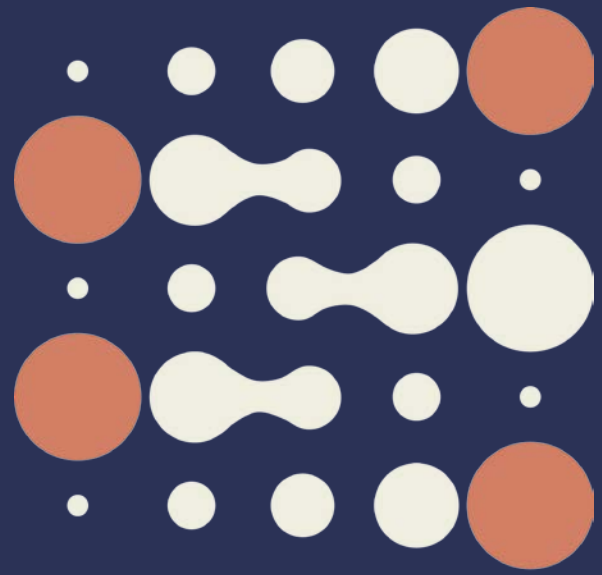
Circulating metabolites associated with gut microbial α-diversity and their associations with risk of colorectal cancer development

Hwayoung Noh[1], Yuhan Zhang[2,3], Chrysovalantou Chatziioannou[2], Hongda Chen[3], Min Dai[3], Hwan-Hee Jang[4], Cristina Menni[5], Daniel Kirk[5], Mazda Jenab[2], Marc J. Gunter[2,6], and Pekka Keski-Rahkonen[2]

[1.] INSERM U1296, Léon Bérard Cancer Centre (CLB), France
[2.] International Agency For Research on Cancer (IARC-WHO), France
[3.] Chinese Academy of Medical Sciences and Peking Union Medical College, China
[4.] National Institute of Agricultural Science (NAS-RDA), Korea
[5.] King's College, UK
[6.] Imperial College London, UK

Related Research Grants
- GMHealthKorea RDA Research Grant (PIs. Marc J. Gunter, Heinz Freisling, Hwayoung Noh)
- CAMS Innovation Fund for Medical Sciences (PI: Min Dai)



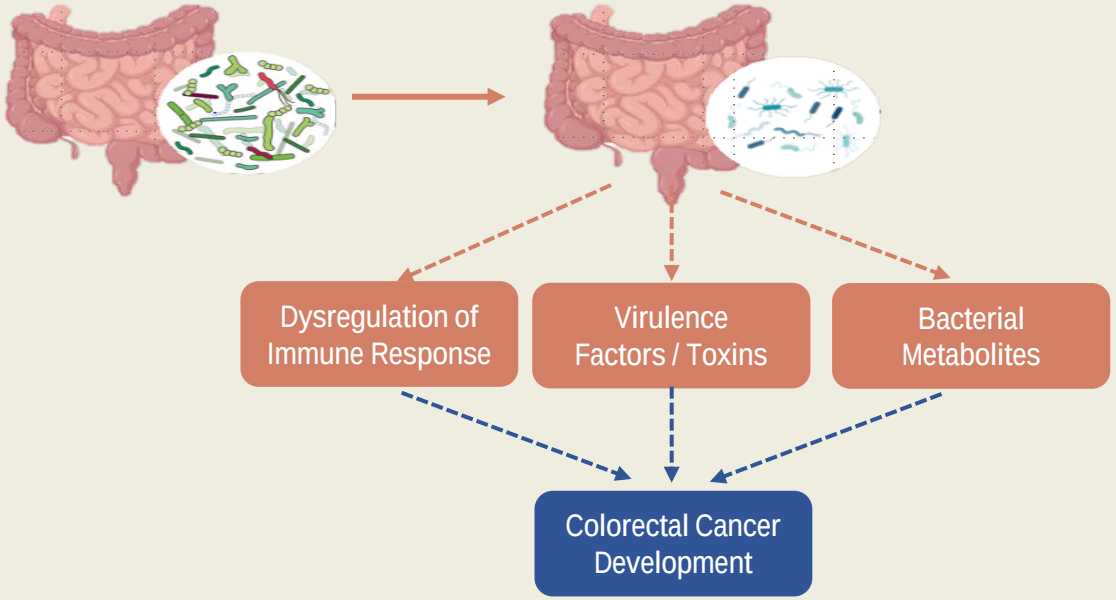
International Agency for Research on Cancer
World Health Organization

Hwayoung.noh@inserm.fr / KeskiP@iarc.who.int

Introduction/Background/Motivation

Gut Microbial Dysbiosis

- Reduced microbial diversity, loss of beneficial bacteria, increase in pathogenic bacteria
- Linked to colorectal cancer development
- Estimated by α-diversity: richness and evenness of species within one single sample



Wang & Li, J. Transl. Med. 2022

Study Hypothesis

- **Circulating metabolites represent gut microbial α-diversity and can be used to explore associations with colorectal cancer development**

Objective 1

- **Identify circulating metabolites associated with gut microbial α-diversity in cross-sectional studies where both gut microbiome and blood metabolomics data are available**

Objective 2

- **Investigate associations of these metabolites with risk of colorectal adenomas and cancers in studies with available blood metabolomics data**

Study Design – Objective 1

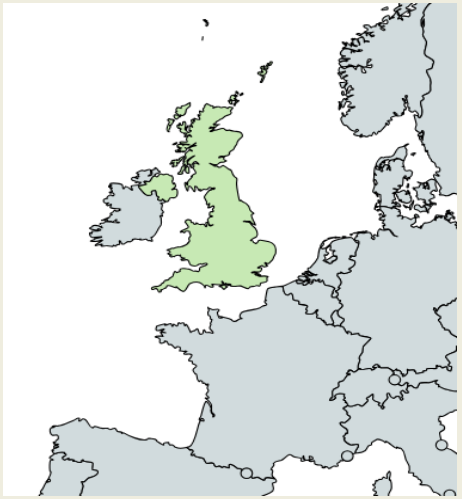
Discovery of circulating metabolites associated with gut microbial α-diversity

TwinsUK

UK population

880 adults

(6.5% men, 42-77 years, BMI: 20-35kg/m^2)



GMHealth 2019 & 2022

Korean population

177/155 adults

(50% men, 20-58 years, BMI:19-34kg/m^2)

TARGET-C

Chinese population

178 adults

(73% men, 52-70 years, BMI: 20-29kg/m^2)

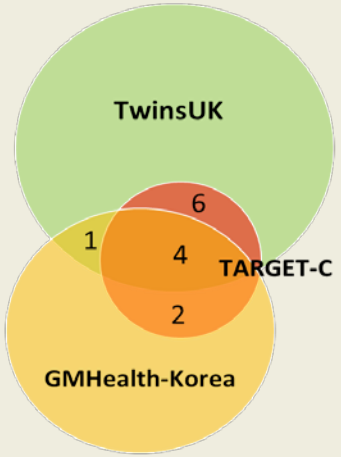
Blood untargeted metabolomics data by high-resolution LC-MS

Gut microbial α-diversity by Shannon index based on stool 16s rRNA gene sequencing data

Results – Objective 1

Circulating metabolites correlated with α-diversity (Shannon index)

Identified circulating metabolites for gut microbial α-diversity[a]		TwinsUK	GMHealth1	GMHealth2	TARGET-C
7-alpha-hydroxy-3-oxo-4-cholestenoate	Bile acid, primary	-0.11[b]	-	-	-0.18
Glycochenodeoxycholic acid	Bile acid, primary and secondary	-0.03	-0.16	-0.27[b]	-0.13
Glycoursodeoxycholic acid	Bile acid, secondary	-0.18[b]	-0.34[b]	-0.44[b]	-0.15
Isoursodeoxycholic acid	Bile acid, secondary	-0.26[b]	-	-	-0.19
Taurolithocholate 3-sulfate	Bile acid, secondary	0.15[b]	-	-	0.22
Indole-3-propionic acid	Tryptophan metabolite	0.19[b]	0.33[b]	0.34[b]	0.15
Hippuric acid	Biomarker of phenolic compound consumption	0.18[b]	0.29[b]	0.34[b]	0.18
Cinnamoylglycine	Plant Food constituent	0.32[b]	-	-	0.16
p-Cresol sulfate	Uremic toxin, tyrosine metabolite	0.27[b]	0.43[b]	0.47[b]	0.23
p-Cresol glucuronide	Uremic toxin, tyrosine metabolite	0.22[b]	-	-	0.20
Phenylacetylglutamine	Uremic toxin	0.23[b]	0.44[b]	0.38[b]	0.07
4-Ethylphenyl sulfate	Uremic toxin	0.13[b]	-	-	0.21
Trimethylamine N-oxide	Pro-inflammatory metabolite	0.07	0.25[b]	0.31[b]	0.18

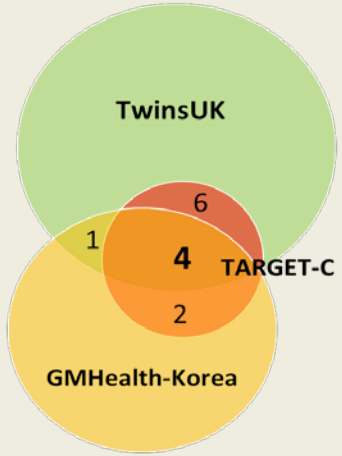


[a]Partial Spearman's rank correlation between Shannon α-diversity index and circulating metabolites adjusted for age, sex, BMI, and study centre (only for TwinsUK & TARGET-C); [b]The significance remained after Benjamini-Hochberg (BH) multiple testing correction

Results – Objective 1

Circulating metabolites correlated with α-diversity (Shannon index)

Identified circulating metabolites for gut microbial α-diversity[a]		TwinsUK	GMHealth1	GMHealth2	TARGET-C
7-alpha-hydroxy-3-oxo-4-cholestenoate	Bile acid, primary	-0.11[b]	-	-	-0.18
Glycochenodeoxycholic acid	Bile acid, primary and secondary	-0.03	-0.16	-0.27[b]	-0.13
Glycoursodeoxycholic acid	Bile acid, secondary	-0.18[b]	-0.34[b]	-0.44[b]	-0.15
Isoursodeoxycholic acid	Bile acid, secondary	-0.26[b]	-	-	-0.19
Taurolithocholate 3-sulfate	Bile acid, secondary	0.15[b]	-	-	0.22
Indole-3-propionic acid	Tryptophan metabolite	0.19[b]	0.33[b]	0.34[b]	0.15
Hippuric acid	Biomarker of phenolic compound consumption	0.18[b]	0.29[b]	0.34[b]	0.18
Cinnamoylglycine	Plant Food constituent	0.32[b]	-	-	0.16
p-Cresol sulfate	Uremic toxin, tyrosine metabolite	0.27[b]	0.43[b]	0.47[b]	0.23
p-Cresol glucuronide	Uremic toxin, tyrosine metabolite	0.22[b]	-	-	0.20
Phenylacetylglutamine	Uremic toxin	0.23[b]	0.44[b]	0.38[b]	0.07
4-Ethylphenyl sulfate	Uremic toxin	0.13[b]	-	-	0.21
Trimethylamine N-oxide	Pro-inflammatory metabolite	0.07	0.25[b]	0.31[b]	0.18



[a]Partial Spearman's rank correlation between Shannon α-diversity index and circulating metabolites adjusted for age, sex, BMI, and study centre (only for TwinsUK & TARGET-C); [b]The significance remained after Benjamini-Hochberg (BH) multiple testing correction

Study Design – Objective 2

Associations of identified metabolites with risk of colorectal adenomas and cancers

TARGET-C case-control study

Chinese population

Advanced colorectal adenomas

384 cases/328 controls
(68% men, 52-71 years, BMI: 20-29kg/m^2)

EPIC nested case-control study

European population (FR, IT, ES, UK, NL, DE, DK)

Colon cancer

1103 cases/1103 matched controls
(45% men, 43-68 years, BMI: 21-36kg/m^2)

Blood untargeted metabolomics data by high-resolution LC-MS

Results – Objective 2 (1)

Metabolites and risk of advanced colorectal adenomas in Target-C

- using 7 metabolites common in the two Asian populations studied

Metabolites of α-diversity	Total (case/control = 384/328)		Men (case/control = 264/227)		Women (case/control = 120/101)	
	OR*	95% CI	OR*	95% CI	OR*	95% CI
Glycochenodeoxycholic acid	1.19	(1.01-1.41)	1.14	(0.92-1.40)	1.33	(0.97-1.84)
Glycoursodeoxycholic acid	1.11	(0.94-1.32)	0.99	(0.80-1.23)	1.39	(1.03-1.89)
Indole-3-propionic acid	0.90	(0.75-1.08)	0.97	(0.79-1.20)	0.81	(0.55-1.17)
Hippuric acid	0.88	(0.71-1.08)	0.91	(0.71-1.18)	0.83	(0.56-1.24)
p-cresol sulfate	0.99	(0.84-1.17)	1.00	(0.82-1.22)	0.99	(0.69-1.41)
Phenylacetylglutamine	1.08	(0.88-1.34)	1.08	(0.83-1.40)	1.15	(0.77-1.73)
Trimethylamine N-oxide (TMAO)	1.03	(0.88-1.22)	0.99	(0.82-1.21)	1.06	(0.77-1.47)

*Odds Ratio (OR) and 95% Confidential Interval (CI) per 1 SD increment in ln-transformed metabolite intensity. Logistic regression models adjusted for study centre, age, sex (only for total), BMI, smoking status, alcohol intake, physical activity, and education

Results – Objective 2 (2)

Metabolites and risk of colon cancers in EPIC

- using 7 metabolites common in the two Asian populations studied

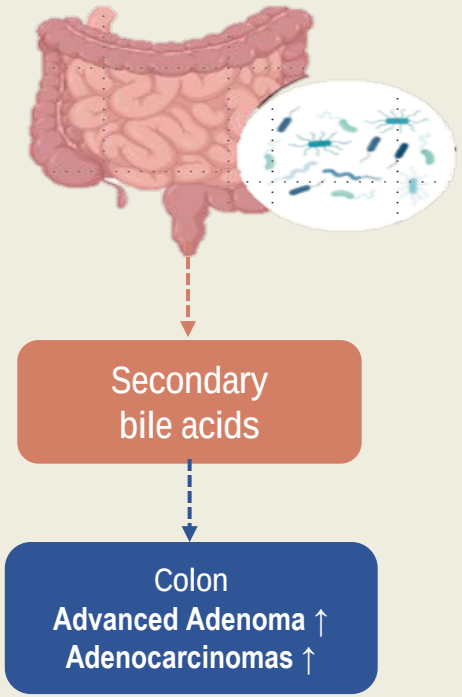
Metabolites of α-diversity	Total (case/control = 1103/1103)		Men (case/control = 491/491)		Women (case/control = 612/612)	
	OR*	95% CI	OR*	95% CI	OR*	95% CI
Glycochenodeoxycholic acid	1.06	(0.95-1.17)	0.90	(0.75-1.08)	1.14	(1.00-1.29)
Glycoursodeoxycholic acid	0.99	(0.90-1.08)	0.89	(0.76-1.03)	1.04	(0.93-1.18)
Indole-3-propionic acid	0.99	(0.88-1.11)	1.16	(0.96-1.39)	0.89	(0.77-1.04)
Hippuric acid	1.02	(0.91-1.14)	1.06	(0.90-1.26)	1.01	(0.87-1.17)
p-cresol sulfate	0.96	(0.88-1.05)	0.97	(0.84-1.12)	1.04	(0.90-1.21)
Phenylacetylglutamine	1.00	(0.89-1.13)	0.98	(0.81-1.18)	1.04	(0.89-1.21)
Trimethylamine N-oxide (TMAO)	1.08	(0.98-1.20)	1.07	(0.92-1.24)	1.11	(0.97-1.27)

*OR and 95% CI per 1 SD increment in ln-transformed metabolite intensity. Conditional logistic regression models stratified by matched case-control pairs with age, sex, study center and adjusted for BMI, smoking status, physical activity, alcohol intake, education

Discussion and Conclusions

- **Circulating metabolites reflect gut microbial α-diversity in distinct populations**
  - Secondary bile acids
  - Tryptophan and other food-derived metabolites
  - Uremic toxins

- **Blood levels of glycochenodeoxycholic acid and glycoursodeoxycholic acid**
  - **negatively correlated with gut microbial α-diversity**
  - **associated with increased risk of colon adenoma and cancers**



Future Directions

- Extension of colon cancer risk associations of identified metabolites to the Northern Sweden Health and Disease Study (NSHDS)
- Examination of the taxonomic profiles of the gut microbiota related to the identified circulating metabolites
- Faecal metabolomics analysis to identify additional metabolites of gut microbial α-diversity and metabolic activity

Key take-home messages

- **Circulating metabolites reflect gut microbial α-diversity**
- **Particularly useful in settings where stool samples are not available**
- **Can serve as tools to explore the role of gut microbial dysbiosis in the development of colorectal and other cancers and contribute to a better understanding of cancer aetiology**