

SUPPLEMENTARY FILE

Title: Evaluation of metabolomics as diagnostic targets in oral squamous cell carcinoma: a systematic review.

Authors: Susanth Alapati¹, Giulio Fortuna², Gordon Ramage¹, and Christopher Delaney^{1*}

*Correspondence: christopher.delaney@glasgow.ac.uk

¹Oral Sciences Research Group, Glasgow Dental School, School of Medicine, Dentistry and Nursing, University of Glasgow, 378 Sauchiehall Street, Glasgow, United Kingdom, G2 3JZ

²Department of Oral Medicine, Glasgow Dental School, School of Medicine, Dentistry and Nursing, University of Glasgow, 378 Sauchiehall Street, Glasgow, United Kingdom, G2 3JZ

Supplementary Table S1: Detailed search strategy across the databases.

Database	No.	Search Statement
Medline (Ovid)	1	(oral or mouth or "oral cavity" or oropharynx* or tongue or "base of tongue" or "floor of mouth" or palat* or lingual or buccal or lip or labial or "oral mucosa*" or retromolar or cheek or gingiva* or intra-oral or vermillion).mp.
	2	(cancer* or tumor* or tumour* or malignant* or neoplasm* or carcinoma* or squamous or squamous cell* or OSCC or soft tissue or HPV-positive or HPV-negative or HPV+ve or HPV-ve).mp.
	3	(metabolomic* OR metabonomic* OR "metabolic profiling" OR metabolome OR "metabolite marker" OR "metabolite biomarker").mp.
	4	1 AND 2 AND 3
Database	No.	Search Statement
Embase (Ovid)	1	(oral or mouth or "oral cavity" or oropharynx* or tongue or "base of tongue" or "floor of mouth" or palat* or lingual or buccal or lip or labial or "oral mucosa*" or retromolar or cheek or gingiva* or "intra-oral" or vermillion).mp.
	2	(cancer* or tumor* or tumour* or malignant* or neoplasm* or carcinoma* or squamous or squamous cell* or OSCC or soft tissue or HPV-positive or HPV-negative or HPV+ve or HPV-ve).mp.
	3	(metabolomic* OR metabonomic* OR 'metabolic profiling' OR metabolome OR 'metabolite marker' OR 'metabolite biomarker').mp
	4	1 and 2 and 3
Database	No.	Search Statement
Cochrane	1	(oral or mouth or "oral cavity" or oropharynx* or tongue or "base of tongue" or "floor of mouth" or palat* or lingual or buccal or lip or labial or "oral mucosa*" or retromolar or cheek or gingiva* or "intra-oral" or vermillion)
	2	(cancer* or tumor* or tumour* or malignant* or neoplasm* or carcinoma* or squamous or squamous cell* or OSCC or soft tissue or HPV-positive or HPV-negative)
	3	(metabolomic* OR metabonomic* OR "metabolic profiling" OR metabolome OR "metabolite marker" OR "metabolite biomarker")
	4	1 and 2 and 3

Database	No.	Search Statement
CINAHL	1	(oral or mouth or "oral cavity" or oropharynx* or tongue or "base of tongue" or "floor of mouth" or palat* or lingual or buccal or lip or labial or "oral mucosa*" or retromolar or cheek or gingiva* or "intra-oral" or vermillion) 'All text'
	AND 2	(cancer* or tumor* or tumour* or malignant* or neoplasm* or carcinoma* or squamous or squamous cell* or OSCC or soft tissue or HPV-positive or HPV-negative or HPV+ve or HPV-ve) 'All text'
	AND 3	(metabolomic* OR metabonomic* OR "metabolic profiling" OR metabolome OR "metabolite marker" OR "metabolite biomarker") 'All text'
	AND 4	1 AND 2 AND 3
Database	Search Statement	
Web of Science Core Collection	<p>TS=(oral or mouth or "oral cavity" or oropharynx* or tongue or "base of tongue" or "floor of mouth" or palat* or lingual or buccal or lip or labial or "oral mucosa*" or retromolar or cheek or gingiva* or "intra-oral" or vermillion) AND</p> <p>TS=(cancer* or tumor* or tumour* or malignant* or neoplasm* or carcinoma* or squamous or squamous cell* or OSCC or soft tissue or HPV-positive or HPV-negative or HPV+ve or HPV-ve) AND</p> <p>TS=(metabolomic* OR metabonomic* OR "metabolic profiling" OR metabolome OR "metabolite marker" OR "metabolite biomarker")</p>	
Database	Search Statement	
ProQuest	<p>(TI,AB,IF(oral) OR TI,AB,IF(mouth) OR TI,AB,IF("oral cavity") OR TI,AB,IF(oropharynx*) OR TI,AB,IF(tongue) OR TI,AB,IF("base of tongue") OR TI,AB,IF("floor of mouth") OR TI,AB,IF(palat*) OR TI,AB,IF(lingual) OR TI,AB,IF(buccal) OR TI,AB,IF(lip) OR TI,AB,IF(labial) OR TI,AB,IF("oral mucosa*") OR TI,AB,IF(retromolar) OR TI,AB,IF(cheek) OR TI,AB,IF(gingiva*) OR TI,AB,IF(intra-oral) OR TI,AB,IF(vermillion)) AND</p> <p>(TI,AB,IF(cancer*) OR TI,AB,IF(tumor*) OR TI,AB,IF(tumour*) OR TI,AB,IF(malignant*) OR TI,AB,IF(neoplasm*) OR TI,AB,IF(carcinoma*) OR TI,AB,IF(squamous) OR TI,AB,IF("squamous cell*") OR TI,AB,IF(OSCC) OR TI,AB,IF("soft tissue") OR TI,AB,IF(HPV-positive) OR TI,AB,IF(HPV-negative) OR TI,AB,IF(HPV+ve) OR TI,AB,IF(HPV-ve)) AND (TI,AB,IF(metabolomic*) OR TI,AB,IF(metabonomic*) OR TI,AB,IF("metabolic profiling") OR TI,AB,IF(metabolome) OR TI,AB,IF("metabolite marker") OR TI,AB,IF("metabolite biomarker"))</p>	

Table S2

	Ishikawa et al., (2016) [35]	Syed et al., (2016) [42]	Ishikawa et al., (2017) [36]	Enomoto et al., (2018) [38]	Ishikawa et al., (2018) [39]	Ishikawa et al., (2019) [40]	Sridharan et al., (2019) [41]	De Sa Alves et al., (2021) [37]	Song et al., (2019) [22]	Tsai et al., (2020) [43]	Yang et al., (2020) [44]	Yang et al., (2021) [20]	Li et al., (2022) [23]
Clearly stated research question and objectives	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Valid inclusion and exclusion criteria	Not clear	Yes	Not clear	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
Spectrum of patients representative of the disease of interest	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Sample size justification	No	No	No	No	No	No	Yes	No	No	No	No	No	No
Type of sample fully described	Yes	Yes	Yes	Not clear	Yes	Yes	Yes	Not clear	Yes	Yes	Not clear	Yes	Not clear
Description of sample collection, handling and storage	Not clear	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Not clear	Yes	Not clear
Confounding factors like smoking, diet interferences addressed	No	No	No	No	No	No	No	Not clear	Not clear	Not clear	No	No	No
Randomisation of samples	No	No	No	Yes	No	No	No	No	No	No	No	No	No
Description of pre-analytical processing of sample	Yes	Yes	Yes	Yes	Yes	Not clear	Yes	Yes	Yes	Yes	Not clear	Yes	Yes
Analytical methods clearly described	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Not clear	Yes	Yes
Clearly defined measures of risk/exposure	Yes	No	Yes	Yes	Yes	Yes	Yes	Not clear	Yes	Yes	Yes	Yes	Yes
Comprehensive details on statistical analysis	Yes	No	Yes	Yes	Yes	Yes	Not clear	Yes	Yes	Yes	Yes	Yes	Yes

R-Script for differentiation of upregulated and downregulated metabolites.

```
#library(dbplyr)
#library(dplyr)
#library(tidyverse)
#Com_FoldChange <- read.csv("filename.csv")
#unique(Com_FoldChange$Comparison)
#Com_FoldChange<-Com_FoldChange %>% mutate(Comparison_order
=Com_FoldChange$`Comparison order`) %>% separate(.,'Comparison order', into = c("Positive",
"Negative"), sep = "_")
#Com_FoldChange<-Com_FoldChange %>% mutate(Comparison_order
=Com_FoldChange$`Comparison.order`) %>% separate(.,'Comparison.order', into = c("Positive",
"Negative"), sep = "_")
#View(Com_FoldChange)
#Com_FoldChange$log2_FoldChange<-log2(as.numeric(Com_FoldChange$`Fold Change`))
#Com_FoldChange$Metaboanalyst_name<-Com_FoldChange$`Metaboanalyst matched name`
#View(Com_FoldChange)
#Com_FoldChange_OSCC<-Com_FoldChange %>% filter(Positive == "OSCC")
#View(Com_FoldChange_OSCC)
#OSCC_UPregulated<- Com_FoldChange_OSCC %>% filter(log2_FoldChange >0)
#Studys_OSCC<-unique(Com_FoldChange_OSCC$Study)
#Com_FoldChange_OSCC<-Com_FoldChange %>% filter(Positive %in% c("OSCC")) %>%
#filter(Negative=="HC")
#View(Com_FoldChange_OSCC)
#OSCC_UPregulated <- Com_FoldChange_OSCC %>% filter(log2_FoldChange >0) %>%
#group_by(Metaboanalyst_name) %>%
#summarise(n = n())
#OSCC_DOWNregulated <- Com_FoldChange_OSCC %>% filter(log2_FoldChange <0) %>%
#group_by(Metaboanalyst_name) %>%
#summarise(n = n())
```

Supplementary Table S3: Metabolite subgrouping into superclass and subclass.

Organic acid derivatives	119
Amino acids, peptides, and analogues	89
Others Carboxylic acid derivatives	15
Hydroxy acids and derivatives	4
Keto acids and derivatives	3
Phosphate esters	3
Carboximidic acids and derivatives	3
Sulfinic acids and derivatives	2
Lipids	94
Fatty acids and Fatty acyls	48
Other lipid derivatives	14
Glycerophospholipids	12
Glycerols	8
Bile acids	6
Steroid esters	6
Organoheterocyclic compounds	46
Purines and purine derivatives	14
Pyrimidines and pyrimidine derivatives	13
Other olines, lignans and ureas	9
Pyridines and derivatives	4
Piperidines	2
Azoles	2
Indoles and derivatives	2
	49
Organic oxygen compounds	
Alcohols and polyols	6
Carbohydrates	43
Benzene and Benzene derivatives	11
Benzene and Benzene derivatives	8
Phenol and Phenol derivatives	3
Organic nitrogen compounds	9
Quaternary ammonium salts	5
Amines	3
Aminoxides	1
Nucleosides	3
Purine and Pyrimidine Nucleosides	2
5'-deoxyribonucleosides	1
Homogeneous non-metal compounds	2
Non-metal phosphates	2
Polyketides	4
Phenylpropanoids	3
Flavonoids	1