

## Kratek opis usposabljanja mladega raziskovalca (*Short description of the Young Researcher's training*)

### 1. Raziskovalna organizacija (*Research organisation*):

Univerza v Ljubljani, Biotehniška fakulteta, Oddelek za biologijo  
University of Ljubljana, Biotechnical faculty, Department of Biology

### 2. Ime, priimek in elektronski naslov mentorja (*Mentor's name, surname and email*):

prof. dr. Darja Žgur Bertok

### 3. Šifra in naziv raziskovalnega področja (*Research field*):

1.05 Biokemija in molekularna biologija  
*1.05 Biochemistry and molecular biology*

### 4. Kratek opis usposabljanja mladega raziskovalca (*Short description of the Young Researcher's training*):

Navedite tudi morebitne druge zahteve, vezane na usposabljanje mladega raziskovalca (npr. znanje angleškega jezika, izkušnje z laboratorijskim delom, potrebne licence za usposabljanje...).

*slo:*

V človeških prebavilih živi več kot  $10^{14}$  mikrobnih celic, ki jih označujemo kot črevesna mikrobiota. Slednja ima pomembno vlogo za razvoj in zdravje človeka, medtem ko je disbioza, neravnovesje mikroorganizmov mikrobiote, povezana z imunskimi in metabolnimi obolenji, vključno z debelostjo in sladkorno boleznijo. Pojavnost obeh v svetovnem merilu ter zlasti v razvitem svetu, skokovito narašča.

Tudi pojavnost nosečnostne sladkorne bolezni (GDM) narašča. Ženske z GDM v 35-60% razvijejo sladkorno bolezen tipa 2 v času 5-10 let po porodu, imajo večje tveganje za pojav zapletov med nosečnostjo, njihovi otroci pa so izpostavljeni večjemu tveganju za pojav debelosti in sladkorne bolezni tipa 2 v obdobju adolescence in odraslosti. Poleg tega, rezultati nekaterih raziskav nakazujejo, da bi GDM lahko bil eden od dejavnikov tveganja za rojstvo otroka s preveliko telesno težo, kar imenujemo makrosomija. Makrosomija predstavlja večjo nevarnost za obporodne poškodbe, ter večje tveganje za pojav debelosti in sladkorne bolezni tipa 2 pri otrocih. Zmanjševanje makrosomije je eden poglavitnih ciljev zdravstvenega varstva nosečnic.

Ker sestava črevesne mikrobiote posameznika vpliva na razvoj debelosti predpostavljam, da vpliva tudi na makrosomijo.

Z namenom razjasnitve pojava makrosomije in izdelave pristopov preprečevanja omenjenega pojava, v raziskovalni skupini že poteka metagenomska raziskava črevesne mikrobiote dveh skupin nosečnic: i) nosečnice z GDM in debelostjo ter ii) nosečnice z GDM in normalno telesno težo. V okviru omenjenih dveh skupin so bile izoblikovane dve podskupini glede na makrosomijo otroka: nosečnice, ki so rodile makrosomnega otroka in nosečnice, ki so rodile otroka z normalno telesno težo.

Doktorsko delo bo potekalo v sodelovanju s Kliničnim oddelkom za endokrinologijo, diabetes in presnovne bolezni Univerzitetnega kliničnega centra, Ljubljana, kjer spremljajo nosečnice z GDM, (z normalno telesno težo ter z debelostjo).

Na Odd. za biologijo, Biotehniške fakultete, UL, bo MR opravil(a) metatranskriptomsko raziskavo

mikrobiote nosečnic z GDM in podrobnejše analize nekaterih bakterijskih pripadnikov mikrobiote:

- I. Iz shranjenih vzorcev blata nosečnic z GDM, bo MR izoliral(a) celokupno bakterijsko RNA in jo z reverzno transkriptazo prepisal(a) v DNA. Sledilo bo sekvenciranje (Illumina) za pripodobitev vpogleda v bakterijske gene, ki so se izražali oz. vpogled v katere funkcije bakterije mikrobiote nosečnic z GDM opravlajo.
- II. Primerjava transkriptomov nosečnic preučevanih skupin in statistična analiza pridobljenih rezultatov. Uporabljena bodo ustrezna orodja bioinformatike.
- III. Najnovejše raziskave razkrivajo ne samo vpliv mikrobiote na zdravje in razvoj človeka, temveč tudi, da gostitelj preko mikroRNA (miRNA), ki vstopajo v bakterije, vpliva na izražanje bakterijskih genov in oblikovanje mikrobiote. Z namenom iskanja označevalcev značilnih za mikrobioto nosečnic s tveganjem za rojstvo makrosomnega otroka bo MR iz zgoraj opisane celokupne RNA karakteriziral(a) in primerjal(a) miRNA.
- IV. Bakterija *Escherichia coli* je pomemben predstavnik fakultativno anaerobnega dela črevesne mikrobiote in tudi lahko pokazatelj disbioze. Iz blata jo zlahka izoliramo, gojimo in karakteriziramo v laboratorijskem okolju. Naravna ekološka niša fakultativno anaerobne bakterije *E. coli* je debelo črevesje in bi lahko bila eden od označevalcev sprememb v sestavi črevesne mikrobiote. Iz blata v raziskavo vključenih nosečnic, bo izoliral(a) in genotipiziral(a) izolate bakterije *E. coli*.
- V okviru bilateralnega projekta Slovenija – ZDA (prof. M. Johnson), bodo sledile raziskave celotnih genomov izbranih izolatov bakterije *E. coli* in analize njihove aktivnosti/patogenosti na celicah urotelija mehurja.

Predlagani projekt bo potekal v okviru prve raziskave povezave mikrobiote nosečnic z GDM in makrosomijo. Iz rezultatov raziskave mikrobiote nosečnic in interakcijah gostitelja ter mikrobiote, bomo lahko izboljšali zdravje ljudi, predvsem vplivali na zmanjšanje pojavnosti makrosomije ter posledično sladkorne bolezni tipa 2 in debelosti.

Znanja kandidata - izkušnje z laboratorijskim delom in znanje angleškega jezika.

eng:

The human gut is inhabited by  $10^{14}$  microorganisms, designated as the gut microbiota. The latter plays a key role in human health and development. On the other hand, dysbiosis or disruption of the microbiota is associated with immune and metabolic diseases, including obesity and diabetes. The prevalence of both is on a global scale increasing; in developed countries at an alarming rate.

30-60% of women with GDM develop diabetes type (diabetes mellitus) 5-10 years after giving birth. They also have a higher risk of complications during pregnancy and their children are at higher risk of developing obesity and diabetes type 2 during adolescence and adulthood. In addition, some studies indicate that GDM could be one of the risk factors for macrosomia, a newborn with an excessive birth weight. Macrosomia represents a greater risk for injury during birth as well as a greater risk for childhood obesity and diabetes type 2. The prevalence of gestational diabetes (GDM) is also increasing. 30-60% of women with GDM develop diabetes type (diabetes mellitus) 5-10 years after giving birth. They also have a higher risk of complications during pregnancy and their children are at higher risk of developing obesity and diabetes type 2 during adolescence and adulthood. In addition, some studies indicate that GDM could be one of the risk factors for macrosomia, a newborn with an excessive birth weight. Macrosomia represents a greater risk for injury during birth as well as a greater risk for childhood obesity and diabetes type 2. Reducing the incidence of macrosomia is one of the main priorities of health care during pregnancy.

As the composition of an individuals' gut microbiota affects the development of obesity we hypothesize, that it is also involved in macrosomia.

To elucidate the appearance of macrosomia and prepare preventive measures, the research group is already performing a metagenomic analysis comparing the gut microbiota of two groups of pregnant women: i) obese pregnant women with GDM and ii) pregnant women with GDM and normal weight.

Within the framework of these two groups two subgroups were formed on the basis of newborn macrosomia: pregnant women that gave birth to a newborn with macrosomia and pregnant women that gave birth to a newborn with normal body weight.

The PhD investigation will be performed in collaboration with the Clinical Department for endocrinology, diabetes and metabolic diseases of the University Clinical Centre, Ljubljana where pregnant women with GDM are routinely examined.

At the Department of Biology, Biotechnical Faculty, University of Ljubljana, the young researcher (MR) will perform a metatranscriptomic analysis of the microbiota of pregnant women with GDM as well as more detailed investigations of some bacterial components of the investigated microbiota :

I. From the stored fecal samples of pregnant women with GDM the young researcher will isolate total bacterial RNA and employing reverse transcriptase cDNA will be isolated. Subsequently, sequencing (Illumina) will be employed to obtain insight into the functions expressed by the microbiota of the investigated women with GDM.

II. Comparison of the metatranscriptomes of the investigated pregnant women and statistical analysis of the obtained results. Appropriate bioinformatics tools will be employed.

III. Recent studies are revealing that not only does the microbiota influence human health and development, but the host via production of specific microRNA (miRNA) that enter bacteria, affects the microbiota. To understand the host →microbiota interaction, the young researcher will from the above isolated total RNA, also characterize and compare miRNA.

IV. *Escherichia coli* is a significant member of the facultative anaerobic gut microbiota. It is easily isolated from fecal samples, cultivated and characterized in the laboratory. The natural ecological niche of *E. coli* is the large intestine and it could serve as a marker for alterations/dysbiosis, of the gut microbiota. To identify new microbial markers, the young researcher will, from the fecal samples, isolate *E. coli* strains.

Within the framework of a bilateral research project, Slovenia-USA (prof. M. Johnson) studies of whole genomes of selected *E.coli* isolates will be investigated as well as their activity/pathogenicity against bladder urothelial cells.

The research performed by the young researcher, will be a significant part of the first investigation of the association of the gut microbiota of pregnant women with GDM and macrosomia. The obtained results on gut microbiota composition and interaction of the host with the microbiota, will be a significant step forward in improving human health, particularly reducing the incidence of macrosomia and subsequently, diabetes type 2 as well as obesity.

Candidate should have experience in the laboratory and knowledge of English.