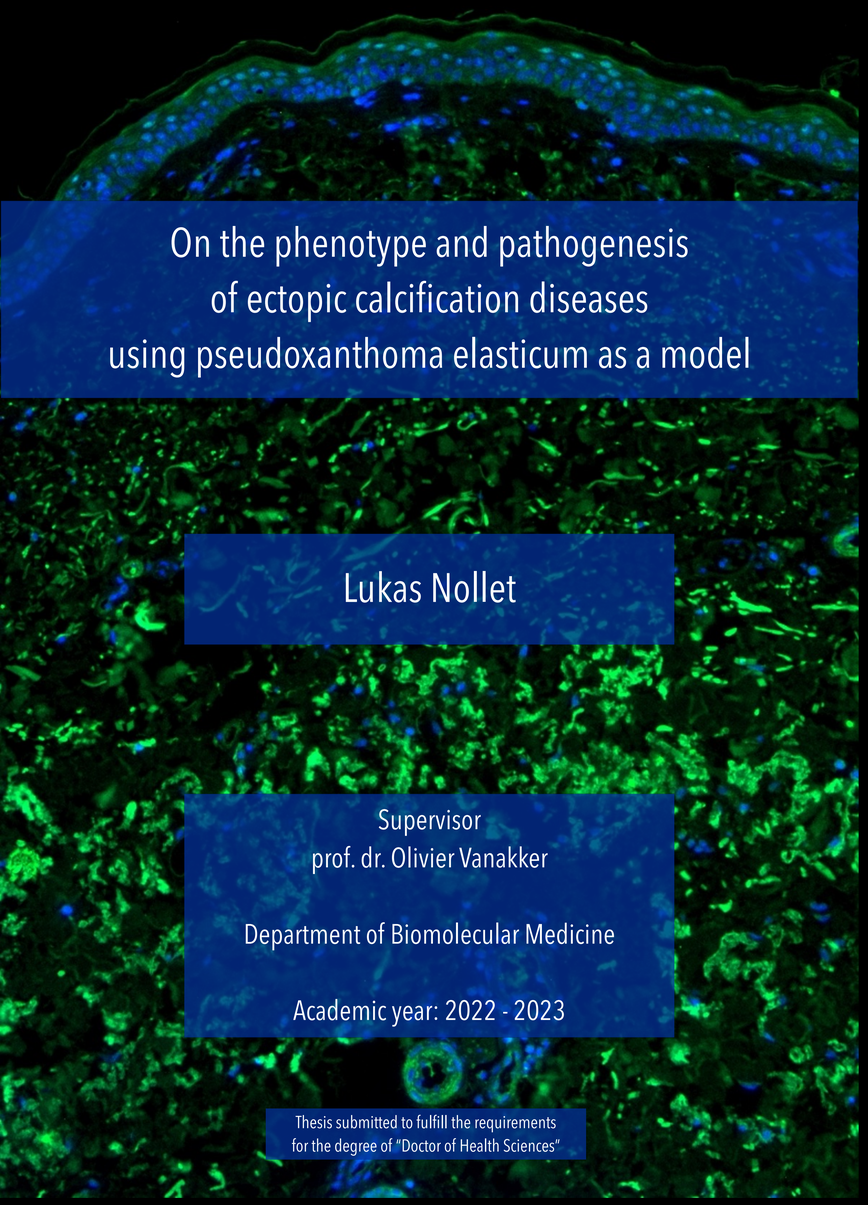
FACULTY OF MEDICINE AND HEALTH SCIENCES



On the phenotype and pathogenesis of ectopic calcification diseases using pseudoxanthoma elasticum as a model

Lukas Nollet

Supervisor
prof. dr. Olivier Vanakker

Department of Biomolecular Medicine

Academic year: 2022 - 2023

Thesis submitted to fulfill the requirements for the degree of "Doctor of Health Sciences"

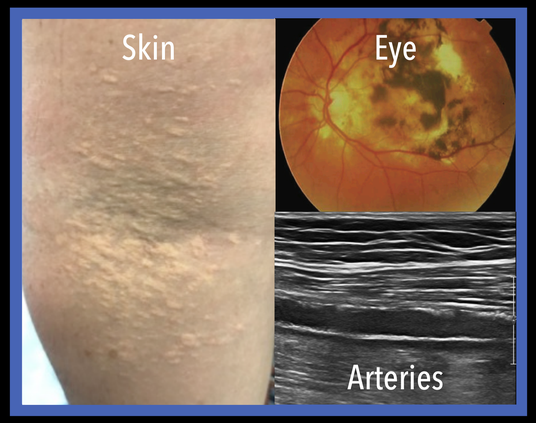
GHENT UNIVERSITY

Summary

Ectopic calcification (EC) refers to the presence of calcium crystals in soft tissues such as skin, arterial blood vessels and internal organs, and is associated with significant morbidity and mortality. EC is present in frequent western disorders such as atherosclerosis, calcific aortic valve disease and osteoarthritis. Currently, no effective treatments preventing, halting or reversing EC exist. Studies in Mendelian EC diseases have been instrumental in understanding important aspects of EC pathophysiology. In this doctoral research project, we therefore studied pseudoxanthoma elasticum (PXE), a multisystem EC disorder caused by bi-allelic pathogenic variants in *ABCC6* resulting in progressive calcification of elastic fibers affecting the skin, eye, and arteries (**Reviews 1-3**).

Similar to the findings in PXE patients, an increased burden of cardio- and cerebrovascular disease has been reported in individuals carrying a single heterozygous *ABCC6* mutation. In **paper 1**, we performed a deep-phenotyping study in a Belgian cohort of *ABCC6* carriers. Clinical, biochemical, imaging and genetic data were analyzed revealing the presence of distinct retinal alterations ('comet-like'), a high prevalence of dyslipidemia, diastolic dysfunction and testicular microlithiasis, as well as accelerated lower limb atherosclerosis, though with highly variable expression. We then created clinical practice guidelines aiming to standardize and improve early diagnosis, treatment and follow-up of *ABCC6*-related health problems in heterozygous carriers.

Identifying novel disease mechanisms and treatment targets in PXE is of utmost importance to accelerate the development of new therapies. In **paper 2**, we demonstrated the involvement of excessive DDR/PARP1 signaling in PXE pathogenesis finding increased activation of the ATM-p21-p53 and PARP1-IL6-STAT3-RUNX2 pathways, which associated with progressive calcification of the extracellular matrix.



The clinical phenotype of PXE

Treatment of PXE patient cells, *abcc6a*$^{-/-}$ zebrafish and *Abcc6*$^{-/-}$ mice (**paper 3**) with the PARP1 inhibitor minocycline attenuated this deleterious mechanism and significantly reduced EC.

Clinical trials in PXE are currently severely hampered by the lack of reliable biomarkers for disease severity, as conventional clinical or radiological outcome measurements cannot be used due to the very slow progression of PXE signs and symptoms. We therefore evaluated the use of serum calcification propensity T50, a measurement of the anti-calcifying buffer capacity of serum, as a novel biomarker for PXE disease severity (**paper 4**). We showed that serum T50 values in PXE are primarily determined by serum fetuin-A, phosphorus and magnesium levels, while no correlations were identified with *ABCC6* genotype. In a multivariate regression analysis, serum T50 was found to be significantly and inversely associated with ocular, vascular and overall clinical disease severity in PXE patients. On the contrary, plasma pyrophosphate - an endogenous calcification inhibitor – was found to be reduced in heterozygous carriers and PXE patients but was not a reliable biomarker for PXE disease severity (**paper 5**).

This doctoral research project further established the existence of a distinct clinical phenotype in heterozygous *ABCC6* carriers and created clinical practice guidelines to aid primary and secondary care physicians in their management of individuals carrying a single *ABCC6* mutation. With the advent of exome-wide genetic screening tests in the general population, an exponential increase in the detection of heterozygous pathogenic *ABCC6* variants is expected and thus our findings will improve genetic counseling of *ABCC6* carriers and their relatives. By demonstrating that DDR/PARP1 activation is a key driver of PXE pathogenesis and that minocycline treatment significantly reduces EC, we greatly increased the translational potential of minocycline and hence clinical trials in PXE patients may be endeavored. We also showed that serum T50 is a clinically relevant biomarker in PXE and may thus be used as a surrogate endpoint in future therapeutic trials. Overall, our research findings may accelerate the development and implementation of safe and effective treatment options for patients with hereditary or acquired EC diseases.

Bibliography

Paper 1: **Nollet L**, Campens L, De Zaeytijd J, Leroy B, Hemelsoet D, Coucke P, Vanakker O. Clinical and subclinical findings in heterozygous ABCC6 carriers: results from a Belgian cohort and clinical practice guidelines. *Journal of Medical Genetics*. 2022;59(5):496–504.

Paper 2: **Nollet L**, Van Gils M, Willaert A, Coucke P, Vanakker O. Minocycline attenuates excessive DNA damage response and reduces ectopic calcification in pseudoxanthoma elasticum. *Journal of Investigative Dermatology*. 2022;142(6):1629-1638.e6.

Paper 3: Bouderlique E*, **Nollet L***, Letavernier E, Vanakker O. Minocycline counteracts ectopic calcification in a murine model of pseudoxanthoma elasticum: a proof-of-concept study. *International Journal of Molecular Sciences*. 2022;23(3):1838.

Paper 4: **Nollet L**, Van Gils M, Fischer S, Campens L, Karthik S, Pasch A, De Zaeytijd J, Leroy B, Devos D, De Backer T, Coucke P, Vanakker O. Serum calcification propensity T50 associates with disease severity in patients with pseudoxanthoma elasticum. *Journal of Clinical Medicine*. 2022;11(13):3727.

Paper 5: Van Gils M, Depauw J, Coucke P, Aerts S, Verschuere S, **Nollet L**, Vanakker O. Inorganic pyrophosphate plasma levels are decreased in pseudoxanthoma elasticum patients and heterozygous carriers but do not correlate with the genotype or phenotype. *Journal of Clinical Medicine*. 2023;12(5):1893.

Review 1: **Nollet L**, Decock A, De Vilder E, Vanakker O. Primaire ectopische mineralisatieaandoeningen: van (vaat)verkalking tot syndroom. *Tijdschrift voor Geneeskunde*. 2020;76(24).

Review 2: Verschuere S*, Van Gils M*, **Nollet L***, Vanakker O. From membrane to mineralization : the curious case of the ABCC6 transporter. *FEBS Letters*. 2020;594(23):4109–33.

Review 3: **Nollet L**, Vanakker O. Mitochondrial dysfunction and oxidative stress in hereditary ectopic calcification diseases. *International Journal of Molecular Sciences*. 2022;23(23):15288.

Funding sources

This doctoral research project was supported by grants from the Research Foundation Flanders (FWO Vlaanderen, 11F2121N and 11F2123N) and the Ghent University Special Research Fund (BOF19/DOC/253) to Lukas Nollet

Doctoral advisory committee

dr. Laurence Campens
Department of Cardiology - Ghent University Hospital
Department of Internal Medicine and Pediatrics - Ghent University

prof. dr. Paul Coucke
Center for Medical Genetics Ghent - Ghent University Hospital
Department of Biomolecular Medicine - Ghent University

Doctoral examination committee

prof. dr. Ernst Rietzschel (chair)
Department of Cardiology - Ghent University Hospital
Department of Internal Medicine and Pediatrics - Ghent University

prof. dr. Tine De Backer (secretary)
Department of Cardiology - Ghent University Hospital
Department of Internal Medicine and Pediatrics - Ghent University

prof. dr. Sofie De Schepper
Department of Dermatology - Ghent University Hospital
Department of Head and Skin - Ghent University

prof. dr. Pieter-Jan Guns
Department of Genetics, Pharmacology and Physiopathology of Heart, Blood Vessels and Skeleton - University of Antwerp

dr. Yvonne Nitschke
Department of General Pediatrics
Münster University Children's Hospital - Germany

prof. dr. Patrick Sips
Center for Medical Genetics Ghent - Ghent University Hospital
Department of Biomolecular Medicine - Ghent University

prof. dr. Paul Eckhard Witten
Department of Biology – Ghent University

Curriculum vitae

Lukas Nollet
°25 July 1995 - Belgium

2019 - present
PhD in Health Sciences, Department of Biomolecular Medicine, Ghent University
Doctoral Training Programme, Faculty of Medicine & Health Sciences, Ghent University
Master after Master in Specialist Medicine: internal medicine, Ghent University

April - June 2023
Internship at Translational Cardiology research group
Karolinska Institutet, Stockholm, Sweden

2021 - 2022
Permanent Education Course in Human Genetics
Belgian Society for Human Genetics

2019 - 2020
Laboratory Animal Science
Faculty of Veterinary Medicine, Ghent University

2016 - 2019
Master of Medicine in Medicine
Ghent University (MD, *summa cum laude*)

2013 - 2016
Bachelor of Science in Medicine
Ghent University (*magna cum laude*)

A digital version of the thesis can be accessed through the following link

INSERT QR