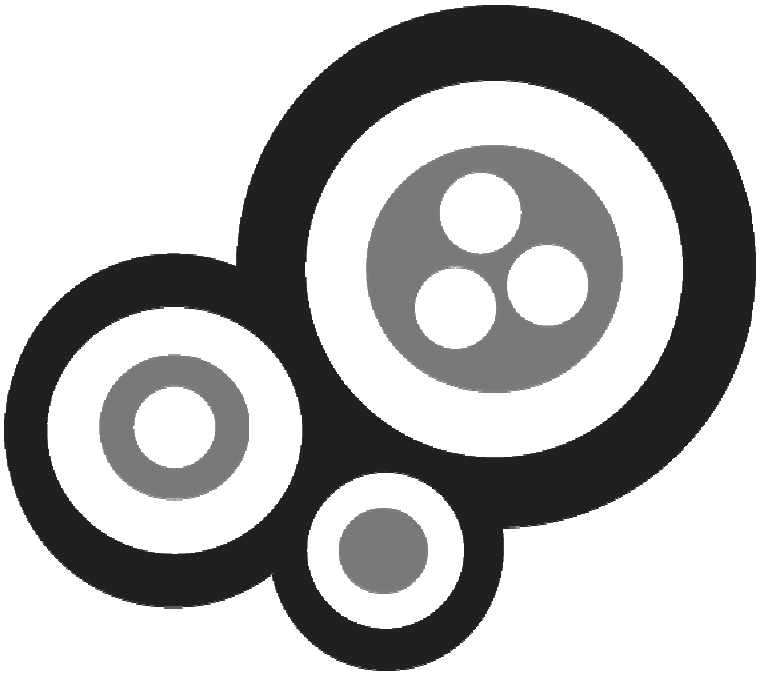

BREAKOUT 1A

FOLIC ACID AND RISK OF CONGENITAL MALFORMATIONS

Moderators:
Pierpaolo Mastroiacovo – Italy
Alina Midro – Poland

FOLATE AND CLEFTS OF THE LIP AND PALATE – A UK BASED CASE-CONTROL STUDY: BIOCHEMICAL AND GENETIC ANALYSIS

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Objectives: We sought to determine the associations between non-syndromic cleft lip with or without cleft palate (CL(P)) and cleft palate only (CPO), biochemical measures of folate status and the *MTHFR* C677T variant in an area where the prevalence at birth of neural tube defects has been high and flour is not fortified with folic acid.

Methods: Interviews regarding periconceptional dietary intake and supplement use were completed with the mothers of 112 CL(P) cases, 78 CPO cases and 248 unaffected infants. DNA extracted from buccal cell samples from the infants and their parents was genotyped for the *MTHFR* C677T variant. Red blood cell (RBC) and serum folate and serum homocysteine levels were determined in 12-month post-partum blood samples from a subset. The data were analysed by logistic and log-linear regression methods.

Results: There was an inverse association between CL(P) and maternal *MTHFR* CT (OR 0.5, 95% CI 0.31-0.95) and TT (OR 0.6, 95% CI 0.21-1.50) genotypes; similar risk estimates were observed for CPO. This effect appeared to be confined to the offspring of mothers who reported higher total folate intake. There was no clear association with infant *MTHFR* genotype. Higher levels of maternal post-partum RBC and serum folate were associated with a lower risk for CL(P), whereas they were associated with an increased risk for CPO. There was a suggestion that higher levels of serum homocysteine were associated with increased risk for both CL(P) and CPO.

Conclusion: While the inverse relation between the mother having the *MTHFR* C677T variant and both CL(P) and CPO suggests perturbation of maternal folate metabolism is of aetiological importance, contrasting relations between maternal post-partum levels of RBC and serum folate and (i) CL(P), and (ii) CPO are difficult to explain and may reflect the play of chance. To understand better whether this is a chance finding or indicates a potential difference in aetiology, it would be valuable to conduct a pooled analysis of clefts and markers of intake and metabolism of folate and related nutrients.

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In addition to the authors, the ITS MAGIC collaboration includes:
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NEURAL TUBE DEFECTS AND MTHFR GENE POLYMORPHISMS – THE INCIDENCE IN SLOVAK POPULATION

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Background: Neural tube defects (NTD) belong to the most debilitating birth anomalies. The geographical & historical differences in NTD incidence varies tremendously - from 8/1000 till 0.21/1000. Discovery of folate preventive effect influenced the research of NTD genetic background focusing to the genes whose products take part in the folic acid metabolism. One of the most important enzymes involved in folate metabolism is methylenetetrahydrofolate reductase (MTHFR) whose´ common termolabile polymorphism C677T in homozygote state reduces MTHFR activity to 30-40% of norm. The second most investigated polymorphism of MTHFR gene, A1298C, reduces MTHFR activity in homozygote state to 60% . The lowered activity might play a crucial role in embryonic development. That is why this polymorphism has been investigated in various NTD populations and in some of them it has been proven as a risk factor for this birth defect. In Slovakia with average natality 50 000 liveborn/year, there are about 10-20 babies annually born with NTD (0.28/1000), mostly meningomyelocele. When including stillborn and selective abortions, the number of NTD pregnancies is some higher (0.35-0.52/1000). To evaluate genetic risk of folate metabolism variations in our population, we investigated MTHFR gene polymorphisms C677T and A1298C in a group of Slovak children with NTD and control population.

Materials, methods: Patients: 91 Slovak children with neural tube defects; material: venous blood in EDTA; population controls: 300 unselected Slovak newborn; material: dry blood spots

Methods: Genotyping of C677T and A1298C in patients - classical PCR, followed by restriction with enzymes Hinf I and MboII, respectively, and visualized after electrophoresis on agarose gel. Genotyping of controls - alternative method due to much lower DNA concentration - real time PCR, with commercial kit (Roche, Tib Mol-Biol).

Results: Distribution of C677T and A1298C polymorphisms genotypes in control population did not differ significantly from expected rates of Hardy-Weinberg equilibrium. T allele frequency was 25.33%, TT homozygotes 7.33%. In A1298C polymorphism, C allele frequency was 35.0%, CC homozygotes 14.0%. Distribution of MTHFR polymorphisms genotypes in our NTD population: C677T - T allele 28.33%, TT homozygotes 8.89%; A1298C - C allele 33.89%, CC homozygotes 6.67%. Comparison of NTD patients and controls genotypes did not not show any significant difference in the prevalence of TT genotype or T allele between the NTD patients and controls (OR=1.22 [95%CI 0.5-2.9]; OR=1.16 [CI 0.8-1.7] respectively). In A1298C polymorphism, there was no excess of C allele in NTD population compared with controls, but CC genotypes were more frequent in controls than in NTD patients (p=0.06, OR= 2.5 [95%CI 1.03-6.05]) and AC genotypes frequency was significantly higher in patients than controls (54.4% vs. 42%, p=0.037, OR-0.60 [95%CI 0.38-0.97]) Combination of both heterozygote genotypes did not differ significantly between patient and control populations – 19.1% vs. 16.7%, p=0.59.

Conclusion: This study did not confirm substantial differences in MTHFR polymorphisms C677T and A1298C between NTD patients and control population in Slovakia. Regardless of genetic background, periconceptional folate supplementation in women is absolutely recommended.

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ELEVATED NEURAL TUBE DEFECTS (NTD) RATES IN UKRAINE – HIGHEST IN POLISSIA

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Background: Population birth defects (BD) surveillance by the OMNI-Net started in 2000 and by 2002 data from Rivne and Volyn oblasts (provinces) of North West Ukraine showed that rates of NTD were sharply elevated (21 per 10000 live births, N. Yuskiv et al., Birth Defects Res. A Clin. Mol. Teratol, 2005). Rivne data suggested that prevalence rates were highest in the Rivne-Polissia, a region impacted by chronic ionizing radiation from the Chornobyl disaster. Polissia has boggy soils with low humus and high peat contents resulting in high soil-to-milk transfer coefficients of Cs-137. The Polissia population is known for self-sufficiency, reliance on locally grown food, consumption of game, wild mushrooms and berries. The likelihood is high that nutrition in Polissia may be deficient in micronutrients and folates, particularly during winters; that nutrients and water are contaminated by Cs-137, including milk and potatoes which are the main dietary staples. Another Cs-137 significant source is reported to be aspiration of contaminated dust and smoke from burning biomass. (See companion abstract by B. Yevtushok and by L. Yevtushok).

Method: Analysis of NTD 2000-2006 data from Rivne Polissia and non-Polissia regions and official background demographic, nutrition and radiodosimetry data..

Results: NTD prevalence rate in Rivne Polissia and non Polissia is 27.0 and 19.6 (21.6 and 16.6 for isolated NTD, contrasts that do not reach statistical significance). However more detailed analysis by raions (counties) is warranted and is ongoing. Every Rivne - Polissia raion is officially designated as impacted by ionizing radiation; the rest are designated as “clean”; there are nearly 200 inhabited points; the largest yearly birthrate is in the city of Rivne (nearly 3000); official Cs-137 monitoring is conducted solely in the Polissia region; available reports show sharp variations by place and time; and in some villages Cs-137 levels in milk have increased with time. Another observation concerns seven instances of conjoined twins of which six were born in the Rivne oblast (one twin had spina bifida) and the other in Khmelnytsky oblast.

Conclusions: Broadening retrospective and prospective investigations of the Rivne Polissia population are warranted.