Does Endothelial Tissue-Nonspecific Alkaline Phosphatase (TNAP) Play a Role in Intracranial Calcification?

Nida Fatima OMS-II*, Shadia N. Ahmed OMS-II*, Gregory Oliva OMS-II, Alexandra Tsivitis OMS-II, Raddy L. Ramos PhD, Olga V. Savinova PhD

Department of Biomedical Sciences, New York Institute of Technology College of Osteopathic Medicine, Old Westbury, NY, USA; * contributed equally

Introduction

Primary Familial Brain Calcification (PFBC), a neurodegenerative disorder, presents with bilateral symmetrical intracranial calcification that clinically leads to motor symptoms. Given the extensive research on the causative effects of TNAP on peripheral vascular calcification we hypothesized that it will similarly play a role in intracranial calcification. TNAP, found in many tissues, is an ecto-phosphatase that degrades inorganic phosphate leading to calcification. We focused primarily on the choroid plexus, which had the highest TNAP expression, by exploring the relationship between TNAP activity and regulation of phosphate levels in the cerebrospinal fluid (CSF) as a possible mechanistic link to intracranial calcification.

Methods

TNAP overexpression, human alkaline phosphatase gene (ALPL) was inserted into a mouse model genome and activated via Cre/lox recombination. We compared it to a TNAP knockout model in which the endogenous gene was knocked out. No correlation was seen between endothelial TNAP gene dosage (knockout, wild type, transgenic) and the level of alkaline phosphatase activity within the choroid plexus.

Results

We found significantly more intracranial calcification within the basal ganglia in our TNAP overexpression model. Collectively these findings suggest that it is unlikely that endothelial TNAP in the choroid plexus is regulating phosphate transport or is responsible for intracranial calcification.

Discussion

After observing the involvement of TNAP expression in intracranial calcification we concluded that TNAP can be a therapeutic target for PFBC. However, the causative role of choroid plexus in intracranial calcification is yet to be determined and requires further research.

Acknowledgements

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Osteopathic Significance

Osteopathic treatment goals include strengthening muscles, increasing range of motion, reducing edema, eliminating motion restriction and stretching soft tissues.

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Research Question

- Primary Familial Brain Calcification (PFBC), a neurodegenerative disorder, presents with bilateral symmetrical intracranial calcification → clinically leads to motor symptom
- We hypothesize that due to the causative effects of TNAP in peripheral vascular calcification, it will similarly play a role in intracranial calcification.
- We focused on the choroid plexus, which had the highest TNAP expression
- Explored the relationship between TNAP activity and regulation of phosphate levels in the cerebrospinal fluid (CSF) as a possible mechanistic link to intracranial calcification.

Osteopathic Significance

- PFBC is often misdiagnosed for hyperparathyroidism or Parkinson’s disease as they share similar clinical manifestations
- We can enhance the practice of diagnosis
- Since no current methods halt the progression of the disease, PFBC treatment primarily targets symptomatic support.
- We hypothesize that Osteopathic Manipulative Medicine (OMM) techniques aimed at treating the Parkinsonian-like symptoms of PFBC, along with traditional pharmaceutical methods (i.e. bisphosphonate, disodium etidronate), can yield better improvements in the quality of life of patients

TNAP Activity

ALPL gene → TNAP protein

Tissue Distribution:
- liver, kidney,
- skeletal tissue,
- nervous system
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Knockout Mouse Model

- TNAP overexpression, human alkaline phosphatase gene (ALPL) was inserted into a mouse model genome and activated via Cre/lox recombination
- Our endothelial TNAP (eTNAP) overexpression mouse model was compared with a TNAP knockout model and wild type
- In this study, we focused on the potential role of vascular TNAP levels on the choroid plexus

Data Analysis

- We quantitatively analyzed intracranial calcification with the Micro-Computed Tomography (micro-CT) instrument SkyScan 1173 and NRecon image reconstruction software
- Reconstructed images were visualized, and quantified, and intracranial calcification was analyzed in 3D via the Dragonfly 4.1 program (Object Research Systems (ORS) Inc., Montreal, Canada, available for academic use at http://www.theobjects.com/dragonfly).

Collection of CSF

- CSF was extracted from the cisterna magna of anesthetized mice
- Inorganic phosphate was measured via a colorimetric assay
- Slides of cryosectioned mouse brains were stained for alkaline phosphatase activity.
- eTNAP knockout and transgenic models were compared with controls under the bright field microscope

CSF collection from mouse models

Data Analysis

Expression patterns of alkaline phosphatase in the brain was analyzed qualitatively from histological images. Data between two groups were compared using a t-test or a Mann Whitney statistics.
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TNAP Calcification

TNAP-induced calcification detected in multiple regions of the brain (basal ganglia, thalamus, ventral tegmental area, brainstem, cerebellar nuclei) with Dragonfly.

We compared levels of alkaline phosphate activity in the vasculature and observed that activity followed the genetic pattern of TNAP expression in a predictable manner. But no correlation was seen between endothelial TNAP gene dosage (knockout, wild type, transgenic) and the level of alkaline phosphatase activity within the choroid plexus. There was also no differences detected in the concentration of inorganic phosphate in CSF between endothelial TNAP overexpression and wild type animals.

Data Analysis

Data was analyzed using GraphPad Prism 8 software. Significance was accepted at p < 0.05

We found no significant changes in both CSF or plasma phosphate expression. Calcification was measured as a percentage of brain volume.
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Sectioned TNAP positive mouse brain with intracranial calcification labeled in blue
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TNAP positive mouse brain imaged in Dragonfly program portraying Intracranial calcification labeled in blue.

PD: 16,412.30 μm
Range Min: 23,610.88 Max: 65,535
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Introduction

PFBC is a rare, autosomal dominant, age related phenomenon primarily seen after the age of 40

Prior studies refer to the intracranial calcifications as “brain stones,” which are mostly composed of hydroxyapatite, the same calcium-phosphate structure found in normal bone

After observing the involvement of TNAP expression in intracranial calcification we concluded that TNAP can be a therapeutic target for PFBC

However, the causative role of choroid plexus in intracranial calcification is yet to be determined and requires further research.

Using OMM

The effectiveness of OMM is difficult to interpret in mouse models and therefore should be done in human trials noting improvement in movement and progression of intracranial calcification via imaging

Osteopathic treatment goals include strengthening muscles, increasing range of motion, reducing edema, eliminating motion restriction and stretching soft tissues
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Future Endeavors

Treatment to improve postural stability and shuffling gait options include:

- myofascial release to eliminate motion restriction
- muscle energy of postural muscles
- myofascial stretch of cervical/thoracic spine

Possible future endeavors include exploring cranial osteopathic manipulations of CSF, pioneered by Dr. Sutherland, to enhance CSF flow and encourage better CSF clearance of potential excess calcification

At the moment, this is still an area of limited research and knowledge