

Deconstructing Fahr's disease/syndrome of brain calcification in the era of new genes

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Abstract

Introduction: There are now a number genes, known to be associated with familial primary brain calcification (PFBC), causing the so called 'Fahr's' disease or syndrome. These are *SCL20A2*, *PDGF-B*, *PDGFRB* and *XPRI*. In this systematic review, we analyse the clinical and radiological features reported in genetically confirmed cases with PFBC. We have additionally reviewed pseudohypoparathyroidism which is a close differential diagnosis of PFBC in clinical presentation and is also genetically determined.

Methods: We performed a Medline search, from 1st Jan 2012 through to 7th November 2016, for publications with confirmed mutations of *SCL20A2*, *PDGF-B*, *PDGFRB*, and *XPRI* and found twenty papers with 137 eligible cases. A second search was done for publications of cases with Pseudohypoparathyroidism or pseudopseudohypoparathyroidism, and found 18 publications with 20 eligible cases.

Results: *SLC20A2* was the most common gene involved with 75 out of 137 cases included with PFBC (55%) followed by *PDGFB* (31%) and *PDGFRB* (11%). Statistically significant correlation was found between the presence of parkinsonism with *SLC20A2* mutations, headache in *PDGFB* and generalised tonic-clonic seizures in patients with pseudohypoparathyroidism.

Conclusion: We combine statistical analysis and clinical inference to suggest a diagnostic algorithm based on the observations in this study to help with investigation of a patient with neurological features and brain calcification.

1. Background

Physiological calcification in brain can be seen in up to 20% of routine CT scans [1, 2]. Pathological brain calcification can be due to parathyroid disorders, phacomatosis, and secondary to infections, inflammation or haemorrhage. Idiopathic calcification, has traditionally been described as ‘Fahr's disease’, based on Theodor Fahr’s report “Idiopathische Verkalkung der Hirngefäße” or idiopathic calcification of cerebral vessels [3]. It is unlikely though that the case described in that paper was ‘primary’ and although the calcification was not mainly in the basal ganglia, Fahr’s name has been associated with primary basal ganglia calcification despite previous, possibly more accurate description by Delacour. The familial basis of primary brain calcification was initially suggested by Boller *et al.* who used the term familial idiopathic cerebral calcification in their 1977 paper with the same name [4]. The understanding of familial ‘idiopathic’ or ‘primary’ brain calcification has advanced dramatically in the recent years with discovery of four causative gene mutations namely *SCL20A2*, *PDGF-B*, *PDGFRB*, and *XPR1* (Figure 1). There is an emerging need for a reappraisal of key concepts in the understanding of brain calcification. These include definition, familial association, nomenclature and disease phenotype that has evolved in the light of new genetic findings.

Nomenclature

The nomenclature of Fahr's disease is complicated by the fact that at least 35 names have been used in publications referring to brain calcification mainly limited to the basal ganglia [5]. “Fahr’s disease” is still the most commonly used term. A similar term “Fahr’s syndrome” first appeared in literature in 1982 [6] to describe the constellation of neuropsychiatric features and calcification. The use of term “disease” for the “primary” or idiopathic calcification and “syndrome” to reflect the clinical/radiological picture, when a secondary

cause is found, has been suggested. “Fahr’s syndrome” and “Fahr’s disease” have also been used interchangeably in some articles[7] and case reports[8, 9]. Some authors ascribe the attribution of Fahr’s name to this disorder a ‘misnomer’[5]. The other names for the same syndrome have been used to reflect location or aetiology such as ‘primary’ or ‘idiopathic’ brain calcification. These include bilateral striatopallidodentate calcification or calcinosis (BSPDC) and idiopathic basal ganglia calcification (IBGC) respectively [5]. Considering the complicated terms used to describe location of the calcification, the term primary familial ‘brain’ calcification (PFBC) has been proposed[10] to replace other terms used such as Fahr's disease and IBGC.[10] For the purpose of the search and analysis we have included a broad range of terms used to describe cases with brain calcification, including “Fahr’s disease” and “Fahr’s syndrome” but for purpose of discussion we prefer to use PFBC which is more contemporary to current literature on the subject [11].

The causes of brain calcification include disorders of parathyroid such as hypoparathyroidism. Some cases with brain calcification are due to other metabolic disturbances [12-15]. Hypoparathyroidism characterized by parathyroid hormone (PTH) deficiency leads to impaired calcium metabolism and has been linked to brain calcification. In cases with pseudohypoparathyroidism and pseudopseudohypoparathyroidism the Parathyroid glands produce PTH but the uptake of PTH is impaired. Both pseudohypoparathyroidism and pseudopseudohypoparathyroidism have been linked to mutations in *GNAS* and *STX16*. There have been very few reports of genetically determined pseudohypoparathyroidism and pseudopseudohypoparathyroidism. There may be other yet unidentified genetic causes of pseudohypoparathyroidism and pseudopseudohypoparathyroidism and it is possible that not all are caused by *GNAS* and *STX16* mutations. The clinical manifestations of these are quite like the cases reported as Fahr’s syndrome or PFBC; such as progressive movement disorders with or without cognitive

decline and psychiatric features. The characteristic metabolic profile associated with hypoparathyroidism is not seen and cases with pseudohypoparathyroidism do not have reduced PTH levels and are difficult to detect clinically. However, one may suspect pseudohypoparathyroidism clinically, based on some characteristic skeletal changes, particularly in the fingers but genetic testing is needed to confirm the diagnosis. Conversely, some other neuro-genetic disorders with DNA repair defects that are associated with basal ganglia calcification but manifest clinical features or demographic profile that is quite unlike PFBC. One such example is Aicardi-Goutières Syndrome which is an early-onset encephalopathy characterized by basal-ganglia calcification, white matter abnormalities, and a chronic cerebrospinal fluid (CSF) lymphocytosis and is characterized by an interferon signature [16]. We included cases with pseudohypoparathyroidism which most closely resemble PFBC in our analysis mainly for differential diagnosis but the cases with phacomatosis, DNA repair defects and hypoparathyroidism were excluded.

Pathophysiology of genetically determined brain calcification

With the discovery of new genes for basal ganglia calcification the understanding of mechanisms of calcification has improved significantly. This understanding though is far from complete. Calcium, like most minerals has quite complex metabolism in human cells and pathogenesis of the brain calcification related to the four reported genes is incompletely understood. A summary of this understanding is presented below.

Located at 8p11.21, the Solute Carrier family 20 (Phosphate Transporter), Member 2 or the *SLC20A2* gene encodes the type III sodium-dependent inorganic phosphate (Pi) transporter 2 (PiT2) [17]. Mutations in *SLC20A2* are inherited in an autosomal dominant manner.

Inorganic phosphate transport is crucial to cellular calcium and phosphate homeostasis and the impairment in the function of PiT2 [17] can contribute to the deposition of calcium

phosphate in the vascular extracellular matrix [18]. Although calcification is limited to brain in cases reported with *SLC20A2*, its role of in homeostasis for inorganic phosphate is evident in several other tissues around the body including bone, parathyroid, and kidneys [19, 20]. The pathophysiology of calcification associated with *SLC20A2* has been studied using *Slc20a2*-knockout (KO) mice. *Slc20a2*-KO mice indeed have a high CSF [Pi] which supports a role of PiT2 in Pi export from the CSF as one of the mechanisms with possible therapeutic implications[21].

Xenotropic and Polytropic Retrovirus receptor or *XPR1* gene is located at 1q25.3. In families, the mutations are inherited as autosomal dominant. The gene is closely linked to PiT2, encoding a retroviral receptor with a role in phosphate export from the cells [18]. It directly affects phosphate homeostasis intracellularly and dysfunction of this mechanism can contribute to calcium deposition.

PDGFRB is another gene implicated in PFBC encodes for one of the two receptors for platelet-derived growth factor (PDGF) with subunit β (*PDGFB*), its major ligand. *PDGFRB* is crucial in maintaining the blood brain barrier (BBB) and loss of function mutations can potentially lead to altered permeability in pericytes surrounding the brain blood vessels that can potentially lead to calcium deposition [22-24]. Mutations in both *PDGFB* and *PDGFRB* have autosomal dominant mode of inheritance. The *PDGFB* is involved in pericyte recruitment, Blood brain barrier (BBB) regulation and angiogenesis [25]. Loss of calcium regulation through the BBB possibly leads to progressive calcinosis [25]. It has also been proposed that the PDGF proteins can have regulatory functions on phosphate transporters *XPR1* and PiT in the brain [23]. The possible interactions between the mechanisms of calcium deposition related to PiT2, pericytes and BBB remain to be elucidated.

Brain calcification can also be seen in disorders with PTH resistance. Loss of function of *GNAS* (also known as Guanine Nucleotide Binding Protein (G Protein), Alpha Stimulating Activity Polypeptide 1) on the maternal allele is known to cause basal ganglia calcification though this is not considered as PFBC. *GNAS* is a complex imprinted locus that produces multiple transcripts through alternative splicing and promoters [26]. *GNAS* mutations can result in a group of pseudohypoparathyroid disorders which include pseudohypoparathyroidism type Ia, pseudohypoparathyroidism type Ic, pseudopseudohypoparathyroidism and McCune-Albright syndrome.

Pseudohypoparathyroidism type Ib is usually due to imprinting/methylation defects in *GNAS* mentioned above leading to loss of function on the maternal allele but can also be seen due to *STX16* mutations [27]. These disorders are known to have distinct clinical features such as brachydactyly, short stature, skeletal abnormalities (except in Pseudohypoparathyroidism type Ib) [28]. This distinction is mainly driven by the understanding that in cases with pseudohypoparathyroidism type 1a with loss of function in *GNAS*, there is generalized hormonal resistance to parathyroid hormone (PTH), TSH and gonadotrophins.

With the clear differences in the genetic pathomechanisms it is reasonable to expect some differences in the clinical features of patients with different genetic mutations. Here in this systematic review we review the clinical presentations in the genetically confirmed PFBC and pseudohypoparathyroidism which is a close differential diagnosis.

2. Methods

Systematic review of the literature was performed per the PRISMA guidelines (Preferred Reporting Items for Systematic Reviews and Meta-analyses). The first search included all publications in English from 1st Jan 2012 (when genes for PFBC were discovered) through to 7th November 2016. The following search terms were applied: Brain calcification, cerebral

calcification, fahr* disease, fahr* syndrome, idiopathic basal ganglia calcification, primary familial basal ganglia calcification, bilateral striatopallidodentate calcification OR calcinosis, IBGC OR PFBC OR BSPDC AND *SLC20A2*, *PDGFB*, *PDGFRB* OR *XPRI*. Inclusion criteria included a positive genetic test result of *SLC20A2*, *PDGFB*, *PDGFRB* or *XPRI* mutation and brain calcification seen on CT or MRI.

We carried out a second search that included all publications in English up until 7th November 2016 using search terms: Pseudohypoparathyroid*, pseudopseudohypoparathyroid*, *GNAS** OR *STX16*; AND brain calcification OR cerebral calcification. Inclusion criteria for a study were clinically confirmed pseudohypoparathyroidism, brain calcification seen on neuroimaging, normal parathyroid testing with or without confirmed *GNAS* mutation. We excluded phacomatosis, DNA repair defects and cases with brain calcification due to other secondary causes.

Demographic data, including age at onset, duration, sex, clinical features at presentation, radiological features on available neuroimaging and blood investigations (parathyroid hormone and calcium) were recorded when available in cases with a genetic diagnosis. Statistical analysis of associations between demographic, clinical and radiological characteristics of *SLC20A2*, *PDGFB*, *PDGFRB*, *XPRI* and pseudohypoparathyroidism was performed using IBM SPSS (version 20). Continuous data was compared between groups using two-tailed *t*-tests (two groups). Comparison of categorical data was performed with Pearson Chi² analysis; threshold for all statistical significance was $p < 0.05$. This article does not contain any studies with human or animal subjects performed by any of the authors.

3. Results

We identified 20 publications which satisfied the inclusion criteria [17, 18, 22, 23, 29-44] for review (Supplementary figure). We collated information on 137 cases, either sporadic or from 34 families, with demographic and clinical characterisation found in Table 1. To further investigate the hypothesis that pseudohypoparathyroidism represents a comparable phenotype as a form of genetic brain calcification, we identified 18 publications with data on 20 patients which satisfied the inclusion criteria for review [12, 15, 45-60]. Apart from two families with two affected individuals in each, all cases of pseudohypoparathyroidism with cerebral brain calcification present were reported as sporadic. There were no clinical reports of pseudopseudohypoparathyroidism secondary to *GNAS* mutations within the search parameters.

3.1. Demographic characteristics and information availability

SLC20A2 was the most common gene involved with 75 out of 137 cases included with PFBC (55%) followed by *PDGFB* (31%) and *PDGFRB* (11%) (Table1). Only 53.5% patients with PFBC had a clearly recorded age of onset. Age at presentation was better recorded with rate of 91% across all cases. The details of PFBC cases and the comparison with Pseudohypoparathyroid cases are presented in Table 1.

3.2. Clinical phenotype and distinctive clinical features

The breakdown of clinical presentation for symptomatic patients, divided into neurological or psychiatric presentation is presented in Table 2. Individual clinical symptoms are presented in Table 2 and Figure 2. Almost a quarter (24%) of cases in the studies included was asymptomatic.

Through group-wise, then individual-wise analysis, we identified certain clinical features occurred significantly more frequently with a genetic abnormality compared to the other

mutations combined (Chi² analysis, $p < 0.05$). Thus, parkinsonism was more commonly observed in *SCL20A2* (21% of cases) and headache was more common in *PDGFB* (32.5% of cases). Generalised tonic-clonic seizures (GTCS) were significantly more in pseudohypoparathyroidism (65% of cases).

Parkinsonism was seen in 16% of all the included cases; however, the details of specific parkinsonian features were sparse. Hyperkinetic movement disorders were reported seen in 20% though a breakdown of hyperkinetic movement disorder was not available in all the cases (details in Figure 2).

Cognitive impairment was seen in 15% of all the cases and although 67% of *XPR1* cases had cognitive impairment, this difference did not reach statistical significance. Depression was the most frequent psychiatric feature in 7% of all cases and present in 33% of *PDGFRB* cases but statistical analysis showed that frequency of depression, and other psychiatric features, was comparable across the groups ($p > 0.05$).

3.3. Radiological phenotype and distinctive features

Basal ganglia calcification was present in all of cases with the PFBC and gene mutations but this was not true for pseudohypoparathyroidism as 95% had calcification of the basal ganglia. Other areas that reported cerebral calcification include the thalamus, cerebellum (and specifically dentate nucleus), subcortical grey matter or grey-white junction and cortical areas (Figure 2). The thalamus and dentate nucleus was significantly more frequently reported as an area of brain calcification in *SCL20A2* cases compared to other mutations combined when tested with Chi² analysis ($p < 0.05$). This result may be affected by reporting bias, which will be discussed below. All other areas of cerebral calcification are comparable across all mutation groups ($p > 0.05$) but only *PDGFB* mutations were noted to have cysts in the white matter with leucodystrophy like presentation in 2 cases[61].

4. Discussion

We discuss here the results from this analysis and summarize the current information for each genetic cause of PFBC and pseudohypoparathyroidism. It has been suggested that clinical features among the commonly reported mutations were psychiatric signs (72.7%, 76.5%, and 80% for *PDGFB*, *SLC20A2*, and *PDGFRB*, respectively), movement disorders (45.5%, 76.5%, and 40%), and cognitive impairment (54.6%, 64.7%, and 40%)[62].

SLC20A2

With regards to age of onset, *SCL20A2* cases were older than *PDGFRB* and pseudohypoparathyroid cases (38.6 vs. 25.3 and 20.1). This observation is limited by the fact that there is only 54.3% data availability for age of onset. Although presentation with hyperkinetic movement disorders is characteristic of all genetic forms of PFBC, parkinsonism (21%) was significantly more common with *SCL20A2* mutations. However, details of parkinsonian features regarding presence of tremor, bradykinesia or gait disorder were not always mentioned. There is marked heterogeneity in presentation, including not otherwise specified “hyperkinetic movement disorder” (19%), dystonia (13%), chorea (12%), and cerebellar ataxia (8%). In the newly redefined IBGC2 kindred linked to *SLC20A2*, gait and upper limb ataxia, slurred speech, hyperreflexia, intellectual impairment has been described[42]. Depression appears to be the most common psychiatric features though anxiety, agitation, psychosis, has been also reported in small number of cases. Headache has been reported in a small number of cases without a clearer classification of the headache characteristics. Involvement of thalamus and dentate nucleus was significantly more frequent in *SCL20A2* cases than in other genetic PFBC on imaging. Thus, patients with late onset movement disorder with signs of parkinsonism and calcification in basal ganglia, thalamus and dentate are possibly best candidates to be screened for this mutation.

PDGFB

The mutations in the *PDGFB* gene are the second most common cause of PFBC. Clinically, cases reported with *PDGFB* mutations have hyperkinetic movements (19%) (not further specified), dystonia (9%), chorea (14%) and ataxia (14%) which appeared more commonly than parkinsonism (7%) and seizures (5%). This difference did not reach statistical significance which could be due to heterogeneity of the clinical description of hyperkinetic movement disorders. Similar to other genetic mutations, basal ganglia and cerebellum are commonly calcified. Subcortical calcification is relatively common (47%) and one of the reported cases have cortical calcification. The cases with *PDGFB* mutations have been most commonly described to have headache (29%) ($p < 0.05$). The implications of this are discussed in greater detail below.

PDGFRB

There is limited number of cases published with clinical details on cases with abnormalities in *PDGFRB* gene. The age of onset and age at presentation is earliest compared to other mutations. Headache and depression seemed commoner in this group but this finding did not reach statistical significance. More studies are needed to better understand characteristics of this group.

XPR1

Recently, mutations in the *XPR1* gene have been identified as a cause of PFBC. *XPR1* mutations seem to present with higher incidence of cognitive dysfunction (66.7%) and cortical calcium deposition (Figure 2). Parkinsonism, dysarthria and chorea have been seen but no cases have been reported with headache, dystonia or cerebellar ataxia. More studies are needed to better understand characteristics of this group.

Pseudohypoparathyroidism

In comparison to PFBC cases, these cases had a higher likelihood of seizures (GTCS) and a notable absence of reported psychiatric features and headache. The reason for including this group in our study and analysis is supported by the overall similarity in neurological presentation of these cases to genetically determined PFBC as there was no statistically significant difference in movement disorders and cognitive changes between PFBC and pseudohypoparathyroidism. There was a trend of higher frequency of hyperkinetic movement disorders in comparison to parkinsonism but that was not statistically significant. However, recognition of seizures as a key clinical feature may help differentiating this disorder when investigating families or sporadic cases with brain calcification and neurological features. It is known that hypocalcaemia is one of the causes of seizures specific to this group [45], but low calcium levels were not recorded as the cause of seizures in most of the reported patients included in this study. The cases with pseudohypoparathyroidism differ from hypoparathyroidism in many ways such as levels of PTH and calcium and presence of skeletal abnormalities. The absence of psychiatric features in this group is interesting, considering that psychiatric complaints are well documented in the literature for cases of hypoparathyroidism and other parathyroid disorders. It may be worthwhile separating this group out from the parathyroid disorders considering the pathomechanism, genetic mutations and clinical features for better identification in clinic and research studies.

PFBC with no known mutations

Nicolas et al reported clinical and radiological profile of 47 cases with no mutations in *PDGFRB* or *SLC20A2* [62]. The cases with no mutations seemed to have a higher chance of seizures and considering our observation in pseudohypoparathyroidism cases, screening all cases with PFBC for this condition may be a useful. Overall the clinical and radiological

features of these cases were quite like the cases with genetically confirmed mutations in *SLC20A2* or *PDGFRB* [62].

Not all cases with PFBC have mutations and the figures vary but up to 65% cases may not have a known mutation [62]. There may be two reasons for this. Firstly, it may be possible that there are still unidentified mutations that are responsible for PFBC. Secondly, it is possible that there has been some inconsistency in how the genes have been tested. For example, the identification that IBGC 1 and more recently IBGC2 loci map to *SLC20A2*, supports the idea that clinical identification is very important [42]. We did not include the PFBC cases without mutations in our study which was mainly to help identify the phenotypic genotypic association, but it comparing the clinical and radiological findings between mutation carriers and non-mutation carriers with PFBC might be useful. Grutz et al., have proposed and tested an algorithm based on sites of calcification and individual's age to predict the chances of positive genetic finding in PFBC [42]. They divided the brain into four sites defined as follows: i) basal ganglia (including the caudate nucleus, putamen, and globus pallidus), ii) thalamus and subthalamic nucleus iii) cerebellum, and iv) cortical region and suggest that presence of

- i) At least one site with bilateral calcification in individuals between 20 and 40 years of age, and
- ii) At least two sites with bilateral calcification in individuals between 41-70 years of age [62].

Together with this algorithm, which helps identify the positive genetic cases, the algorithm we propose below enhances the possibility of finding the right gene based on the clinical features.

Analysis

Here we confirm and extend previous descriptions including a comprehensive review of a large patient cohort with *SCL20A2* or *PDGFRB* mutation by the French IBG group.[63] Tadic *et al.* (2015) performed a similar systematic, thorough review of *SCL20A2*, *PDGFB* and *PDGFRB* cases, but not the *XPRI* mutation, discussing the need for more robust and uniform data-collection in future PFBC cases. There are some small differences in frequency of clinical features reported in both studies compared to this systematic review and likely represent different patient selection.

This study has some limitations and we acknowledge the potential errors of genotype-phenotype characterisation using systematic review but feel it is a robust technique when reviewing such rare diseases. This method should be used to continually update our clinical knowledge as new cases are discovered and more data can be included in analysis. We support previous suggestions for a more uniform reporting style with key clinical data fields included in future research studies. The low number of cases for certain mutations, including *XPRI* and pseudopseudohypoparathyroidism, is a form of statistical bias. Selection bias is another avenue for error within a systematic review. [11, 64]

It has been suggested that the functional abnormalities leading to movement disorders, cognitive and psychiatric features in brain calcification probably result from the disruption of the basal ganglia-thalamocortical circuits.[65] In terms of genotype-phenotype correlation, one may hypothesise that the clinical presentation can vary depending on location of calcium deposits (e.g. striatum, cortical areas or dentate nucleus). This correlation does not seem absolute. There has been at least one study using [18F] FDG-PET that found areas of cortical hypometabolism, in the areas that did not have calcifications or other morphological changes and this correlated to the patient's neuropsychological symptoms [29]. It is therefore

plausible that calcium deposition may not be the only pathophysiological mechanism in patients with the genetic mutations and highlights the importance of future studies investigating asymptomatic patients.

It has been noted that patients with the *PDGFB* mutations had headache. However, on closer analysis, this symptom did not co-segregate in most of the families suggesting an absence of causal relationship. There is quite marked clinical heterogeneity of familial PFBC cases and ~40% of the patients carrying basal ganglia calcification did not show any symptoms as reported on a large case series[66].

Although this co-segregation may be incidental, or reflective of data collection (selective bias), one may bear in mind that calcium and parathyroid abnormalities have been recorded in patients of headache with Idiopathic intracranial hypertension (IIH) [67, 68]. In one study ten percent of IIH patients had abnormalities in calcium (Ca) serum level: six had hypocalcemia and 1 had hypercalcemia[68]. A better documentation of calcium, parathyroid hormone status and CSF pressure or at least fundus examination might help with better characterization of headaches in genetic mutations leading to brain calcification.

There are some key limitations in the current understanding of brain calcification that this review highlights. Interestingly none of the genes involved in the calcium metabolic pathway have been described as a cause of the PFBC syndrome [69]. Phosphate transport seems to be an important part of the process of calcification in PFBC. *SLC20A2* gene encodes type III sodium-dependent inorganic phosphate (Pi) transporter 2 (PiT2)[17] and the impairment in the function of PiT2 [17] can contribute to the deposition of calcium phosphate in the vascular extracellular matrix [18]. A recent study in *Slc20a2*-knockout (KO) mice demonstrated high CSF [Pi]. The hyperphosphatemia in CSF possibly reflects impaired

phosphate export from the CSF [21]. These studies support a pathophysiological link between *SLC20A2* mutations and defective phosphate transport that might be responsible for PFBC.

XPR1 also closely links to PiT2 [18] and considering the observation of CSF increase of phosphate in *Slc20a2*-knockout animal models, it is quite likely that impairment in the function of PiT2 and inorganic phosphate (Pi) transport is the main cause of PFBC at least in IBGC 1, 2, 3, and 6.

PDGFRB is another gene implicated in PFBC encodes for one of the two receptors for platelet-derived growth factor (PDGF) with subunit β (*PDGFB*), its major ligand. *PDGFB* is a cell-surface tyrosine kinase receptor, which plays an essential role in various signalling pathways involved in the regulation of cell proliferation, differentiation, survival, and migration. The PDGF in the endothelial cells and pericytes seem to have a different mechanism of action with the same consequence of calcium deposition around the blood vessels in brain[70]. It has been proposed that the integrity of the BBB is compromised in *PDGFRB*, which secondarily induces vascular and perivascular calcium depositions [22]. In a study of the correlation between calcification induced by Pi, PDGF-BB was shown to increase the expression of PiT-1 in the endoplasmic reticulum in primary cultures of rat aortic vascular smooth muscle cells. This could thereby mediate an increase in the Pi influx into the smooth muscle cell, therefore facilitating the formation of calcium phosphate deposits in the new generated matrix vesicles[71]. Although PiT1 is possible more in abundance in the vascular endothelium and possibly equally or more important for phosphate transportation, interestingly, *SLC20A1* gene, which codes for the phosphate transporter 1 (PiT1), has been screened but not been found in PFBC patients [69].

The calcium deposition in PFBC starts in the endothelial and smooth muscle cells of blood vessels in the Globus pallidus which are most susceptible to build calcium deposits in

response to various metabolic triggers [72]. Calcifications limited to Globus pallidum can be also be linked to ageing and are prevalent in people over 60 years of age (5.5–20 %)[2].

Drawing from some studies on vascular smooth muscle cells and aortic valve calcification, hyperphosphatemia is an important contributor to vascular calcification [72, 73]. Elevated phosphate induces calcification of smooth muscle cells (SMC) in vitro and inhibition of phosphate transport by phosphonoformic acid blocks phosphate-induced calcification, implicating sodium-dependent phosphate cotransporters (PiT 1 and PiT 2) in this process. This can have potential therapeutic implications considering that *Slc20a2*-knockout (KO) mice have already been shown to have hyperphosphatemia in the CSF.

It seems that there is interplay of the genetic factors contributing to calcium deposition but more experimental work is needed to understand this.

5. Conclusion

Our analysis of genotype-phenotype correlation in brain calcification related to genetic mutations suggests that although there is significant overlap in terms of clinical and radiological features, there may be certain features significantly associated with specific mutations.

With regards to significant distinctive neurological features, parkinsonism was more common with *SLC20A2* mutations, headache with *PDGFRB* and generalised tonic-clonic seizures were seen in pseudohypoparathyroidism. Radiologically, calcification of the thalamus was significantly more common with *SCL20A2* mutations.

Some features that differed across groups were not statistically significance however we feel the association may still be noteworthy. For example, with regards to psychiatric features, depression was more often reported in *PDGFRB* ($p>0.05$). Cognitive impairment and

parkinsonism tended to occur with late onset of disease (>45 years) while younger onset cases more commonly had hyperkinetic movement disorders such as chorea and dystonia. Like other neurodegenerative conditions it is possible that patients with PFBC may have different clinical presentation at different ages with the same pathological process.

In summary, using systematic review we have identified several distinct features which may aid the clinical diagnostic process. Based on the existing knowledge (figure 3a) and observations made in this analysis we propose a schematic plan (figure 3b) to investigate patients with brain calcification and neurological or psychiatric features.

Author roles

(1) Research Project: A. Conception, B. Organization, C. Execution; (2) Statistical Analysis: A. Design, B. Execution, C. Review and Critique; (3) Manuscript: A. Writing of the First Draft, B. Review and Critique.

A.B.: 1A, 1B, 1C, 2A, 2B, 2C, 3A, 3B

X.T.: 1C, 2A, 2B, 2C, 3A, 3B

R.E. 1C, 2C, 3B

B.B: 1C, 2C, 3B

K.P.B.: 1A, 2C, 3B

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Legend for figures:

Figure 1a Simple schematic representation of mechanisms responsible for brain calcification

Figure 1 b Schematic representation of the postulated mechanisms in genetically mediated microvascular calcium deposition in the brain. The scheme shows a cross section through a blood vessel in the brain demonstrating the location of pericytes and neural tissue. PDGF B and PDGF RB are located in the pericytes and loss of gene function can cause age-dependent phenotypic change in pericytes that ultimately provokes the formation of microvascular calcification. Phosphate uptake through the inorganic phosphate transporter type III is impaired in *SLC20A2* and *XPR1* mutations. Parathormone uptake is facilitated through G Protein coupled cyclic AMP activity which can be lost in *GNAS1* mutations.

PiT2 – inorganic phosphate transporter 2, PTH- Parathormone

Figure 2 Histogram showing (A) clinical presentation by category (neurological, psychiatric or asymptomatic), (B) psychiatric presentation by symptom, and (C) neurological presentation by symptom. (D). Histogram showing calcification on CT head imaging by anatomical location.

Genetic mutations leading to cerebral calcification present with neurological symptoms more frequently than psychiatric symptoms or being asymptomatic. Depression is the most common psychiatric symptom. Parkinsonism (14.7%), a hyperkinetic movement disorder (20.5%) and cognitive impairment (13.9%) were the most frequent neurological symptoms across all mutations. GTCS, parkinsonism and headache were associated with Pseudohypoparathyroidism, *SLC20A2* and *PDGFB* mutations, respectively, at statistically significant higher frequency compared to other mutations combined when tested with Chi² analysis (« indicating **p <0.05**). Note that chorea and dystonia represent further breakdown of the hyperkinetic movement disorder category.

Basal ganglia calcification is most consistently observed (99.26% of all cases). Cerebellar calcification is the next most frequently calcified area across all mutations (50% of all cases). Dentate nucleus calcification is described with *SCL20A2* mutations at statistically significant higher frequency compared to other mutations combined (***p <0.05**).

CT – computerised tomography GTCS = generalised tonic clonic seizures

Figure 3a. Causes of brain calcification

Figure 3b. Schematic plan of investigation of brain calcification.

Supplementary figure 1 Search strategy and included studies.