February 25, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 15578-review.doc).

Title: From variome to phenome: Pathogenesis, diagnosis and management of ectopic mineralization disorders

Author: Eva Yvette Gerona De Vilder, Olivier Madeleine Vanakker

Name of Journal: *World Journal of Clinical Cases*

ESPS Manuscript NO: 15578

The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

Editor

Ad.1 *The full names of the authors should be given. Thank you!*
This has been changed accordingly.

Ad. 2 To enhance your academic influence, we request that the first author make a video core tip, in which the most interesting aspects of your research process (as related to your article) are described. And please write a core tip of less than 100 words for the contents of your video to attract readers. This video file and the core tip will be published online, along with your article. Please submit video files according to the following specifications:
Acceptable file formats: .mov, .wmv, .mpg, .mpeg, .mp4, or .avi
Maximum file size: 10 MB
Minimum dimensions: 320 pixels wide by 240 pixels deep
Maximum length: 5 minute
Verify that the videos are viewable in QuickTime or Windows Media Player.

We agree with the Editor that a video core tip is a good idea to improve the attractiveness of a manuscript. However, currently we do not have the resources nor the necessary equipment and software at our disposal to make a video of sufficient quality for the *World Journal of Clinical Cases*.

Reviewer 1.

Ad. 1 *Unnecessary and excessive of hyphen (-) used throughout the manuscript is inappropriate and need to be deleted.*
We agree with the reviewer and have completely revised the manuscript, using a.o. the Oxford English Dictionary for correct spelling. Moreover, we have restructured some of our sentences hereby omitting the use of unnecessary hyphens.

Ad. 2 *All abbreviations should be mentioned with their full forms where they have been used initially and later can be used with their full forms (e.g. ECM, NADPH, ….etc).*
We agree with the reviewer. All abbreviations are now explained where they are first introduced, and further on in the manuscript only the abbreviations are used.

Ad. 3 *"Tissue-Nonspecific Alkaline phosphatase" should be written as "tissue-nonspecific alkaline phosphatase" (removing undue upper case).*
We agree with the reviewer and we have removed the capital letters. The sentence now reads as "Initial formation of hydroxyapatite in the MV itself: after budding from the plasma membrane, tissue-nonspecific alkaline phosphatase (TNAP; OMIM*171760) activity induces an increase of extracellular Pi concentration, which then enters the vesicles via sodium-dependent inorganic phosphate transporters (PiTs)."

Ad.4 *Classification of ectopic mineralization should be written clearly, removing 'and' between metastatic and dystrophic (minor language editing).*
We agree with the reviewer and have changed the "and" into "or". The classification paragraph now reads as "Ectopic mineralization disorders are conventionally classified based on the mechanism through which the mineralization takes place: i.e. metastatic or dystrophic calcification or ectopic ossification (Table 1) [14] […]"

Ad. 5. *All tables need re-formatting. The title of the tables should be on the top of tables and abbreviation descriptions in the footnote.*
All tables have been changed accordingly.

Ad.6 I*n most of the tables and figure 1, author/s did not mention all abbreviations used in the tables and figure. Moreover explanation of some abbreviations is given that are not present in the tables/figure. There is a need to go through the legends carefully and make the necessary amendments. The figure 1. Should be explained briefly in the foot note.*

"Hepatocyte: impairment of ABCC6 function leads to upregulation of pro-osteogenic pathways (MSX2-WNT, TGFβ-Smad 2/3, BMP2-Smad-RUNX2), upregulation of their downstream targets and eventually to ectopic mineralization. GGCX carboxylates and hence activates multiple targets, such as coagulation factors and MGP, the latter being a potent BMP2-inhibitor and hence mineralization inhibitor. When GGCX function is impaired, these targets stay inactive, leading to increased mineralization. ENPP1 converts ATP to AMP and PPi, the latter being a mineralization inhibitor. Impairment of this conversion and hence a decrease in the PPi level leads to increased in ectopic mineralization. Peripheral cell: After glycosylation by GALNT3, FGF23 forms a complex with FGFR1 and KL (coreceptor) which leads to increased renal excretion of Pi, a pro-mineralizing agent and decreased 1,25 dihydroxyvitamin D_3,, causing a decrease in intestinal Pi absorption. NT5E converts AMP to Pi and adenosine, which inhibits the pro-mineralizing TNAP. Impairment of NT5E function leads to increased TNAP activity and decreased PPi concentration, hence leading to ectopic mineralization. Pi is internalized into the peripheral cell by PiT2 and leaves the cell through apoptotic bodies, which cause ectopic mineralization through apoptotic pathways (not shown). In MVs an influx occurs of Pi via PiT2 and of Ca^{2+}, which is facilitated by A and PS. This leads to an accumulation of growing hydroxyapatite crystals, eventually causing the MVs to burst and the crystals to grow in the extracellular matrix."

It is however impossible to summarize this figure in a few lines, since it is used as a backbone for the whole

review. Furthermore, it seems against the journal's guidelines to provide detailed legends under the figures. We leave it at the Editor's discretion whether or not to keep the figure legend as it is now.

Ad.*7 Remove 'Zeigler SG et al, oral communication, ASHG 2014' from the tekst.*
We agree with the reviewer. We have turned this comment into a reference, with reference number 32. The revised sentence now reads as "The metabolic hypothesis was reinforced several times, until very recently Ziegler et al. reported that a conditional, liver-specific Abcc6-/- mouse model does not develop ectopic mineralization and concluded that mineralization in PXE occurs through a liver-independent mechanism [32]."

Ad.8 *Change from upper case to lower case in Dystrophic Cardiac …. And Ectonucleotide to ecto….*
We agree with the reviewer and we have removed the capital letters.
In addition to these changes, the whole paper was thoroughly revised and capital letters where changed into lower case letter where necessary, e.g. in protein names.

Ad.9 *Write down the age correctly in years or months in the clinical characteristics of PXE.*
This has been changed accordingly. The sentences now read as "CV symptoms, usually arising when patients are 30-40 years old, include accelerated coronary and peripheral artery disease (hypertension, myocardial infarction, intermittent claudication), diastolic cardiac dysfunction and gastrointestinal hemorrhage [24]. In 15% of PXE patients ischemic stroke may occur, at an average age of 49 [1,24]. Heterozygous carriers usually develop neither skin nor eye symptoms but can suffer from accelerated atherosclerosis and (mild) diastolic dysfunction of the heart [24]."

Ad.10 *Please explain the meaning of (AD;?) written in table 4.*
(AD; ?) means: (autosomal dominant; causal gene unknown). We understand the confusion and have changed this in table 4.

Ad.11 *The definitions of Keutel syndrome and IBGC written in very long sentences and therefore difficult to read and comprehend. Moreover seem to be copy-pasted from some other text.*
These definitions were not copy-pasted from another text, but we agree with the reviewer that the sentences are long and difficult to comprehend. Therefore we have altered the definitions, which now read as:

- Keutel syndrome: Since its first identification by Keutel et al. in 1971, approximately 30 cases have been described of Keutel syndrome (OMIM#245150), which is an autosomal recessive multisystem disease with an age of onset in childhood (5-15 years) [97,98].
- IBGC: Idiopathic basal ganglia calcification (IBGC) is a rare neurodegenerative disorder with unknown prevalence. The disease is sometimes referred to as Fahr's disease, although the patient he described primarily had mineralization in blood vessels of the white matter of the brain [113]. IBGC affects young to middle aged adults, with an average onset in the 3rd or 4th life decade; however the disease has also been described in childhood [114-116].

Ad.12 *Correct spellings of 'fundo' and 'residu'.*
We agree with the reviewer and the spelling has been corrected.

Ad.13 *Lastly and not the least, the author/s did not mark page numbers in their manuscript that created problem in writing the comments.*
We sincerely apologize for this inconvenience. We have added page numbers to the manuscript.

Reviewer 2.

Ad.14. *Firstly as regards some of your abbreviations I do not know what they mean, although some I think you are put in full in table etc.. Could I suggest you just check your paper through and make sure all abbreviations are defined in the prose (as one should be able to read it without looking at tables) and similarly a table is understandable on its own without the prose so again without abbreviations or with abbreviations explained in the legend.*

We agree with the reviewer and have made the necessary changes, for which we would like to refer to the answers to comment Ad. 2 and Ad. 6 of reviewer 1.

Ad.15 *Secondly the plural of fundus is fundi, not fundo.*

This has been changed accordingly.

Ad.16 *Also I would change anxiolytica to anxiolytics as the anglicized plural is more commonly used nowadays.*

This has been changed accordingly.

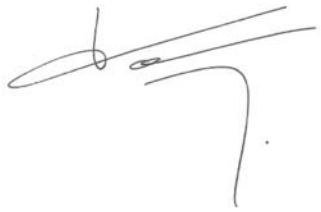
Ad.17 *Finally in your introductory comments about cellular biochemistry and physiology and the pathogenesis behind ossification - I was not sure if all the processes were extracellular, intracellular or mixed. It was my understanding that intracellular calcium is low and highly controlled and so I am assuming these processes are extracellular but if you are able to clarify that would be most appreciated.*

The ectopic mineralization takes place in the extracellular matrix, however some mechanisms underlying the mineralization take place intracellularly. This is depicted in figure 1. However, we agree with the reviewer that this is not written down clearly in the introduction text of the manuscript. We have revised this and the sentence now reads as "Physiological biomineralization is a complex multifactorial metabolic process, which in normal conditions is restricted to the extracellular matrix (ECM) of specific body structures, namely the bones, teeth, hypertrophic growth plate cartilage and calcified articular cartilage [1,2]. The intracellular and extracellular mechanisms, underlying physiological biomineralization, rely on a balanced interplay between mineralization inhibitors and propagators (Figure 1) [2,3]**."**

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Clinical Cases*.

Sincerely yours,



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