Research report ‘Genetic factors of host glycogen metabolism and its relationship with the vaginal microbiome’

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Summary

In this retrospective data analysis project we aim to answer the question whether single nucleotide polymorphisms in the TCF7L2 gene (rs7903146-T and rs7903146-T) associated with type 2 diabetes are linked with composition of the vaginal microbiome in a multi-ethnic cohort of premenopausal women.

We received data of 199 female participants of the HELIUS project and excluded 5 participants because of antibiotic use or diabetes status. The data consisted of vaginal microbiome (VMB) composition, genotypes for SNP rs7903146 and rs12255372, fasting blood glucose level, ethnicity, age and immigration status. We posted our research proposal online prior to data analysis. To answer the main research questions we classified the various VMB groups as either *Lactobacillus* dominant (*L. crispatus, L. iners* or other *Lactobacillus*) or low *Lactobacillus*. No difference was detected between the two vaginal microbiome groups and alleles at either SNP.

Next we further specified *Lactobacillus*-dominant in either *Lactobacillus crispatus* or *Lactobacillus iners* and grouped all other VMB groups as ‘other’. Again, we saw no significant differences between *Lactobacillus* dominant microbiota and the type 2 diabetes risk alleles rs7903146-TT and rs12255732-TT.

We conclude that there is no significant association between two known type 2 diabetes risk alleles and vaginal microbial composition.

Introduction

Evidence is accumulating that glycogen released by endometrium and the cervicovaginal epithelium functions as an important carbohydrate for vaginal bacteria. Luminal glycogen is found to vary both with hormonal status and with the bacterial make-up of the reproductive tract. Here we propose to study whether host genetic factors that are involved in glycogen metabolism regulation show correlation with vaginal microbial signatures.

The multi-ethnic HELIUS cohort of Amsterdam is uniquely positioned for this analysis (Snijder et al. 2017). The vaginal microbiome of several participants was characterized previously (Borgdorff et al. 2017), showing a variation in *Lactobacillus*-dominated microbiota and *Lactobacillus*-depleted dysbiosis. This latter microbial state is associated with an increased risk of preterm labor and acquisition of sexually transmitted infection. Last year we proposed to analyze whether women with *Lactobacillus*-dominated vaginal microbiota are significantly more likely to carry certain alleles in the most prominent SNP’s of the TCF7L2 gene that are associated with type 2 diabetes. This preliminary analysis of (already existing) data could inform novel studies within this same HELIUS cohort to study the correlations between host vaginal glycogen synthesis, lactate concentration and vaginal microbiome.

Hypothesis

Glycogen functions as a carbon source for *Lactobacillus* species colonizing and acidifying the human vagina. We hypothesize that genetic changes in the host glycogen/glucose homeostasis and its regulatory factors can affect the ability of *Lactobacillus* species to acidify the vagina and prevent transitioning to dysbiotic microbial state. Moreover, unpublished experiments have shown that bacterial members of this dysbiotic state such as *Gardnerella* and *Prevotella* can utilize glycogen to as a carbon and energy source, and may use it to produce biogenic amines (odor), cause inflammation (recruiting target cells for HIV to the mucosal surfaces), desialylation of the mucosa and exfoliation of the vaginal epithelium (abnormal discharge). We speculate that differences in

glycogen stores may explain the diversity in symptoms such as odor, discharge and inflammation that are associated with vaginal dysbiosis.

Background

During reproductive years glycogen is synthesized by the tissues of the reproductive tract (Milwidsky, Palti and Gutman 1980, Mirmonsef et al. 2016) and is essential for early embryo implantation and development. Glycogen shed into the vaginal lumen functions as a carbon source for vaginal lactobacilli such as *Lactobacillus crispatus* (Hertzberger, Brandt and Kort 2018, van der Veer et al. 2018) and *Lactobacillus iners* but also for *Gardnerella vaginalis* and *Prevotella bivia* (unpublished data). Glycogen levels are reduced in women who have *Lactobacillus*-depleted microbial states (Mirmonsef et al. 2014) which could be explained by the interplay between host factors (glycogen synthesis) and bacterial factors (glycogen breakdown) (Vaneechoutte 2017).

It has been well-established that ethnicity is an important factor determining the odds of a woman to have a low-*Lactobacillus* vaginal microbiota. Women of African descent are found to have more *Lactobacillus iners* than *Lactobacillus crispatus* and are more often colonized by a *Lactobacillus*-depleted community (Fettweis et al. 2014, Ma, Forney and Ravel 2012, Ravel et al. 2011, Borgdorff et al. 2017). These women often have symptoms such as discharge and odor, and are at higher risk of acquiring sexually transmitted infection (Gosmann et al. 2017, Sewankambo et al. 1997), are more likely to have persistent HPV colonization (Kero et al. 2017, Brown et al. 2018) and are more likely to give birth prematurely (Brown et al. 2018, Donati et al. 2010, Martius and Eschenbach 1990).

There has been considerable effort to understand the variation of health outcomes and symptoms amongst women with comparable vaginal microbiota. Most studies were directed at understanding this variation from a bacterial perspective, for instance by looking at genetic variation amongst bacterial isolates of *Gardnerella vaginalis* (Schellenberg, Patterson and Hill 2017, Janulaitiene et al. 2018) and *Lactobacillus crispatus* (van der Veer et al. 2018, France, Mendes-Soares and Forney 2016). We believe that the dataset we propose to analyze provides a unique opportunity to also take host factors into consideration.

Variations in glycogen metabolism due to host genetic factors may affect both hepatic glycogen accumulation as well as endometrial and vaginal glycogen accumulation. For a long time it was thought that progesterone was the main hormone controlling glycogen synthesis in the endometrium (Jaffe, Stevens and Verhage 1985, Mimori et al. 1981). A recent study has now found that the progesterone/glycogen link is indirect and insulin is required for inactivation of glycogen synthase kinase 3β thereby activating glycogen synthase, similarly in liver tissue as well as in endometrial cells (Flannery et al. 2018).

These commonalities lead to our hypothesis that there may be a genetic relationship between bacterial vaginosis and type 2 diabetes. In genome-wide association studies into genetic risk factors for type 2 diabetes several SNP's have been identified. The most robust and consistent SNP's are found in the TCF7L2 gene. Certain SNP's in this transcription factor (elsewhere referred to as a TCF/LEF or TCF7) confer up to twofold increased risk of developing type 2 diabetes (Florez et al. 2006). TCF7L2 knockout mice had reduced glycogen stores in the liver (Boj et al. 2012). People in whom the CC nucleotides have been replaced by CT or TT at location rs7903146 of the TCF7L2 gene synthesized 1.4 or 2.0 times less insulin in response to a glucose challenge test respectively (Jainandunsing et al. 2018, Loos et al. 2007). This lower insulin production may have consequences for glycogen accumulation in the reproductive tract. Less glycogen synthesized means less carbohydrate for vaginal *Lactobacillus* species to acidify and *Gardnerella* to cause symptoms, odor and discharge. As of yet the ethnic differences in type 2 diabetes (where African ethnicity are at higher risk) were not able to be traced back to any SNP's. The link between one specific TCF7L2 SNP's (rs7903146) and type 2 diabetes was reported in various regions of the world. A second polymorphism found in this same gene (rs12255372G>T) is often found to be associated with a similar increased risk of type 2 diabetes.

There are only few studies to date taking host genetic factors into account when studying vaginal colonization . Our cohort includes people from European heritage but includes a variety of groups with Asian, African and European roots. Clues from this study may inform bigger studies where we could look at the influence of genetic factors on lactate and glycogen taking into account the different ethnic groups and vaginal colonization patterns. If glycogen metabolic commonalities are found between diabetes and BV, this opens up a wide range of new treatment options for BV and related conditions, and possibly novel applications for established diabetes drugs including amylase inhibitors, insulin and metformin.

Data

Helius VMB source: Janneke van de Wijgert, previously published (Borgdorff et al. 2017)	546
Helius metadata	231
genotype data (rs7903146/rs12255372)	199
diabetes status (excluded), based on self reported, or increased fasting glucose (>=7 mM) or increased HBA1C (>=48mM/mM) use of glucose lowering meds	3
antibiotic use (excluded)	2
# Helius participants used in this data analysis	**194**
average age	26,9
average fasting blood glucose (mmol/L)	4,9

Ethnic groups	
Dutch	9
South-Asian Surinamese	35
African Surinamese	10
Ghanaian	3
Turkish	60
Moroccan	77

rs7903146 genotype	
CC	88
TC	85
TT	21

rs12255372 genotype	
GG	79
TG	84
TT	31

VMB groups	
Coryne/*bifidobact*-dominated	8
Gardnerella-dominated	8
L. crispatus-dominated	36
L. iners-dominated	82
Other lacto-dominated	6
Other polybacterial	7
Polybacterial+*Gardnerella*	38
uropathobiont-dominated	9

Results

We received data of 231 female subjects between 19 and 34 years old. For 199 women we had complete genotype and vaginal microbiome data. Three women had diabetes (self-reported, by fasting glucose, abnormal HBAC1 levels or reported use of glucose lowering medication) and two women had taken antibiotics. These five samples were excluded from the data analysis since we believed that both characteristics could alter the microbiome data. The HELIUS cohort is a multi-ethnic cohort. In this subset the largest group was of Moroccan (40%) ethnicity, followed by Turkish (31%), South Asian-Surinamese (18%), African Surinamese (5%) and Dutch descent (5%).

Vaginal microbiome data included analysis of richness, diversity, number of reads and grouping into twenty clusters and eight VMB-groups. Data analysis was performed by the group of Janneke van de Wijgert and published (Borgdorff et al. 2017).

Data analysis 1:

We classified the VMB-groups as either *'Lactobacillus*-dominated' (124 women) or 'other' (70 women). Minor allele frequency (MAF) for rs7903146 (T), which is the type 2 diabetes risk allele, was 0,33 and we detected no significant differences between women with (MAF 0,34) or without a *Lactobacillus*-dominated microbiome ('other', MAF 0,31) (by Fisher's exact in R, see separate wordpad file).

Minor allele frequency for rs12255372 (T) was 0,38 and there were no significant differences between women without a *Lactobacillus*-dominated (MAF 0,35) and with a *Lactobacillus*-dominated microbiome (MAF 0,39). (by Fisher's exact in R, see separate wordpad file)

Of the rs7903146-T carriers 34% had a low-*Lactobacillus* profile compared to 38% in the rs7903146-C group. A similar distribution was found for rs1122573: carriers of the T allele had 34% low-*Lactobacillus* profile compared to 38% amongst the carriers of the G allele. None of these differences reached statistical significance.

Due to the small sample size and the fact that several articles have reported a copy number effect we also compared women homozygous for either allele. We found that 38% of homozygous rs7903146-TT-women had a low-*Lactobacillus* VMB compared to 40% of the rs7903146-CC group. In the heterozygous group 31% had a low-*Lactobacillus* VMB indicating no effect of either genotype.

Data analysis 2: distinguishing between *Lactobacillus* species

Although we did not propose this in the initial study design we further stratified the VMB in three cluster: *L. crispatus* dominant (36 women), *L. iners* dominant (82 women) or 'other' (76 women). We found that in both SNP's slightly more women with the TT genotype had a *L. crispatus* dominated microbiome, however these differences did not reach significance (by Fisher's exact in R, see separate wordpad file).

rs7903146	*L. crispatus* dominated	*L. iners* dominated	other
CC	0,15	0,44	0,41
TC	0,21	0,41	0,38
TT	0,24	0,38	0,38

rs12255372	*L. crispatus* dominated	*L. iners* dominated	other

GG	0,15	0,46	0,39
TG	0,19	0,38	0,43
TT	0,26	0,42	0,29

Interpretation

Our primary hypothesis is not supported by these data. There is no significant difference between TCF7L2 polymorphisms, which are associated with increased risk of type 2 diabetes, and vaginal microbiome. We hypothesized an association between host glucose/glycogen homeostasis and a *Lactobacillus*-dominated vaginal microbiome due to similar response of glycogen synthesis in the reproductive tract and the postulated importance of this glycan to bacterial metabolism in the vagina.

Our interest was partially sparked because of the evolutionary perspective. Evolutionary relationships of haplotype A, consisting of 65 SNP's in the TCF7L2 genetic region as well as a microsattelite, indicates that it was introduced by a positive selective sweep around the time of the agricultural revolution 10,000 – 5,000 years ago .

The human vaginal environment is -as far as is known- unique. No other primates or mammals have been found with such dominant levels of *Lactobacillus* and such low pH. There are many factors in physiology, endocrinology and immunology that could help explain this phenomenon but metabolism may also have played a role. The introduction of the carbohydrate-rich diet and the frequent and increased insulin peaks could have resulted in the positive selection of a genotype that results in reduced blood glucose levels and increased insulin respons.

The low levels of glycogen during bacterial vaginosis as well as the glycogen degradation metabolism of vaginosis species *Gardnerella* and *Prevotella* means that the ancestral vaginal microbiome may wreak havoc with an increased supply of carbon and energy due to a starch-rich diet. The association between *Lactobacillus* absence and preterm birth indicate that there may be very strong fitness costs attached to genital infection and inflammation. Hence, the acquisition of a *Lactobacillus*-dominant microbiome could be protective in case of a high-carbohydrate environment and even be a strong driver of evolution of glucose/glycogen homeostasis. However, the lack of significant difference and the tendency that women with the "ancestral" genotype (TT/TT) slightly more often (insignificantly so) have 'protective' *Lactobacillus crispatus* is opposing this hypothesis.

One possible explanation is that glycogen is not the most important source of carbohydrate or that glycogen synthesis in the reproductive tract is not connected to the insulin regulated glucose/glycogen homeostasis or is not in any meaningful way.

Literature

Boj, S. F., J. H. van Es, M. Huch, V. S. Li, A. Jose, P. Hatzis, M. Mokry, A. Haegebarth, M. van den Born, P. Chambon, P. Voshol, Y. Dor, E. Cuppen, C. Fillat & H. Clevers (2012) Diabetes risk gene and Wnt effector Tcf7l2/TCF4 controls hepatic response to perinatal and adult metabolic demand. *Cell,* 151**,** 1595-607.

Borgdorff, H., C. van der Veer, R. van Houdt, C. J. Alberts, H. J. de Vries, S. M. Bruisten, M. B. Snijder, M. Prins, S. E. Geerlings, M. F. Schim van der Loeff & J. H. H. M. van de Wijgert (2017) The association between ethnicity and vaginal microbiota composition in Amsterdam, the Netherlands. *PLOS ONE,* 12**,** e0181135.

Brown, R. G., M. Al-Memar, J. R. Marchesi, Y. S. Lee, A. Smith, D. Chan, H. Lewis, L. Kindinger, V. Terzidou, T. Bourne, P. R. Bennett & D. A. MacIntyre (2018) Establishment of vaginal microbiota composition in early pregnancy and its

association with subsequent preterm prelabour rupture of the fetal membranes. *Translational Research*.

Donati, L., A. Di Vico, M. Nucci, L. Quagliozzi, T. Spagnuolo, A. Labianca, M. Bracaglia, F. Ianniello, A. Caruso & G. Paradisi (2010) Vaginal microbial flora and outcome of pregnancy. *Archives of Gynecology and Obstetrics,* 281**,** 589-600.

Fettweis, J. M., J. P. Brooks, M. G. Serrano, N. U. Sheth, P. H. Girerd, D. J. Edwards, J. F. Strauss, C. The Vaginal Microbiome, K. K. Jefferson & G. A. Buck (2014) Differences in vaginal microbiome in African American women versus women of European ancestry. *Microbiology,* 160**,** 2272-2282.

Flannery, C. A., G. H. Choe, K. M. Cooke, A. G. Fleming, C. C. Radford, P. H. Kodaman, M. J. Jurczak, R. G. Kibbey & H. S. Taylor (2018) Insulin Regulates Glycogen Synthesis in Human Endometrial Glands Through Increased GYS2. *J Clin Endocrinol Metab,* 103**,** 2843-2850.

Florez, J. C., K. A. Jablonski, N. Bayley, T. I. Pollin, P. I. de Bakker, A. R. Shuldiner, W. C. Knowler, D. M. Nathan & D. Altshuler (2006) TCF7L2 polymorphisms and progression to diabetes in the Diabetes Prevention Program. *N Engl J Med,* 355**,** 241-50.

France, M. T., H. Mendes-Soares & L. J. Forney (2016) Genomic Comparisons of Lactobacillus crispatus and Lactobacillus iners Reveal Potential Ecological Drivers of Community Composition in the Vagina. *Appl Environ Microbiol,* 82**,** 7063-7073.

Gosmann, C., M. N. Anahtar, S. A. Handley, M. Farcasanu, G. Abu-Ali, B. A. Bowman, N. Padavattan, C. Desai, L. Droit, A. Moodley, M. Dong, Y. Chen, N. Ismail, T. Ndung'u, M. S. Ghebremichael, D. R. Wesemann, C. Mitchell, K. L. Dong, C. Huttenhower, B. D. Walker, H. W. Virgin & D. S. Kwon (2017) Lactobacillus-Deficient Cervicovaginal Bacterial Communities Are Associated with Increased HIV Acquisition in Young South African Women. *Immunity,* 46**,** 29-37.

Hertzberger, R. Y., A. Brandt & R. Kort (2018) Carbohydrate active enzymes in Lactobacillus crispatus - a possible link between the pullulanase gene and growth on glycogen. *Figshare*.

Jaffe, R. C., D. M. Stevens & H. G. Verhage (1985) The effects of estrogen and progesterone on glycogen and the enzymes involved in its metabolism in the cat uterus. *Steroids,* 45**,** 453-62.

Jainandunsing, S., H. R. Koole, J. N. I. van Miert, T. Rietveld, J. L. D. Wattimena, E. J. G. Sijbrands & F. W. M. de Rooij (2018) Transcription factor 7-like 2 gene links increased in vivo insulin synthesis to type 2 diabetes. *EBioMedicine,* 30**,** 295-302.

Janulaitiene, M., V. Gegzna, L. Baranauskiene, A. Bulavaite, M. Simanavicius & M. Pleckaityte (2018) Phenotypic characterization of Gardnerella vaginalis subgroups suggests differences in their virulence potential. *PLoS One,* 13**,** e0200625.

Kero, K., J. Rautava, K. Syrjanen, S. Grenman & S. Syrjanen (2017) Association of asymptomatic bacterial vaginosis with persistence of female genital human papillomavirus infection. *Eur J Clin Microbiol Infect Dis,* 36**,** 2215-2219.

Loos, R. J., P. W. Franks, R. W. Francis, I. Barroso, F. M. Gribble, D. B. Savage, K. K. Ong, S. O'Rahilly & N. J. Wareham (2007) TCF7L2 polymorphisms modulate proinsulin levels and beta-cell function in a British Europid population. *Diabetes,* 56**,** 1943-7.

Ma, B., L. J. Forney & J. Ravel (2012) Vaginal microbiome: rethinking health and disease. *Annual Review of Microbiology,* 66**,** 371-389.

Martius, J. & D. A. Eschenbach (1990) The role of bacterial vaginosis as a cause of amniotic fluid infection, chorioamnionitis and prematurity--a review. *Archives of Gynecology and Obstetrics,* 247**,** 1-13.

Milwidsky, A., Z. Palti & A. Gutman (1980) Glycogen metabolism of the human endometrium. *J Clin Endocrinol Metab,* 51**,** 765-70.

Mimori, H., K. Fukuma, I. Matsuo, K. Nakahara & M. Maeyama (1981) Effect of progestogen on glycogen metabolism in the endometrium of infertile patients during the menstrual cycle. *Fertil Steril,* 35**,** 289-95.

Mirmonsef, P., A. L. Hotton, D. Gilbert, D. Burgad, A. Landay, K. M. Weber, M. Cohen, J. Ravel & G. T. Spear (2014) Free glycogen in vaginal fluids is associated with Lactobacillus colonization and low vaginal pH. *PloS one,* 9**,** e102467.

Mirmonsef, P., A. L. Hotton, D. Gilbert, C. J. Gioia, D. Maric, T. J. Hope, A. L. Landay & G. T. Spear (2016) Glycogen Levels in Undiluted Genital Fluid and Their Relationship to Vaginal pH, Estrogen, and Progesterone. *PLoS One,* 11**,** e0153553.

Ravel, J., P. Gajer, Z. Abdo, G. M. Schneider, S. S. Koenig, S. L. McCulle, S. Karlebach, R. Gorle, J. Russell, C. O. Tacket, R. M. Brotman, C. C. Davis, K. Ault, L. Peralta & L. J. Forney (2011) Vaginal microbiome of reproductive-age women. *Proceedings of the National Academy of Sciences of the United States of America,* 108 Suppl 1**,** 4680-4687.

Schellenberg, J. J., M. H. Patterson & J. E. Hill (2017) Gardnerella vaginalis diversity and ecology in relation to vaginal symptoms. *Res Microbiol,* 168**,** 837-844.

Sewankambo, N., R. H. Gray, M. J. Wawer, L. Paxton, D. McNaim, F. Wabwire-Mangen, D. Serwadda, C. Li, N. Kiwanuka, S. L. Hillier, L. Rabe, C. A. Gaydos, T. C. Quinn & J. Konde-Lule (1997) HIV-1 infection associated with abnormal vaginal flora morphology and bacterial vaginosis. *Lancet (London, England),* 350**,** 546-550.

Snijder, M. B., H. Galenkamp, M. Prins, E. M. Derks, R. J. G. Peters, A. H. Zwinderman & K. Stronks (2017) Cohort profile: the Healthy Life in an Urban Setting (HELIUS) study in Amsterdam, The Netherlands. *BMJ Open,* 7**,** e017873.

van der Veer, C., R. Hertzberger, S. Bruisten, H. Tytgat, J. Swanenburg, A. de Kat Angelino-Bart, F. Schuren, D. Molenaar, G. Reid, H. de Vries & R. Kort (2018) Comparative genomics of human *Lactobacillus crispatus* isolates reveals genes for glycosylation and glycogen degradation: Implications for in vivo dominance of the vaginal microbiota. *bioRxiv***,** 441972.

Vaneechoutte, M. (2017) The human vaginal microbial community. *Res Microbiol,* 168**,** 811-825.