Neural tube defects and MTHFR gene polymorphisms - the incidence in the Slovak population

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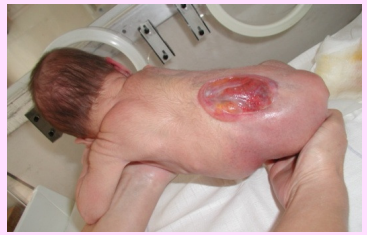
Folate & NTD(Neural Tube Defects)Prevention

- **NTD recurrence prevention**
  - periconceptional vitamins
  0,7 % vs. 4,7 % ***Smithells, 1981, ArchDisChild***



- **Double blind study - multivitamins vs. placebo**
  - vitamins: significantly ↓incidence of BD and NTD
  Czeizel, 1992, NEJM, 1993, BMJ

- **MRC Vitamin Study Research Group**
  - protective effect of folic acid ***Lancet, 1991***

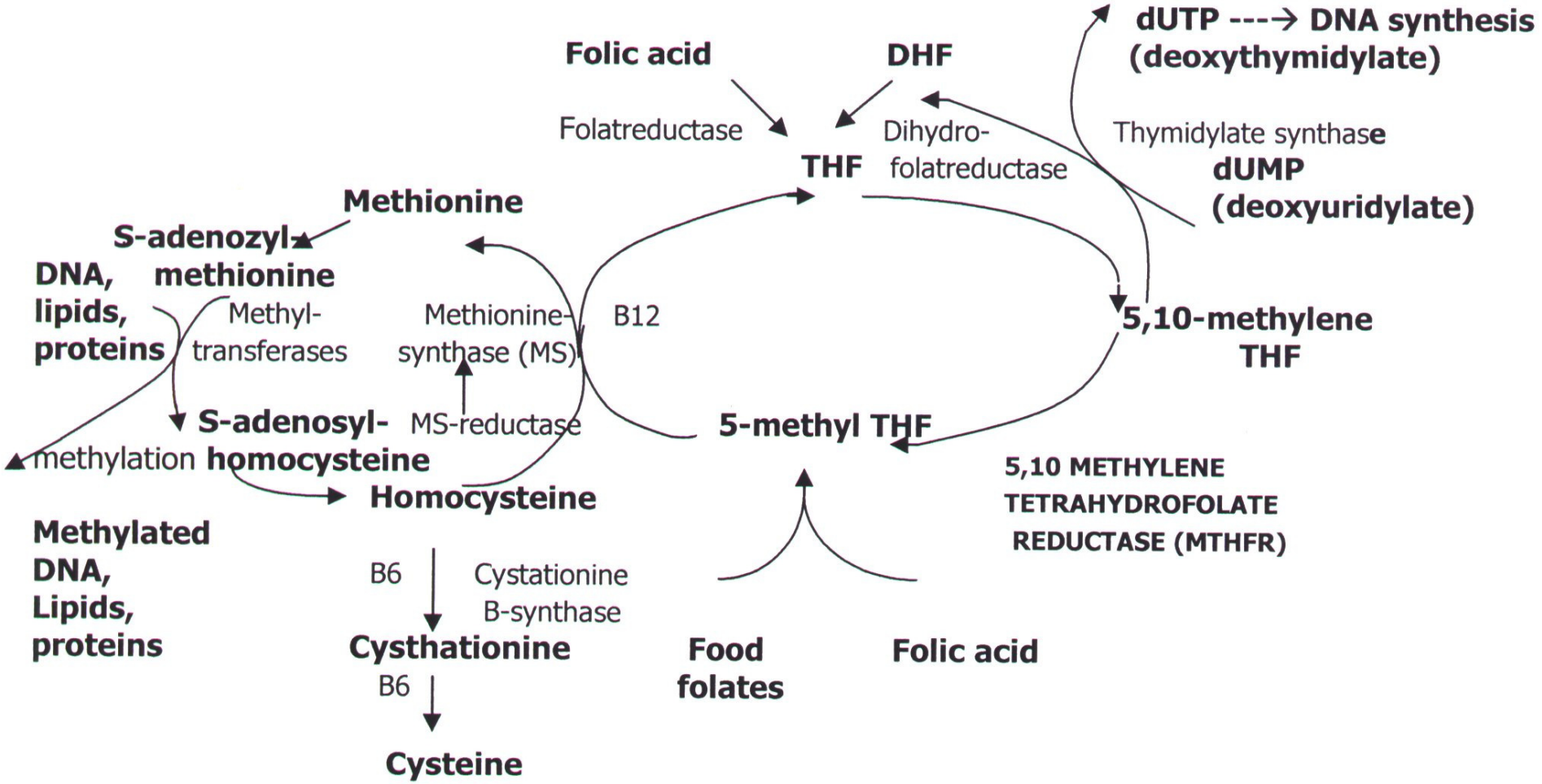
- **Berry, 1999, double blind study (folate - placebo)**
  ~ 250 000 gestations, folate: ↓ NTD in 50-70 % ! ***NEJM***

↓

Preventive mechanism???

↑Thymidine? ↑Methionine? ↓Homocysteine?

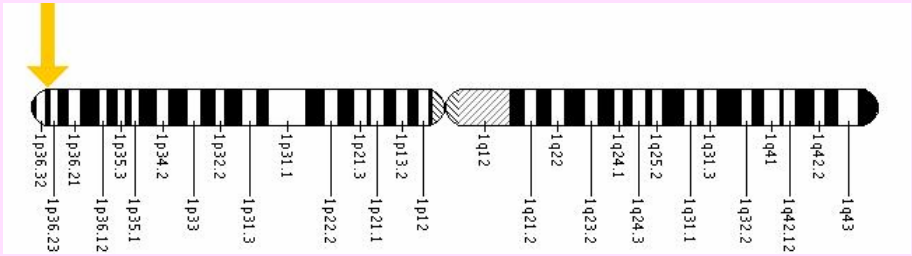
Main biochemical roles of folate



** thymidine nucleotids synthesis
** homocysteine -› methionine -›
** -› methylation reactions

MTHFR gene and its gene product

- **Chromosome locus: 1p36.3**



- **Coding DNA: 2,2kb, 11 exons**

- **Gene product - enzyme MTHFR 656 AA 74,5 kDa**
  - dimer, 2 main domains: N-terminalna catalytic
  C-terminal regulative
- **Severe mutations : homocystinuria**

Goyette, 1994, Nat Genet 7:195-200
Goyette, 1998, Mamm Genome, 9:652-656

C677T polymorphism of MTHFR gene

- **Exone 4: 677 C ---> T, alanine --- > valine**

  Frosst, 1996, Nat Genet, 10:111-113

- **The consequence: thermolabile enzyme form**
- **TT homozygotes: MTHFR activity - 35-50% of norm**
  => significantly higher homocysteinemia
- **TT homozygotes - various populations: 0 - 23%**
- **T allele - various populations:**
  0,005 (Africa)..0,2 (Norway)..0,4-0,5 (Italy, Spain...)

A1298C polymorphism of MTHFR gene

- **Exone 7: 1298 A--->C, glutamate--->alanine**
- **CC homozygotes - slightly ↓ MTHFR activity (60%)**
  - homocysteine not increased
- **Consequence: combination with C677T**
  - both polymorphisms heterozygotes:
  significantly ↑ Hcy and ↓ folate
- **C allele prevalence: 0,27-0,35**

Van Der Put, 1998, Am J Hum Genet, 62

Neural tube defects - Slovakia

- population > 5 000 000
- total liveborns ≈ 50 000 / year
- total NTD gestations - 16-33 /year
  - 0,31-0,59 / 1000 liveborn
- liveborn NTD - 8 - 21/ year
  - 0,14-0,31 /1000 liveborn

	Slovakia								
years	1996	1997	1998	1999	2000	2001	2002	2003	2004
note	Number of cases: Liveborn / Stillborn / Induced abortions								
anenceph.	1 /2/ 10	2 / 0 / 13	1 / 0 / 7	2 / 0 / 9	0 / 1 / 5	2 / 0 / 6	2 / 0 / 4	1 / 1 / 7	0 / 0 / 5
encephaloc.	3 / 0 / 3	2 / 0 / 2	4 / 0 / 3	3 / 1 / 2	0 / 0 / 2	2 / 0 / 5	1 / 0 / 0	4 / 0 / 1	2 / 1 / 1
spina bifida	10 / 0 / 2	8 / 1 / 0	13 / 0 / 2	16 / 0 / 0	8 / 1 / 0	10 / 0 / 0	7 / 1 / 1	7 / 0 / 1	10 / 0 / 0
LbNTD/all NTD per1000Lb	0,23 / 0,52	0,2 / 0,47	0,31 / 0,52	0,37 / 0,59	0,14 / 0,31	0,27 / 0,49	0,20/ 0,31	0,23/0,43	0,22 / 0,35

Neural tube defects incidence - Slovakia & world history

Slovakia								
1996	1997	1998	1999	2000	2001	2002	2003	2004
Liveborn NTD / all NTD per 1000 Liveborn								
0,23 / 0,52	0,2 / 0,47	0,31 / 0,52	0,37 / 0,59	0,14 / 0,31	0,27 / 0,49	0,20/ 0,31	0,23/0,43	0,22 / 0,35

state	Ireland	China			Britain		Mexico	Canada		Hungary	Japan	USA			
regio		North China			Engl+Wales			New Scotia				Most states average			
years	60-s	87	93-5		72	91		92-7	98-9	70	60-s	95-6		98-9	
note			FA -	FA +									no PC		no PC
NTD/ 1000	8	7.0	4,8	1,3	4,03	0,76	3,6	2,9	1,4	1,4	0,65	0,38	0,53	0,3	0,46

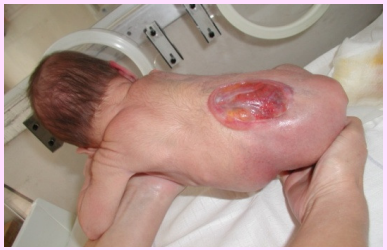
Objectives

- MTHFR gene polymorphisms C677T and A1298C in Slovak newborn population
- MTHFR gene polymorphisms C677T and A1298C in Slovak patients with NTD and their mothers
- MTHFR genotypes & ethnicity - caucasian vs. gypsy newborn groups

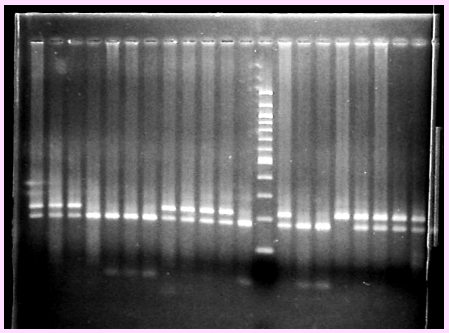
- New - MTHFR gene polymorphisms in patients with uropoetic system anomalies

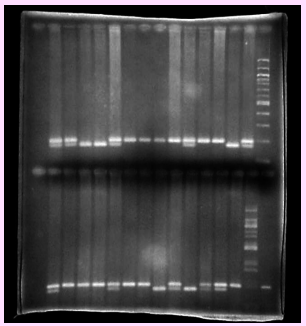
Methods

- **NTD patients n=93 and their mothers n=31**
  - venous blood, DNA isolation (Promega kit)
  - PCR + enzyme restriction - Hinf I, resp. Mbo II



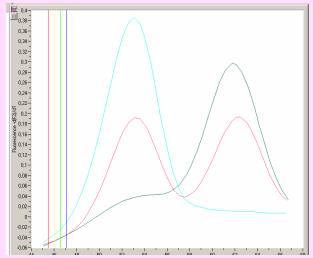
C677T





A1298C

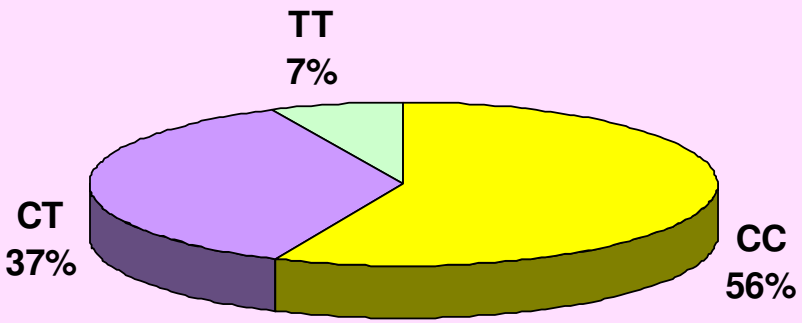
- **Controls - unselected newborn population n= 290**
  - dry blood spot, DNA isolation - Machery Nagel kit
  - RT PCR - Real time light cycler 1,5 Roche, reaction Mix and MTHFR probes (Roche and Tib MolBiol)



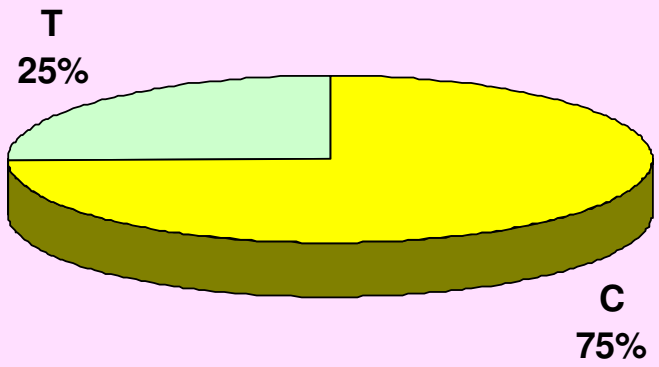
MTHFR polymorphisms - Slovak population

C677T

genotypes

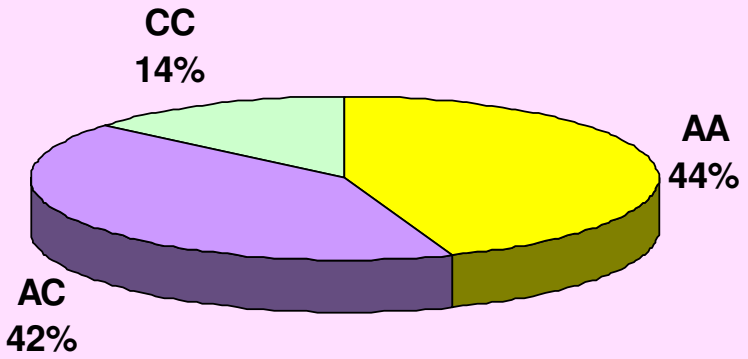


alleles

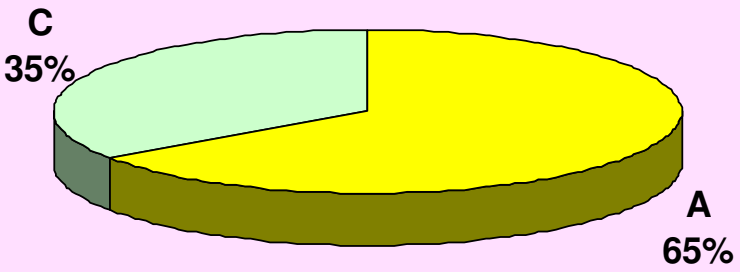


A1298C

genotypes

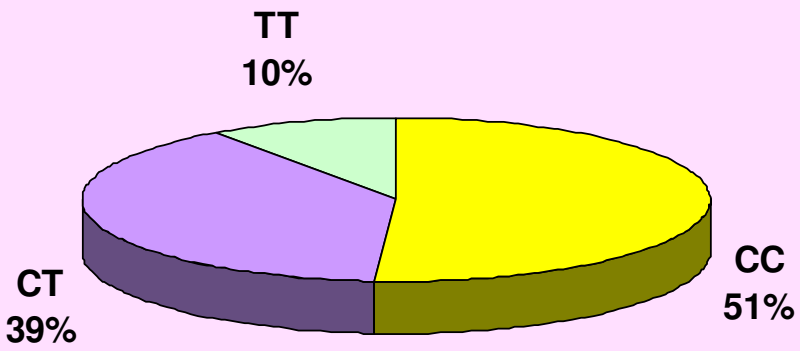


alleles

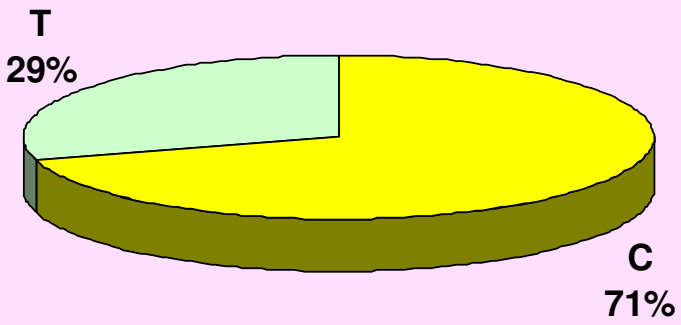


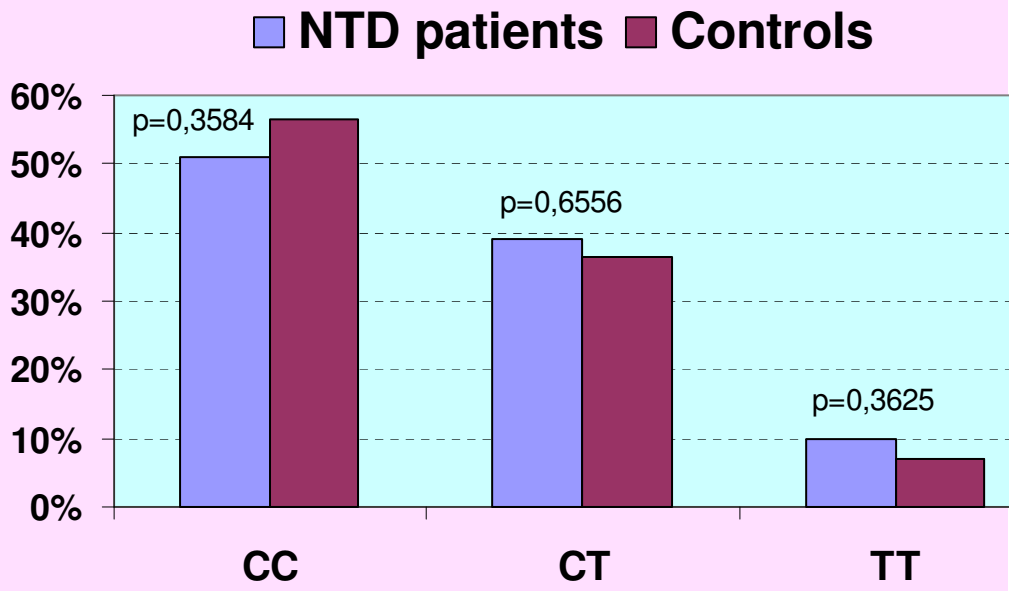
C677T polymorphism - NTD patients

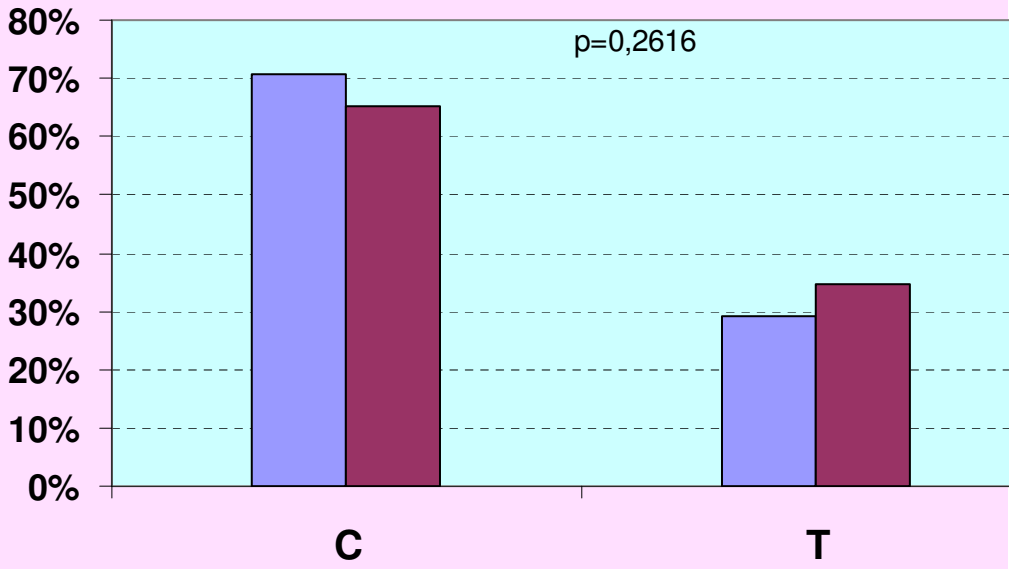
genotypes



alleles

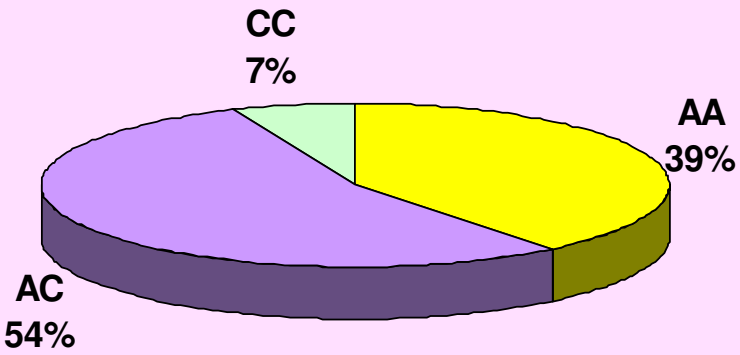




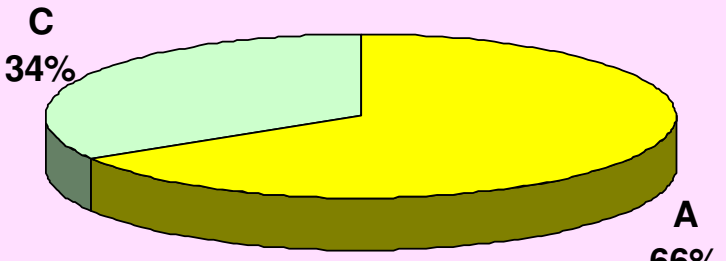


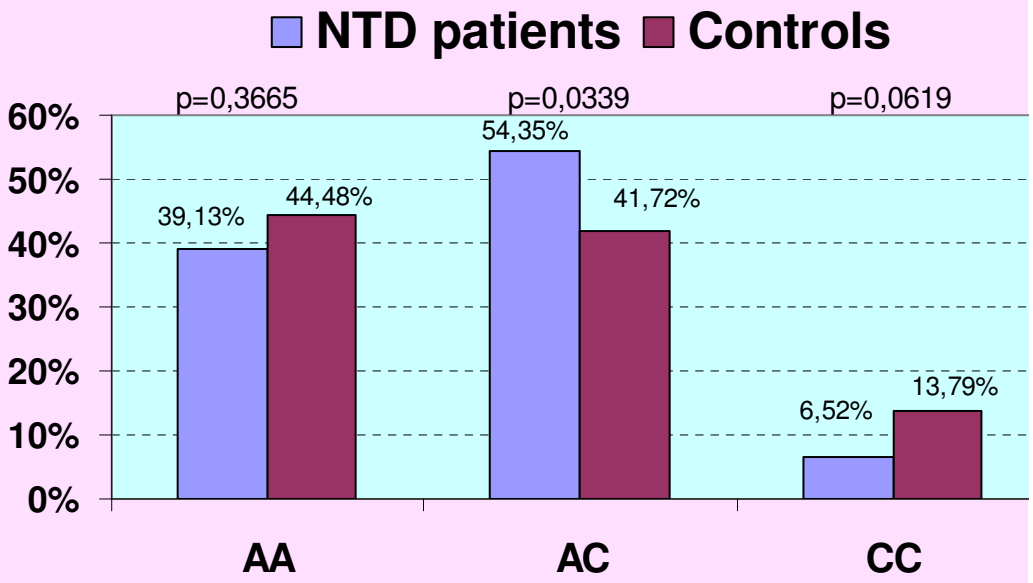
A1298C polymorphism - NTD patients

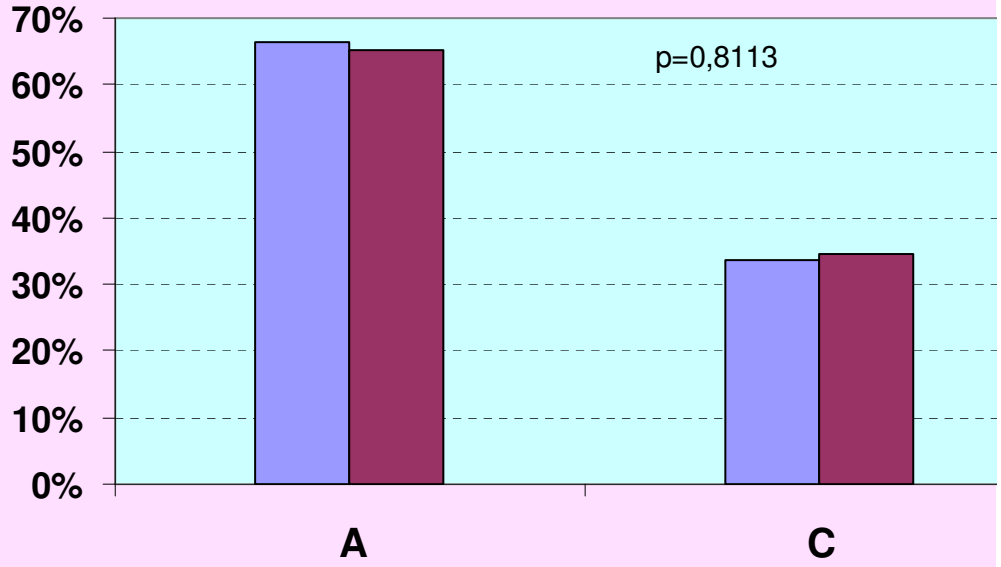
genotypes



alleles







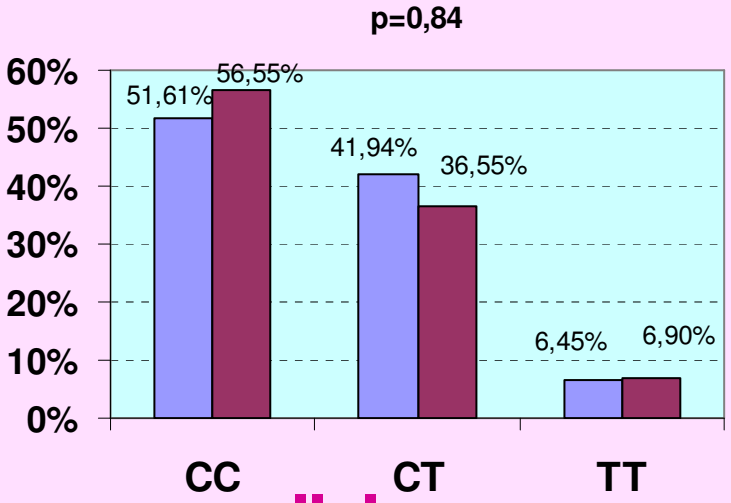
MTHFR genotypes combinations - NTD patients vs. controls

A1298C / C677T	AA		AC		CC		
	No / %	p vs control	No / %	p vs control	No / %	p vs control	all
CC	10 / 11,0%	p=0,1343	31 / 34,1%	p=0,0966	6 / 6,6 %	p=0,0659	47 / 51,6 %
CT	17 / 18,7%	p=0,7825	18 / 19,8 %	p=0,4770			35 / 38,5 %
TT	9 / 9,9%	p=0,3475					9 / 9,9 %
all	36 / 39,6%		49 / 53,8 %		6 / 6,6 %		91 / 100,0 %

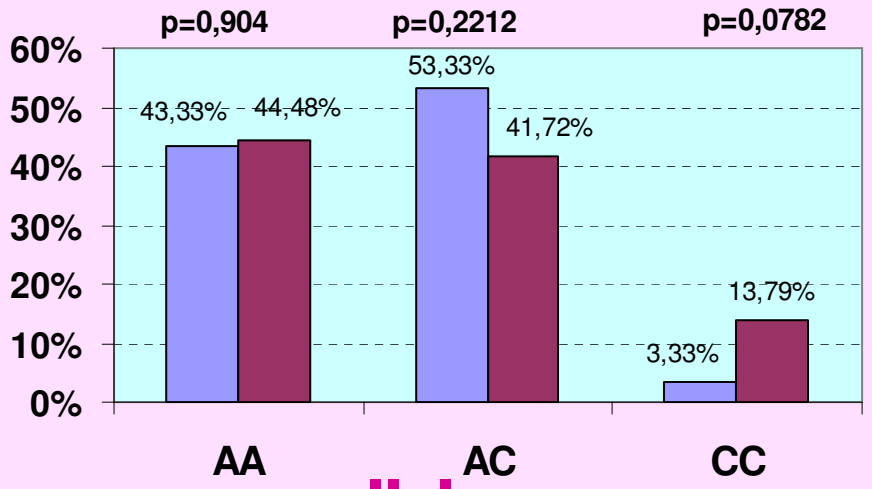
- No significant differences
- No mutant homozgygotes / homozygotes + heterozygotes

NTD patients mothers vs. controls

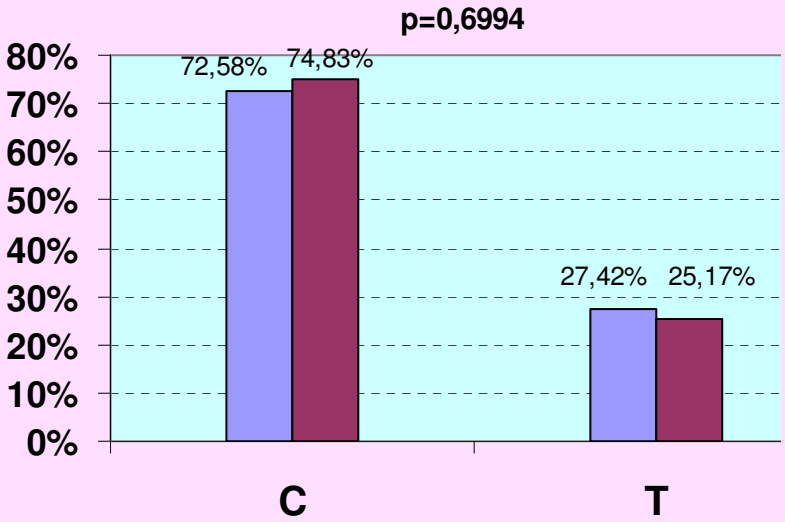
C677T genotypes



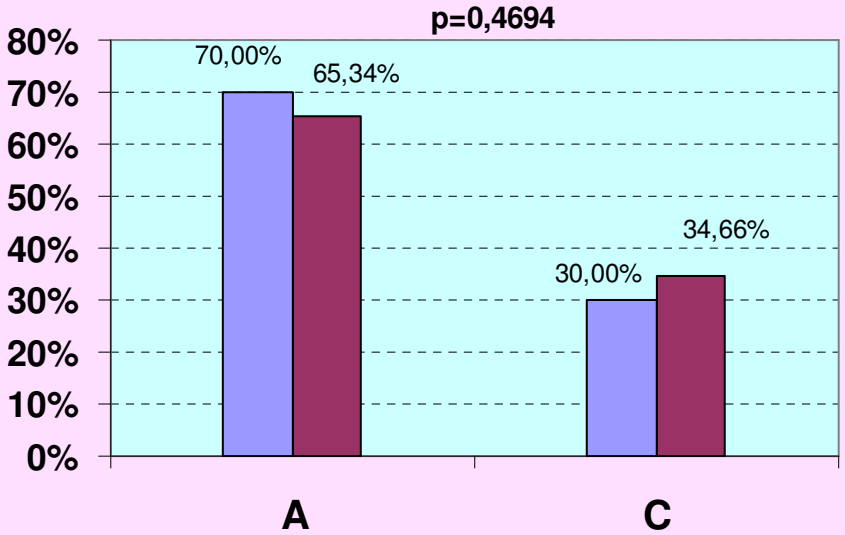
A1298C genotypes



C677T alleles

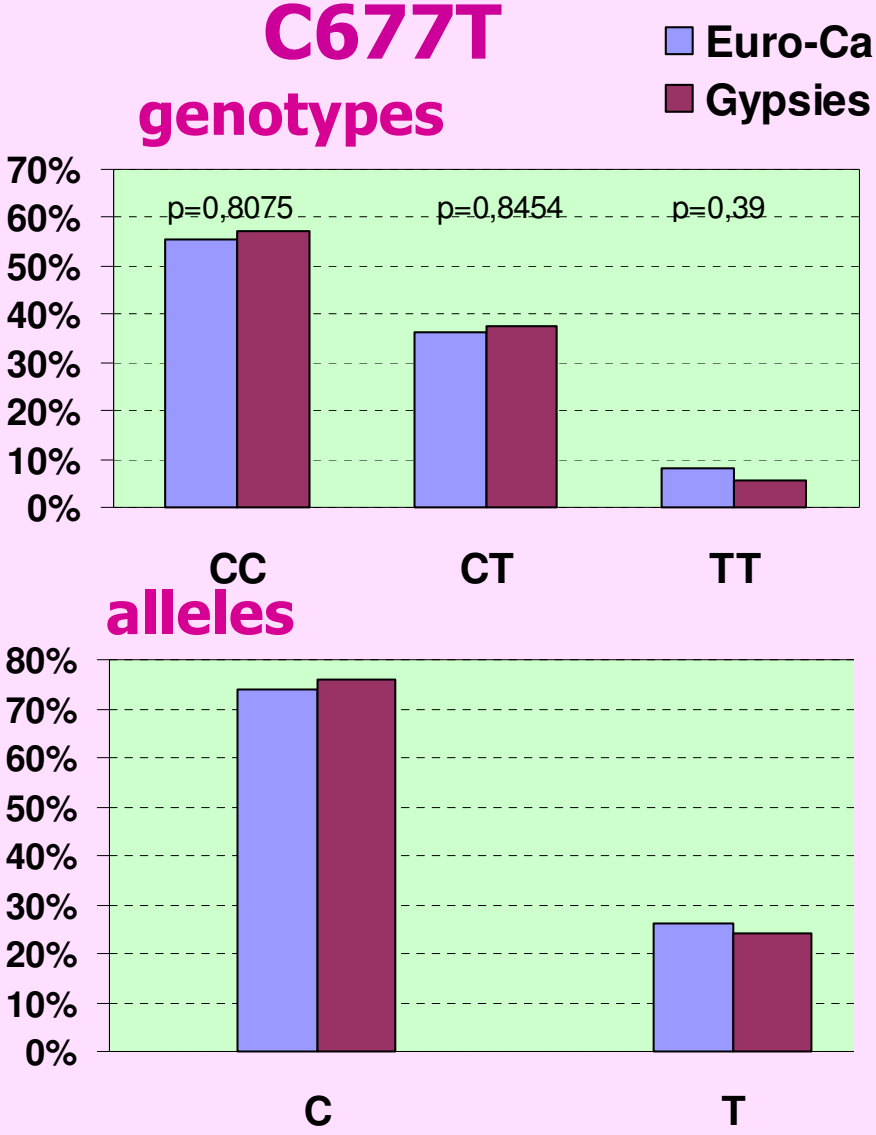


A1298C alleles

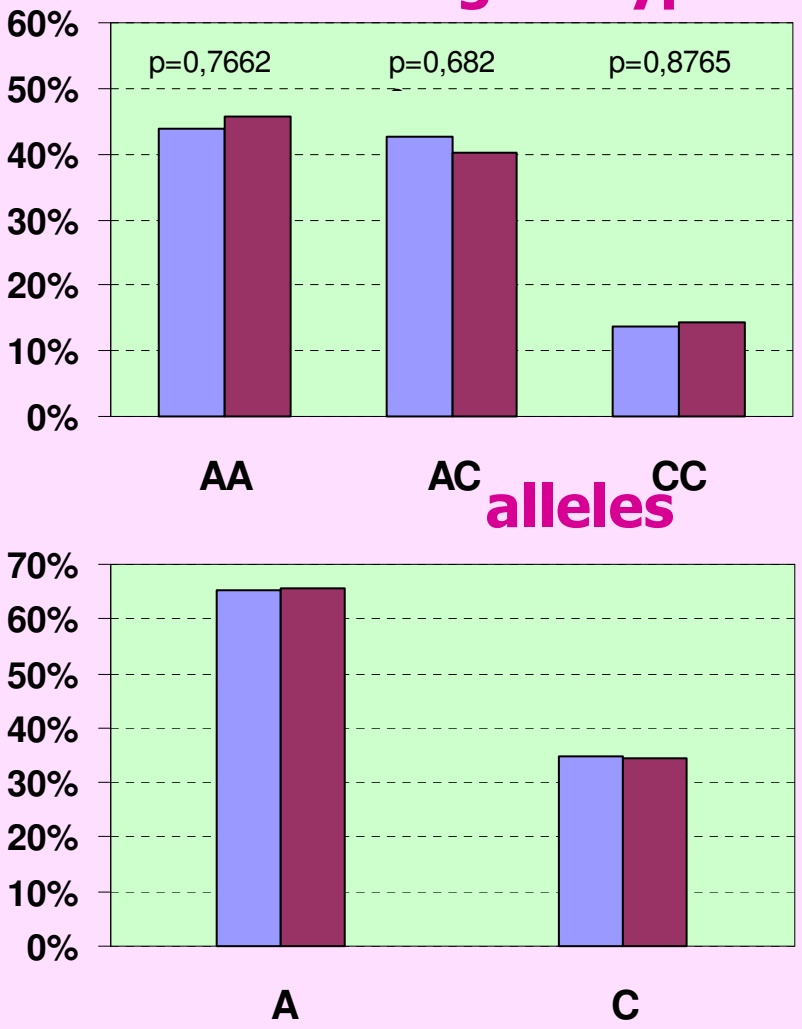


MTHFR polymorphisms: euro-caucasians vs. gypsies (controls)

C677T



A1298C



S U M M A R Y

- **Control Slovak population:**
  C677T MTHFR gene polymorphism
  T allele and TT genotype: 25% resp 7,3%
  A1298C polymorphism
  C allele and CC genotype: 35% resp. 14%

- **Slovak NTD patients vs. controls:**
  no significant differences - C677T & A1298C

- **NTD patients ´ mothers vs. control - no difference**

- **Caucasian vs. Gypsy ethnic groups - no difference**

C O N C L U S I O N

- **Premise:multiple mechanisms of folate preventing NTD**

- **External factors influence**
  (e.g.:TT genotype C677T MTHFR => 16 % ↑ risk of HS
  smoking => 200 % ↑ risk of HS)
  (HS - heart stroke)

- **Essential: large groups of patients + meta-analyses**

- **Limited value of solitary gene polymorphisms**

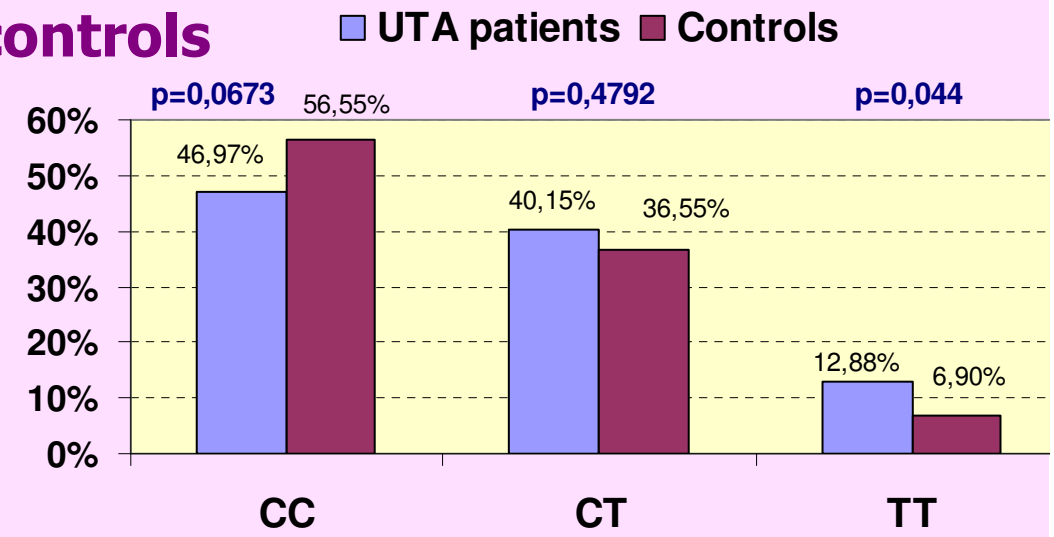
- **Future: panels of numerous gene polymorphisms**
  => polygene chips, gene and protein families

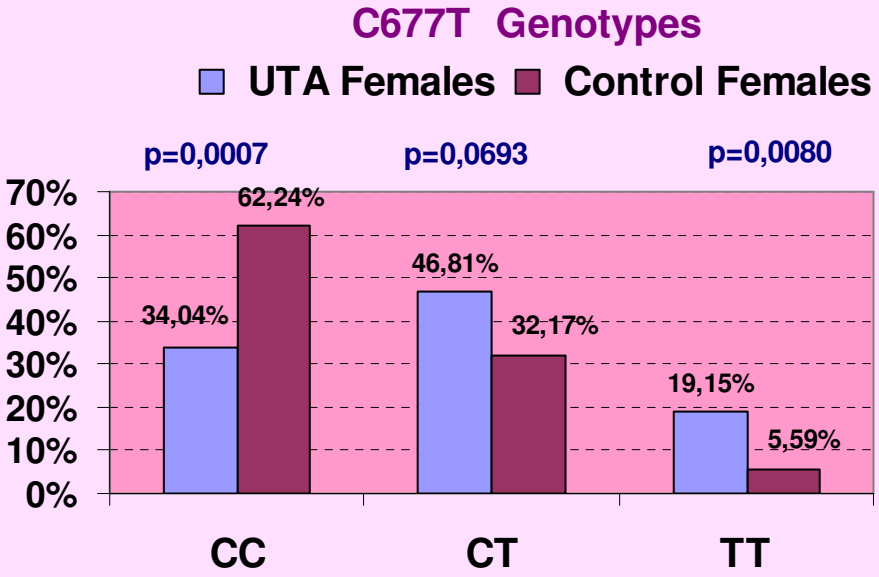
Annex: MTHFR gene polymorphisms in children with urinary tract anomalies (UTA)

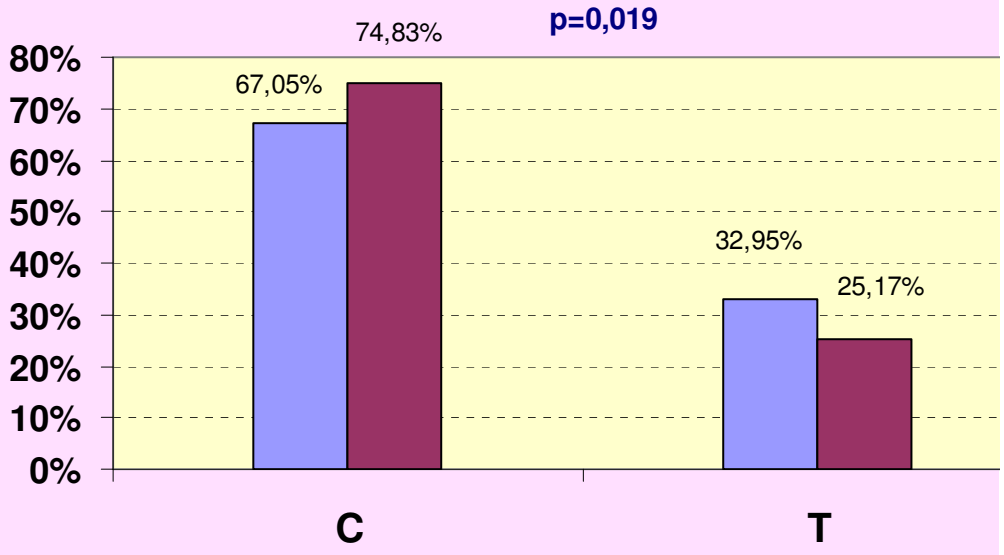
- **UTA Patients (N=132)**

  significant differences vs. controls

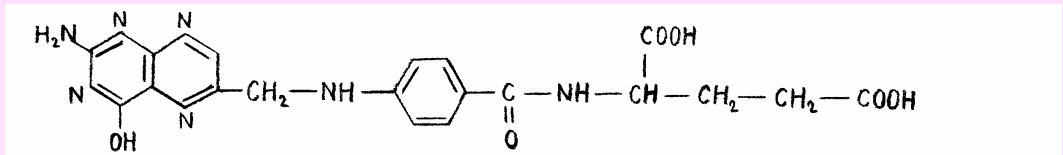
  C677T ↑ T & ↑ TT
- **p=0,019 resp. 0,044**
- **More pronounced genotype differences: sex**







Presence: - need of folate campaign in Slovakia
- fortification?



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