Discovery and characterization of new glucosylated metabolites: pathophysiological consequences

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About the author – Curriculum Vitae

I was born in Goes, the Netherlands on May 2nd 1990. My secondary education was at Het Goese Lyceum, including subjects such as biology, chemistry, physics, mathematics and the international Baccalaureate English, IB certificate English. In 2009, I started the bachelor Life Science and Technology at Leiden University and Delft University of Technology. During my bachelor thesis I had the opportunity to work at the Leiden University Medical Centre, at the department of diagnostics. Here I worked on my first, disease and patient related project. During the internship I developed an analysis pipeline for Whole Exome Sequencing (WES) data and a Single Nucleotide Polymorphism (SNP) validation test for Next Generation Sequencing (NGS) sample identification. The developed validation allows detection of sample switches. The used analysis method was a Melting Curve Analysis to determine the genotype of the samples. After this internship I realized that I wanted to do more research, so I decided to start the Research and Development master of Life Science and Technology at University of Leiden in 2013.

During my master thesis, I did my internship at the Leiden University Medical Centre, department of Human Genetics – Polycystic Kidney Disease. The research group focuses on Autosomal Dominant Polycystic Kidney Disease. The disease is characterized by enlargement of the kidneys and occurrence of large fluid filled cysts, which results in progressive deterioration of kidney function. The disease is caused by mutations in the PKD1 or PKD2 genes, encoding polycystin-1 (PC1) and polycystin-2 (PC2). When either one of these proteins is lost a complex network of signaling pathways is disrupted. The PKD research group researches the signaling pathways involved in PKD, with the aim to develop treatment. During this research project I worked on signaling pathways in Polycystic Kidney Disease (a kidney disease where many fluid filled cysts destroy renal function). The focus of my project was on two important transcriptional coactivators, YAP and TAZ. These proteins are potential modulators in Polycystic Kidney Disease. During the project I studied the expression levels of YAP and TAZ on RNA and protein level during disease progression and worked with antisense oligonucleotide (ASOs) technology, in order to down-regulate YAP and TAZ expression. During the project I practiced my skills in culturing and transfecting cells, qPCR and Western blot analysis. I also developed skills in immunofluorescence techniques, when I studied the localisation of the proteins within cells.

While doing my master, I worked at 'Reizend DNAlab', an organization that organizes specialized practicals for high school students. The main topic of these practicals was protein folding and disease. During these practicals the students perform immunofluorescence experiments, and depending on the educational level, study protein function or create protein crystals. This job showed me that I have, besides research, a passion for education. Therefore, I applied in 2015 for this special PhD program, where I could combine both research and teaching. During this PhD program I did my research and work of this thesis at the Leiden Institute of Chemistry, in the Medical Biochemistry department under the supervision of Prof. dr. J.M.F.G. Aerts. Meanwhile I obtained my Master of Education - first degree chemistry teacher at Interfacultair Centrum voor Lerarenopleiding (ICLON) – Leiden University. My thesis for my master of Education covered research on cooperative learning for high school chemistry students. The research was nominated for the ROS Rijnland research price, and received the second price.

List of publications

1. Marques AR, Mirzaian M, Akiyama H, Wisse P, Ferraz MJ, Gaspar P, Ghauharali-van der Vlugt K, **Meijer R**, Giraldo P, Alfonso P, Irún P, Dahl M, Karlsson S, Pavlova EV, Cox TM, Scheij S, Verhoek M, Ottenhoff R, van Roomen CP, Pannu NS, van Eijk M, Dekker N, Boot RG, Overkleeft HS, Blommaart E, Hirabayashi Y, Aerts JM. **Glucosylated cholesterol in mammalian cells and tissues: formation and degradation by multiple cellular β-glucosidases.** J Lipid Res. 2016 Mar;57(3):451-63. Doi: 10.1194/jlr.M064923. Epub 2016 Jan 2. PMID: 26724485; PMCID: PMC4766994.

2. Boer DEC, Mirzaian M, Ferraz MJ, Zwiers KC, Baks MV, Hazeu MD, Ottenhoff R, Marques ARA, **Meijer R**, Roos JCP, Cox TM, Boot RG, Pannu N, Overkleeft HS, Artola M, Aerts JM. **Human glucocerebrosidase mediates formation of xylosyl-cholesterol by β-xylosidase and transxylosidase reactions.** J Lipid Res. 2021;62:100018. Doi: 10.1194/jlr.RA120001043. Epub 2021 Jan 6. PMID: 33361282; PMCID: PMC7903134.

3. Aerts JM, Ferraz MJ, Mirzaian M, Gaspar P, van Oussoren S, Wisse P, Kuo CL, Lelieveld LT, Kytidou K, Hazeu MD, Boer DEC, **Meijer R**, van der Lienden MJC Lienden, Chao DHM, Gabriel TL, Aten J, Overkleeft HS, van Eijk M, Boot RG, Marques ARA.**Lysosomal Storage Diseases. For Better or Worse: Adapting to Defective Lysosomal Glycosphingolipid Breakdown.** eLs John Wiley & Sons, Ltd., Chichester, UK, 2017, pp. 1-13.

Addendum I

Marques AR, Mirzaian M, Akiyama H, Wisse P, Ferraz MJ, Gaspar P, Ghauharali-van der Vlugt K, **Meijer R**, Giraldo P, Alfonso P, Irún P, Dahl M, Karlsson S, Pavlova EV, Cox TM, Scheij S, Verhoek M, Ottenhoff R, van Roomen CP, Pannu NS, van Eijk M, Dekker N, Boot RG, Overkleeft HS, Blommaart E, Hirabayashi Y, Aerts JM. **Glucosylated cholesterol in mammalian cells and tissues: formation and degradation by multiple cellular β-glucosidases.** J Lipid Res. 2016 Mar;57(3):451-63. Doi: 10.1194/jlr.M064923. Epub 2016 Jan 2. PMID: 26724485; PMCID: PMC4766994.

The membrane lipid glucosylceramide (GlcCer) is continuously formed and degraded. Cells express two GlcCer-degrading β-glucosidases, GBA and GBA2, located in and outside the lysosome, respectively. Here we demonstrate that through transglucosylation both GBA and GBA2 are able to catalyze *in vitro* the transfer of glucosyl-moieties from GlcCer to cholesterol, and vice versa. Furthermore, the natural occurrence of 1-O-cholesteryl-β-D-glucopyranoside (GlcChol) in mouse tissues and human plasma is demonstrated using LC-MS/MS and $^{13}C_6$-labelled GlcChol as internal standard. In cells the inhibition of GBA increases GlcChol, whereas inhibition of GBA2 decreases glucosylated sterol. Similarly, in GBA2-deficient mice GlcChol is reduced. Depletion of GlcCer by inhibition of GlcCer synthase decreases GlcChol in cells and likewise in plasma of inhibitor-treated Gaucher disease patients. In tissues of mice with Niemann-Pick type C, a condition characterized by intralysosomal accumulation of cholesterol, marked elevations in GlcChol occur as well. When lysosomal accumulation of cholesterol is induced in cultured cells, GlcChol is formed via lysosomal GBA. This illustrates that reversible transglucosylation reactions are highly dependent on local availability of suitable acceptors. In conclusion, mammalian tissues contain GlcChol formed by transglucosylation through β-glucosidases using GlcCer as donor. Our findings reveal a novel metabolic function for GlcCer.

Addendum II

Boer DE, Mirzaian M, Ferraz MJ, Zwiers KC, Baks MV, Hazeu MD, Ottenhoff R, Marques ARA, **Meijer R**, Roos JCP, Cox TM, Boot RG, Pannu N, Overkleeft HS, Artola M, Aerts JM. **Human glucocerebrosidase mediates formation of xylosyl-cholesterol by β-xylosidase and transxylosidase reactions.** J Lipid Res. 2021;62:100018. Doi: 10.1194/jlr.RA120001043. Epub 2021 Jan 6. PMID: 33361282; PMCID: PMC7903134.

Deficiency of glucocerebrosidase (GBA), a lysosomal β-glucosidase, causes Gaucher disease. The enzyme hydrolyzes β-glucosidic substrates and transglucosylates cholesterol to cholesterol-β-glucoside. Here we show that recombinant human GBA also cleaves β-xylosides and transxylosylates cholesterol. The xylosyl-cholesterol formed acts as acceptor for subsequent formation of di-xylosylcholesterol. Common mutant forms of GBA from patients with Gaucher disease with reduced β-glucosidase activity were similarly impaired in β-xylosidase, transglucosidase and transxylosidase activities, except for a slightly reduced xylosidase/glucosidase activity ratio of N370S GBA and a slightly reduced transglucosylation/glucosidase activity ratio of D409H GBA. XylChol was found to be reduced in spleen from Gaucher disease patients. The origin of newly identified XylChol in mouse and human tissues was investigated. Cultured human cells exposed to exogenous β-xylosides generated XylChol in a manner dependent on active lysosomal GBA but not the cytosol-facing β-glucosidase GBA2. We later sought an endogenous β-xyloside acting as donor in transxylosylation reactions, identifying xylosylated ceramide (XylCer) in cells and tissues that serve as donor in the formation of XylChol. UDPglucosylceramide synthase (GCS) was unable to synthesize XylChol but could catalyse formation of XylCer. Thus, food-derived β-D-xyloside and XylCer are potential donors for the GBA-mediated formation of XylChol in cells. The enzyme GCS produces XylCer at a low rate. Our findings point to further catalytic versatility of GBA and prompt a systematic exploration of the distribution and role of xylosylated lipids.

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Next I would like to thank my supervisor and promotor, Prof. dr. Hans Aerts. As he gave me the opportunity to work in his research group and he made me a better researcher by supervising and discussing my research. Also would like to thank my co-promotor dr. Rolf Boot, for the good talks at the end of my PhD. Also many thanks to dr. Marta Artola, for all the support in reading my work and correcting my chemical structures.

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