

1. Raziskovalna organizacija (*Research organisation*):

Univerza v Ljubljani, *Biotehniška fakulteta*

2. Ime in priimek mentorja (*Name and surname of a mentor*):

prof. dr. Darja Žgur Bertok

3. Področje znanosti iz šifranta ARRS (*Primary research field*):

1.05 Biokemija in molekularna biologija

4. Kontaktni e-naslov mentorja (*Contact of a mentor*):

darja.zgur.bertok@bf.uni-lj.si

5. Kratek opis programa usposabljanja (*Short description of the program*):

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 pglavitnih ciljev zdravstvenega varstva nosečnic.

Ker sestava črevesne mikrobiote posameznika vpliva na razvoj debelosti predpostavljamo, da vpliva tudi na makrosomijo. Z namenom razjasnitve pojava makrosomije in izdelave pristopov preprečevanja omenjenega pojava, bo mladi(a) raziskovalec(ka) (MR) z metagenomsko raziskavo in raziskavo miRNA, preučil(a) in primerjal(a) črevesno mikrobioto dveh skupin nosečnic: i) nosečnice z GDM in debelostjo ter ii) nosečnice z GDM in normalno telesno težo. V okviru omenjenih dveh skupin bodo izoblikovane še 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), od katerih bo MR pridobil vzorce blata (v 2. in 3. trimesstru nosečnosti) ter oceno poporodne teže otrok in morebitnih zapletov ob in po porodu.

Na Odd. za biologijo, Biotehniške fakultete, UL, bo MR opravil sledeče:

I. Vzorce blata bo nosečnic ustrezno obdelal(a) za shranjevanje pri -80°C .

II. Sledila bo izolacija celokupne DNA iz shranjenih vzorcev blata; nato s PCR pomnožitev regij V3 in V4 gena za 16S rRNA na izolirani celokupni DNA; pirosekvenciranje; bioinformacijska analiza pridobljenih nukleotidnih zaporedij ter metagenomska analiza – taksonomska uvrstitev zaporedij z ustreznimi orodji. Primerjava metagenomov nosečnic preučevanih skupin in statistična analiza pridobljenih rezultatov.

III. Najnovejše raziskave razkrivajo ne samo vpliv mikrobiote na zdravje in razvoj človeka, temveč tudi, da gostitelj preko mikroRNA (miRNA), ki vstopajo v bakterije in vplivajo na izražanje genov in oblikovanje mikrobiote. Z namenom iskanja označevalcev značilnih za mikrobioto nosečnic s tveganjem za rojstvo makrosomnega otroka bo MR iz vzorcev blata vseh proučevanih skupin nosečnic izoliral(a) tudi celokupno RNA in 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 pred zamrzovanjem, izoliral(a) in genotipiziral(a) izolate bakterije *E. coli*.

Predlagani projekt bo prva raziskava povezave mikrobiote nosečnic z GDM in makrosomijo. Iz rezultatov raziskave sestave 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.

ANG

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. 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 young researcher (MR) will perform a metagenomic and miRNA investigation 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 will be 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 and from whom fecal samples will be collected (during the 2. and 3. trimester). The newborns' weight will also be determined and possible complications during and following delivery will be assessed.

At the Department of Biology, Biotechnical Faculty, University of Ljubljana, the young researcher (MR) will perform the following:

I. Appropriately prepare fecal samples for storage at -80°C .

II. Isolation of total DNA from the stored fecal samples; PCR amplification of the V3 and V4 regions of the 16S rRNA gene on the isolated total DNA; pyrosequencing; bioinformatic analysis of the obtained nucleotide sequences and metagenomic analysis employing appropriate pipelines. Comparison of the gut metagenomes of the investigated groups of pregnant women and statistical analysis of the obtained results.

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 stored fecal samples of all studied groups of pregnant women, also isolate total RNA and characterize as well as 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 prior to its storage at -80°C.

The research that will be performed by the young researcher, will be 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, we 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.