. There is some evidence that intracranial artery calcifications are associated with cognitive related outcomes as a smaller total brain volume, a smaller volume of the white matter, worse cognitive function and dementia.[7, 8, 9, 10]

In summary, most previous studies on clinical outcomes of arterial calcifications have focused on CAC. Furthermore, few studies on clinical outcomes have taken the differences between intimal and medial calcifications into account. As mentioned, a lot of patients receive imaging during their life, but the implications of possible found arterial calcifications are unclear. For example, most patients with cognitive complaints referred to a memory clinic receive cerebral imaging, usually with magnetic resonance imaging (MRI) or computed tomography (CT). Especially with CT it is possible to measure arterial calcifications. Therefore, the memory clinic population can be used to investigate the presence of intimal and medial intracranial artery calcifications, as well as risk

factors and clinical outcomes of these calcifications.

In this study, we aimed to investigate the risk factors of intracranial artery calcifications and the association of these calcifications with cardiovascular disease and cognitive function. Furthermore, we investigated whether these associations are different for intimal and medial calcifications.

Methods

Patients were recruited from the memory clinic in Tergooi Hospital, the Netherlands, between April 2009 and April 2015, into this cross-sectional study. This study population was previously described.[11] Patients were excluded if there was no CT-scan performed. All patients were examined by the memory clinic team consisting of a geriatrician, neurologist, neuropsychologist and nurse. All patients underwent a standard diagnostic work-up including medical and neurological examinations, assessment of vital functions, assessment of education level, cognitive testing, laboratory tests, electrocardiography and imaging of the brain. A CT-scan of the brain was the standard imaging performed and only rarely a CT-scan was not performed.

Imaging variables

A 64-detector row CT-scanner (Somatom Definition AS; Siemens Healthineers, Erlangen, Germany) was used to scan patients from the base of the skull to the vertex. The acquisition parameters were as follows: 12 kV, 260 mAs, collimation 64 × 0.6 mm, pitch 0.55, window center 40 HU and window width 80 HU. The CARE kV tool (dose optimization slider for non-contrast examinations) was used. Scans were reconstructed as oblique coronal sections of 3.0 mm, axial sections of 5 mm with soft tissue window and axial sections of 1.5 mm in bone window.

All CT-scans were assessed for calcification in the iICA and in the basilar artery. The iICA calcifications were scored using a previously developed scoring model by Kockelkoren et al.[1] Points were awarded for different morphological aspects of calcifications (circularity, thickness and continuity of calcification). Based on the total score, the calcifications were defined as predominantly intimal (score <7), predominantly medial (score ≥7) or absent/indistinguishable.[1] The severity of the calcifications were scored according to a four-point scoring system (none, mild, moderate or severe), previously described by Woodcock et al.[12] The basilar artery calcifications were divided into absence or presence of calcifications. All CT-scans were analyzed for calcifications by one of the authors (NG or EdB) who was blinded for the risk factors and outcomes. Before independently analysing the CT-scans, an adequate weighted kappa (>0.5) between the observers was achieved.

Clinical variables

The following data was retrieved from the patient files: age, sex, cardiovascular risk factors (including hypertension, diabetes mellitus, hypercholesterolemia and smoking) and previous cardiovascular diseases (including myocardial infarction, stroke, TIA and peripheral arterial disease).

Hypertension was defined as hypertension noted in the medical history, using antihypertensive drugs, having a systolic blood pressure of ≥160 mmHg or diastolic blood pressure of ≥90 mmHg. Diabetes mellitus was defined as using antidiabetic drugs or diabetes mellitus in the medical history. Hypercholesterolemia was defined as hypercholesterolemia in the medical history or using cholesterol lowering drugs. Smoking was categorized in smoking and non-smoking. Stroke was defined as either ischemic or hemorrhagic stroke noted in the medical history or cortical infarcts visible on the CT-scan. Myocardial infarction, TIA and peripheral arterial disease (defined as symptoms of vascular claudication and/or interventions because of peripheral arterial disease) were defined as noted in the medical history.

Cognitive outcome variables

Cognitive function was measured with the Cambridge Cognitive Examination (CAMCOG). The CAMCOG is a well-validated cognitive evaluation, which contains the Mini-Mental State Examination (MMSE) but also tests on other cognitive domains as apraxia, executive functions and so on. The CAMCOG ranges from 0 to 107 points and the cut-off point is variable with adjustment for age and education. The CAMCOG was divided in a memory section, non-memory section and total score. It has a high sensitivity and specificity for diagnosing dementia (97% and 91%, respectively).[13]

The possible diagnoses were subjective cognitive impairment (SCI), mild cognitive impairment (MCI), dementia or other diagnosis (including psychiatric disorders as depression and other neurologic disorders as brain tumors). MCI was diagnosed using the criteria first proposed by Petersen et al.[14] Dementia was diagnosed using the Diagnostic and Statistical Manual of Mental Disorders (DSM) IV criteria. From all patients with dementia, the type of dementia was noted and included Alzheimer's dementia, vascular dementia, mixed dementia (coexistence of Alzheimer and vascular dementia) and other types of dementia (including Lewy Body dementia, frontotemporal dementia and Parkinson's disease dementia). Alzheimer's disease was defined using the National Institute of Neurological and Communicative Disorders and Stroke—Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria[15] and vascular dementia using the National Institute of Neurological Disorders and Stroke—Association Internationale pour la Recherche et l'Enseignement en Neurosciences (NINDS-AIREN) criteria.[16] The diagnosis was established by the memory clinic team. In case of dementia, the stage of dementia was measured with the clinical dementia rating (CDR) scale.[17]

Statistical analyses

Baseline characteristics were analyzed by descriptive statistics. All continuous descriptive statistics were noted as mean and standard deviation or, in case of a skewed distribution, as median and range. Categorical data were noted as numbers and percentages.

We used logistic regression to investigate which variables were associated with the dichotomous outcome measures. Ordinal logistic regression was used for ordinal outcome measurements with more than two categories (Woodcock score and CDR-score). The results were noted as an odds ratio (OR) with 95%-confidence interval (95%-CI). Linear regression was used for scale outcome measurements (CAMCOG) and results were noted as B with 95%-CI. The CAMCOG-scores were normally distributed (skewness CAMCOG memory −0.450, CAMCOG total −0.813) except for the CAMCOG non-memory (skewness −1.089). However, with multiple transformations the distribution of the CAMCOG non-memory did not improve so we accepted the slightly skewed distribution. After univariate analysis, the results were adjusted with different multivariate logistic regression models. In model 1, the results were adjusted for age and sex. Model 2 consists of further adjustment for cardiovascular risk factors in case of significant results in model 1. These variables were chosen in the models, because based on clinical reasoning and earlier studies they could be possible confounders for both cognitive and cardiovascular outcomes.[2, 18-20] We entered all these variables into the models to correct for confounders, because this was an etiological study.

We used SPSS software, version 25.0.0.2 (SPSS Inc., Chicago, Illinois) for the analyses. A p-value <0.05 was considered statistically significant.

Ethics

All patients included in this study have given written informed consent and the study was approved by the local medical ethical committee.[11]

Results

A total of 1992 patients were included, 8 patients were excluded because of the lack of a CT-scan of the brain. Of the included patients, 803 (±40%) were male and the median age was 78.2 years. Almost half of the patients (±47%) were diagnosed with dementia, with Alzheimer's disease being the most common type of dementia. The majority of patients had at least some calcifications in the iICA, ±95%. Calcifications in the basilar artery were less prevalent (±8%). These and other baseline characteristics are shown in table 1. For all variables the amount of missing data was <1%, except for the CDR-score where in almost 12% of patients with dementia the score was missing.

After individually assessing the CT of 100 patients (NG, EdB) and jointly discussing any discrepancies (NG, EdB, PdJ), an adequate interobserver agreement was achieved for basilar artery calcification (weighted kappa 0.65). The scoring of the iICA was more difficult to learn. After 200 patients, an adequate interobserver agreement was achieved for the Kockelkoren score (weighted kappa 0.74) and for the Woodcock score (weighted kappa 0.56).

Risk factors for intracranial calcifications

Risk factors for predominantly intimal calcifications in iICA were age (adjusted OR 1.11, 95%-CI 1.09–1.13), hypertension (adjusted OR 1.84, 95%-CI 1.34–2.52) and smoking (adjusted OR 1.88, 95%-CI 1.18–3.00). Risk factors for predominantly medial calcifications in iICA were age (adjusted OR 1.13, 95%-CI 1.11–1.15) and diabetes mellitus (adjusted OR 2.14, 95%-CI 1.27–3.60). Risk factors for severity of calcifications in iICA were age (adjusted OR 1.08, 95%-CI 1.07–1.10), diabetes mellitus (adjusted OR 2.21, 95%-CI 1.73–2.83) and smoking (adjusted OR 1.47, 95%-CI 1.11–1.95). Age (adjusted OR 1.06, 95%-CI 1.04–1.09) and hypertension (adjusted OR 1.67, 95%-CI 1.09–2.57) were also risk factors for basilar artery calcifications. For these and other results on risk factors for arterial calcifications see table 2.

Association between intracranial artery calcifications and cardiovascular disease

Predominantly intimal calcifications, predominantly medial calcifications and severity of calcifications in iICA were independently associated with stroke (adjusted OR 1.84, 1.88 and 2.88, respectively). Predominantly intimal calcifications and severity of calcifications in iICA were also independently associated with myocardial infarction (adjusted OR 2.27 and 4.45, respectively). There were no significant associations between intracranial artery calcifications and TIA or peripheral artery disease found. These results are shown in table 3.

Association between intracranial artery calcifications and cognitive function

The associations between cognitive function and intracranial artery calcifications were almost completely explained by the effect of age and sex. After adjustment for these two factors there was no association between intracranial artery calcifications and type of cognitive disorder or cognitive function, see table 4.

Also, type of dementia was not significantly associated with intracranial arterial calcifications after adjustment for age and sex, except for a significant association between mixed dementia and severity of calcifications in iICA (see table 5). The latter association lost significance after further adjustment for cardiovascular risk factors (data not shown). There was also no association between intracranial artery calcifications and CDR-score in case of dementia (data not shown).

Male (n,%)	**803 (40.3)**
Age in years (median, range)	**78.2 (45-96)**
Cardiovascular risk factors (n,%)	
• Hypertension	1360 (68.3)
• Diabetes mellitus	329 (16.5)
• Hypercholesterolemia	217 (10.9)
• Smoking	227 (11.4)
Cardiovascular comorbidities (n,%)	
• Myocardial infarction	202 (10.1)
• Stroke	200 (10.0)
• TIA	116 (5.8)
• Peripheral arterial disease	179 (9.0)
Intracranial calcifications (n,%)	
• Kockelkoren iICA	
Absent/indistinguishable	254 (12.8)
Predominantly intimal	996 (50.0)
Predominantly medial	742 (37.2)
• Woodcock iICA	
None	107 (5.4)
Mild	282 (14.2)
Moderate	1239 (62.2)
Severe	364 (18.3)
• Basilar artery	153 (7.7)
Cognitive function (median, range)	
• CAMCOG memory	22.2 (0-36)
• CAMCOG non-memory	50.6 (0-67)
• CAMCOG total	72.9 (1-102)
Type of cognitive disorder (n,%)	
• Subjective cognitive impairment	332 (16.7)
• Mild cognitive impairment	491 (24.6)
• Dementia	939 (47.1)
• Other	230 (11.5)
Type of dementia (n,%)	
• Alzheimer's dementia	702 (74.8)
• Vascular dementia	56 (6.0)
• Mixed dementia	128 (13.6)
• Other types of dementia	53 (5.6)
CDR-score* (median, range)	1.29 (1-3)

TABLE 1. Baseline characteristics (n = 1992)

N = number of patients, * = CDR-score only in case of dementia, **CT** = computed tomography, **iICA** = intracranial internal carotid artery, **CDR-SCORE** = clinical dementia rating score

	Kockelkoren iICA[a]			Woodcock iICA	Basilar artery calcifications
	Predominantly intimal calcifications	Predominantly medial calcifications			
Crude OR (95%-CI)					
Sex					
Male	1.10 (0.84 – 1.47)	0.70 (0.53 – 0.94)‡		0.81 (0.68 – 0.97)‡	0.95 (0.68 – 1.33)
Age	1.11 (1.10 – 1.13)‡	1.13 (1.11 – 1.15)‡		1.08 (1.07 – 1.10)‡	1.07 (1.04 – 1.09)‡
Cardiovascular risk factors present					
Hypertension	2.63 (1.98 – 3.49)‡	2.04 (1.53 – 2.73)‡		1.70 (1.40 – 2.05)‡	2.09 (1.38 – 3.16)‡
Diabetes mellitus	1.88 (1.19 – 2.98)‡	2.52 (1.59 – 4.02)‡		2.25 (1.77 – 2.85)‡	1.37 (0.91 – 2.07)
Hypercholesterolemia	1.66 (1.02 – 2.73)‡	1.28 (0.76 – 2.14)		1.19 (0.89 – 1.57)	1.18 (0.71 – 1.94)
Smoking	1.25 (0.82 – 1.91)	0.59 (0.37 – 0.94)‡		1.07 (0.81 – 1.41)	0.46 (0.23 – 0.92)‡
Model 1: OR (95%-CI) adjusted for age and sex					
Sex					
Male	1.42 (1.04 – 1.94)‡	0.95 (0.68 – 1.33)		0.96 (0.80 – 1.15)	1.10 (0.78 – 1.55)
Age	1.12 (1.10 – 1.14)‡	1.13 (1.11 – 1.15)‡		1.08 (1.07 – 1.10)‡	1.07 (1.05 – 1.10)‡
Cardiovascular risk factors present					
Hypertension	1.43 (1.05 – 1.96)‡	1.33 (0.95 – 1.86)		1.31 (1.08 – 1.60)‡	1.74 (1.14 – 2.65)‡
Diabetes mellitus	1.79 (1.09 – 2.94)‡	2.26 (1.35 – 3.74)‡		2.29 (1.79 – 2.92)‡	1.35 (0.89 – 2.05)
Hypercholesterolemia	1.62 (0.95 – 2.75)	1.32 (0.75 – 2.33)		1.21 (0.91 – 1.61)	1.19 (0.72 – 1.98)
Smoking	1.90 (1.20 – 3.02)‡	0.99 (0.57 – 1.71)		1.48 (1.11 – 1.96)‡	0.59 (0.29 – 1.18)
Model 2: OR (95%-CI) adjusted for age, sex and cardiovascular risk factors					
Sex					
Male	1.40 (1.02 – 1.91)	0.93 (0.66 – 1.30)		0.92 (0.77 – 1.10)	1.14 (0.80 – 1.61)
Age	1.11 (1.09 – 1.13)‡	1.13 (1.11 – 1.15)‡		1.08 (1.07 – 1.10)‡	1.06 (1.04 – 1.09)‡
Cardiovascular risk factors present					
Hypertension	1.84 (1.34 – 2.52)‡	1.22 (0.87 – 1.72)		1.19 (0.98 – 1.45)	1.67 (1.09 – 2.57)‡
Diabetes mellitus	1.52 (0.92 – 2.53)	2.14 (1.27 – 3.60)‡		2.21 (1.73 – 2.83)‡	1.25 (0.82 – 1.90)
Hypercholesterolemia	1.45 (0.85 – 2.48)	1.10 (0.61 – 1.99)		1.04 (0.78 – 1.39)	1.10 (0.66 – 1.85)
Smoking	1.88 (1.18 – 3.00)‡	0.95 (0.55 – 1.64)		1.47 (1.11 – 1.95)‡	0.59 (0.29 – 1.18)

TABLE 2. Risk factors for intracranial artery calcifications

OR = odds ratio, **95%-CI** = 95%-confidence interval, **CT** = computed tomography, **iICA** = intracranial internal carotid artery, **a** = compared to indistinguishable + absent, **‡** = p-value <0.05

	Myocardial infarction	Stroke		TIA	Peripheral arterial disease
Crude OR (95%-CI)					
Kockelkoren iICA					
Indistinguishable + absent	Ref.	Ref.		Ref.	Ref.
Predominantly intimal calcifications	3.44 (1.78 – 6.65)‡	2.11 (1.19 – 3.74)‡		1.34 (0.71 – 2.52)	2.22 (1.25 – 3.93)‡
Predominantly medial calcifications	2.50 (1.27 – 4.93)‡	1.99 (1.10 – 3.58)‡		1.21 (0.63 – 2.34)	1.27 (0.69 – 2.33)
Woodcock iICA					
None	Ref.	Ref.		Ref.	Ref.
Mild	3.79 (0.87 – 16.57)	1.56 (0.57 – 4.26)		1.81 (0.51 – 6.43)	1.15 (0.41 – 3.23)
Moderate	6.58 (1.61 – 26.96)‡	2.35 (0.94 – 5.88)		2.04 (0.63 – 6.61)	2.33 (0.93 – 5.83)
Severe	7.03 (1.68 – 29.53)‡	3.03 (1.17 – 7.81)‡		3.11 (0.93 – 10.41)	1.97 (0.75 – 5.18)
Basilar artery calcifications					
Present	1.64 (1.03 – 2.62)‡	1.13 (0.67 – 1.92)		1.14 (0.59 – 2.24)	0.94 (0.52 – 1.69)
Model 1: OR (95%-CI) adjusted for age and sex					
Kockelkoren iICA					
Indistinguishable + absent	Ref.	Ref.		Ref.	Ref.
Predominantly intimal calcifications	2.73 (1.38 – 5.39)‡	1.95 (1.07 – 3.54)‡		0.94 (0.48 – 1.83)	1.82 (1.00 – 3.31)
Predominantly medial calcifications	2.12 (1.05 – 4.28)‡	1.91 (1.03 – 3.54)‡		0.85 (0.42 – 1.70)	1.11 (0.58 – 2.09)
Woodcock iICA					
None	Ref.	Ref.		Ref.	Ref.
Mild	3.37 (0.76 – 14.87)	1.54 (0.56 – 4.25)		1.40 (0.39 – 5.07)	1.05 (0.37 – 3.01)
Moderate	5.19 (1.24 – 21.65)‡	2.23 (0.87 – 5.72)		1.38 (0.41 – 4.62)	1.94 (0.75 – 4.99)
Severe	5.75 (1.34 – 24.77)‡	2.97 (1.11 – 7.96)‡		2.01 (0.57 – 7.03)	1.70 (0.62 – 4.68)
Basilar artery calcifications					
Present	1.49 (0.92 – 2.40)	1.07 (0.63 – 1.83)		1.00 (0.51 – 1.96)	0.86 (0.47 – 1.56)
Model 2: OR (95%-CI) adjusted for age, sex and cardiovascular risk factors					
Kockelkoren iICA					
Indistinguishable + absent	Ref.	Ref.		Ref.	Ref.
Predominantly intimal calcifications	2.27 (1.14 – 4.52)‡	1.84 (1.01 – 3.36)‡		0.79 (0.40 – 1.55)	1.62 (0.88 – 2.97)
Predominantly medial calcifications	1.86 (0.94 – 3.80)	1.88 (1.01 – 3.49)‡		0.78 (0.39 – 1.58)	1.04 (0.55 – 1.99)
Woodcock iICA					
None	Ref.	Ref.		Ref.	Ref.
Mild	2.99 (0.67 – 13.30)	1.54 (0.55 – 4.27)		1.22 (0.34 – 4.44)	1.02 (0.35 – 2.94)
Moderate	4.23 (1.01 – 17.77)‡	2.11 (0.82 – 5.46)		1.11 (0.33 – 3.77)	1.82 (0.70 – 4.74)
Severe	4.45 (1.02 – 19.34)‡	2.88 (1.06 – 7.77)‡		1.65 (0.47 – 5.85)	1.60 (0.58 – 4.44)
Basilar artery calcifications					
Present	1.39 (0.86 – 2.26)	1.07 (0.62 – 1.82)		0.98 (0.50 – 1.94)	0.82 (0.45 – 1.49)

TABLE 3. Association between intracranial artery calcifications and cardiovascular disease

OR = odds ratio, **95%-CI** = 95%-confidence interval, **iICA** = intracranial internal carotid artery, **Ref.** = reference, ‡ = p-value <0.05

	SCI	MCI	Dementia		Other diagnosis	CAMCOG-M	CAMCOG-NM	CAMCOG-T
Crude OR (95%-CI)								
Kockelkoren iICA								
Indistinguishable + absent	Ref.	Ref.	Ref.		Ref.	Ref.	Ref.	Ref.
Predominantly intimal calcifications	0.44 (0.32 – 0.61)‡	1.25 (0.90 – 1.74)	2.22 (1.65 – 2.97)‡		0.46 (0.32 – 0.67)‡	-1.95 (-3.02 – -0.89)‡	-2.61 (-4.03 – -1.20)‡	-4.54 (-6.82 – -2.27)‡
Predominantly medial calcifications	0.47 (0.34 – 0.66)‡	1.24 (0.88 – 1.75)	2.11 (1.56 – 2.86)‡		0.49 (0.33 – 0.72)‡	-2.01 (-3.13 – -0.90)‡	-3.08 (-4.62 – -1.55)‡	-5.08 (-7.54 – -2.62)‡
Woodcock iICA								
None	Ref.	Ref.	Ref.		Ref.	Ref.	Ref.	Ref.
Mild	0.77 (0.47 – 1.26)	1.46 (0.84 – 2.55)	2.07 (1.24 – 3.43)‡		0.32 (0.18 – 0.57)‡	-1.19 (-2.91 – 0.53)	-0.11 (-2.46 – 2.24)	-1.33 (-5.11 – 2.45)
Moderate	0.39 (0.25 – 0.60)‡	1.46 (0.88 – 2.41)	3.22 (2.03 – 5.11)‡		0.32 (0.20 – 0.51)‡	-3.01 (-4.53 – -1.48)‡	-3.00 (-5.08 – -0.92)‡	-5.93 (-9.26 – -2.61)‡
Severe	0.32 (0.19 – 0.53)‡	1.41 (0.82 – 2.42)	3.62 (2.21 – 5.93)‡		0.32 (0.19 – 0.55)‡	-3.57 (-5.24 – -1.90)‡	-4.07 (-6.41 – -1.73)‡	-7.55 (-11.27 – -3.83)‡
Basilar artery calcifications								
Present	0.56 (0.33 – 0.96)‡	1.30 (0.91 – 1.87)	1.22 (0.87 – 1.69)		0.69 (0.39 – 1.25)	-0.78 (-2.06 – 0.51)	0.01 (-1.73 – 1.74)	-0.75 (-3.54 – 2.03)
Model 1: OR (95%-CI) adjusted for age and sex								
Kockelkoren iICA								
Indistinguishable + absent	Ref.	Ref.	Ref.		Ref.	Ref.	Ref.	Ref.
Predominantly intimal calcifications	1.07 (0.73 – 1.58)	1.02 (0.71 – 1.47)	1.11 (0.79 – 1.55)		0.90 (0.59 – 1.38)	0.97 (-0.10 – 2.03)	0.35 (-1.13 – 1.82)	1.34 (-0.97 – 3.65)
Predominantly medial calcifications	1.20 (0.79 – 1.83)	0.94 (0.64 – 1.38)	0.95 (0.67 – 1.36)		1.08 (0.68 – 1.71)	1.10 (-0.08 – 2.28)	0.73 (-0.93 – 2.38)	1.79 (-0.82 – 4.40)
Woodcock iICA								
None	Ref.	Ref.	Ref.		Ref.	Ref.	Ref.	Ref.
Mild	1.84 (1.02 – 3.31)‡	1.04 (0.57 – 1.89)	1.07 (0.60 – 1.89)		0.47 (0.26 – 0.88)	1.72 (1.06 – 3.38)‡	3.16 (0.80 – 5.52)	4.85 (1.17 – 8.52)‡
Moderate	1.04 (0.63 – 1.74)	1.11 (0.65 – 1.90)	1.35 (0.81 – 2.50)		0.90 (0.52 – 1.54)	0.60 (-0.94 – 2.14)	0.70 (-1.47 – 2.86)	1.37 (-2.02 – 4.76)
Severe	1.16 (0.56 – 2.40)	1.16 (0.54 – 2.10)	1.23 (0.67 – 2.27)		0.75 (0.36 – 1.56)	0.56 (-1.49 – 2.60)	1.63 (-1.24 – 4.51)	2.21 (-2.32 – 6.73)
Basilar artery calcifications								
Present	0.89 (0.51 – 1.54)	1.19 (0.83 – 1.74)	0.87 (0.62 – 1.23)		1.03 (0.56 – 1.87)	0.48 (-0.72 – 1.68)	1.42 (-0.24 – 3.08)	1.91 (-0.71 – 4.54)

TABLE 4. Association between intracranial artery calcifications and type of cognitive disorder and cognitive function

OR = odds ratio, **95%-CI** = 95%-confidence interval, **SCI** = subjective memory complaints, **MCI** = mild cognitive impairment, **CAMCOG** = Cambridge Cognitive Examination, **CAMCOG-M** = CAMCOG memory, **CAMCOG-NM** = CAMCOG non-memory, **CAMCOG-T** = CAMCOG total, **iICA** = intracranial internal carotid artery, **Ref.** = reference, ‡ = p-value <0.05

	Kockelkoren iICAa			Woodcock iICA	Basilar artery calcifications
	Predominantly intimal calcifications	Predominantly medial calcifications			
Crude OR (95%-CI)					
Type of dementia					
Alzheimer's dementia	2.95 (1.99 – 4.37)‡	2.86 (1.91 – 4.30)‡		2.35 (1.80 – 3.06)‡	1.71 (0.97 – 3.03)
Vascular dementia	6.16 (1.84 – 20.64)‡	3.09 (0.86 – 11.04)		2.05 (1.15 – 3.64)‡	1.52 (0.49 – 4.72)
Mixed dementia	5.92 (2.46 – 14.26)‡	5.04 (2.05 – 12.38)‡		3.41 (2.25 – 5.16)‡	3.03 (1.48 – 6.19)‡
Other types of dementia	1.12 (0.51 – 2.48)	1.24 (0.55 – 2.79)		1.23 (0.69 – 2.20)	0.78 (0.17 – 3.47)
Subjective memory complaints	Ref.	Ref.		Ref.	Ref.
Model 1: OR (95%-CI) adjusted for age and sex					
Type of dementia					
Alzheimer's dementia	1.10 (0.68 – 1.78)	0.86 (0.51 – 1.44)		1.21 (0.90 – 1.63)	0.95 (0.51 – 1.75)
Vascular dementia	2.76 (0.79 – 9.71)	1.07 (0.27 – 4.19)		1.29 (0.72 – 2.33)	0.91 (0.28 – 2.90)
Mixed dementia	1.81 (0.70 – 4.68)	1.29 (0.47 – 3.57)		1.57 (1.03 – 2.45)‡	1.46 (0.68 – 3.16)
Other types of dementia	0.69 (0.30 – 1.60)	0.69 (0.28 – 1.68)		0.99 (0.55 – 1.77)	0.63 (0.14 – 2.84)
Subjective memory complaints	Ref.	Ref.		Ref.	Ref.

TABLE 5. Association between intracranial artery calcifications and type of dementia
OR = odds ratio, **95%-CI** = 95%-confidence interval, **iICA** = intracranial internal carotid artery, **Ref.** = reference, **a** = compared to indistinguishable + absent, ‡ = p-value <0.05

Discussion

This study showed that calcifications in the iICA were highly prevalent in memory clinic patients and that risk factor profiles differed between intimal and medial dominant calcification patterns. Stroke was associated with both intimal and medial calcification patterns and severity of calcifications in iICA. Myocardial infarction was associated with intimal calcifications and severity of calcifications in iICA. The association between intracranial artery calcifications, type of cognitive disorder or cognitive function were largely explained by age and sex. Our study adds to the understanding that various types of intracranial artery calcifications exist, which appear to be relevant for major cardiovascular diseases, although their role in dementia remains uncertain.

Earlier studies found similar risk factors for intracranial arterial calcifications as we did. A previous study, also using the Kockelkoren and Woodcock score on the iICA on CT, investigated the difference in risk factors in patients suspected of acute ischemic stroke. This study found that higher age, smoking and hypertension were risk factors for predominantly intimal calcifications, whereas higher age and diabetes mellitus were risk factors for predominantly medial calcifications.[2] A study investigating the vertebrobasilar arteries found that higher age, diabetes mellitus and obesity were significant risk factors for presence of calcifications.[18] A difference with our study is that in this study all included patient had either a risk factor for or manifest vascular disease. A study using ^{18}F-sodium fluoride accumulation on positron emission tomography/computed tomography (PET/CT) to measure medial calcification in the femoral arteries in oncology patients found an association between medial calcifications with age and diabetes mellitus, but also with hypercholesterolemia and hypertension.[19] A systematic review and meta-analysis on the risk factors of breast arterial calcifications, generally considered to be exclusively medial calcifications, also showed a positive association between age and diabetes mellitus with these calcifications.[20]

There have been earlier studies that investigated the association between intracranial artery calcifications and stroke. In summary, these studies suggest that there is a higher risk of ischemic stroke in patients with intracranial artery calcifications.[5] We also found an association between myocardial infarction and iICA. There were no other studies that investigated this association.

A few other studies have been performed to investigate an association between artery calcifications and cognitive function or dementia and they found varied results. An Italian study found that patients with common carotid artery calcifications on ultrasound had a lower MMSE-score than patients without these calcifications (23.7 ± 0.3 versus 25.5 ± 0.8; p = 0.02) after adjustment for a number of possible confounders in a study population that consisted of patients visiting a Hypertension Center.[10] A study performed in Taiwan that recruited subjects with non-contrast brain CT obtained for a variety of clinical indications (such as headache, vertigo, sinusitis, head injury, and suspicion of malignancy) found a significant association between iICA calcifications and a lower score on the MMSE after adjustment for age, education year, hypertension and diabetes mellitus (adjusted OR 1.06, 95%-CI 1.00–1.13).[9] However, a longitudinal population based Dutch study with nondemented participants found no significant association between iICA calcifications and cognitive function or dementia on follow-up after adjustment for presence of stroke.[7] Moreover, a study with a random sample of this population based Dutch study with non-demented persons also found no association with iICA calcifications and worse cognitive performance after adjustment for potential confounders like cardiovascular risk factors.[8]

There were five important strengths of this study. Firstly, this is the first study that investigated the differences between medial and intimal calcifications in a memory clinic cohort. Secondly, it is a large study population. Thirdly, it concerns a memory clinic population, which is a very relevant setting, as these patients are at risk for both intracranial artery calcifications and cognitive decline. Fourthly, we used CT-scans to assess intracranial artery calcifications and CT is widely available. However, not in all memory clinics a CT-scan of the brain is the standard choice for brain imaging. Lastly, the results of this study are generalizable to the general patient population of the memory clinic, because of the lack of exclusion criteria in this study, except for the absence of a CT-scan of the brain (n = 8).

A limitation of this study was that most patients included in our cohort had intracranial artery calcifications. Nevertheless, our smallest reference group had over 100 subjects. Calcifications in the basilar artery were far less common in our study. A population-based study in the Netherlands investigated the presence of vertebrobasilar artery calcifications in the general population and also found a low presence of basilar artery calcifications (1.5%).[21] It is a limitation that we were unable to measure the vertebral artery calcifications. A second limitation was that we had no information on the serum cholesterol levels, fasting glucose levels, HbA1c or electrocardiogram. Therefore, we could have missed some patients with hypercholesterolemia, diabetes mellitus or silent myocardial infarction. On the other hand, because we also took medication use into account it is possible this overestimated the number of people with hypercholesterolemia. Furthermore, we used CT to establish the calcifications, while CT does not have the resolution to differentiate between the intimal and medial arterial layer. Also, the sensitivity for microcalcification is poor. Nevertheless, our calcification score was able to make an estimation of the predominant intimal or medial macrocalcifications.[1] The assessment of the calcification scores was more difficult for the iICA than it was for the basilar artery. After 200 patients the weighted kappa was adequate for iICA although the kappa for the Woodcock score was lower than for the Kockelkoren score. This means that the use of these calcification scores in clinical practice requires some learning time. Lastly, although our results were adjusted for most relevant confounders, residual confounding might be present in this observational study. Also, a possible causal relationship could not be derived given the cross-sectional design.

In this study we found that vascular risk factors are differentially related to predominantly medial and intimal iICA calcifications. Also, the associations with cardiovascular diseases were different. This could indicate that medial and intimal located calcifications have different consequences and might need different approaches, which makes it important to identify in which layer the calcifications occur. Future, longitudinal studies are needed to further elaborate the causes and consequences of intracranial artery calcifications in memory clinic patients and other settings.

Conclusion

This study showed that intracranial artery calcifications are a very common phenomenon in memory clinic patients. Vascular risk factors are differentially related to predominantly medial and intimal located iICA calcifications and both types of calcifications are associated with stroke. Predominantly intimal iICA calcifications were also associated with myocardial infarction. No association was found between intracranial artery calcifications and cognitive function or type of cognitive disorder and the relevance of large artery calcifications to dementia remains uncertain.

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CHAPTER 9

General Discussion

General discussion

In this thesis, we focused on intracranial calcifications, mainly in the basal ganglia and hippocampus. In Part 1, we examined the relationship between computed tomography (CT) images and histology findings to understand the histological nature of the calcifications seen on the CT scans of our patients. Part 2 comprised an overview of the pathophysiology and consequences of calcifications using Fahr disease as a model for basal ganglia calcifications. In Part 3, we aimed to expand current knowledge of risk factors for intracranial calcifications and the potential relevance of these calcifications to cognitive dysfunction in two large cohorts.

Main findings and interpretation

Part 1: Intracranial calcifications on computed tomography: what do we see?

In general, arterial calcifications can be divided into two dominant types: calcifications in the tunica intima, which are predominantly atherosclerotic, and calcifications in the tunica media and internal elastic lamina (also called Monckeberg sclerosis), which are non-atherosclerotic.[1] Each type is associated with its own risk factors and physiological and clinical consequences. Calcifications can be found in most of the intracranial arteries, including large arteries such as the carotid artery and basilar artery, and in small arteries in the basal ganglia and hippocampus.

As mentioned above, our research focused mainly on calcifications in the basal ganglia and hippocampus. In Chapters 2 and 3, we investigated whether the calcifications in the basal ganglia and hippocampus are, as expected, vascular in nature and whether CT scans correspond with histology preparations. In Chapter 2, we reported that calcifications were indeed found in the arterioles of the basal ganglia in 8 of 22 adult patients with unenhanced CT scans of the brain and for whom brain autopsy findings were available. These calcifications started in the internal elastic lamina and spread to the media, which corresponds favorably with a non-atherosclerotic origin. They were located in the internal globus pallidus and sometimes also in the external globus pallidus, following the vascularization pattern. Our findings are in line with earlier case reports in patients with Fahr disease in whom calcifications were located in the tunica media and no atherosclerosis was found.[2,3]

In Chapter 3, we described a histological investigation of nine hippocampi. Calcifications were found in the tail, body, and sometimes head of six of the hippocampi, and were localized in precapillaries, capillaries, and arteries of the molecular and granular layers of the dentate gyrus and cornu ammonis 1 (CA1). The arterial calcifications were located in the tunica media and tunica adventitia. Since only the head of the hippocampus is sampled for most histological examinations, many calcifications will be missed based on our results. A study by Wegiel and colleagues[4] investigating the histology of hippocampal calcifications in patients with Alzheimer's disease, with Down syndrome, and control patients also described the localization of calcifications in the molecular, granular, and polymorphic layers of the dentate gyrus, spreading from the tail to the body and, in some cases, to the head of the hippocampus. Moreover, this study reported calcifications localized in the tunica media and tunica adventitia just as our study.[4]

In conclusion, both studies showed that calcifications in the basal ganglia and hippocampus seen with CT correspond with those seen in histology samples and that the medial localization in the vessel wall suggests that the calcifications are non-atherosclerotic. The precise pathophysiology of calcifications in the tunica media is not clear, but these calcifications are associated with aging, diabetes mellitus, and chronic kidney disease.[5] Therapy with antihypertensive drugs or statins is probably not effective in preventing or delaying the development of these calcifications because of their presumed non-atherosclerotic origin.

Part 2: The pathophysiology of intracranial artery calcifications: lessons from Fahr disease

Chapter 4 included a review of the underlying pathophysiology of Fahr disease, a rare disorder characterized by bilateral calcifications in the basal ganglia and often also in the thalami and cerebellum. Patients with Fahr disease can suffer from movement disorders, psychiatric disorders, and cognitive problems. Since our histopathology findings in patients with incidental basal ganglia calcifications (Chapter 2) corresponded with those in patients with Fahr disease, we hypothesized that the symptoms of patients with Fahr disease correspond with the similar but milder symptoms seen in patients with incidental calcifications in the basal ganglia and possibly other intracranial sites.

Mutations in six genes are known to cause Fahr disease. Mutations in *SLC20A2* and *PDGFB* lead to calcifications in the vascular extracellular matrix, mutations in *XPR1* lead to calcifications in endothelial cells, and mutations in *PDGFRB* lead to calcifications in smooth muscle cells. The underlying mechanism in the other two genes are not yet clear. *SLC20A2* and *XPR1* encode for inorganic phosphate transporters in endothelial cells that have opposite functions. The transporter encoded for by *SLC20A2* (PiT2) provides the transport of inorganic phosphate from the vascular extracellular matrix/cerebral spinal fluid into the blood, whereas the transporter encoded for by *XPR1* ensures the efflux of inorganic phosphate to the vascular extracellular matrix. Mutations in these genes mostly lead to a dysfunctional transporter that causes the accumulation of inorganic phosphate and the formation of calcium phosphate depositions in the form of hydroxyapatite in the vascular extracellular matrix or in the endothelial cells. *PDGFB* and *PDGFRB* are important for the regulation of pericyte formation and migration during angiogenesis; mutations in these two genes lead to dysfunction of the blood–brain barrier.

This chapter also highlighted the important role played by the balance of inorganic phosphate and pyrophosphate. Calcium phosphate salts are formed in the presence of calcium, with the final product being hydroxyapatite – a major component of vascular calcification. Because pyrophosphate is a potent inhibitor of hydroxyapatite formation, the bisphosphonate etidronate (a homologue of pyrophosphate) could be used as a potential treatment not only for patients with Fahr disease, but also for those with incidental calcifications.

In conclusion, the pathology findings in patients with Fahr disease correspond with our findings in patients with incidental basal ganglia calcifications (Chapter 2). Therefore, perhaps the symptoms of patients with Fahr disease correspond with similar but milder symptoms in patients with incidental intracranial calcifications, especially in the basal ganglia. Although there is yet no cure, potential treatments for Fahr disease have been identified. We hope that, in the future, the same potential therapeutic options for Fahr disease can be used for incidental calcifications.

Part 3: Risk factors and clinical relevance of incidental intracranial artery calcifications

An aim of our research was to expand the existing knowledge of the prevalence of intracranial calcifications. To do this, we studied two large cohorts. Chapter 5 discussed the first cohort, comprising 1133 patients suspected of acute ischemic stroke. These patients had a mean age of 67 years and 30% had basal ganglia calcifications. Chapters 6–8 examined the second cohort (1992 patients who visited a memory clinic) for the presence of calcifications in the basal ganglia, hippocampus, carotid artery siphon, and basilar artery. The median age of these patients was 80 years. Basal ganglia calcifications were found in 43%, hippocampal calcifications in 19%, basilar artery calcifications in 8%, and carotid artery siphon calcifications in 95%. Intracranial calcifications are, therefore, a frequent finding on CT scans of older patients, especially in the carotid artery siphon and the basal ganglia.

In Chapters 5 and 6, we investigated the risk factors for basal ganglia calcifications in both of the abovementioned cohorts. No association with standard vascular risk factors (smoking, diabetes mellitus, hypertension, hyperlipidemia) was found in either cohort. In addition, no association was found with renal function in patients suspected of ischemic stroke. These findings suggest that calcifications in the basal ganglia are non-atherosclerotic, which corresponds with the histological results of the study described in Chapter 2. However, we did not find an association with diabetes or with renal function, which are known risk factors for medial – i.e., non-atherosclerotic – calcifications. There was an association with older age and, unexpectedly, with a lower body mass index (BMI). The explanation for this inverse relationship with BMI is not yet clear, but it is possible that either bone demineralization in patients with a low BMI plays a role or the loss of fat and muscle mass in older people is an indicator of global deterioration in health. We also found an association with female gender and an inverse association with hypertension. A possible explanation for this inverse relationship could be the under-representation of patients with hypertension and severe cardiovascular disease because of selective mortality. Another explanation could be the use of antihypertensive medication, specifically calcium-channel blockers and inhibitors of the renin–angiotensin–aldosterone system, which could lead to less arterial calcification because of their effect on vascular smooth muscle cells.[6] One could hypothesize that hypotension in the elderly is harmful and that hypertension in these individuals could be favorable because it leads to a better perfusion of the brain and thereby to less calcification.[7]

Hippocampal calcifications, as described in Chapter 7, are associated with older age, diabetes mellitus, and smoking. Older age and diabetes mellitus are known risk factors for medial calcifications, while the role of smoking in medial calcifications remains uncertain. Histological examination of hippocampal calcifications, as described in Chapter 3, supports these associations. It showed that these calcifications were located in the tunica media and that there were no signs of atherosclerosis.

In Chapter 8, we identified the risk factors for basilar artery calcifications as being age and hypertension. This is in line with expectations since calcifications in the vertebrobasilar arteries are often atherosclerotic in origin.[8] Risk factors for predominantly intimal calcifications in the carotid artery siphon were older age, hypertension, and smoking, while those for predominantly medial calcifications were older age and diabetes mellitus.

In conclusion, intracranial calcifications in the basal ganglia and hippocampus are non-atherosclerotic with risk factors mostly matching those of medial calcifications, while basilar calcifications are atherosclerotic and are mostly associated with risk factors for atherosclerosis. The calcifications in the carotid artery siphon can be either atherosclerotic or non-atherosclerotic in origin, with risk factors matching the location of the calcifications: tunica intima or tunica media, respectively. This knowledge is important, because treatment of these risk factors with either lifestyle interventions or medication could help prevent intracranial arterial calcifications. This should be further investigated and include the effect on disease outcome.

An explanation for this atherosclerotic/non-atherosclerotic difference between the deeper brain structures and the larger arteries is that atherosclerosis mostly affects large- to medium-sized arteries such as the carotid arteries and the basilar artery, whereas medial (i.e., non-atherosclerotic) calcifications are also found in arterioles.

In the abovementioned cohort of 1992 patients visiting a memory clinic, all patients underwent a standard workup including a brain CT and the Cambridge Cognitive Examination (CAMCOG).[9] Our primary goal with this cohort – and an important aim of the research described in this thesis – was to investigate whether intracranial calcifications are associated with cognitive function. Depending on the location of the calcifications, we hypothesized different underlying mechanisms and cognitive problems. In patients with basal ganglia calcifications, for example, we expected to find executive dysfunction similar to that seen in patients with Fahr disease or Parkinson's disease. Both of these disorders involve the basal ganglia, and have as symptoms executive dysfunction and mental slowness.[10-12] We also hypothesized that hippocampal calcifications lead to memory complaints in patients with Alzheimer's disease since the hippocampus is an important area for learning and memory.

Finally, calcifications in the carotid artery siphon were theorized to cause cognitive problems because arterial stiffness alters brain microcirculation and leads to blood-brain-barrier dysfunction and/or reduced cerebral blood supply.[13]

Arterioles at the border of vascular territories of the anterior and middle cerebral arteries, which are vulnerable to reduced blood supply, are responsible for the blood supply of the white matter, and therefore reduced cerebral blood supply can also lead to white matter disease.[14, 15] The blood supply to the basal ganglia comes from arterioles arising directly from the Circle of Willis and its proximal branches and are thereby susceptible to damage caused by stiffening of large arteries.[16, 17] Thereby, in calcifications in the carotid artery siphon we expected to find impairment in executive function and processing speed, also delayed recall.

In contrast to our expectations, however, we did not find an association between calcifications in these three locations and cognition. It is possible that subtle cognitive decline was missed since the CAMCOG is a "rough" screening tool to diagnose cognitive decline. Moreover, the CAMCOG tests the executive domain only to a limited extend, as a result of which cognitive impairment by calcifications in the carotid artery siphon or basal ganglia may not be properly detected. It is possible that calcifications in the basal ganglia, for example, only lead to cognitive problems when they are extensive, like in patients with Fahr disease.

As for the hippocampus, it is a complex structure with functionally distinct subregions. In Chapter 3, we saw that hippocampal calcifications are found in the dentate gyrus and CA1, and sometimes in the subiculum. The literature suggests that the dentate gyrus is associated with the aging process and CA1 with vascular disease; where Alzheimer's disease is associated with a diffuse atrophy pattern.[18]

In conclusion, our studies do not support a relationship between intracranial artery calcifications and cognitive function. Nevertheless, more research should be conducted in this field and studies investigating the executive and other cognitive domains should use more sensitive cognitive tests.

Future perspectives

In the introduction of this thesis, we stated an unconventional hypothesis: namely, that intracranial calcifications are related to neurodegenerative disease and may even be a cause of brain dysfunction and cognitive decline. To prove the hypothesis, we started our scientific journey with the histopathological validation of what we see on CT scans and the interesting literature from many decades ago. Although several of our investigations had negative results, we learned a lot and stress the importance of being open-minded and observant as a scientist and a physician.

Intracranial calcifications are a frequent finding on CT scans in older patients, especially in the carotid artery siphon and the basal ganglia. Moreover, they are predominantly of vascular origin. A distinction – atherosclerotic or non-atherosclerotic – can be made based on the location of the calcification in the vessel wall and thereby the underlying pathophysiology. Calcifications in the basilar artery are mainly atherosclerotic, calcifications in the basal ganglia and hippocampus are non-atherosclerotic, and calcifications in the carotid artery siphon can be either. As the calcifications in the basilar artery and, partly, in the carotid artery siphon are atherosclerotic in origin, they are associated with the classic risk factors for cardiovascular disease, e.g., hypertension and smoking. This means that treating these risk factors with lifestyle interventions or medication could help prevent intracranial arterial calcifications. This should be further investigated, including its effect on disease outcome. Since calcifications in the basal ganglia and hippocampus, and sometimes in the carotid artery siphon, are non-atherosclerotic, classic cardiovascular or atherosclerotic risk factors do not play a role in their development and treatment with, for example, statins is not expected to be useful. The treatment and prevention of diabetes mellitus and renal failure may be useful because these diseases seem to be related to medial calcifications; however, scientific substantiation is lacking.

Further research should focus on treatment options for medial calcifications not only because of their ability to modify the calcification burden, but also because of the clinical outcomes for the patient. Such research is a major focus of the center for patients with Fahr disease, which is located in the UMC Utrecht. One promising therapeutic option for patients with Fahr disease is the bisphosphonate etidronate. Currently, preparatory steps are being taken to start a trial with etidronate for patients with Fahr disease. If the results of this trial are positive, patients with incidental basal ganglia calcifications may also benefit from treatment with this drug.

While fundamental research is the well-known standard, we would like to stress the importance of (investigator-initiated) innovative drug trials in etiological research. Seeing something on imaging or with histology does not mean that it is a cause of disease, not even when it makes sense based on clinical reasoning or physiological insights. A good example of this is the field of amyloid and tau in dementia. So far, in trials using scans that showed that amyloid was no longer in the brain after treatment, they were unable to link this to a significant slowdown in cognitive decline and dementia development. Much remains to be done regarding the calcifications that were investigated for this thesis. Knowledge gaps in the clinical relevance of intracranial calcifications to mobility/falls or neuropsychiatric symptoms, for example, still need to be filled. Currently, a study investigating the association between intracranial calcification and mobility in patients visiting a mobility/fall clinic is being prepared.

It is crucial that all physicians look at their patients individually and focus on a personal/tailored plan of diagnostic and therapeutic approach for each one. This is especially true for patients with a heterogeneous disease like Fahr disease, and for the multiple and complex problems that occur with aging.

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CHAPTER 10

Summary

Summary

Intracranial artery calcifications are a common finding with computed tomography (CT). Most research on intracranial artery calcifications so far focusses on the carotid artery siphon. Carotid artery siphon calcifications are associated with cerebrovascular disease, such as stroke. Some studies also suggest a possible relation with cognitive impairment. However, besides calcifications in the larger arteries in the brain, such as the carotid artery siphon and the vertebrobasilar artery, calcifications can also be found histologically and radiologically in the smaller arteries or arterioles in the basal ganglia and hippocampus. In this thesis, we mainly focused on calcifications in the basal ganglia and hippocampus. We aimed to better understand what we see on CT scans in our routine practice by comparing CT images with histology. Next, we described the mechanisms of calcification in patients with Fahr disease as a model disease for intracranial calcifications. Finally, we performed several large cohort studies to increase our knowledge on risk factors for intracranial calcifications in the hippocampus and basal ganglia and their potential relevance for cognitive dysfunction.

Intracranial calcifications on computed tomography: what do we see?

In Chapter 2, we investigated the histological characteristics of basal ganglia calcifications and compared histology findings with CT. For this study, we identified 22 adult patients of whom unenhanced CT scans of the brain, at maximum 1 year before autopsy, and brain autopsy findings were available. Histologic examination showed calcifications in the internal globus pallidus and sometimes additionally in the external globus pallidus. Calcification in the putamen was not seen. The deposits were, in accordance with our hypothesis, located in the vessel wall. They seemed to arise along the internal elastic lamina in an early stage, merging with more peripheral calcifications in or along the tunica media in a later stage, ultimately forming a single circular deposit. This distribution started in the ventral striatopallidum and fanned out posterolaterally into the external half of the globus pallidus, seemingly following the vascular tree downstream. Eight patients had basal ganglia calcifications on histology and six patients had calcifications on CT. In four patients, the basal ganglia calcifications seen on CT correlated with the histological findings, suggesting that the calcifications identified on CT scan are vascular in nature. Since the calcifications are located in the internal elastic lamina and the tunica media, and not in the tunica intima, the calcifications are probably non-atherosclerotic.

In Chapter 3, we determined the histological basis of hippocampal calcifications in seven post-mortem brains, of which in total nine hippocampi were available, to find out whether hippocampal calcifications are vascular in nature. We also compared histology findings with CT. In four hippocampi, the tail, body and head, and in five hippocampi only the body was examined. CT and histology showed calcification in six of nine hippocampi. Calcifications were always found in the tail, three times in the body and twice also in the head of the hippocampus. Mild calcifications were found

in precapillaries and capillaries of the molecular layer of the Cornu Ammonis 1 and dentate gyrus, in a more severe stage also in arteries, and in the granular layer of the dentate gyrus, and spreading out over the border of Cornu Ammonis 1 into the molecular layer of the subiculum. Calcification was found in the tunica media and tunica adventitia, which suggest that they are non-atherosclerotic. Thus, hippocampal calcifications as observed on CT are located in the hippocampal vasculature and the results indicate that calcifications start in the tail, and spread to the body and finally when calcifications are severe even to the head of the hippocampus.

The pathophysiology of intracranial artery calcifications: lessons from Fahr disease

In Chapter 4, we provided an overview of the literature on the underlying mechanisms of basal ganglia calcification in Fahr disease. It is possible that what we learn from Fahr disease can give us insight into the origin of calcifications and possible treatments, also in patients with incidental intracranial calcifications. Fahr disease is a rare, either autosomal dominant or recessive inherited disease, characterized by extensive calcifications in the basal ganglia. Patients can be asymptomatic, but they can also suffer from movement disorders, psychiatric symptoms and cognitive impairment. There are four genes so far recognized to cause autosomal dominant inherited Fahr disease. Mutations in *SLC20A2*, the first gene linked to Fahr disease, lead to inhibition of inorganic phosphate uptake in the cell by an inorganic phosphate transporter and thereby to deposition of calcium phosphate in the extracellular matrix. Mutations in the gene *XPR1*, encoding a retroviral receptor with phosphate export function, leads to intracellular calcium deposition. Mutations in *PDGFB*, a growth factor with an important role in the recruitment of pericytes during angiogenesis, and *PDGFRB*, the receptor for the growth factor, lead to pericyte deficiency and thereby to disruption of the blood-brain barrier which can lead to calcium deposition in the vascular extracellular matrix. Besides mutations in these four genes, recently the *MYORG* gene, which causes Fahr disease with an autosomal recessive pattern of inheritance, is found. The pathophysiologic mechanism behind the calcifications of this gene is not yet clarified. There are currently no therapies available for Fahr disease, however, we propose to try treatment with etidronate in a research setting. Etidronate is a molecular homologue of the circulating calcification inhibitor inorganic pyrophosphate. The potential of etidronate for the treatment of vascular calcification is convincingly shown in patients with other calcification disorders, such as pseudoxanthoma elasticum. A case series showed improvement of symptoms in some patients with Fahr disease. Also, etidronate is a safe and inexpensive drug that is well tolerated.

Risk factors and clinical relevance of incidental intracranial artery calcifications

In two large cohort studies, we investigated the risk factors for intracranial artery calcifications to find out whether we can influence or prevent the development of these calcifications.

In Chapter 5, we examined the prevalence of basal ganglia calcifications with CT and the association with vascular risk factors in a large cohort of 1133 patients suspected of acute ischemic stroke participating in the DUtch acute Stroke Trial (DUST). We found a prevalence of 30% of these

calcifications, of which 58% were mild, 31% were moderate and 11% were severe. The calcifications were significantly associated with age and lower body mass index. No association was found with vascular risk factors as hypertension, hyperlipidemia, diabetes mellitus and smoking, suggesting a non-atherosclerotic origin.

In Chapter 6, 7 and 8, we investigated the prevalence of intracranial artery calcifications, vascular risk factors, and the association with cognitive function in a large, well-characterized cohort. This cohort consists of 1992 patients who visited the memory clinic of Tergooi Medical Center, a Dutch general hospital, between April 2009 and April 2015 because of cognitive complaints. All patients underwent a standard diagnostic workup including the Cambridge Cognitive Examination, which also includes the Mini Mental State Examination, and a brain CT.

In Chapter 6, we investigated whether basal ganglia calcifications are associated with cognitive function and with vascular risk factors in the above mentioned cohort. Calcifications in the basal ganglia were found in 43% of the patients; in 20% of patients mild, in 20% of patients moderate and in 3% of patients severe. We found no association between presence or severity of these calcifications and cognitive function. The basal ganglia calcifications were associated with female gender. Hypertension and antihypertensive drug use were associated with a lower risk of calcifications. These findings also suggest that these calcifications are non-atherosclerotic, which corresponds with the results of the studies described in chapter 2 and chapter 5.

In Chapter 7, also in the above mentioned memory clinic cohort, the association between hippocampal calcifications on CT and cognitive function was investigated, and the association with vascular risk factors. Hippocampal calcifications were present in 19% of the patients. No association between presence or severity of hippocampal calcification and cognitive function was found. Hippocampal calcifications were associated with older age, diabetes mellitus and smoking.

In Chapter 8, calcifications in the intracranial carotid artery and basilar artery were studied in the same cohort as mentioned above. In the carotid syphon, calcifications were assessed on severity and location in the tunica intima and tunica media. Carotid calcifications were present in 95% of the patients; in 50% predominantly intimal, in 37% predominantly medial, in 8% indistinguishable, and in 5% absent. Basilar artery calcifications were present in 8%. Risk factors for calcifications in the siphon in predominantly intimal calcifications were age, hypertension and smoking, and in the predominantly medial calcifications age and diabetes mellitus. Siphon calcifications (intimal and medial) were associated with stroke and intimal calcifications also with myocardial infarction. Basilar artery calcifications were associated with age and hypertension. There was no association with cardiovascular disease. No association with cognitive function was found.

In Chapter 9, we discussed the results and implications of the studies described above. We concluded that intracranial calcifications are a frequent finding in older patients with CT scan, especially in the carotid artery siphon and the basal ganglia, and that they are predominantly of vascular origin. A distinction can be made based on the location of the calcification in the vessel wall, intimal or medial, and thereby the underlying pathophysiology and possible preventive measures. Our studies do not support a relation between intracranial artery calcifications and cognitive function. However, further research should focus on the clinical relevance of intracranial calcifications and treatment options.

ADDENDA

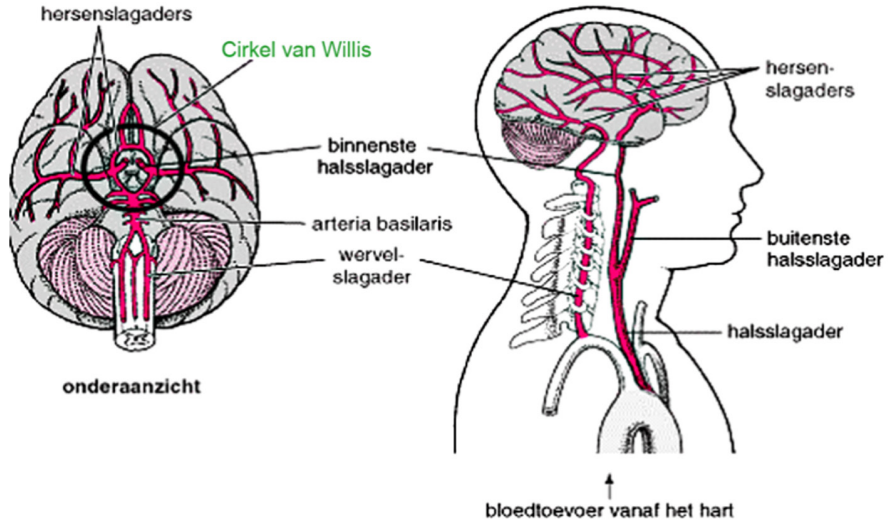
Samenvatting

Dankwoord

Curriculum vitae

Samenvatting

Dit proefschrift gaat over de aanwezigheid en gevolgen van afzettingen van kalk in het hoofd. Verkalkingen in het hoofd worden regelmatig gezien op computer tomografie (CT)-scans van de hersenen, maar de betekenis van deze verkalkingen is nog onduidelijk. In dit proefschrift hebben we gekeken naar verkalkingen in de halsslagader waar die net het hoofd binnenkomt, ook wel de sifon van de binnenste halsslagader (arteria carotis interna) genoemd, de basilaire slagader (arteria basilaris; een slagader achter in de hersenen) en naar verkalkingen diep in het hersenweefsel.

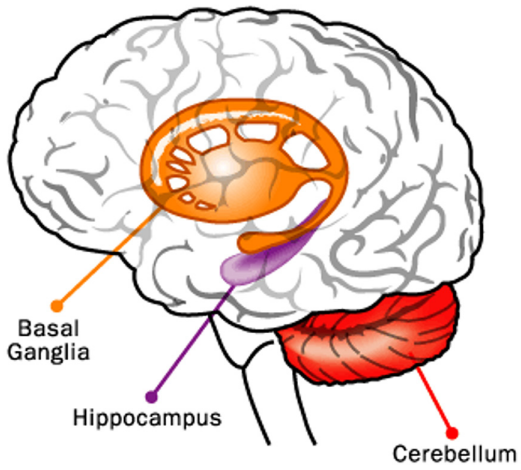


FIGUUR 1. Afbeelding die de binnenste halsslagader en basilaire slagader (arteria basilaris) toont. Uit De Merck Manual Medisch Handboek

Een slagader bestaat uit 3 hele dunne laagjes. De binnenste laag die direct in contact staat met het bloed heet tunica intima. De middelste laag, een soort hele dunne spier, heet tunica media. De buitenste laag heet tunica adventitia. Verkalkingen in de bloedvaten kunnen voor zover we nu weten in twee hoofdgroepen worden onderverdeeld: verkalkingen in de binnenste laag van het bloedvat en verkalkingen in de middelste laag van het bloedvat. Verkalkingen in de binnenste laag van het bloedvat (tunica intima) ontstaan door atherosclerose. Atherosclerose ontstaat door vetdeeltjes en ontsteking aan de binnenkant van de grote slagaders. In een later stadium treedt er verkalking op. Deze vorm van verkalkingen is het meest bekend en wordt vaak aderverkalking genoemd (terwijl een betere naam slagadervervetting zou zijn). Risicofactoren hiervoor zijn oudere leeftijd, mannelijk

geslacht, hoge bloeddruk (hypertensie), roken, hoge cholesterolwaarde in het bloed en suikerziekte (diabetes mellitus). Verkalkingen in de middelste laag van bloedvaten (tunica media) ontstaan niet door atherosclerose, maar de kennis over deze verkalkingen is beperkt. Deze verkalkingen worden in verband gebracht met hogere leeftijd, genetische of erfelijke ziekten, suikerziekte (diabetes mellitus) en nierschade, maar de precieze oorzaak is niet bekend.

Naast verkalkingen in grote bloedvaten hebben we in dit proefschrift ook gekeken naar verkalkingen diep in het hersenweefsel, met name in de basale kernen en de hippocampus. De basale kernen zijn vooral van belang bij het controleren van bewegingen. Ook hebben ze invloed op emoties en denkvermogen (cognitie). Cognitie is het vermogen om kennis op te nemen en te verwerken. Het is een breed begrip waar onder andere waarnemen, snelheid van denken, taal, geheugen, aandacht en concentratie onder vallen. De hippocampus is een hersengebied dat belangrijk is bij het opslaan van informatie en hiermee het geheugen. Aan het begin van dit promotieonderzoek was niet bekend waar de verkalkingen in de basale kernen en hippocampus zich precies bevinden; in of tussen de hersencellen of in de kleinere bloedvaten.



FIGUUR 2. Afbeelding die de basale kernen (basal ganglia) en hippocampus toont
Afbeelding gemaakt door Kevin Bonsor, Copyrighted free use, via Wikimedia Commons

In het proefschrift hebben we ook een zeldzame genetische ziekte onderzocht om meer te leren over verkalkingen diep in de hersenen. De ziekte van Fahr is een erfelijke aandoening waarbij patiënten uitgebreide verkalkingen hebben in met name de basale kernen, maar ook in andere gebieden van de hersenen. Sommige patiënten met de ziekte van Fahr hebben geen ziekteverschijnselen, maar andere patiënten hebben bewegingsstoornissen, psychische klachten en problemen met het denkvermogen.

In dit proefschrift hebben we de volgende aspecten van verkalkingen in het hoofd onderzocht:

- of de verkalkingen die we op een CT-scan zien in de basale kernen en de hippocampus zich in de bloedvaten bevinden;
- wat het onderliggende mechanisme is van verkalkingen bij patiënten met de ziekte van Fahr;
- wat de risicofactoren zijn voor verkalkingen in de basale kernen, de hippocampus, de binnenste halsslagaders en de wervelslagaders;
- of verkalkingen in de basale kernen, de hippocampus, de binnenste halsslagaders en de wervelslagaders invloed hebben op het denkvermogen.

Deel 1. Verkalkingen in de hersenen op een CT scan: wat zien we?

Deel 1 van dit proefschrift richt zich op de vraag of verkalkingen in de basale kernen en de hippocampus in de kleinere bloedvaten in de basale kernen en hippocampus zitten of in/tussen de hersencellen. Dit deden we door beelden van CT-scans te vergelijken met microscopisch onderzoek van de hersenen van overleden mensen.

In Hoofdstuk 2 onderzochten we verkalkingen in de basale kernen. We gebruikten hiervoor gegevens van 22 patiënten van wie een CT-scan van de hersenen was gemaakt en later hersenobductie (onderzoek na overlijden) plaatsvond. Bij microscopisch onderzoek werden bij acht van de 22 patiënten verkalkingen in het middelste deel van de vaatwand gezien. In vier patiënten kwamen de verkalkingen in de basale kernen op de CT-scan overeen met de bevindingen onder de microscoop, wat suggereert dat de verkalkingen die op een CT-scan gezien worden zich inderdaad in de bloedvaten in de basale kernen bevinden. Kleine verkalkingen kunnen op een CT-scan gemist worden, wat waarschijnlijk bij de overige vier patiënten waar onder de microscoop wel verkalkingen gezien werden, het geval was. De locatie in het middelste deel van de vaatwand past niet bij atherosclerose als onderliggende oorzaak van deze verkalkingen.

In Hoofdstuk 3 onderzochten we verkalkingen in de hippocampus onder de microscoop, om te onderzoeken of deze verkalkingen zich in de bloedvaten bevinden en overeenkomen met de bevindingen op een CT-scan. Bij microscopisch onderzoek en met CT-scan werden verkalkingen in zes van de negen onderzochte hippocampi gevonden. Verkalkingen werden gevonden in de middelste laag en de buitenste laag van het bloedvat (tunica adventitia). Deze verkalkingen lijken dus ook niet te zijn ontstaan door atherosclerose, omdat deze verkalkingen zich eveneens niet in de binnenste laag van de bloedvaten bevinden.

Deel 2. De ziekteleer van verkalkingen in de hersenen: lessen van de ziekte van Fahr

In Deel 2 (Hoofdstuk 4) geven we een overzicht van de literatuur over de ontstaanswijze van verkalkingen in de basale kernen bij de ziekte van Fahr. Dit geeft ons inzicht in het ontstaan van verkalkingen en mogelijke behandelingen, ook bij patiënten met niet-erfelijke verkalkingen in de hersenen. Er zijn zes genetische afwijkingen bekend bij patiënten met de ziekte van Fahr die op verschillende manieren tot kalkophopingen in de bloedvaten leiden. Er is momenteel nog geen behandeling voor de ziekte van Fahr die het ziekteproces kan afremmen of genezen. Inmiddels hebben we de Hersenstichting bereid gevonden om een onderzoek naar de werkzaamheid van het medicijn etidronaat bij de ziekte van Fahr financieel te ondersteunen. Etidronaat heeft eigenschappen die gelijk zijn aan pyrofosfaat, een lichaamseigen stof die het verkalkingsproces remt en oorspronkelijk was gemaakt om wasmachines te ontkalken. Bij andere aandoeningen waar verkalkingen in bloedvaten optreden, zoals de zeldzame ziekte pseudoxanthoma elasticum, zijn hier al goede resultaten mee behaald. Een klein onderzoek in het buitenland heeft ook verbetering van ziekteverschijnselen laten zien bij patiënten met de ziekte van Fahr. Het medicijn is in het verleden veel voorgeschreven aan mensen met botontkalking waardoor bekend is dat het een veilig en goedkoop medicijn is, dat goed verdragen wordt.

Deel 3. Risicofactoren en betekenis van verkalkingen in de hersenen

In Deel 3 onderzochten we in twee grote patiëntengroepen de risicofactoren voor en gevolgen van verkalkingen in de hersenen om te beoordelen of de ontwikkeling van deze verkalkingen kan worden beïnvloed of voorkomen.

In Hoofdstuk 5 onderzochten we hoe vaak verkalkingen in de basale kernen op een CT-scan gezien worden en het verband met risicofactoren voor vaatziekten in een groep van meer dan 1000 patiënten die verdacht werden van een herseninfarct. In 30% van de patiënten werden verkalkingen in de basale kernen gezien. De verkalkingen hielden verband met een hogere leeftijd en lagere 'body mass index' (BMI; maat om vast te stellen of iemand een gezond lichaamsgewicht heeft in verhouding tot de lichaamslengte). Er werd geen verband gevonden met andere risicofactoren voor vaatziekten, zoals hoge bloeddruk, hoog cholesterol, suikerziekte en roken. Dit suggereert dat deze verkalkingen niet ontstaan door atherosclerose en komt overeen met de bevindingen van Hoofdstuk 2.

In Hoofdstuk 6, 7 en 8 beschrijven we onze onderzoeken onder een groep van bijna 2000 patiënten die de geheugenpolikliniek van Tergooi Medisch Centrum bezochten tussen 2009 en 2015. Alle patiënten ondergingen dezelfde onderzoeken, waaronder een test van het denkvermogen (Cambridge Cognitive Examination) en een CT-scan van de hersenen.

In Hoofdstuk 6 onderzochten we of verkalkingen in de basale kernen verband houden met het denkvermogen en met risicofactoren voor vaatziekten. Verkalkingen in de basale kernen werden gevonden in 43% van de patiënten. We vonden geen samenhang met het denkvermogen. Verkalkingen in basale kernen kwamen vaker voor bij vrouwen dan bij mannen. Patiënten die bekend waren met een hoge bloeddruk of hiervoor medicijnen gebruikten, hadden minder vaak verkalkingen.

In Hoofdstuk 7 werd het verband tussen verkalkingen in de hippocampus op CT-scan en het denkvermogen onderzocht en het verband met risicofactoren voor vaatziekten. Hippocampusverkalkingen werden gevonden bij 19% van de patiënten. Een verband met het denkvermogen werd niet gevonden. Hippocampusverkalkingen hielden verband met hogere leeftijd, suikerziekte en roken.

In Hoofdstuk 8 werden in dezelfde groep patiënten verkalkingen in de sifon van de binnenste halsslagader en de basilaire slagader onderzocht. Bij 95% van de patiënten werden verkalkingen in de sifon gevonden; bij 50% in de binnenste laag van het bloedvat, bij 37% in de middelste laag van het bloedvat en bij 8 procent was het onderscheid moeilijk te maken.

Verkalkingen in de basilaire slagader waren bij 8% van de patiënten aanwezig. Risicofactoren voor verkalkingen in de binnenste laag van de sifon waren leeftijd, hoge bloeddruk en roken. Leeftijd en suikerziekte waren risicofactoren voor verkalkingen in de middelste laag van de sifon. Verkalkingen in de binnenste en middelste laag van de sifon hielden beiden verband met beroerte en verkalkingen in de binnenste laag ook met hartinfarct. Verkalkingen in de basilaire slagader hielden verband met hogere leeftijd en hoge bloeddruk. Er werd geen verband met het denkvermogen gevonden.

In Hoofdstuk 9 bespraken we de resultaten en betekenis van de bovenstaande onderzoeken. We concludeerden dat verkalkingen in de hersenen vaak voorkomen bij oudere patiënten op CT-scan, vooral in de sifon van de binnenste halsslagader en de basale kernen, en dat ze in de bloedvaten zitten.

Er kan onderscheid gemaakt worden op basis van de locatie in de bloedvaten van de verkalkingen en daarmee de onderliggende ontstaanswijze en mogelijke preventieve maatregelen. Zo zagen we dat in de basale kernen en de hippocampus verkalkingen zich in de tunica media bevinden. Deze verkalkingen houden geen verband met atherosclerose, maar wel met suikerziekte en oudere leeftijd. Preventie en goede behandeling van suikerziekte kan mogelijk invloed hebben op het ontstaan van deze verkalkingen.

Verkalkingen in de grote bloedvaten, de sifon van de binnenste halsslagader en de basilaire slagader, bevinden zich vaak in de tunica intima en houden verband met atherosclerose. Preventie en behandeling van risicofactoren voor atherosclerose, zoals hoge bloeddruk, roken, hoog cholesterol en suikerziekte hebben mogelijk invloed op het ontstaan van deze verkalkingen. Bij verkalkingen in de sifon van de binnenste halsslagader zien we naast verkalkingen in de tunica intima ook verkalkingen in de tunica media.

Onze onderzoeken ondersteunen geen verband tussen deze verkalkingen in de hersenen en het denkvermogen, maar mogelijk komt dit door de selectie van onze patiënten. Onze patiënten zijn namelijk allemaal oudere mensen die vanwege geheugenklachten bij een geheugenpolikliniek kwamen. Er kon dus geen vergelijking plaatsvinden met mensen zonder geheugenklachten. Verder onderzoek zou zich moeten richten op het belang en de behandelopties van verkalkingen in de hersenen. Zo adviseren wij onderzoek te doen naar het verband tussen verkalkingen in de hersenen en het denkvermogen met andere tests die het denkvermogen onderzoeken, maar ook te onderzoeken of verkalkingen in de hersenen samenhangen met psychische klachten en bewegingsstoornissen. Momenteel worden de voorbereidende stappen genomen om een onderzoek naar de werkzaamheid van etidronaat bij patiënten met de ziekte van Fahr te starten.

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Curriculum vitae

Esther de Brouwer was born on December 24, 1986, in Lettele, the Netherlands, where she also grew up. She completed high school at the Etty Hillesum Lyceum in Deventer in 2005 after which she attended medical school at the University of Groningen. Her interest in geriatrics became clear during her last internship at the Deventer Hospital. After obtaining her master's degree in December 2011, she began working at the Department of Geriatric Medicine at Tergooi Medical Center in Blaricum. She started the residency training in December 2012 with rotations in internal medicine (hospital Gelderse Vallei, Ede, supervisor dr. R. Heijligenberg), psychiatry (GGZ Altrecht, Utrecht, supervisor dr. J. Sanders) and geriatrics (University Medical Center Utrecht, supervisor dr. H.J.J. Verhaar, and Tergooi Medical Center, Blaricum, supervisor C. van Rees). During the final years of her residency she studied the association between hippocampal calcifications and cognition, which resulted in this thesis. In 2018 she finished her residency and started working as clinical geriatrician at the Deventer Hospital. Esther lives with her partner and two children in Deventer.