

# Research Activities in Dentistry – Practical Course

**Mgr. Tereza Deissová<sup>1,2</sup>**

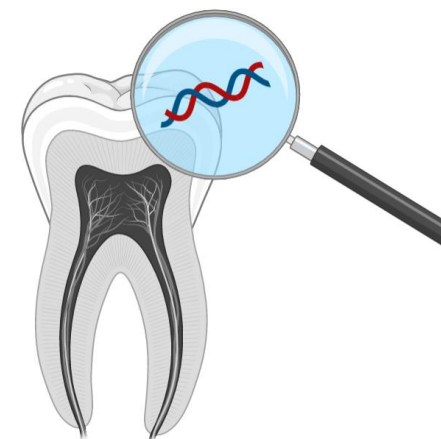
**Assoc. Prof. RNDr. Petra Bořilová Linhartová, Ph.D., MBA<sup>1,2,3,4</sup>**

<sup>1</sup> Clinic of Stomatology, Faculty of Medicine, Masaryk University Brno

<sup>2</sup> Department of Patophysiology, Faculty of Medicine, Masaryk University Brno

<sup>3</sup> Institute of Medical Genetics, Faculty of Medicine, Masaryk University Brno

<sup>4</sup> Clinic of Maxillofacial Surgery, Fakultní nemocnice Brno



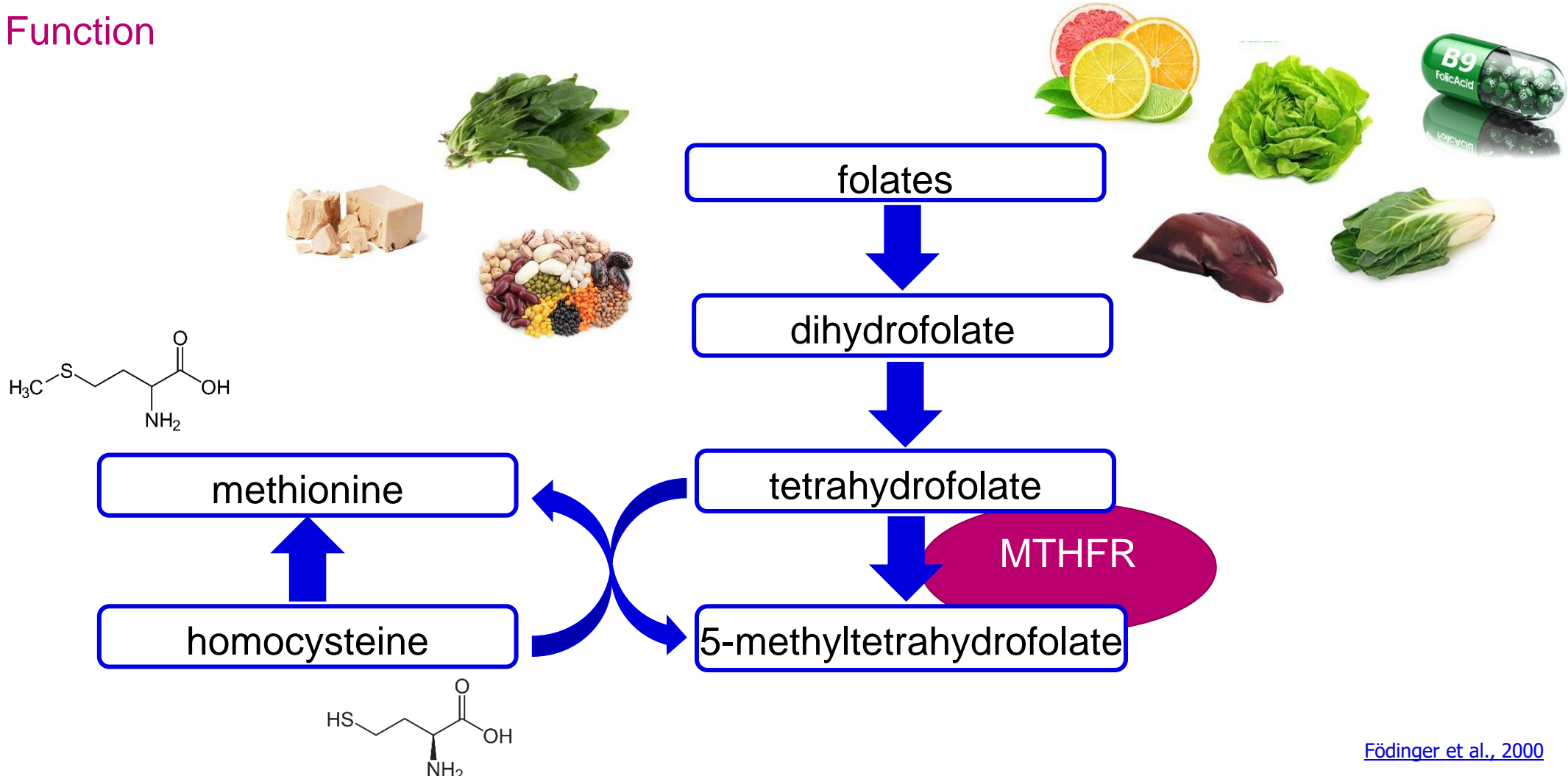
# Variability in the *MTHFR* gene and oral diseases

## Content of the practical course

- Enzyme methylenetetrahydrofolate reductase (MTHFR) – functional effect and its polymorphisms
- Oral diseases associated with the variability in gene for MTHFR and our studies results
- Analysis of polymorphisms in the gene for MTHFR (laboratory practice)
- Evaluation of results

# Methylenetetrahydrofolate reductase (MTHFR)

## Function



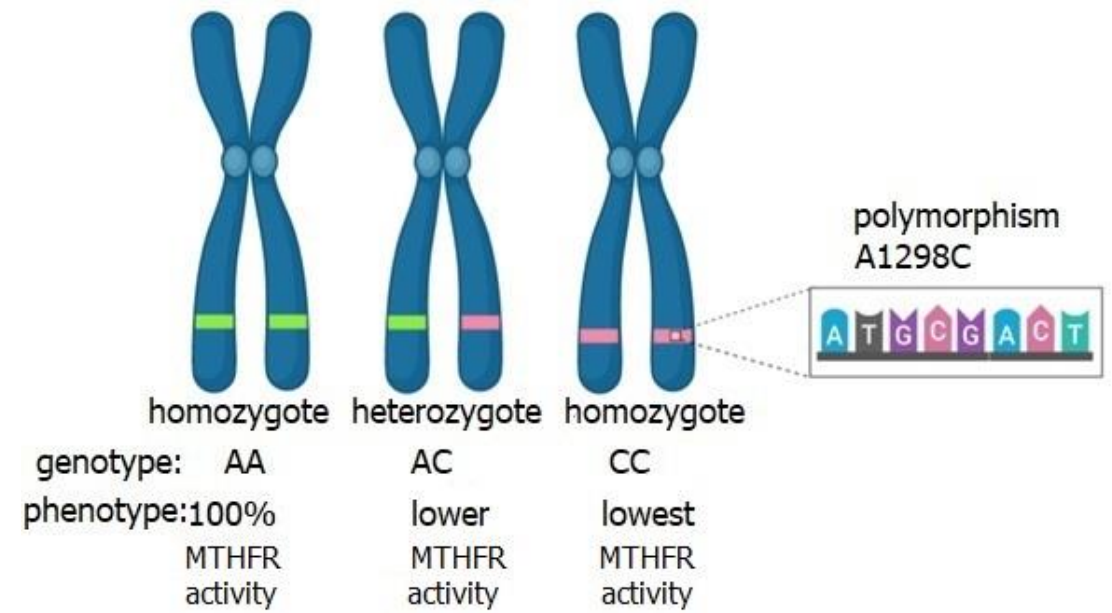
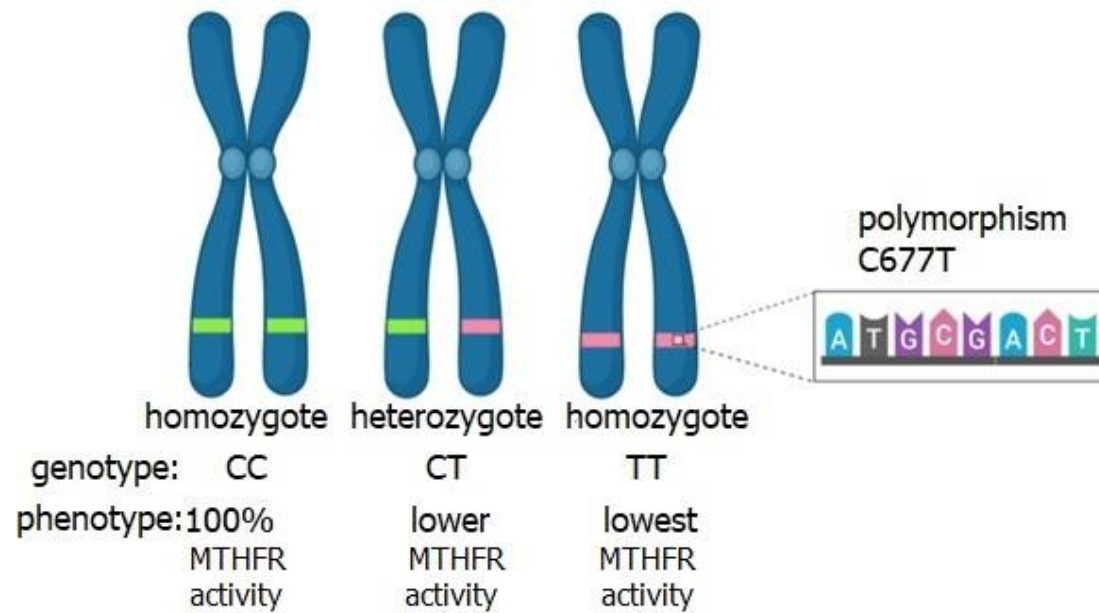
Födingen et al., 2000

MUNI  
MED

# Methylentetrahydrofolate reductase (MTHFR)

## Genetic variability

- gene *MTHFR* and single nucleotide polymorphisms C677T and A1298C

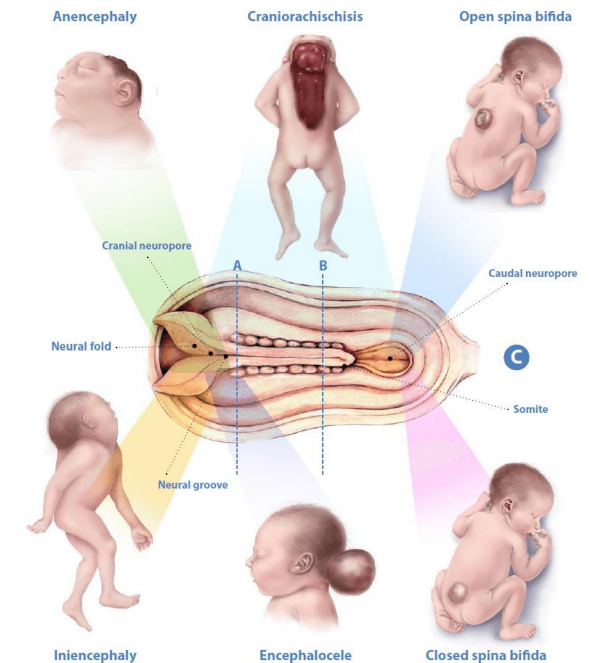


[Weisberg et al., 1998](#)

# Methylentetrahydrofolate reductase (MTHFR)

## Function of polymorphisms C677T and A1298C

- genetically determined reduction of MTHFR enzymatic activity
  - reduced methylation of homocysteine to methionine
  - accumulation of homocysteine in plasma
  - risk of congenital malformations, cardiovascular disorders
- Increased in prevalence of thromboembolic disorders and in risk of spontaneous abortion in homozygous mothers with mutant alleles
- increased toxicity of cytostatic CMFs (cyclofosamid, MTX, 5-FU)



[Doležálková et al., 2014](#)  
[Shere et al., 2015](#)  
[Wang et al., 2019](#)

**MUNI**  
**MED**

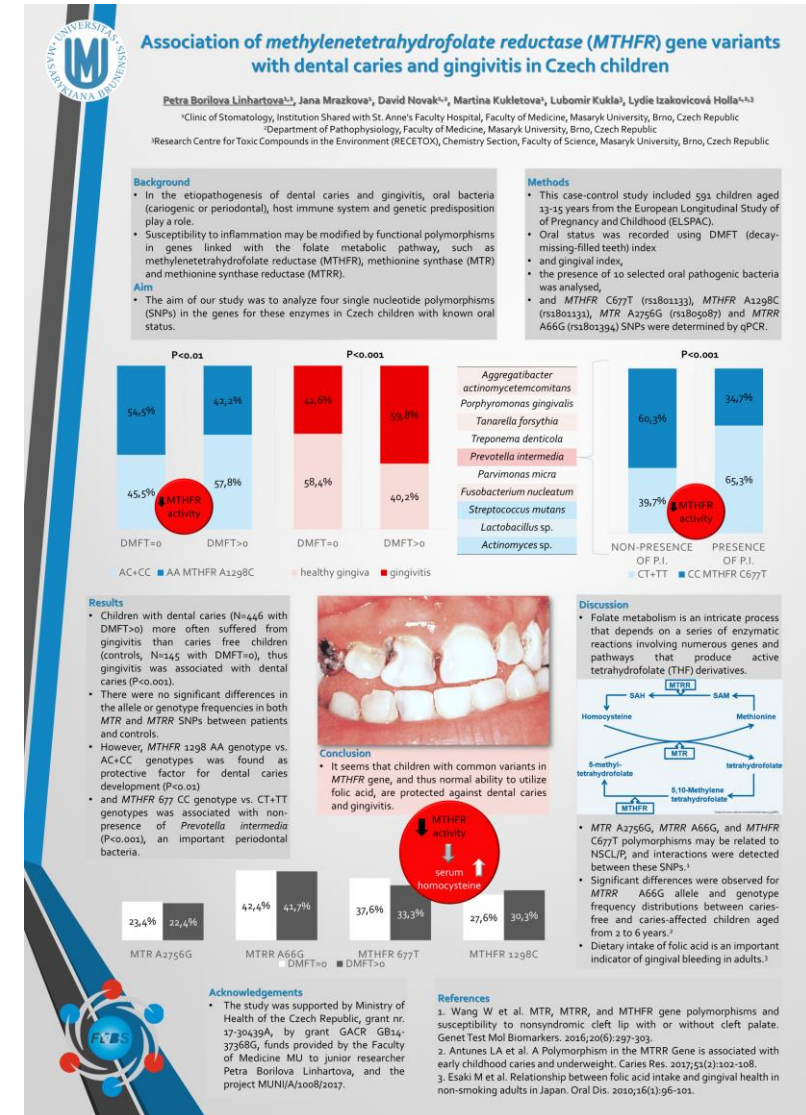
# Tooth caries and gingivitis

## Etiopathogenesis

- ↑ homocysteine in saliva = oxidative marker
  - oral mucosa inflammation
  - cariogenic proces
- 2x daily 1 mg/ 1 mL of folate in mouthwash for 14 days = ↓gingivitis symptoms



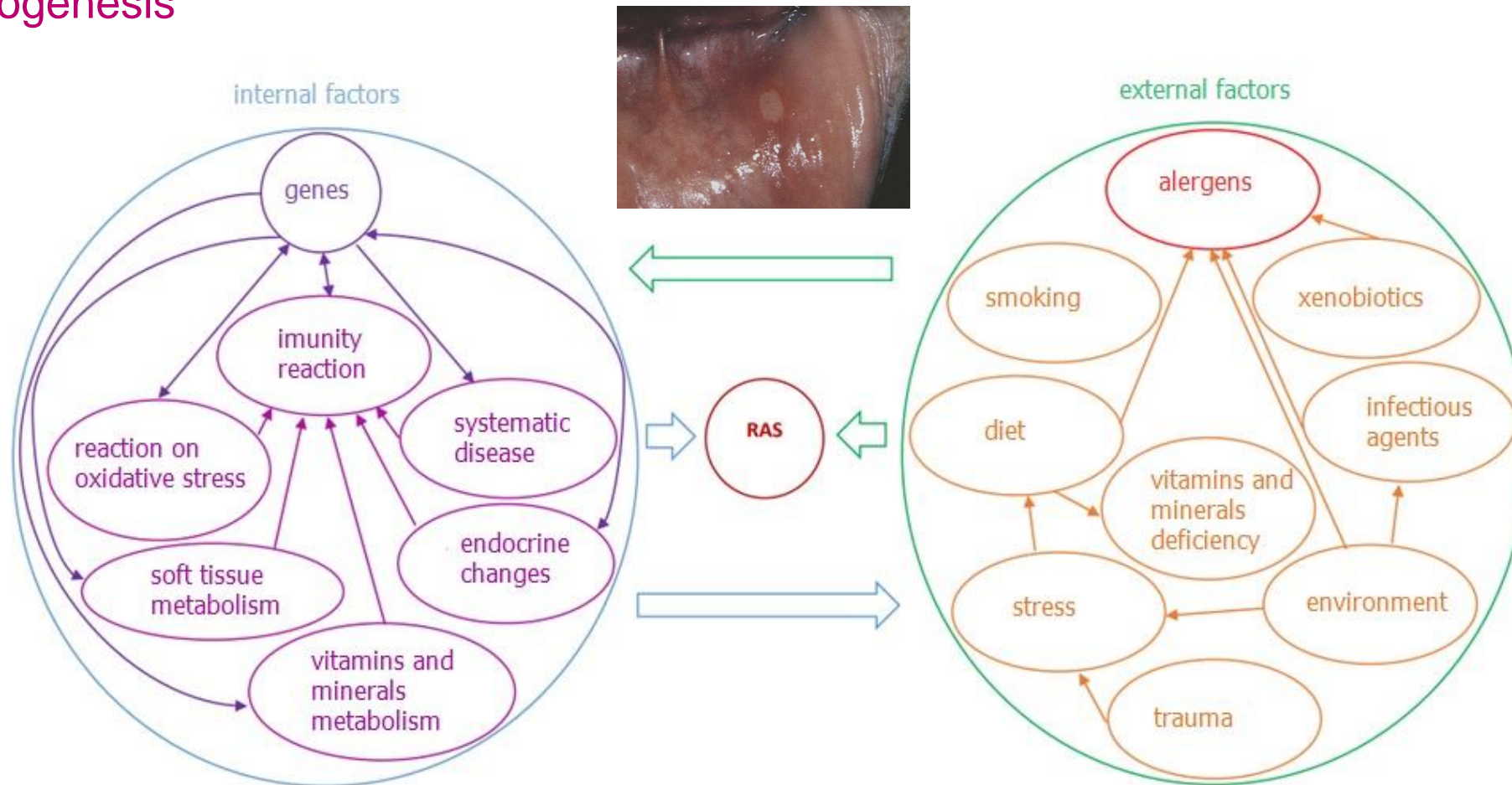
Pack 1984  
Cope et al., 2011  
Kumar et al., 2011  
Bořilová Linhartová et al., 2018





# Recurrent aphthous stomatitis

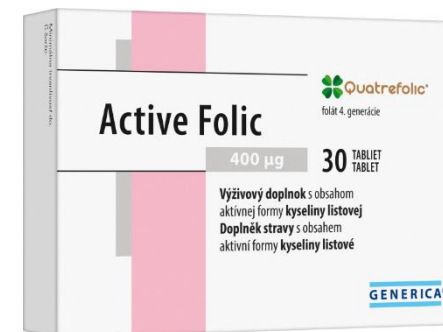
## Etiopathogenesis



# Recurrent aphthous stomatitis

## Therapy

- symptomatic therapy, supportive therapy – **Škach's vitamin treatment**  
**folic acid, pyridoxine, B12**
- folic acid is only a precursor of the biologically active substance – vitamin B9 (a particular folate)
- glucosamine salt of 5-methylfolate (4<sup>th</sup> generation) – compared to the 3<sup>rd</sup> generation, it is stable for a long time, highly water-soluble, with better bioavailability and safety

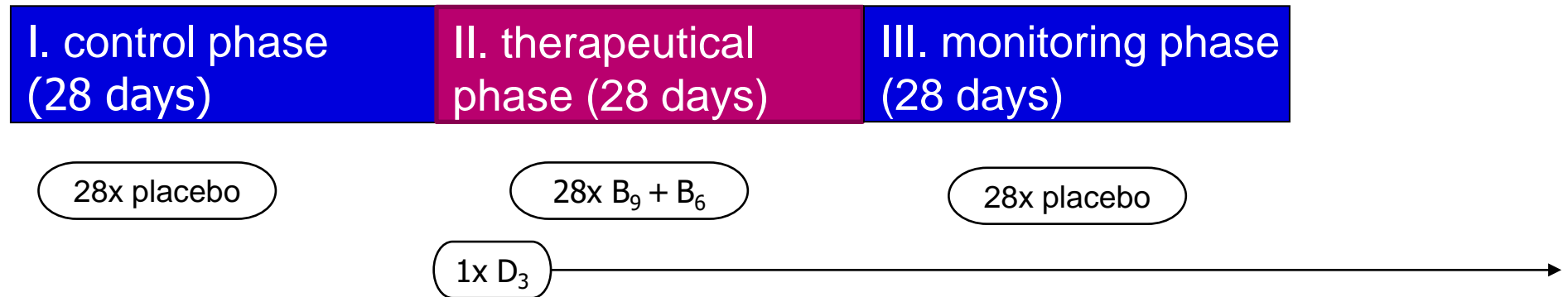




# Recurrent aphthous stomatitis

## Design of a pilot clinical experimental study

- 10 patients – genotype analysis for haplotypes in the *MTHFR* gene (IM and PM)
- pharmacotherapy in a modified design of a double-blind cross-over study (cross-over design) took place from the spring 2018 for of 3 months
- patients received active folate (glucosamine salt of 5-methylfolate), vitamins B6 and D3 in a three-phase schedule



# Recurrent aphthous stomatitis

## Pilot clinical experimental study

ČESKÁ STOMATOLOGIE A PRAKTICKÉ ZUBNÍ LÉKAŘSTVÍ 1/2019  
4 | PŮVODNÍ PRÁCE






FARMAKOTERAPIE RECIDIVUJÍCÍ AFTÓZNÍ  
STOMATITIDY U PACIENTŮ S GENETICKY PODMÍNĚNOU  
SNÍŽENOU SCHOPNOSTÍ METABOLIZOVAT KYSELINU  
LISTOVOU – PILOTNÍ STUDIE

Původní práce – experimentální klinická studie

PHARMACOTHERAPY OF RECURRENT APHTHOUS  
STOMATITIS IN PATIENTS WITH GENETICALLY  
IMPAIRED ABILITY TO METABOLIZE FOLIC ACID  
– PILOT STUDY

Original article – experimental clinical study

Bořilová Linhartová P.<sup>1, 2</sup>, Fassmann A.<sup>1</sup>, Linhartová J.<sup>1, 3</sup>,  
Izakovičová Holá L.<sup>1, 2</sup>

SNP		A1298C <i>MTHFR</i> (rs1801131) MAF (C)=34 %		
	genotype MTHFR enzymatic function frequency in EUR population	AA 100 % 43 %	AC 80-100 % 45 %	CC 60 % 12 %
C677T <i>MTHFR</i> (rs1801133) MAF (T)=31 %	CC	--/--	--/+	--/++
	100 %	100%	80%	60%
	46 %	EM	EM	IM 
	CT	+/-	+/-	+/-
	65 %	65%	50%	30%
	44 %	IM 	IM 	PM
	TT	++/-	++/+	++/++
	20 %-30 %	<30%	<30%	<10%
	10 %	PM 	PM 	PM

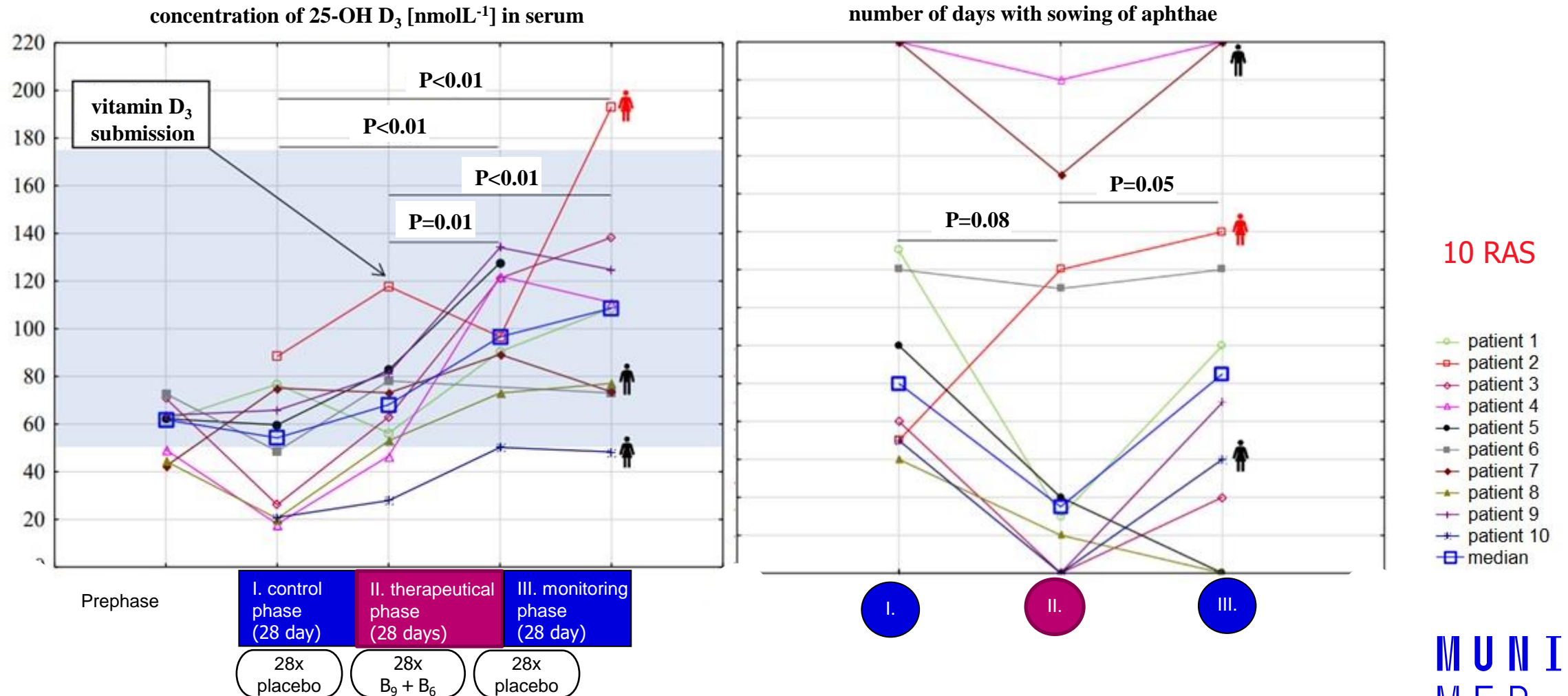
haplogenotype  
MTHFR enzymatic function  
presumed phenotype  
individuals with RAS in our study

[Bořilová Linhartová et al., 2019](#)

MUNI  
MED

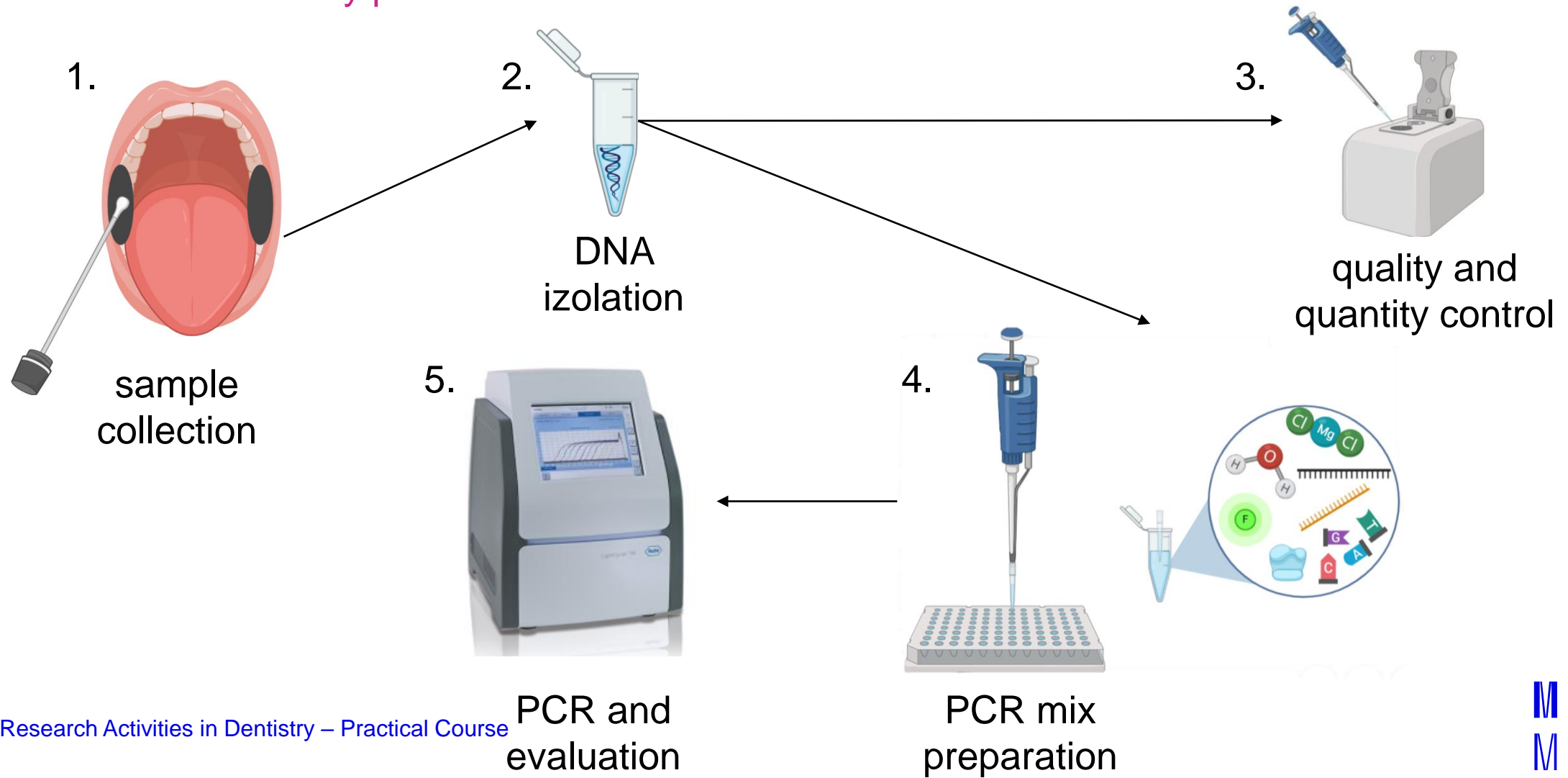
# Recurrent aphthous stomatitis

## Pilot clinical experimental study



# Analysis of polymorphisms in gene *MTHFR*

Manual for laboratory practice



# DNA isolation from buccal swabs

Manual for laboratory practice

Sampling of the buccal mucosa → brush rotation 1-2 minutes → lytic solution



1



2



3



# DNA isolation from buccal swabs

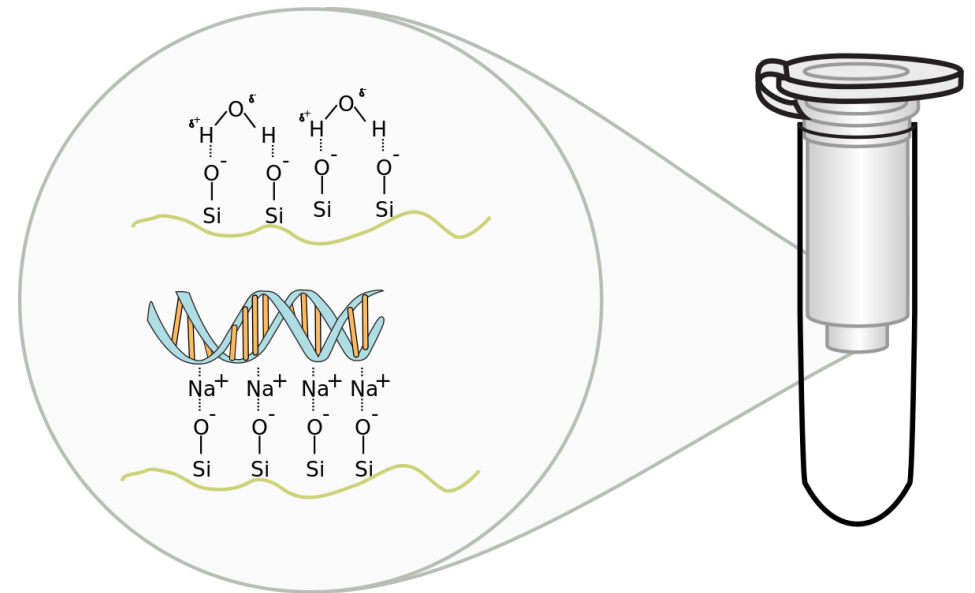
Manual for laboratory practice

DNA isolation using silicate columns →

disruption of plasma and nuclear membranes →

proteinase K, chaotropic agent, detergent

- 1) ethanol precipitation
- 2) DNA binding to the silicate column
- 3) washing
- 4) elution of pure DNA from the column



# DNA isolation from buccal swabs

Manual for laboratory practice

Evaluation of isolated DNA quality by spectrophotometry (NanoDrop)

→ absorbance measurement, nucleic acids have a maximum absorbance at 260 nm

a) concentration in ng /  $\mu$ L

b) purity:

absorbance ratio 260/280

<1.8 protein contamination by proteins (aromatic)

absorbance ratio 260/230

<2 contamination by organic agents



# PCR – reaction mix preparation

Manual for laboratory practice

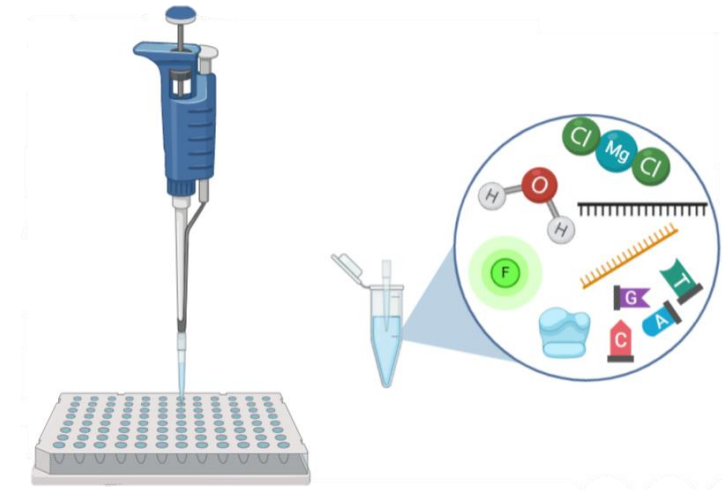
MasterMix contains:

buffer

nucleotides (adenine, thymine, guanine, cytosine)

magnesium chloride / magnesium sulphate

DNA-dependent DNA polymerase



TaqMan assay contains:

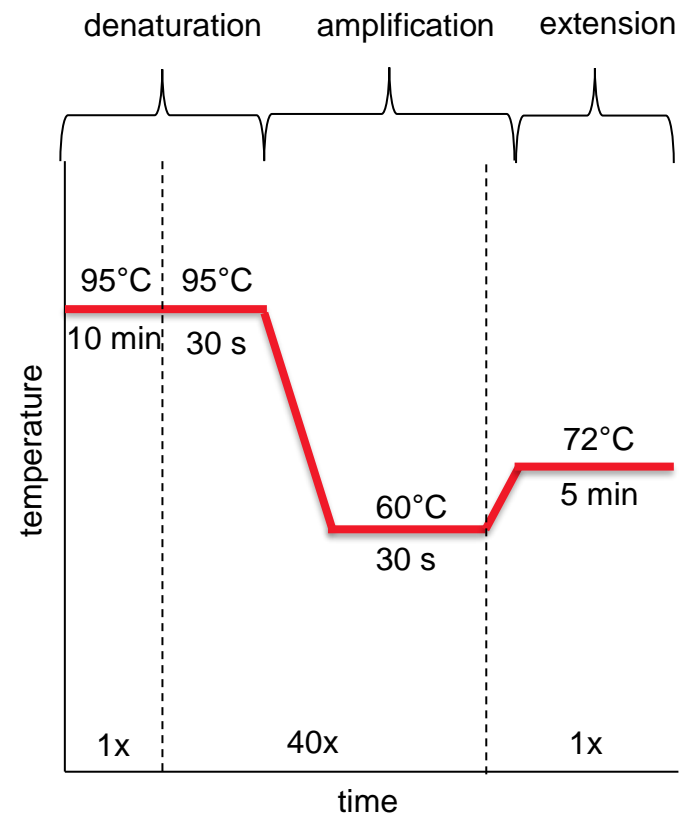
forward and reverse primers

VIC fluorophore-labeled oligonucleotide and FAM fluorophore-labeled oligonucleotide

reaction mix = MasterMix +  
TaqMan assay + template DNA

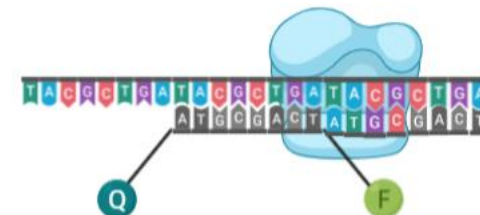
# qPCR

## Manual for laboratory practice



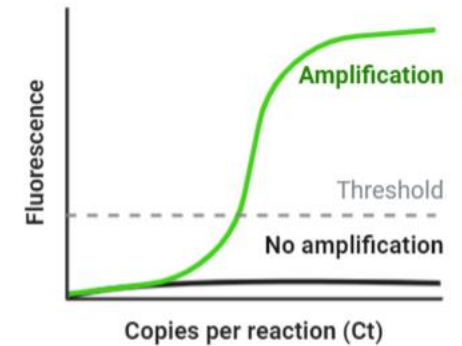
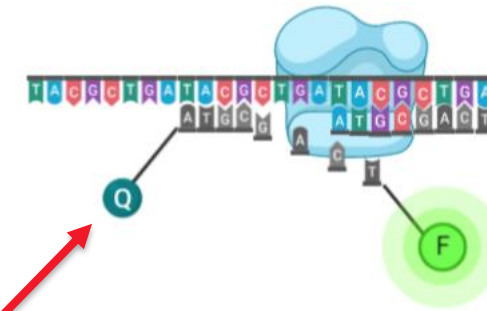
DNA polymerase

primer



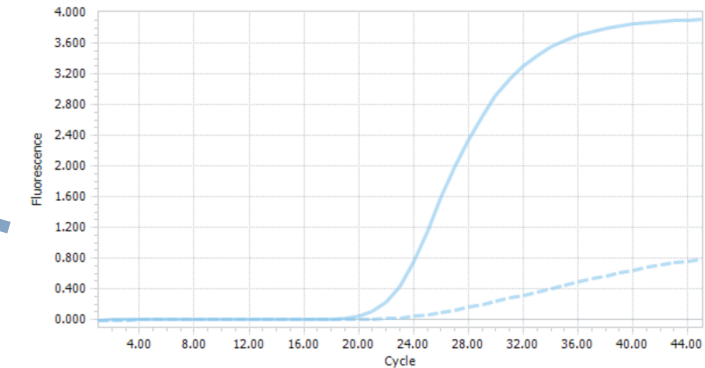
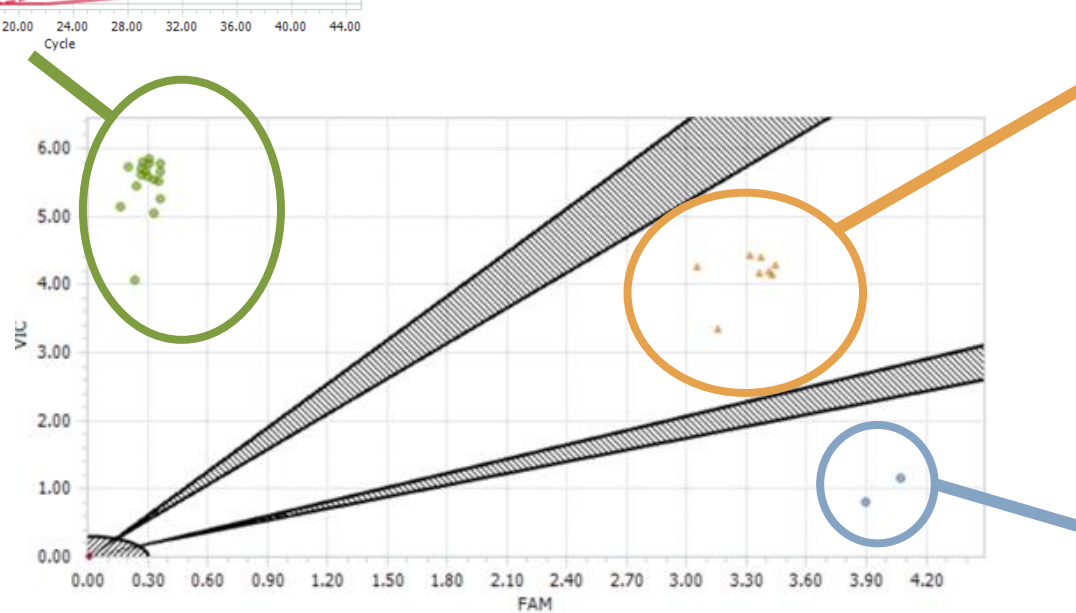
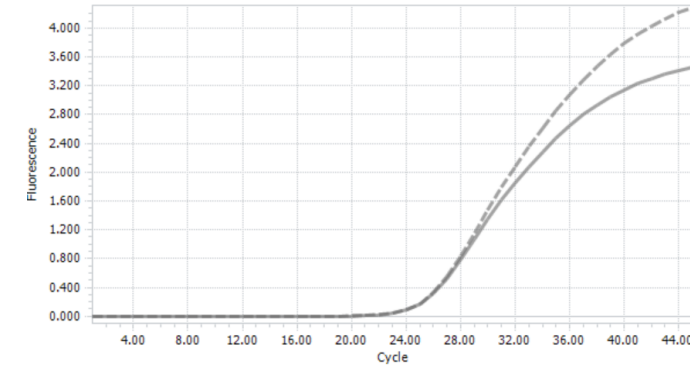
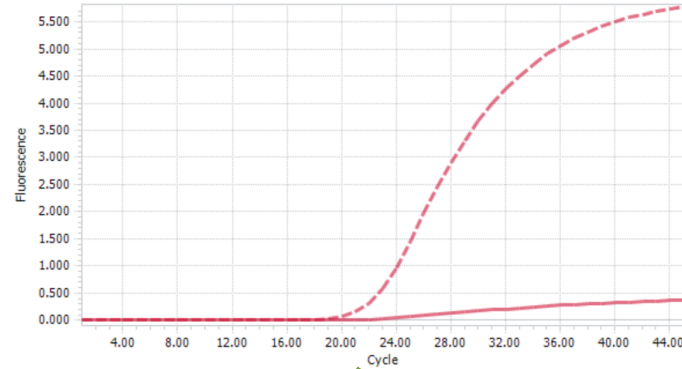
quencher

fluorophore



# Evaluation of the results

## Manual for laboratory practice





# Protocol

## Manual for laboratory practice

<b>Title: Analysis of polymorphisms C677T and A1289C of the <i>MTHFR</i> gene</b>			
Method: TaqMan PCR			
Name:		Surname:	
Input material:			
DNA concentration:		DNA purity (260/280):	
		DNA purity (260/230):	
Genotype:			
C677T:		A1289C:	
Haplogenotype (C677T/A1289C):			
Phenotype:			
Recommendation:			

Table 1: Individual haplogenotypes resulting from a combination of genotypes of both SNPs

SNP	A1298T genotype AA	A1298T genotype AC	A1298T genotype CC
C677T genotype CC	CCAA 100% MTHFR activity	CCAC 80%	CCCC 60%
C677T genotype CT	CTAA 65%	CTAC 50%	CTCC 30%
C677T genotype TT	TTAA <30%	TTAC <30%	TTCC <10% MTHFR activity

# Acknowledgement

- Supported by the project **MUNI/FR/1406/2019 Research Activities in Dentistry**.
- The authors would like to thank Prof. MUDr. Lydie Izakovičová Hollá, Ph.D. (Clinic of Stomatology, Faculty of Medicine, Masaryk University Brno) for her critical review of this text.

# Opponents

- **RNDr. Mária Hovořáková, Ph.D.**

Institute of Histology and Embryology, First Faculty of Medicine, Charles University

- **Assoc. Prof. MUDr. Jakub Suchánek, Ph.D.**

Department of Dentistry, Faculty of Medicine in Hradec Kralove, Charles University

# References

Bořilová Linhartová, P.; Fassmann, A.; Linhartová, J.; Izakovičová Hollá, L. Farmakoterapie Recidivující Aftózní Stomatitidy u Pacientů s Geneticky Podmíněnou Sníženou Schopností Metabolizovat Kyselinu Listovou - Pilotní Studie. *Pharmacother. Recurr. Aphthous Stomatitis Patients Genet. Impair. Abil. Metab. Folic Acid - Pilot Study* **2019**, 119 (1), 4–11.

Bořilová Linhartová, P.; Mrázková, J.; Novák, D.; Kukletová, M.; Kukla, L.; Izakovičová Hollá, L. Association of methylenetetrahydrofolate reductase (MTHFR) gene variants with dental caries and gingivitis in Czech children. In The 43rd FEBS Congress, Prague **2018**. 2018. ISSN 2211-5463.

Borilova Linhartova, P.; Valová; Izakovicova Holla, L. Vrozená Náchylnost k Recidivující Aftózní Stomatitidě (Přehledový Článek) Genetic Predisposition to Recurrent Aphthous Stomatitis (Review). *Ceskoslovenská Stomatol.* **2017**, 117, 27–34.

Cope, G.; Cope, A. Gingivitis: Symptoms, Causes and Treatment. *Dent. Nurs.* **2011**, 7 (8), 436–439.

Doležálková, E.; Unzeitig, V. Kyselina listová a prevence rozštěpových vad centrálního nervového systému. *Čes Gynek* 2014 (2), 134–139.

Födinger, M.; Hörl, W. H.; Sunder-Plassmann, G. Molecular Biology of 5,10-Methylenetetrahydrofolate Reductase. *J. Nephrol.* **2000**, 13 (1), 20–33.

Kumar, D.; Pandey, R. K.; Agrawal, D.; Agrawal, D. An Estimation and Evaluation of Total Antioxidant Capacity of Saliva in Children with Severe Early Childhood Caries. *Int. J. Paediatr. Dent.* **2011**, 21 (6), 459–464.

Shere, M.; Bapat, P.; Nickel, C.; Kapur, B.; Koren, G. Association Between Use of Oral Contraceptives and Folate Status: A Systematic Review and Meta-Analysis. *J. Obstet. Gynaecol. Can.* **2015**, 37 (5), 430–438.

Pack, A. R. C. Folate Mouthwash: Effects on Established Gingivitis in Periodontal Patients. *J. Clin. Periodontol.* **1984**, 11 (9), 619–628.

Wang, Y.; Jin, Y.; Wang, Y.; Li, L.; Liao, Y.; Zhang, Y.; Yu, D. The Effect of Folic Acid in Patients with Cardiovascular Disease. *Medicine (Baltimore)* **2019**, 98 (37).

Weisberg, I.; Tran, P.; Christensen, B.; Sibani, S.; Rozen, R. A Second Genetic Polymorphism in Methylenetetrahydrofolate Reductase (MTHFR) Associated with Decreased Enzyme Activity. *Mol. Genet. Metab.* **1998**, 64 (3), 169–172.

# Copyright notice

- This material is copyrighted work created by employees of Masaryk University.
- Students are allowed to make copies for learning purposes only.
- Any unauthorised reproduction or distribution of this material or its part is against the law.



**MASARYKOVA  
UNIVERZITA**