Glycomics based biomarkers of the rate of aging : development and applications of high-throughput N-glycan analysis

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Abbreviations

2-AA	2-aminobenzoic acid
2-AB	2-aminobenzamide
AAL	Aurelia aurantia lectin
AAT	alpha-1-antitrypsin
ABEE	2-aminobenzoic acid ethyl ether
ACN	acetonitrile
AD	Alzheimer's disease
ADCC	antibody dependent cellular cytotoxicity
AEX	anion exchange chromatography
AGP	alpha-1-acid glycoprotein
ANTS	2-aminonaphthalene trisulfonic acid
APTS	1-aminopyrene-3,6,8-trisulfonic acid
AUC	area under the curve
BACH	6-(biotinyl)-aminocaproyl hydrazide
BGE	background electrolyte
BMI	body-mass index
CDG	congenital disorder of glycosylation
CE	capillary electrophoresis
CGE	capillary gel electrophoresis
CHCA	α-cyano-4-hydroxycinnamic acid
CID	collision induced dissociation
COW	correlation optimized warping
CRP	C-reactive protein
CSF	cerebrospinal fluid
CVA	cerebrovascular accident
DAP	2,6-diaminopyridine
DEAEAB	4-amino-N-[2-(diethylamino)ethyl] benzamide
DHB	2,5-dihydroxybenzoic acid

DMSO	dimethylsulfoxide
EOF	electroosmotic flow
ER	endoplasmatic reticulum
ESI	electrospray ionozation
ESR2	estrogen receptor beta
ETD	electron transfer dissociation
F	fucose
FL	fluorescence detection
FTICR	Fourier Transform Ion Cyclotron Resonance
Fuc	fucose
FUT8	fucosyltransferase 8
Gal	galactose
GalNAc	N-acetylgalactosamine
GHRKO	growth hormone receptor knock-out
Glc	glucose
GlcNAc	N-acetylglucosamine
GU	glucose units
GWAS	genome-wide association scan
H	hexose
HDL	high density lipoprotein
Hex	hexose
HexNAc	N-acetylhexosamine
HILIC	hydrophilic interaction liquid chromatography
HL	hydrazide labeling
HPAEC	high-pH anion exchange chromatography
HPLC	high performance liquid chromatography
IgA	immunoglobulin A
IGF-1	insulin-like growth factor 1
IGFBP$_3$	insulin-like growth factor binding protein 3
IgG	immunoglobulin G
IIS	insulin-IGF signaling

IT	ion trap
LCA	Lens culinaris agglutinin
LDL	low-density lipoprotein
LIF	laser induced fluorescence
LLS	leiden longevity study
MA	Michael addition.
MALDI	matrix assisted laser desorption ionization
Man	mannose
MI	myocardial infarction
MS	mass spectrometry
N	N-acetylhexosamine
$NaBH_3CN$	sodium cyanoborohydride
$NaBH(OAc)_3$	sodium triacetoxyborohydride
NANA	N-acetylneuraminic acid
NeuAc	N-acetylneuraminic acid
OR	odds ratio
OST	oligosaccharyltransferase
PA	2-aminopyridine
PAA	polyacrylamide
PCA	principal component analysis
PGC	porous graphitic carbon
PMP	1-phenyl-3-methyl-5-pyrazolone
PTM	post translational modification
Q-TOF	quadrupole-time of flight
RA	rheumatoid arthritis
RedAm	reductive amination
ROC	receiver operating characteristic
RP	reverse phase
RSD	relative standard deviation
S	N-acetylneuraminic acid
SEC	size exclusion chromatography

SNPs	single nucleotide polymorphisms
SPE	solid phase extraction
Std	standarddeviation
TFA	trifluoroacetic acid
TEA	triethylamine
TOF	time-of-flight
TOR	target of rapamycin
ZIC	zwitterionic interaction chromatography

Curriculum Vitae

Renee Ruhaak was born in Leiden on May 9th 1982. She attended high school at the Visser 't Hooft Lyceum in Leiden, from which she graduated in 2000. She then started her studies at Leiden University where she earned her M.Sc. degree in Biopharmaceutical Sciences in August 2005. The research for her master thesis, which was performed in the Plant Metabolomics group of Prof. Dr. R. Verpoorte, concerned the validation of a novel vaporizing device (the Volcano) for the administration of medicinal cannabis. The project was awarded the van Os award from the Dutch Society for research on herbal medicines (NVGO). Her second internship of 12 months took place at the Pharmacognosy group of Prof. Dr. L. Bohlin at Uppsala University in Sweden where she performed a project on the cyclooxygenase (COX) inhibiting effects of purified cannabinoids. After graduation in 2005, she started to work for Storz Bickel GmbH as a contract researcher investigating the possibilities to use the Volcano vaporizer for applications other than medicinal cannabis.

In July 2006 she started her research as a PhD student under supervision of Prof. Dr. A.M. Deelder, Prof. Dr. P.E. Slagboom and Dr. M. Wuhrer in the biomolecular Mass Spectrometry Unit, part of the devision of Parasitology at the Leiden University Medical Center. In 2009-2010 she spent 10 weeks in the group of Prof. Dr. C.B. Lebrilla at University of California, Davis, U.S.A. Her work on the role of N-glycosylation in familial longevity and the analysis techniques necessary to study this relation has culminated in this thesis, which is entitled "Glycomics based biomarkers of the rate of aging".

In 2010 she moved to Davis, U.S.A. to start a post-doc at University of California, Davis, in the group of Prof. Dr. C.B. Lebrilla, where she will continue her research in the field of glyco-analytics.

List of publications

1. Ruhaak LR, UH H-W, Beekman M, Hokke CH, Westendorp RGJ, Houwing-duistermaat JJ, Wuhrer M, Deelder AM, Slagboom PE. Plasma protein N-glycan profiles are associated with calendar age, familial longevity and health. J. Proteome Res., in press

2. Ruhaak LR, Huhn C, Koeleman CAM, Wuhrer M, Deelder AM. Robust and high-throughput sample preparation for (semi-)quantitative analysis of N-glycosylation profiles from plasma samples. Methods Mol. Biol., in press

3. Ruhaak LR, Hennig R, Huhn C, Borowiak M, Dolhain RJEM, Deelder AM, Rapp E, Wuhrer M. Optimized workflow for preparation of APTS-labeled N-glycans allowing high-throughput analysis of human plasma glycomes using 48-channel multiplexed CGE-LIF. J. Proteome Res. (2010) 9;6655-64.

4. Ruhaak LR, UH H-W, Beekman M, Koeleman CAM, Hokke CH, Westendorp RGJ, Wuhrer M, Houwing-Duistermaat JJ, Slagboom PE, Deelder AM. Decreased levels of bisecting GlcNAc glycoforms of IgG are associated with human longevity. PLoS One (2010) 5;e12566

5. Ruhaak LR, Steenvoorden E, Koeleman CAM, Deelder AM, Wuhrer M. 2-picoline-borane: a non-toxic reducing agent for oligosaccharide labeling by reductive amination. Proteomics (2010) 10:2330-6.

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11. Hazekamp A, Ruhaak LR, Zuurman L, van Gerven J, Verpoorte R. Evaluation of a vaporizing device (Volcano) for the pulmonary administration of tetrahydro-cannabinol. J. Pharm. Sci. (2006) 95:1308-17.

12. Huhn C, Ruhaak LR, Mannhardt J, Wuhrer M, Meyer H. Direct comparison of detection traces using a scale of effective electrophoretic mobility in the coupling of capillary electrophoresis to laser-induced fluorescence and mass spectrometric detection: CE-LIF-MS analysis of labeled N-glycans. Submitted

13. Ruhaak LR, Felth J, Karlsson PC, Rafter JJ, Verpoorte R, Bohlin L. Evaluation of the cyclooxygenase inhibiting effects of six major cannabinoids isolated from Cannabis sativa. Submitted

Patent EP 09252309.1 (Reductive animation of carbohydrates, LR Ruhaak & M Wuhrer) was filed by the LUMC patent office on 30 September 2009.

Dankwoord

Having arrived at the final stage of my PhD there is time for a little bit of reflection. Going back to my first steps in science there was Rob Verpoorte who led the pharmacognosy department at Leiden University and made it possible for me to join his multicultural group. Supervised by ever-enthusiastic Arno Hazekamp I was introduced to science and the magical world of medicinal plants, particularly Cannabis sativa L. In the group of Lars Bohlin at Uppsala University, I continued my studies and learned the value of method optimization and independent research. Rob, Arno, Anneke, Lars, Jenny and all other members of the pharmacognosy groups, you showed me what science is like, and made me realize I wanted to start a PhD; thanks for that.

I will never forget the job interview I had with André Deelder and Ron Hokke as the first topic addressed was the political situation of our home village Oegstgeest; Andre, I still believe Oegstgeest one day will merge with Leiden. After the job interview I got a tour through the lab and got overwhelmed by the vast amount of state of the art instrumentation – now after 4 years the collection is thoroughly expanded but is not as impressive to me anymore, as I got used to work with a lot of the instruments. Next to all these instrumentation, I was introduced to Manfred Wuhrer, the expert on glyco-analysis who later became my supervisor.

After a crash course in glyco-analysis by reading a bucket-load of literature, I started off with practical work in the lab supervised by Manfred. Soon I realized the first landmarks for the methodology had already been set, but during my first year, I learned inns and outs on sample preparation of labeled N-glycans and their analysis. After another year of analyzing the full cohort on HPLC, I got my first dataset complete. Eline, Jeanine, Marian and especially Hae-Won were of great help during the following discussions on data processing, statistics and the epidemiological consequences. Thanks ladies, I really learned a lot, and hope I did not take too much of your time and effort.

More development of analytical techniques followed as well as more measurements of loads of samples. Luckily, I was helped in parts of the method development by several students: Willem-Jan, Evelyne, René and Matthias. Thanks for the time you

invested in my project and the good time we had together. Off course, with such large sample- and eventually data- sets, the analysis was not always easy, and several times I got stuck with all kinds of problems. My roommates Arjen, Maurice, Gerhild, Moniek, Carolin, Niels and Marco could usually help me out or cheer me up; and if they couldn't there was always someone else from the department to do so. In this light I would also like to express my special thanks to the 'second' and 'third' (OK, you know there is no following order) Caroline and Carolien.

Especially in the last period of my PhD, time got limited and I was often to be found in the lab late and/or in the weekend. Cees and Carolin gave me company several times, and I do remember this Sunday when Carolin, Cees, Helen, Maarten and I were in the lab. Maarten, by now you must be an expert in filling the boxes with pipette tips, which I emptied in large pace.

Before finally writing this thesis, I visited the lab of Carlito Lebrilla in Davis, California to learn myself some additional glyco-analytical techniques, and to learn to adapt myself to a new scientific environment. Carlito, Hyun-Joo, Mariana, Cora, Caroline, John, Hae-Young, Scott, Kyle, Larry, Grace, Süreyya and all other groupies, I really appreciated it that you allowed me to work together with you and showed me what science is like on the other side of the Atlantic.

Overall, It would not have been possible to write this thesis without the support of all people mentioned, especially André, Eline and Manfred and more importantly my parents, my brother and sister and Maarten. Thanks to all of you for allowing me to finish this thesis and continue my career in science.