

## 9th EUROCAT European Symposium Naples, Italy, 7 May 2007

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### **Impact of Folic Acid Food Fortification on the Prevalence of Neural Tube Defects in Canada**

*P de Wals, F Tairou, MI Van Allen, S-H Uh, RB Lowry, B Sibbald, JA Evans, MC Van den Hof, P Zimmer, M Crowley, B Fernandez*

#### Background

In 1988, fortification of a large variety of cereal products with folic acid became mandatory in Canada, a country where there was an East to West decreasing gradient in neural tube defect (NTD) prevalence.

#### Objective

To assess the impact of food fortification on NTD prevalence, according to the provincial baseline rate.

#### Material and Methods

The study population included livebirths, stillbirths and terminations of pregnancies because of fetal anomaly to women resident in 7 Canadian provinces, from 1993 to 2002. In each province, the ascertainment of NTD cases relied on multiple sources, and in addition all medical charts were reviewed in order to verify diagnoses. Based on the results of red blood cell folate tests, the study period was divided into pre-fortification, partial fortification and full fortification periods. A theoretical birth date was calculated for each NTD case assuming a gestation of 40 weeks. The relationship between NTD baseline rate in each province and the magnitude of the decrease after fortification was modelled, testing a series of mathematical functions.

#### Results

A total of 2,446 NTD cases were recorded in 1.9 million births. NTD prevalence decreased from 1.58/1,000 before fortification to 0.86/1,000 during the full fortification period, a 46% reduction. The magnitude of the decrease was higher for spina bifida (53%) than for anencephaly (38%) and encephalocele (31%). There was a quasilinear relationship between the baseline NTD rate in each province and the absolute reduction in rate subsequent to fortification ( $\text{rate difference}/1,000 = -0.466 + 0.777 \text{ pre-fortification rate}/1,000$ ). The effect was higher for upper than for lower spina bifida. Interestingly, no significant decrease in rate was observed for lipomyelomeningocele.

#### Conclusion

Food fortification has clearly been successful in Canada and benefits were greatest where the baseline NTD rate was high. In the meantime, the public health policy in Europe to promote vitamin supplement use has been largely unsuccessful.

## **The Ciliopathies: The Example of the Oral-Facial-Digital Type 1 Syndrome**

*B Franco*

Primary cilia have been recognised on nearly all mammalian cells and emerging data suggest they have diverse motility and sensory functions acting as cellular antennae sensing a wide variety of signals. As such, defects in cilia formation or function have profound effects on the development of body pattern and the physiology of multiple organ systems. Cilia dysfunction has been implicated in a wide spectrum of genetic disorders such as Bardet-Biedl, Joubert, Meckel, Alstrom, oral-facial-digital type 1 syndromes, nephronophthisis and polycystic kidney diseases.

The molecular data linking clinical entities, which are apparently unrelated, are beginning to highlight a common theme, where defects in ciliary structure and function may lead to a predictable phenotypic pattern.

In particular, our laboratory has been focusing on the study of the OFD1 syndrome, which is transmitted as an X-linked dominant condition with lethality in males and displays craniofacial, limbs and brain abnormalities and polycystic kidneys with a highly variable expressivity even within the same family. We previously identified the responsible gene, named OFD1, and demonstrated the OFD1 is a centrosomal/basal body protein. We recently generated OFD1-knockout anomalies, which reproduced the main features of the human disease, albeit with increased severity, possibly due to differences of X-inactivation patterns between human and mouse. Characterisation of this animal model allowed us to demonstrate that OFD1 is required for primary cilia formation, for specification of the left-right asymmetry and it is also implicated in the hedgehog (Hh) signalling pathway. Our data definitively places OFD1 in the increasing number of genetic disorders associated to primary ciliary dysfunction.

We propose to use the Oral-facial-digital type 1 syndrome as a model to study primary cilia function and dysfunction in both physiology and pathological conditions and to this aim we are exploiting the power of conditional inactivation to specially inactivate the OFD1 transcript in the genito-urinary tract and in the limbs where cilia have been hypothesised to play an important role. Preliminary data in this direction are very encouraging and we believe that the biological relevance and significance of the results we will obtain will have implications far beyond the rare Oral-facial-digital type 1 syndrome patients and may shed light on the mechanisms underlying other rare birth defects due to primary ciliary dysfunction.

## **Anomalies of Sex Differentiation**

*A Bignon-Lauber*

Differentiation of a testis or an ovary from the bipotential gonad is a complex developmental process involving various genes and hormones. Additional elements of the reproductive tract develop from an indeterminate stage via the differentiation of Wolffian (male reproductive tract anlage) and Mullerian (female reproductive tract anlage) ducts. Whereas factors involved in male sex differentiation are well studied, the pathways that regulate female sexual differentiation remain incompletely defined. To date, no genes have been demonstrated to play an equivalent role to that of SRY or SOX9 genes in testes development. I will mention the role of factors such as SF1, BMP15 and FOXL2 on ovarian determination and differentiation, but mainly focus on WNT4. WNT4, a secreted protein that acts as a repressor of the male differentiation, is a member of the WNT family of secreted molecules that function in a paracrine manner to effect several developmental changes. WNTs bind to members of the Frizzled family of cell surface receptors and activate a cascade of intracellular signals leading to the transcriptional activation of target genes. Vainio et al

studied a mouse model in which WNT4 is ablated development by Stark et al and observed that, whereas both male and female WNT4-knockout mice exhibit similar defects in kidney development and in adrenal function, gonadal development and steroidogenic function are affected exclusively in the WNT4<sup>-/-</sup> females. WNT4-knockout females are masculinised, as demonstrated by the absence of Mullerian ducts and the presence of Wolffian ducts, and express the steroidogenic enzymes 3 $\beta$ -hydroxysteroid dehydrogenase (HSD17B2) and 17 $\alpha$ -hydroxylase (CYP17) required for production of testosterone, which are normally repressed in the female ovary [2]. WNT4 upregulates Dax1, a gene known to antagonise Sf-1, and thereby inhibits steroidogenic enzymes. In the XX gonads WNT4 represses mesonephric endothelial and steroidogenic cell migration, preventing the formation of a male-specific coelomic blood vessels and the production of steroids. Collectively, these data suggest that WNT4 normally functions to repress gonadal androgen biosynthesis in females.

The identification of three patients with absence of uterus (and not other Mullerian abnormalities) and ovarian androgen excess are caused by WNT4 loss-of-function mutations, identifies WNT4 as a major player in the development and maintenance of the female phenotype in women, by regulating Mullerian duct formation and controlling steroidogenesis in the ovary.

### **Rare Diseases: Where do we Stand in Europe?**

*S Ayme*

Most rare diseases are life-threatening and chronically debilitating and the vast majority of them are genetically determined. Their low prevalence requires special combined efforts to address them so as to improve diagnosis, care and maybe prevention.

A disease is considered as rare if it affects less than 5 per 10,000 persons in the European Union which translates into approximately 246,000 persons in the EU with 27 countries. If it is impossible to develop a public health policy specific to each rare diseases, it is possible to have a global rather than a piecemeal approach in the areas of scientific and biomedical research, drug research and development, industry policy, information and training, social benefits, hospitalisation and outpatient care. However there is no specific public health policy for rare diseases in most European countries, except in Denmark, France, Italy and Sweden, or research policy for rare diseases in most countries except France, Germany, Italy and Spain. In a recent past, several initiatives at EU and Member State level have been taken and proved efficient to develop suitable solutions which are now impacting very positively on the quality of life of patients. These initiatives will be presented. They include the establishment of Orphanet, a database of rare diseases and orphan drugs providing an encyclopaedia of rare diseases and a directory of associated expert services, which is serving daily 20,000 users from 150 countries. They also include the funding of research networks to boost the collaboration between research teams and the funding of six networks of clinical centres of reference to better serve the patients and contribute to developing clinical research. The European orphan drug regulation has been in place for 5 years now; it proved to act as an incentive for the development of new drugs for rare diseases, showing that a policy can act for the benefit of the disadvantaged citizens. 20% of the new drugs which got recently a market approval were orphan drugs. Rare diseases are a field where action has to take place at the European level as no member state has the capacity to handle optimally all the cases in their diversity. Actions to be promoted in the coming years in the following areas: promotion of awareness about rare diseases; availability and accessibility of diagnostic tests in quality-controlled labs; establishment of repositories of cases and of electronic diagnostic and management networks; improvement of coding and classification systems to handle rare diseases appropriately, funding of non-industry lead clinical trials among others.

## **Prenatal Screening for Birth Defects in Europe - A EUROCAT Study of the Impact of Different National Policies**

*P Boyd, C de Vigan, B Khoshnood, M Loane, E Garne, H Dolk*

### Background

Over the last 20 years there have been rapid advances in the field of prenatal screening and diagnosis for Down's Syndrome and in ultrasound scanning for the detection of fetal anomaly. The aim of such screening is to give parents choices. The uptake and impact of different programmes will depend on social and cultural factors as well as on the availability of different resources and laws regarding termination of pregnancy for fetal anomaly.

### Aim

This study aims to "map" the current state of prenatal diagnosis in those countries in European which are members of EUROCAT and assess its impact.

### Methods

A questionnaire covering policies/official recommendations in place in 2004 for prenatal screening for Down's Syndrome, ultrasound scans and termination of pregnancy laws was sent to a EUROCAT register leader in 18 European countries. Data on rates for prenatal detection and termination of pregnancy for fetal anomaly for Down's Syndrome and Neural Tube Defects (NTDs) were extracted from the EUROCAT database.

### Results

Screening for Down's Syndrome-National Policy:

16/18 countries had some form of Down's Syndrome screening in place with 10/18 having an official country wide policy. Over all (in the EUROCAT registry areas from 12 countries providing data) 68% of Down's Syndrome cases were detected prenatally and 60% of all affected pregnancies results in termination of pregnancy (TOP). Countries with a first trimester screening policy were 13% more likely to detect a Down's Syndrome case prenatally than those countries with a mixed first and second trimester screening policy. Countries with no country-wide policy but some form of Down's screening in place were 11% less likely to detect a Down's Syndrome case.

Screening for Structural Anomalies by Ultrasound Scanning:

14/18 countries had a national policy/recommendation regarding fetal ultrasound scanning in place in 2004. These policies recommended an anomaly scan (mostly at 18-22 weeks). Over all 88% of cases of NTD were prenatally detected and 77% of all NTD affected pregnancies results in TOP. registry areas from countries with no national scan policy detected 17% less NTDs compared to those with a national ultrasound policy.

Termination of Pregnancy for Fetal Anomaly (TOPFA):

There is very wide variation in the laws regarding TOPFA. In two countries termination of pregnancy is illegal. In the remaining 16, the gestation limit for TOPFA varies from 22 weeks gestation to no limit. Having a legal gestational age limit did not significantly alter the number of pregnancies resulting in TOP for Down's Syndrome or for a Neural Tube Defect.

### Conclusion

There is wide variation in the screening policies in place in 2004 in different countries in Europe but most offer some form of screening for Down's Syndrome and for structural anomalies. Having a national Down's Syndrome policy has a measurable effect but factors other than policy are important.

## **Antenatal Screening: The Experience of Women in Northern Ireland**

*F Alderdice , J McNeill , R Rowe , D Martin, J Dornan*

### Background

Many antenatal screening tests have been routinised through a universal offer policy in the NHS in England and Wales. However the offer and uptake of tests, especially screening tests for Down's syndrome and neural tube defects (NTD), remains fragmented, inconsistent and influenced by many factors. Previous work suggests that social and ethnic inequalities exist in the offer and uptake of tests in the UK but no data were available to indicate if this was the case in Northern Ireland.

### Objectives

To investigate social inequalities and variation regarding the offer and uptake of antenatal screening tests and to explore the decision making process reported by the women being offered Down's screening.

### Design

A prospective survey of women attending two hospitals in Northern Ireland using semi structured interviews on two occasions.

### Main Outcome Measures

The pattern of offer and uptake of antenatal screening tests including screening for Down's syndrome across social groups including education, religious denomination, deprivation scores, type of antenatal care, parity and car ownership. Content analysis of qualitative data regarding women's decision to accept or decline screening tests offered.

### Results

Variations in offer and uptake of Down's syndrome and NTD screening were observed across social class and educational groupings. No variations were observed in other maternal screening tests. Women's reasons for declining screening included unconditional acceptance of the baby and not wanting to know. Reasons given by women who accepted screening included their family history or age. Responses from both groups of women indicated that health professionals were influential in women's decision-making.

### Conclusions

Variation in offer and uptake exist within a universal offer policy of screening for Down's syndrome. Qualitative data from women suggest a gap in their understanding, which may have arisen from lack of good discussion between professionals and women at the time of offering screening.

## **Prenatal Diagnosis and Outcomes for Babies Born Alive with Spina Bifida from One Tertiary Centre, John Radcliffe Hospital, Oxford 1991-2003**

*MY Anthony, JK Calvert, PA Boyd, PC Chamberlain, M Redshaw , JJ Kurinczuk*

### Objectives

To document the outcome of pregnancies with an affected fetus. To assess the accuracy of prenatal ultrasound. To document the outcome of babies born alive with isolated spina bifida.

### Design

All cases of spina bifida 1991-2003 cared for at one centre were obtained from the congenital anomalies register (CAROBB), the Neonatal Unit register and histopathology reports. Pre & postnatal details were obtained from the case notes.

### Participants

All affected pregnancies 1991-2003, and all surviving live born infants with isolated spina bifida.

### Main Outcome Measures

1. Outcome of the pregnancies.
2. Accuracy of prenatal ultrasound.
3. Postnatal developmental, mobility and continence.
4. Influence of level of lesion on outcome
5. Influence of ventriculomegaly on outcome.

### Results

139 pregnancies, 105 terminated, 2 still births, 8 post-natal deaths and 24 live births. 131 (96%) were diagnosed antenatally of which 118 (90%) were suspected to be isolated. Antenatal diagnosis of ventriculomegaly had a sensitivity of 91% and specificity of 67%. The distribution of the antenatal level was similar to postnatal findings. Of 24 live births mobility data were available for 21. Seven were in wheelchairs, 3 could stand, 7 walk and 4 run. Developmental data were available for 20. One had severe problems, 5 moderate problems and 14 were normal. Of 20, 16 were incontinent of urine and 13 incontinent of faeces. The level affected mobility but did not predict the need for later complex spinal surgery. The presence of ventriculomegaly was an important prognostic feature. The median age at last assessment was 8 years. There were no post neonatal deaths from any cause.

### Conclusions

The majority of pregnancies were terminated. The antenatal scans had a moderate to high accuracy. Incontinence was numerically the greatest current problem for survivors. Ventriculomegaly is an important marker. Shunt and renal deaths were less than previously reported.

### **Evaluation of Prenatal Diagnosis of Congenital Malformations in Poland (2000-2004)**

*A Latos-Bielenska, A Materna-Kirylyuk, J Mejnartowics, K Wisniewska, M Badura, A Balcar-Boron, B Borszewska-Kornacka, G Breborowicz, M Czerwionka-Szaflarska, A Dobrzanska, J Gadinowski, E Gajewska, U Godula-Stuglik, E Helwich, M Krawczynski, J Limon, H Saawulicka-Ole-Warowicka, J Szczapa, J Swietlinski, M Walczak*

### Objective

Evaluation of prenatal diagnosis of congenital malformations in Poland. It is the first report on state of prenatal diagnosis of congenital malformations in Poland.

### Methods

Source of information was Polish Registry of Congenital Malformations (PRCM), data for Wielkopolska Registry (EUROCAT Registry No 67) and Poland Registry (EUROCAT Registry No 76), 2000-2004. Data on the malformation, gestational age at diagnosis, place of residence of the mother, age and education of the mother were analysed. Information on organization and methodology of PRCM is available at [www.rejestrwad.pl](http://www.rejestrwad.pl). (also J.Appl.Genet.46, 341-348, 2005).

### Results

During the study period (2000-2004), 1,210,974 children were born, of these 22,900 children with congenital malformations were identified (prevalence 189.10/10,000 births). The overall prenatal detection rate was 8.9 % in 2000, 9.6 % in 2001, 10.8 % in 2002, 12.0 % in 2003 and 15.4 % in 2004.

Number prenatally diagnosed cases/total number of cases (% prenatal detection rate) in 2004: Q86-87 Multiple malformations 143/476 (30.0%), Q90-99 Chromosome aberrations 452/428 (12.1%), Q90 Down syndrome 27/358 (7.5%). Isolated malformations: Q00-07 Nervous system anomalies 172/320 (53.8%), Q05 Spina bifida 59/131 (45.0%), Q03 Hydrocephaly 50/70 (71.4%), Q20-28 Heart defects 67/1541 (4.3%), Q35-37 Cleft lip/cleft palate 30/353 (8.5%), Q38-45 Digestive system anomalies 16/141 (11.3%).

Detection rate depended on the place of residence of the mother (town >100,000 13.2%; country area 10.5%) and on the mother's education (higher education 13.1-14.4%, elementary school 10.2-11.5%).

### Conclusion

Prenatal detection rate of congenital malformation is in Poland still unsatisfactory. Prenatal care should be improved - special attention must be paid to poorly educated women, especially those living at the country.

### **Birth Defects Prenatal Diagnostics Status in Ukraine**

*OV Gorina, GG Zinchenko, TM Pokanevych, NV Brezitska, OV Lynchak, OI Tymchenko*

### Objective

To estimate birth defects (BD) prenatal diagnostics status in Ukraine.

### Design

Data of ultrasound examination of pregnancy women, qualification of doctors, availability and quality of ultrasound equipment were analysed in Kyiv, Oblast and Ukraine (2000-2004).

### Results

Observation by obstetrician and double ultrasound diagnostics is obligatory for pregnancy women. If BD is suspected pregnancy women undergoes medical-genetic consulting. Ultrasound screening (2000-2004) underwent 89% of women (2004-92.4%), so more than 30,000 women are not examined this way per year.

Decision about fetus elimination belongs to family (in 2000-2004 up to 28 gestation week, since 2005 up to 22). When diagnosis of BD was determined by specialist of medical-genetic care service before 28 gestation week, it was confirmed in 97.9% of eliminated fetuses (more than 2,000 cases per year). In BD structure there was majority of central nervous system BD (30.6%), kidneys and urinary tracts BD (22.9%), multiple BD (15.9%), cardio-vascular system BD (7.9%).

Detection of biochemical BD markers was limited. Some biochemical BD markers were detected only among 16% of pregnancy women. Only 1,500 invasive investigations were performed.

In Kyiv region there were eliminated all fetuses with suspicion of front abdominal BD, 96.24% with central nervous system BD, 95.39% with multiple BD. There were eliminated 62.5% of fetuses with cardio-vascular system BD and 66.67% with reduction BD, 38.85% with kidneys and urinary tracts BD, 42.86% with face BD and 44.44% with hereditary skeleton diseases were eliminated; diagnosis of BD was determined by geneticist. There was 98.29% dissection confirmed diagnosis of BD among pregnancies which were cut for genetic causes.

Analysis of material and personnel basis of ultrasound service showed correlation between BD frequency and qualification of doctors ( $r$  from -0.76 till -0.52 for different BD).



## Conclusion

Given data show that there are reserves for improvements of BD diagnostics.

## **The Biological Bases of Congenital Heart Diseases**

*B Dallapiccola*

Congenital Heart Disease (CHD) affects about 1 in 110 newborns. Family studies have proved that most of these defects have a genetic component, which either consists in a mendelian mutation or is related to a chromosome imbalance or, more commonly, is consistent with a multifactorial model. Linkage studies, animal models, analysis of candidate genes and genes located in critical regions have shed light onto the genetic mechanisms underlying some CHDs.

Atrial Septal Defect (ASD) accounts for about 10% of all CHDs. Although most of them are sporadic, some familial cases show an autosomal dominant inheritance, either in the presence of an Atrio-Ventricular Block (AVB), or with normal conduction. NKX2.5, a transcription factor (TF) located in chromosome 5q35, is mutated within the gene homeodomain in 4% of sporadic and 12% of familial ASDs with AVB. Different gene mutations outside the homeodomain can result in Tetralogy of Fallot (ToF, 4%) with AVB, or tricuspid and mitral valve anomalies, or hypoplastic left heart. Mutations in another TF, GATA4 have been found in a subset (12%) of familial ASD with pulmonary stenosis (PS), ASD, Ventricular Septal Defect (VSD), Atrioventricular Canal Defect (AVCD), ToF and AV conduction defects occur in Holt-Oram syndrome. In about one third of these patients the TF TBX5 is mutated. Interestingly, interaction between NKX2.5, GATA4 and TBX5 genes has been proved. In a few families with autosomal dominant ASD, linkage analysis have proved an association with chromosome 14q12 and pathogenic mutations have been found in MYH6 gene, coding for the myosin heavy chain 6.

A similar heterogeneity has been proved for AVSD, which accounts for about 7% of all CHDs. The complete form of AVSD is often associated with chromosome imbalances. Notable examples are trisomy 21 (Down syndrome - DS), del 8p23 and del3p25 syndromes. AVSD occurs in 20% of the children with DS; the complete form is prevailing (75%); additional CHDs are rare, except ToF. Within the distal 12q region, spanning for about 4 Mb a number of candidate genes have been tested, including COL6A1, COL6A2, because they are overexpressed in DS fetuses; DS cultured skin fibroblasts show some differences in adhesion to collagen 6, linkage disequilibrium was found between specific COL6A1 alleles and CHD in DS. However at present no simple mechanism seems capable of explaining this association. Complete AVSD is also a major feature of del8p syndrome. The GATA4 gene, which maps close to the deleted region has been considered a good candidate, being mutated in a mouse model of AVSD. However, analysis of several sporadic and familial cases have not corroborated this results. AVSD occurs in about one third of the patients with del3p25 syndrome. CRELD1 gene, encoding for a matricellular protein involved in cell adhesion processes has been considered a likely candidate and is mutated in 6% of patients with partial AVSD. An additional locus for AVSD has been mapped to the 1p21-p31 region, based on an extended USA family. The identification of the Ellis-Van Creveld genes (EVC1, EVC2), a rare osteochondrodysplasia with cardiac involvement, including partial AVSD, common atrium, persistent left upper vena cava, has added further evidence to the biological complexity of the cardiac septation process.

Heterotaxy is a class of birth defects due to the incomplete or failed left-right embryonic patterning. Unpaired organs of chest and abdomen begin their development in the midline and move to their final asymmetric positions following an ordered cascade of patterning genetic

signals. These defects accounts for about 3% of all CHD. Based on animal models, a number of candidate genes have been investigated and mutations have been found in ZIC3, NODAL, CFC1, LEFTYA, ACVR2B genes, which account only for a minority of these cases. Two of these genes (CFC1, ZIC2) have been found to be mutated, together with PROSIT240 and NKX2.5 genes, in a few cases of transposition of the great arteries (TGA).

Conotruncal Heart Defects (CTHD) represent a major group of septal CHD. Most are sporadic and the multifactorial inheritance has been considered a likely mechanism. DiGeorge/Velocardiofacial syndrome (DGS/VCFS) associated with del22q11.2 represents the most important human model for CTHDs. Manipulation of the homologous mouse region (chromosome 16) has indicated that the TBX1 gene, a TF, is required for development of the pharyngeal apparatus and its deletion results in CTHD. Based on a few patients with the DGS/VCFS phenotype presenting with a point mutation in the TBX1 gene as the sole genetic anomaly, it was concluded that this gene accounts for most of the features occurring in this disorder. However, this gene is not responsible for the isolated CTHDs. Other genes have been found mutated in sporadic cases of CTHD, including ISK1, JAG1 (6%) responsible also for Alagille syndrome, FOG2 (5%) a GATA4 co-factor, and GATA4 (rare) and NKX2.5 (6%).

Recent advances in molecular genetics have also provided clues to the understanding of mechanisms responsible for some forms of syndromic CHD. For example, about 40% of patients with Noonan syndrome (NS) are due to mutation of the PTPN11 gene encoding for a non-receptor tyrosine phosphatase SHP2. The same gene is mutated in about 90% of LEOPARD syndrome individuals. A genotype-phenotype correlation was established, with a distinct association between PS and exon 8 mutations, ASD and exon 3 mutations, and hypertrophic cardiomyopathy and exons 7, 12 and 13 mutations. In addition, subsets of patients with NS and related disorders have been associated with mutations of different genes within the RAS cascade, including the KRAS, BRAK, MEK, ERK, SOS1, RAF1 genes.

In conclusion, progress in understanding the complex pathways of CHD development and diseases suggests that multiple factors are contributing to their susceptibility. Deciphering the molecular basis of CHDs also explains their incomplete penetrance, genetic heterogeneity and variable expression, and provides some hint to the genetic counselling.

### **Screening for Major Congenital Heart Defects: What is Achievable?**

*D Tucker*

#### Objective

To use antenatal detection rates and outcome data to inform antenatal screening policy for congenital heart anomalies.

#### Design

Antenatal Screening Wales quotes a 25% chance of detecting a major heart defect by a detailed ultrasound antenatal scan at 18-20 weeks. In Wales we are developing a list of conditions that would be expected to be detected more frequently. Four cardiac conditions were assessed. These were chosen because they are likely to be detected as an abnormal 4 chamber view or an abnormal view of the outflow tracts. Outcome data was assessed to inform parental counselling.

#### Participants

The Congenital Anomaly Register and Information Service for Wales (CARIS)

### Main outcome measures

Antenatal detection rates, trends and outcome data.

### Results

Hypoplastic left heart syndrome: Antenatal detection has risen to 90% where this is an isolated anomaly (2005 data). Outcome has shown a steady improvement. 80% of livebirths survived to the end of the first year of life. (2005 data).

Hypoplastic right heart: Antenatal detection has risen to 83% in isolated anomalies (2005). Outcome to the first year of life was 75% of livebirths.

Truncus arteriosus: Antenatal detection has risen to 65% in isolated anomalies (2005 data). Outcome has fluctuated. 50% of livebirths survived to the end of the first year of life (2005 data).

Transposition of the great vessels: Antenatal detection has not improved over time with a detection rate of 25% for isolated cases in 2005. Outcome to the end of the first year of life was 92% (2005 data).

### Conclusion

This shows general improvement in antenatal detection rates and outcomes for these serious conditions. Training in imaging of the outflow tracts should further improve detection of truncus arteriosus and transposition. This gives a baseline against which to measure further improvements in antenatal detection and outcomes of congenital cardiac anomalies.

## **Oral Clefts in Alsace, North-Eastern France (1995-2004): Epidemiological and Genetic Study of 266 Case and Evaluation of Prenatal Diagnosis**

*B Doray, B Dott, R Rinkenbach, H Dollfus*

### Objective

The aim of our study was to review clinical and epidemiologic data of oral clefts and to evaluate the impact of prenatal diagnosis.

### Design and Participants

The authors performed a large retrospective study among 127,626 malformed infants and fetuses reported to the registry of Alsace, North-Eastern France over a period between 1995 and 2004; 266 cases with oral clefts were collected.

### Main Outcome Measures

Incidence, anatomical types and associated birth defects were analysed. Prenatal diagnosis was studied, particularly detection rate, gestational age and accuracy of diagnosis.

### Results

The incidence of oral clefts was 2.08 per 1,000, with cleft lip/palate whereas (cp) sex ratio was 0.86. 1.41 oral clefts were isolated (53%) and 125 (47%) were associated with other birth defects. 36% of (cl/p) were associated contrasting with 67% of associated (cp). Isolated and associated Pierre Robin sequences were estimated. Among associated oral clefts, chromosomal anomalies, monogenic syndromes and other multiple congenital anomalies were described. Prenatal diagnosis of oral clefts was studied. The mean gestational age of sonographic

diagnosis was 22 weeks of gestation. The rate of sonographic detection was highly variable, with extreme values of 22% in 1998 and 70% in 2000 and we estimated the prenatal/postnatal concordance for the anatomical type of oral clefts.

### Conclusion

This study constitutes a large retrospective study about oral clefts in North-Eastern France over a 10-year period. It emphasises a high incidence of oral clefts, the embryological and genetic differences between cleft lip/palate (cl/p) and cleft palate (cp) and demonstrates the difficulties of prenatal diagnosis.

## **Congenital Heart Defects in Cornelia de Lange, Fraser and Goldenhar Syndrome - Epidemiological Survey of EUROCAT Registries**

*I Barisic, V Tokic, M Loane, F Bainchi, E Calzolari, E Garne, D Wellesley, H Dolk and EUROCAT Working Group*

### Objectives

To determine the presence of congenital heart defects in the three selected rare dysmorphic syndromes (Cornelia de Lange syndrome (CdLS), oculo-auriculo-vertebral spectrum (OAVS) including Goldenhar syndrome, and Fraser syndrome (FS)).

### Design and Participants

The data were extracted from the database of EUROCAT (European Surveillance of Congenital Anomalies), a large European network of population-based congenital anomaly registries that use the same epidemiological methodologies.

### Main Outcome Measures

According to EUROCAT data, congenital heart defects represent the most frequent subgroup of anomalies (54/10 000 births) and we anticipate the high occurrence of cardiac malformations in the rare syndrome cases as well.

### Results

Cardiac anomalies were the most common in CdLS, being present in 46% (42/93) of patients. Congenital heart defects were found in 25% (57/224) of OAVS cases and in 20 % (3/15) of Fraser patients. Ventricular septal defect was the most frequent anomaly in all analysed syndromes and was recorded in 14 % (13/93) of CdLS, 12% (27/224) of OAVS patients and 7% (1/15) of FS patients. Atrial septal defect followed, with 11% (10/93), 8% (17/224) and 7% (1/15) of cases, respectively. Pulmonary valve stenosis was also frequent in CdLS (10% - 9/93). Dextrocardia was noted in 4 patients with OAVS (2%).

### Conclusion

Considering the relatively high proportion of cases with congenital heart disease in three analysed dysmorphic syndromes, we emphasise the need that the detection of a cardiac anomaly on prenatal ultrasound is followed by a detailed examination of the fetus, including a thorough examination of facial profile, extremities and the search for other associated anomalies.

## **Outcome and the Changes in the Spectrum of Prenatally Detected CHD in Tertiary Care Centre**

*MG Russo, D Paladini, C Ricci, F Fratta, F Castaldo, M Felicetti, L Di Pietto, A Tartaglione, G Capozzi, R Calabro*

### Introduction

Congenital Heart Disease (CHD) are the most common malformations pre and postnatally. Nowadays, fetal echocardiography is a widely practised technique, however, the impact of prenatal diagnosis on prognosis of the newborns affected by CHD remains uncertain.

### Objective

The aim of our study was to assess the outcome and the changes in the spectrum of prenatally detected CHD in our tertiary care centre in an eleven-years activity.

### Methods and Results

The study group was composed of 1,493 mothers, 71% came to our observation because of suspicion of a cardiac anomaly during an obstetric scan. Mean gestational age was 26.7 weeks (range 15-41 w). A congenital heart diseases was diagnosed in 705 cases (47%): 32% (223) were CHD associated with extracardiac and/or chromosomal anomalies (aCHD) and 68% (482) were isolated (iCHD). Termination of pregnancy was chosen in 53% of cases in which a diagnosis was obtained before 24 weeks, 81% for aCHD and 37% for iCHD ( $p < 0.001$ ). Of these, more than one third occurred in fetuses affected by Hypoplastic left heart syndrome. The general survival rate is 72% it is significantly lower for aCHD if compared to the survival rate of iCHD (46% vs 80%,  $p < 0.001$ ). Over the period of observation we noticed a changing in the spectrum of fetal CHD, resulting in a reduction of the aCHD and of the number of Hypoplastic left heart syndrome and an higher number of Aortic arch anomalies detected. Almost half of the 705 CHD were observed during the first six years of activity, when the survival rate resulted 55%. Survival has significantly increased to 84% ( $p < 0.05$ ) from 2000 to 2006. The same trend has been observed for the termination rate which has significantly decreased from 35% to 14% ( $p < 0.001$ ). The number of neonatal deaths has significantly decreased from 39% to 10% ( $p < 0.001$ ).

### Conclusions

The survival of the prenatally detected CHD and the voluntary termination of the pregnancies are strongly influenced by their severity and by the associated extracardiac and/or chromosomal anomalies. Over the period of observation we noticed a changing in the spectrum of fetal CHD and a significant improvement in the outcome.

## **Environment-Gene Interactions**

*E Giavini*

Embryonic development is under the control of several genes regulating the main processes typical of development such as cell proliferation, differentiation, migration and death (apoptosis). This control is very complication and involves the interaction of different genes, in particular those encoding transcription factors, ie. proteins that binding the enhancer or promoter regions, interact to activate or repress the transaction of particular genes. The tissue specific expression of particular gene may be the result of the presence of a constellation of transcription factors are activated by other transcription factors. Furthermore, for a precise co-ordination of developmental processes, interaction is necessary between cells and tissues through chemical signal (embryonic induction): growth and differentiation factors like FGF, TGF- $\beta$ , Hedgehog, and morphogens (eg. retinoic acid) play a crucial role in these processes. All these factors, interacting with specific membrane or cytoplasmatic receptors of the responding cells, transmit the signal through complex signal

transduction pathways to the nucleus. Here, inactive transcription factors are transformed by phosphorylation in active transcription factors which activate or repress particular genes. Environmental chemicals could be teratogenic by interfering with transcriptional response or with proteins involved in the signal transduction pathways that activate the transcriptional regulation of developmental genes.

Chemicals like retinoic acid, inhibitors of histone deacetylase, inhibitors of retinoic acid metabolic pathways etc. represent some example of different ways to interfere with embryonic gene expression in the induction of congenital malformations.

### **Congenital Anomalies in Areas of Campania (Italy) Characterised by Multiple Dumping Sites**

*F Bianchi, F Minichilli, A Pierini, G Scarano, R Pizzuti, M Santoro, M Martuzzi, F Mitis, P Comba, L Fazzo*

#### Introduction

Increased risk of congenital anomalies (CAs) in residents in the vicinity of waste landfill sites was repeatedly reported in scientific literature and also by a pilot study conducted in Campania. In 2000 a large area of the Campania Region including 61 municipalities was declared as reclamation site of national interest. To investigate the impact on health of residing near waste disposal sites, a working group including WHO E&H Centre, National Institute of Health, National Research Council, Environment Protection Agency and Epidemiological Observatory of Campania Region was activated in 2004 on behalf of the Department of Civil Protection.

#### Materials and Methods

Considering the main characteristics of the waste sites as well as the type of waste and management, a score index of environmental impact for each site has been developed. The environmental indicator, developed on the basis of environmental characterisation performed by GIS, was used as a proxy of the exposure from hazardous waste sites at municipality level. This indicator was categorised in 5 classes by cluster analysis method: from class I, including unexposed municipalities (reference category) to class V, including more exposed municipalities. Eleven groups of CAs observed in total births and terminations of pregnancy over 1996-2002 in 192 municipalities were considered. To calculate the correlation between the environmental hazard index and 11 groups of CAs, Poisson regression methods and hierarchical Bayesian models were used.

#### Results

Comparing class V (higher risk municipalities) to class I (unexposed municipalities) statistically significant results were found for all CAs (411 cases) RR=1.29 (95% CI 0.97-1.73), nervous system anomalies (46 cases) RR=1.83 (95% CI 1.25-2.70) and urogenital malformations (49 cases) RR=1.83 (95% CI 1.26-2.66). For the same groups of CAs significant increasing trends from lower to higher class of environmental index were found.

#### Conclusions

The hypothesis of a reproductive risk associated to waste sites exposure is confirmed by the increased risks observed for total and selected CAs in higher exposed municipalities. Further studies by adopting analytical design with individual exposure assessment appear opportune.

## **Epidemiology of Congenital Malformations Sensitive to Folic Acid in Italy**

*F Bianchi, F Minichilli, A Pierini, M Rial, S Bianca, E Calzolari, G Scarano, R Tenconi, D Taruscio*

### Objectives

In 2005 a national network to improve the consumption of folic acid (FA) in order to reduce the risk of serious congenital malformations (CMs) was launched by the Italian Institute of Health. Recommendation established a FA dose of 0.4 mg/day to be taken in the periconceptional period. Since 2006 FA tablets are free of charge for women planning a pregnancy, their cost being paid by the National Health Service.

To establish an adequate baseline to allow a FA impact assessment in the next years and to investigate spatial differences among CMs registries, time trends and time-space interactions, a study based on the Italian registries of CMs was carried out.

### Design

Multicentre descriptive study on data collected over 1996-2003 by the Italian Registries members of EUROCAT and ICBDSR concerning live births/stillbirths (LB-SB) and induced abortions (IA) with selected CMs.

### Participants

The Italian CMs registries of North-East, Emilia Romagna, Tuscany, Campania, Sicily.

### Main outcomes measures

Total prevalence (LB, SB, IA) of neural tube defects, ano-rectal atresia, omphalocele, oral clefts, cardiovascular, limb reduction and urinary system defects.

### Results

**All the CMs showed statistically significant differences among registries with the exception of ano-rectal atresia. The majority of CMs by registry showed stable or increasing trends over time.**

### Conclusions

**The few uncoordinated campaigns conducted in Italy to improve awareness and knowledge of FA in preventing CMs up to 2006 do not seem to be effective. The study design adopted to detect the impact of FA on a small proportion of women that consume FA in periconceptional period must be taken into account. Differences among registries indicate the need of having a baseline for each registry to follow trends over time. The recognized importance of FA to prevent numerous CMs calls for a communication strategy on supplementation and consumption by natural and fortified food at national level, that is today in progress.**

## **Rare Diseases: What Italy is Doing at Institutional level?**

*D Taruscio, E Agazio, F De Angelie, Y Kodra, D Pierannunzio, S Pulciani, P Salerno, A Trama*

### Objectives

To provide an institutional and scientific response to rare diseases in Italy.

### Design

The Italian Government promulgated a Regulation on Rare Diseases (Ministerial Decree 279/2001, «Institution of the national network of rare diseases»). The National Network includes Regional centres and the National Registry of Rare Diseases. The Italian Drug Agency (AIFA) in 2005, launched the first call for proposals on orphan drugs. In addition, the Ministry of Health (MoH) and the Istituto Superiore di Sanità (ISS) launched 2 calls for proposals (in 2004 and in 2006) on rare diseases.

### Participants

Regions, ISS, MoH, AIFA, Research Institutes.

### Main Outcome Measures

**Improve scientific knowledge, develop a surveillance system, improve prevention, diagnosis and access to treatment and information on rare diseases.**

### Results

Regional centres have been identified in nearly the entire national territory. The list is available on: <http://www.iss.it/cnmr/>. A permanent inter-regional technical group was established including Regional Representatives, the MoH and the ISS to optimise the function of the national network. The National Registry of Rare Diseases was established at the ISS with the aim of defining the prevalence/incidence of rare diseases, identifying possible risk factors and fostering collaboration among healthcare providers. The National registry of Orphan Drugs was established at the ISS with the aim of establishing a post marketing surveillance system of orphan drugs approved with centralised procedures and available in Italy. Twenty research protocols have been selected for funding by the AIFA. Fifty four research projects were supported by the MoH/ISS call in 2004 and 82 in the 2006 call.

### Conclusions

The described initiatives demonstrate the Government's commitment to rare diseases. However, additional activities are needed:

- provide further support to research;
- **strengthen citizens and patients engagement;**
- **promote stronger collaboration among all stakeholders;**
- **ensure continue monitoring of the impact of the activities of the national network**

### **Cranioectodermal Dysplasia (Sensenbrenner's Syndrome) as an Example of Rare Diseases. Evolution of Phenotypic Features in Two Siblings**

***A Latos-Bielenska, J walczak-Sztulpa, M Szczepanska, M Krawczynski, J Zachwieja, D Zwolinska***  
**Objectives**

To present the Cranioectodermal dysplasia - a very rare malformation syndrome with tubulointerstitial nephropathy and to present evolution of phenotypic features in two siblings from the neonatal period.

### Case Report

Cranioectodermal dysplasia (CED, Sensenbrenner's syndrome, OMIM 218330) is a very rare malformation syndrome characterized by rhizomelic dwarfism, dolichocephaly, characteristic face, dental and nail dysplasia, sparse hair and tubulointerstitial nephropathy. Only about 20 cases were reported to date, including these with only some features of CED. We present detailed clinical features of the sibs in comparison to previously reported cases, as well as prenatal features of the syndrome in the ultrasound beginning from 17<sup>th</sup> week of the 2<sup>nd</sup> pregnancy.

The girl was consulted at the age of 3.5 years, the boy at the age of three days. The sibs have been under our observation for two years now. Their parents (healthy, 16- and 25-year-old at the birth of the first child) are consanguineous which supports the assumption of autosomal recessive inheritance. Clinical features in both sibs are severe and include short stature with rhizomelic shortening of limbs, brachydactyly, narrow chest, dolichocephaly, full cheeks, telecanthus, broad nasal bridge, small and widely spaced teeth, dysplastic auricles, fine, sparse hair, very large bilateral inguinal hernia and hyperelastic skin. Intelligence is normal.



Early diagnosis of CED payed our attention to the kidney. Both children suffer now from tubulointerstitial nephropathy, more severe in the brother and are under regular medical care. Bilateral inguinal hernia was operated on early to prepare the children for the peritoneal dialysis in the future.

#### Conclusion

The phenotype of the patients with severe form of the CED changes for the worse. The case pictures the problems of rare diseases: importance of early diagnosis and collaboration of many specialists to provide the children with proper medical care.

### **Nervous System Birth Defects Frequency Among Newborns in Ukraine and the Prophylaxis**

*O Lynchak, O Polka, E Omelchenko, O Tymchenko*

#### Objective

Determination of nervous system birth defects (BD) frequency among newborns in Ukraine and possibilities of prophylaxis.

#### Design

BD were coded according to ICD10. BD incidence was estimated among borne alive, stillborn and abortions after 28 weeks according to documents of geneticists and pathoanatomists in Kyiv oblast.

#### Results

Nervous system BD frequency among newborns in Ukraine decreases due to reducing of anencephaly and spina bifida (SB) level. Anencephaly frequency (1993-1999) was 0.170‰, during next six years it was 0.008‰; SB was 0.41‰ and 0,331‰ accordingly. BD frequency (2001-2005) among born alive was 0,787‰, its proportion in structure was 2.98%. In Kyiv oblast (1999-2003) nervous system BD frequency was 2.47 per 1000 among borne alive, stillborns and abortions. Anencephaly frequency was 0.29‰ (0.00‰ among born alive; 1.71‰ among stillborns; 4.09‰ among abortions); SB frequency was 0.64‰ (0,14 among born alive; 5.15 among stillborns; 5.81‰ among abortions). Given figures show that there are still a lot of prophylaxis possibilities (especially preconception prophylaxis). An important prophylactic measure is folic acid usage, in some countries folic acid is added in bakery. At the same time it is known that tumor cells have much more receptors to folic acid than normal cells. In Ukraine more than 400 thousand cases of cancer are diagnosed per year; cancer risk group are people older than 60 years (20% among 47 million); a big part of population contacts with carcinogenic factors. There are different ethnic groups in our country, with have different food allowance. That is why there are some doubts as to necessity of folic acid food fortification.

#### Conclusion

It is possible to reduce pathology burden by interdisciplinary approach and primary prophylaxis measures (mutagens and teratogens control, risk factors detection and elimination, health life stile propaganda, creation and use of special products for people of reproductive age).

### **Influence of External Risk Factors on Birth of Child with Birth Vascular Skin Defects**

*Y Shcherbak, V Galagan, O Tymchenko, O Lynchak*

The frequency of congenital vascular skin anomalies (VSA) among born alive babies comprises five per 1,000 among population of the city of Kyiv. It is supposed that environmental factors are the probably cause of these anomalies. The role of pathologies of pregnancy and deliveries, unhealthy

lifestyle of both parents, women's sexual inflammatory diseases, parturient women age, endocrinopathy, medication on pregnancy is discussed.

### Objective

To estimate of risk beginning cutaneous vascular defects among new-born under the influence of parents' chronic infections and extragenital diseases, drugs taken by women before and during pregnancy, parents' unhealthy lifestyle (including smoking tobacco), damages caused by professional activities.

### Design

Every case of new-born VSA between 1999 and 2003 was registered with special form filled out (634 cases had been analysed). Birth defects were coded according to ICD10, 2,407 forms were filled for same age mother delivered healthy babies. Odds ratio (OR) calculated with 95% confidence intervals (CI).

### Results

The chronic infections diseases of mother (OR = 1.53; CI 1.10-2.13) and father (OR = 1.93; CI 1.07-3.60); extragenital diseases of woman (OR = 1.61; CI 1.20-2.17), including endocrine (OR = 2.13; CI 1.37-3.30); taking drugs during preconception period and the first term of the pregnancy (OR = 2.36; CI 1.35-4.11 and OR = 1.65, CI 1.17-2.34 accordingly); smoking women (OR = 1.44; CI 1.00-2.07); alcohol use by women (OR = 1.96; CI 1.03-3.70), and by men (OR = 1.45; CI 1.06-1.97); unhealthy labour conditions for women (OR = 1.14; CI 1.05-2.00) increase the risk of appearance VSA among new-born.

### Conclusion

Harmful influence of mentioned factors can be prevented by family planning measures and promoting of healthy lifestyles.

## **Elevated Prevalence of NTD and a Cluster of Conjoined Twins in Ukraine**

*L Yevtushok, N Zymak-Zakutnya, S Polishuk, N Yuskiv, S Lapchenko, G Oakley, W Wertelecki*

The prevalence rate (PR) of neural tube defects (NTD) in northwestern Ukraine (Rivne and Volyn oblasts) is persistently elevated. During 2000-2002 and in 2005 the PR was 21 and 22 per 10,000 live births, respectively. Higher PR (28) was observed in the Chernobyl-impacted northern raions (counties) collectively known as Polissia. We also observed a cluster of conjoined twins solely in the Rivne oblast (~ 14,000 live births yearly). Occurrences were documented twice during 2000, and once during, 2002, 2003 (1 twin had spina bifida), 2004 and 2006. No conjoined twins were detected in five other oblasts except for Kherson, which is distant from Rivne (one instance in 2005 in the period 2003-2006). The conjoined twins were born in four different villages, two impacted and two not impacted by Chernobyl. The NTD PR in the first two villages was 38 and 34 and in the other two was 15 and 18.

The persistent and clearly elevated NTD rates led the Ukrainian Academy of Medical Sciences, in partnership with UNICEF-Kyiv, to recommend prompt implementation of a national folic acid flour fortification program. Experts estimated that ~ 500 infant deaths could be prevented each year due to folic acid preventable acquired NTD. Concurrently, a "Polissia Initiative" emerged to foster national and international partnerships to further investigate the role, if any, of folic acid deficiency, chronic low dose radiation and other teratogenic factors resulting in conjoined twins and other birth defects in Polissia.

### **The Italian Network for Folic Acid Promotion**

*D Taruscio, E Agazio, F De Angelis, Y Kodra, D Pierannunzio, S Ruggeri, P Salerno, M Salvatore, A Trama, G Ugolini and the Italian EUROCAT Registries of Malformations*

#### Objectives

To promote and coordinate actions in favour of a greater intake of folic acid in the periconceptual period through programmes of primary prevention of congenital defects.

#### Design

The Italian Network for the Promotion of Folic Acid is a synergy among institutions: universities, scientific societies, registries, regional offices, patient associations, Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione, local health units. It is coordinated by the National Centre of Rare Diseases of the Istituto Superiore di Sanità (ISS) in collaboration with coordinators of the working groups established within the network. The list of the working groups and activities follow:

- Advocacy
- Training of health operators
- Information of the general population
- Surveillance and outcome evaluation
- Risk to benefit assessment
- Health education on folate supplementation

#### Main Outcome Measure

Reduction of neural tube defects assessed by the Italian congenital malformation registries.

#### Results

- Elaboration of the national recommendations
- Development of Information Education and Communication materials
- Organisation of an awareness week on prevention on neural defects
- Risk and benefit analysis of the folic acid intake
- Evaluation of the prevalence of congenital malformation preventable by the intake of folic acid (Italy 1996-2002)
- Development of an education model for the promotion of acid folic in schools
- Inclusion of the folic acid among the list of medicinal products charged to the Italian National Health Service

#### Conclusions

The Network is actively working since 2004. In Italy we are experiencing an increasing and important commitment of the Ministry of Health, the Italian Drug Agency (AIFA), the ISS and other Public Institutions to the folic acid promotion as a primary prevention strategy to reduce congenital defects. In this context, Italy is also actively involved at European level in the discussion on the best approach for folic acid promotion.

## Outcome of Prenatally Detected Tetralogy of Fallot and Pulmonary Atresia with Ventricular Septal Defect

*M Russo, C Ricci, D Paladini, M Felicetti, G Capozzi, F Fratta, M Volpe, M Palladino, G Santoro, G Cainiello, C Vosa, R Calabro*

### Introduction

It has been reported that genetic syndrome worsen the outcome of Tetralogy of Fallot (TOF) and of Pulmonary atresia with ventricular septal defect (PAVSD). Aim of our study was to assess the outcome of prenatally detected isolated TOF (iTOF) and associated (aTOF) with extracardiac anomalies and/or chromosomalopathies and iPAVSD and aPAVSD.

### Methods

Since 1995 to 2006 we detected in utero 689 congenital heart disease, there were 60 (8.7%) TOF: 36 (60%) were iTOF and 24 (40%) were aTOF; moreover, 11 (1.6%) fetuses had PAVSD: 7 (64%) iPAVSD and 4 (36%) aPAVSD.

### Outcome

Out of 36 iTOF, 2 were interrupted. out of 24 aTOF, 6 were interrupted (5.5% vs 25%). Out of 7 iPAVSD, 3 were interrupted (43%). out of 4 aPAVSD, 1 was interrupted. The table shows the outcome of the continued pregnancies:

IUFD	AeW	NND pre	NND post
2 (5%)	32 (84%)	1 (3%)	3 (8%)
3 (14%)	9 (44%)	4 (19%)	5 (23%)

IUFD: intrauterine fetal deaths

AeW: alive and well after surgery

NND pre: neonatal deaths before surgery

NND post: neonatal deaths after surgery

There's a statistically significant difference in survival between the two groups (84% vs 44%;  $p < 0.001$ ). 1 aTOF AeW showed a right sided aortic arch 16/60 (27%) fetuses only presented a ventricular septal defect and an overriding aorta, they developed various degrees of right ventricle tract obstruction and right ventricular hypertrophy after birth.

Surgical repair was performed in 42 TOF (median age:  $11 \pm 5$  months). in 1 case a stent had been previously positioned in the patent ductus arteriosus, in 5 cases a systemic-pulmonary shunt had been previously implanted. 7/42 (17%) babies died after surgery [4/7 (57%) were aTOF], 4/15 (27%) liveborn aTOF died before surgery.

The 7 liveborn PAVSD were operated: 3 were aPAVSD (2 are still alive and 1 died) and 4 were iPAVSD, they are all still alive.

### Conclusions

The survival of prenatally detected TOF and the voluntary termination of pregnancies are strongly influenced by the association with extracardiac malformations and/or chromosomalopathies. A malalignant ventricular septal defect with an overriding aorta can evolve in TOF. The mortality rate after surgery is lower for iTOF. Death before surgery is more likely to happen for aTOF. The post-surgical outcome of iPAVSD is better than the outcome of aPAVSD.

### **Prenatal and Postnatal Sex Ratio in Trisomy 18**

*B Barrano, C Ingegnesi, R Cunsolo, B Ciancio, A Catallioti, G Ettore, S Bianca*

#### Objectives

Such determinants may influence the sex ratio (SR) either before fertilisation, by differential production or the fertilisation efficiency of X and Y carrying spermatozoa, or after fertilisation, by sex differential mortality in utero. A SR imbalance for autosomal trisomies has been debated in the literature also with changes in sex ratio during intrauterine life. The purpose of our study was to verify the changes in sex ratio in trisomy 18 cases during intrauterine life and a possible relationship with maternal age.

#### Design

Retrospective study.

#### Participants

We analysed data on 100 cases (57 males and 43 females) of which: 11 Spontaneous Abortions, 55 Terminations of Pregnancy followed prenatal diagnosis and 34 livebirths.

#### Results

The SR for all cases was 1.32 and the mean of maternal age was  $35 \pm 7$  with no difference between male and female. Our results showed an excess of males detected in spontaneously aborted fetuses (registered before 12 gestational week) and prenatal diagnosed cases (performed by amniocentesis at 16-18 gestational week) in contrast to the karyotypes detected in the newborn cases. If we stratify cases by maternal age classes we found an excess of cases in the age  $\geq 35$  years ( $p: 0.000$ ) due to an increase of trisomy 18 risk related to advanced maternal age. When we stratify for sex and maternal age we found statistical significant values for SR related to advanced maternal age  $\geq$  ( $p: 0.005$ ) and no statistical significant values for the single classes.

#### Conclusion

Our study points to a mechanism working to abort trisomy 18 males embryos during second and third trimester of pregnancy through differential intrauterine selection with a mechanism not influenced by maternal age. Moreover, the evidence of different sex ratio survival in intrauterine life may be used at genetic counselling when prenatal diagnosis discovered fetal trisomy 18.

### **Reproductive and Environmental Risk Factors for Hypospadias**

*R Cunsolo, C Ingegnesi, B Barrano, B Ciancio, A Cataliotti, G Ettore, S Bianca*

#### Objectives

Report our experience in search for possible reproductive risk factors for hypospadias.

#### Design

Data from two case control studies.

#### Participants

In the first study we evaluated 415 hypospadias cases compared with 812 controls. In the second study (68 cases versus 211 controls), we evaluated cases identified in two towns in South Eastern Sicily, which have intense industrial (Augusta) and agricultural activities (Vittoria).

### Results

The results of the first study suggest that women at the extremes of the age distribution ( $p < 0.000$  and  $0.026$  respectively) relative to women in the middle of the distribution. The results of the second study showed an incidence that was 3.8 and 2.3 times higher, respectively, than expected (3.2 per 1,000 male live births in South Eastern Sicily). Fathers job exposure (working in oil-refinery) alone gave an odds ratio of 5.5 in Augusta and (working in hothouses) of 2.9 in Vittoria.

### Conclusion

It has been postulated that changes in concentrations of sex hormones during the fetal critical period of genital development (weeks 8-14), caused by endogenous or exogenous factors, may play a role in the development of hypospadias. Mothers at the extremes of the age distribution may be more susceptible to this hormonal disruption, that the associated between maternal age, both for younger than for older women, may be explained as a "defect in nature's quality control" with a reduction of defensive maternal mechanisms in the hypospadias's mothers that would be less efficient in the elimination of malformed fetuses. For the environmental causes our study suggests that exposure to large amounts of industrial and agricultural pollutants is sufficient to increase the risk of hypospadias.

### **Hypoplastic Left Heart Syndrome: Possible Relationship between Intrauterine Sex Selection and Chromosomal Imbalance**

*C Ingegrosi, B Barrano, R Cunsolo, B Ciancio, A Cataliotti, G Ettore, S Bianca*

### Objectives

Evaluation of sex ratio and reproductive history in Hypoplastic Left Heart Syndrome (HLHS) cases.

### Design

Retrospective study.

### Participants

We evaluated the sex ratio and reproductive history of 40 HLHS cases compared with a group of 1,000 consecutive newborns with the same geographic origin used as control.

### Results

The SR was 2.3 and there is a statistical association with  $\geq 3$  previous spontaneous abortions.

### Conclusions

Hypoplastic left heart syndrome (HLHS) is a severe congenital heart disease consisting of a severely underdeveloped left heart ventricle. The mean birth incidence of HLHS has been estimated to be 0.1-0.25/1,000 livebirths and it has been suggested that HLHS presents with a male sex ratio imbalance. There is good evidence that a high percentage of malformed cases are conceived than birth with a very large proportion of malformed embryos eliminated prenatally.

Our data suggests an association between an high multiparity and an high frequency of fetal loss and the occurrence of an HLHS newborn. Then it is possible to support the hypothesis that the genetic and/or environmental factors causing HLHS are closely related to those causing embryonic deaths or that embryonic deaths are a result of conception of malformed embryos.

The HLHS is a congenital malformation that may be associated with 45,XO karyotype; this chromosomal anomaly is one of the most frequent cause of spontaneous abortion in the first trimester of pregnancy, than it may be plausible that a number of conceived 45,XO fetuses with

HLHS are spontaneously aborted with a predominance of male cases at birth. This hypothesis points the indication to perform chromosomal analysis in all prenatal diagnosed cases of HLHS to search for possible association with 45,XO or mosaic for this condition and may be useful in search for a possible etiological mechanism responsible of this congenital anomaly.

### **Pre-Conceptional Screening for Rubella Infection**

*A Agangi, S Simioli, A Votino, G Tessitore, G Ciavolino, L Mazzarelli, A Iannaccone P Martinelli*

#### Objective

To verify women's compliance with the pre-conceptional screening and the vaccination programs for the prevention of the congenital rubella.

#### Design

Perspective study. A questionnaire was submitted to women in order to verify their compliance with the pre-conceptional visit. Moreover their immunological state with respect to rubella infection was estimated.

#### Participants

Department of Obstetrics and Gynaecology of University of Naples Federico II, between January 2006 and April 2006.

#### Main Outcome Measure

78.8% of the women had a natural immunity against rubella, 7% received the vaccine and 11.7% were susceptible to the infection. Between those who were susceptible, only 3 (4.2%) were vaccinated after delivery.

#### Results

Among the 604 women enrolled, 42 (7%) were vaccinated, 71 (11.7%) were susceptible, 476 (78.8%) had natural immunity and 15 (2.4%) did not exhibit any proof about their immunity state. Of the 42 vaccinated women: 4 were vaccinated at age 0-15 months, 28 at 15 months - 14 years, 10 between puberty and childbearing age. About the plans for pregnancy, 366 out of 604 (60.5%) had planned pregnancy and 151 (25%) had practiced the pre-conceptional counselling but only in 61 (40.3%) cases it included the research of laboratory evidence of immunity for rubella. Despite 8 women (13.1%) were not protected against rubella infection, only in 3 cases (37.5%) physicians suggested to vaccinate. Before discharge, the 71 susceptible women have been advised to vaccinate, but among those only 3 (4.2%) had joined to the vaccination program.

#### Conclusions

Our results show that a strong effort must be done to improve compliance to the vaccination program. Not only women but also physicians should be better informed. In fact an incomplete vaccination program may have more harm than good since the shift in the age distribution with a higher risk to contract rubella during pregnancy.

### **Obesity in Pregnancy and Congenital Anomalies: A Systematic Review**

*K Stothard, R Bell, J Rankin*

#### Objective

To assess current available evidence relating to the association between maternal overweight and obesity and the risk of congenital anomaly.

### Design

Systematic literature review.

### Participants

Pregnant women.

### Methods

Medline, Embase and Scopus literature databases were searched using a comprehensive list of keywords for the years 1966-2006. Titles and abstracts were read by two members of the research team and included in the review if they met our inclusion criteria: observational studies published in English between January 1966 and December 2006, including data on body mass index in pregnancy and congenital anomalies as the outcome measure. All included articles were obtained and read in full by two members of the research team and data extracted using a piloted data extraction form.

### Main Outcome Measures

All types of congenital anomalies were considered including multiple anomalies and different sub-groups.

### Results

Thirty-four studies were included. Twenty studies were from the USA, six from Sweden, two from Spain and one from Australia. Twenty-two studies included a case control design and 12 were cohort studies. Twelve studies reported neural tube defects as their outcome measure, 11 multiple anomalies, six cardiac anomalies and one each for cryptorchidism, gastroschisis, renal, orofacial clefts and diaphragmatic hernia. Many of the reported studies had methodological weaknesses including sample sizes have been too small to evaluate individual congenital anomaly subtypes, not including cases of congenital anomaly occurring in termination of pregnancy and inconsistency in the definition of obesity.

### Conclusion

This is the first systematic review of the effect of obesity in pregnancy on congenital anomaly risk. The rapidly rising prevalence of obesity in pregnancy means that any increased risk will have important implications for fetal health. Congenital anomalies may be increased in obese women due to hyperglycaemia, reduced detection due to technical difficulties with screening, or nutritional deficits such as reduced folate.

## **Congenital Anomalies in Twin Pregnancies: A Population-Based Register Study**

*SV Glinianaia, C Wright, J Rankin*

### Objective

To describe the prevalence of congenital anomalies in twin pregnancies by chorionicity and major sub-type.

### Participants

All twin pregnancies notified to the Northern Multiple Pregnancy Registry (MPR) from 1st January 1998 to 31st December 2002.

### Methods

The MPR was established in 1998 to capture details on all multiple pregnancies in the former Northern Region of England. Ascertainment is from the earliest antenatal scan on which a multiple



pregnancy is detected. The records are linked to the population based Northern Congenital Abnormality Survey (NorCAS). Chorionicity is ascertained where there is appropriate antenatal ultrasound determination and subsequently by placental examination and histology

#### Main Outcome Measures

Congenital anomaly rates by chorionicity; congenital anomaly subtypes; pregnancy outcomes in twins with congenital anomalies.

#### Results

There was a total of 2,167 twin maternities during the five years, giving an average twinning rate of 14.5 per 1,000 maternities. Congenital anomalies complicated 162 pregnancies, involving 182 individuals (4% of all twins). Of these, there were 12 terminated pregnancies; two selective reductions; and 135 liveborn twins with 114 (84.4% still alive at one year). This compares with a background congenital anomaly rate of 2.5%. In twins with known chorionicity, the prevalence of congenital anomalies was higher in monochorionic (6.2%) than dichorionic twins (3.3%) (OR = 1.9, 95% CI 1.3-2.7). The most common types of congenital anomalies were: cardiovascular anomaly (47 cases, 25.8%), anomalies of central nervous system (23, 12.6%), chromosomal anomaly (22, 12.1%), renal/urinary tract anomalies (18, 9.9%) and other (56, 30.8%). Of anomalies specific twins, there were four sets of conjoined twins and three individuals affected by the TRAP sequence.

#### Conclusion

The rate of multiple pregnancies continues to increase due to the combined effect of increases in maternal age and the use of assisted reproductive technology. This is of concern given the higher risk of adverse outcome associated with these pregnancies.

### **Prenatal Diagnosis and Counselling of Major and Rare Diseases: A south Italian Referent Centre's Experience and Evaluation of 822 Cases of Chorionic Villus Sampling (cvs)**

*G Maruotti, R Napolitano, S Simioli, A Votino, G Tessitore, G Ciacolino, F Quaglia, P Martinelli*

#### Objective

To evaluate the performance of chorionic villus sampling (cvs) as a first trimester diagnostic procedure and counselling for fetal karyotyping, major and rare disease.

#### Design

Fourteen year retrospective of invasive procedure of prenatal diagnosis performance and counselling.

#### Participants

All women to receive invasive procedure of prenatal diagnosis by cvs at University Federico II (Naples) between January 1994 and March 2007. The population included 822 women of ages variable from 16 to 44 years. Of these 6 had a twin pregnancy.

#### Main Outcome Measures

Women booked into the clinic at increased risk of carrying a fetus with major or rare disease were offered counselling and an invasive diagnostic procedure (cvs). Follow up of the outcome of pregnancies was carried out.

### Results

Since 1994, at prenatal diagnosis centre in collaboration with ceinge we have performed 822 cvs for prenatal diagnosis of rare and severe diseases. The results of the molecular diagnosis were gotten in our laboratories or in international laboratories for the realisation of an international network.

### Conclusions

Clinical trials and our experience suggest that cvs carries a very low risk of pregnancy loss, a risk that is comparable to that of second trimester amniocentesis. The role of genetic counselling for the inherited diseases is debated. An international collaborative network with some centre in the world assure the possibility of prenatal diagnosis for all diseases whose genetic test is available. After fetal diagnosis of the most severe and rare disease, next objective of our centre in these years of advances in early genetic analysis and in human genome investigations will be the applications of methods for curing genetic disease either by in utero stem-cell transplantation or by genetic therapy with the insertion of functional genes into the germline.