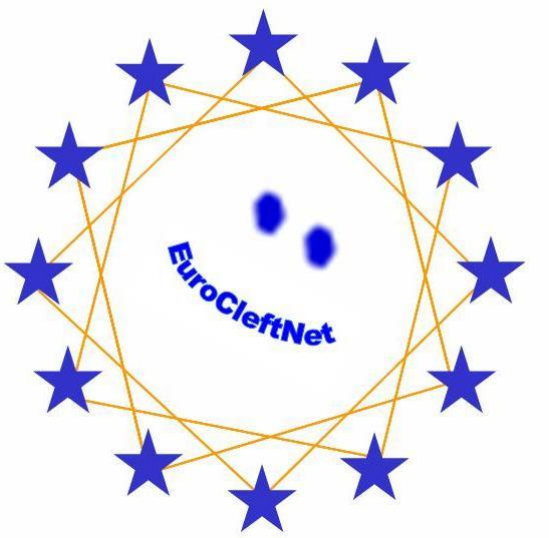
REPORT



EUROCleftNet Research Conference

Genetics, Environment and Prevention

30th June – 1st July 2016

Apex City Quay Hotel

West Victoria Dock Road, Dundee, Scotland

EUROPEAN SCIENCE FOUNDATION
SETTING SCIENCE AGENDAS FOR EUROPE

UNIVERSITY OF DUNDEE

CONTENTS

The final EUROCleftNet Research Conference was held in Dundee, Scotland from Thursday 30th June to Friday 1st July 2016.

Day 1: Thursday 30th June
Session 1: Prediction - identifying cleft loci

Session 2: Prediction - identifying cleft-causing variants

Session 3: Early career researchers discuss their projects

Session 4: Personalization – identifying cleft-causing gene-environment interactions

Day 2: Friday 1st July
Session 5: Prevention - monitoring cleft occurrence and recurrence

Session 6: Prevention - reducing cleft occurrence and recurrence

Session 7: **Workshops** - the future of cleft research

Session 8: Participation – synergies between patients/parents and scientists/clinicians

This document acts as a summary report on conference content and proceedings, and contains abstracts from all speakers - including our early career researchers. It also provides feedback from our **Workshops** and, importantly, **action points** to continue to build on the work of EUROCleftNet.

Note: the full conference programme can be downloaded from our website at https://eurocleftnet.org/

Peter A. Mossey
Professor of Craniofacial Development and Associate Dean for Research, University of Dundee, Scotland

Abstract 1: ***The evidence base for intervention with respect to smoking and consanguinity.*** Meta-analyses reveal that passive smoking and consanguinity ARE both risk factors in OFC. Mossey, P.A.

"*Passive smoking in the etiology of non-syndromic orofacial clefts: a systematic review and meta-analysis*"**.** Sabbagh HJ, Hassan MH, Innes NP, Elkodary HM, Little J, Mossey PA

Data extraction from studies reporting maternal passive smoking and NSOFC was implemented without language restrictions. Risks of bias in the identified studies were assessed and this information was used in sensitivity analyses to explain heterogeneity. Meta-analysis and meta-regression of the extracted data were performed. Egger's test was used to test for small study effects. Fourteen eligible articles were identified. Maternal passive smoking exposure was associated with a twofold increase in risk of NSOFC (odds ratio: 2.11, 95% confidence interval: 1.54–2.89); this was apparent for both cleft lip with and without palate (OR: 2.05, 95% CI: 1.27–3.3) and cleft palate (OR: 2.11, 95% CI: 1.23–3.62).

There was substantial heterogeneity between studies. In the studies that provided data enabling crude and adjusted odd ratios to be compared, adjustment for potential confounders attenuated the magnitude of association to about a 1.5-fold increase in risk.

"*Parental Consanguinity and Nonsyndromic Orofacial Clefts in Children: A Systematic Review and Meta-Analyses*". Sabbagh HJ, Hassan MH, Innes NP, Al Baik A, Mossey PA

Objective: To assess whether individuals born to consanguineous parents had a higher frequency of nonsyndromic orofacial clefts compared with those with no parental consanguinity.

Design: A prespecified plan for a search strategy, inclusion/exclusion criteria, and data extraction from studies reporting consanguinity in relation to nonsyndromic orofacial clefts (NSOFC) was carried out. Papers reporting observational studies with control populations were included, without language restrictions, and these reports were assessed for quality. Sensitivity analyses using subgroups, homogeneity evaluation, and assessment of publication bias were carried out, and meta-analyses of extracted data were performed.
Results: Sixteen studies fulfilled the selection criteria and were included in the metaanalyses. There were statistically significant relationships between consanguinity and NSOFC for all 16 studies combined (P = .0003), with odds ratio (OR) = 1.83 and 95% confidence interval (CI) (1.31, 2.54); 10

case-control studies (P = .006), with OR = 2.06 and 95% CI (1.23, 3.46); six cross-sectional studies (P = .03), with OR = 1.34 and 95% CI (1.02, 1.76); first cousins consanguineous marriages (P = .04), with OR = 1.40 and 95% CI (1.01, 1.93); cleft palate alone (P =.01), with OR = 1.89 and 95% CI (1.14, 3.13); and cleft lip with or without cleft palate cases (P = .002), with OR = 1.56 and 95% CI (1.18, 2.07).
Conclusion: Although there was a high level of study heterogeneity, the evidence is consistent in suggesting that consanguinity is a risk factor for NSOFC, with an overall OR of 1.83 (95% CI, 1.31 to 2.54), implying that there was almost twice the risk of a child with NSOFC being born if there was parental consanguinity.

Abstract 2: ***Clefts and craniofacial anomalies: alternative funding strategies in light of global priorities***. We often look to the large funding bodies and stakeholders when seeking guidance on fundable strategies in health. Recent WHO priorities have included non-communicable diseases and disorders, and the Global burden of disease (GBD) is an attempt to quantify the mortality and morbidity is in relation to a range of disorders and diseases. Fortunately a number of the more prevalent birth defects are included in the 2010 WHO recommendations to Member states. The sustainable development goals (SDGs) are a call to action for low income and developing countries, and it is incumbent on the international community to assist with these. The EU Horizon 2020 priorities for funding in health include rare diseases and it is prudent to explore the landscape of Craniofacial disorders including clefts when considering future strategy.

NIH / NIDCR are one of the prime sources of funding for Craniofacial research and post GWAS, there may well be opportunities for cleft research under a recent call on “Genetic sustainability and variability of human structural birth defects”. The NIDCR FY 2015 emphasised a focus on inequalities and the value of a global approach to "apply rigorous, multidisciplinary research approaches to overcome disparities in Dental, Oral and Craniofacial health". This also mentioned the building of a strong evidence base for cost-effective implementation with health economics, cost-effectiveness and cost benefit of health interventions requiring more emphasis with a focus on prevention. Upstream social determinants of health and the common risk factor approach to a number of Do we have the robust evidence base, standardised measuring instruments and costings to be able to make a strong case for craniofacial anomalies and OFC using the health economics and health inequalities agendas?

Michele Rubini
Medical Genetics Unit - Dept. of Biomedical and Specialty Surgical Sciences, University of Ferrara, Italy

Abstract 1: ***P4-Cleft: Translating scientific achievement into applications.*** Research into the genetic causes of non-syndromic orofacial cleft (OFC) has recently made great strides and led to the identification of major OFC loci. New research developments are now directed to track down of variants with functional significance, to unravel how exposure to environmental factors interacts with genotype in determining OFC risk, and to identify endophenotypes and epigenomic modifications that can be used for diagnostic and predictive purposes. To better care for children with OFC scientific discoveries must be translated into practical clinical applications. Translational research is needed to provide clinicians with safer, more effective, and more powerful tools for both diagnosis, treatment and management of patients with OFC. However, translational research cannot rely only on the contribution of scientists and clinicians, but strongly needs also active participation of proband's families, and a shift from reactive symptoms-centered medicine to a proactive and patient-centered approach. Prediction, Prevention, Personalization, and Participation are the fundamental components of a proactive approach for CL/P patients (the P4-Cleft approach).

Abstract 2: ***Folic acid and nutrigenetics of orofacial clefts.*** There is evidence that maternal exposure to folate deficiency or to folic acid antagonists during early pregnancy increases the risk of orofacial cleft in the offspring, while supplementation with folic acid in the periconceptional period is generally considered a mild protective factor. Inter-individual differences in the response to folic acid supplementation could be ascribed – at least in part - to common functional variants in genes encoding one-carbon metabolism enzymes or folate transporters, and some evidence supports interaction between folate gene polymorphisms and folic acid supplementation as factor influencing the risk of developing orofacial cleft. Increasing our knowledge on such interactions could potentially lead to developing personalized preventive measures, with higher effectiveness in avoiding cleft recurrence.

Elizabeth J. Leslie
Assistant Professor, School of Dental Medicine, University of Pittsburgh, United States of America

Abstract: ***Taking a genome-wide multiethnic view of orofacial cleft risk.*** Orofacial clefts (OFCs) are caused by incomplete fusion of the upper lip and/or palate, resulting in three major subtypes: cleft lip (CL), cleft palate (CP), and CL plus CP (CLP). Combined they represent the most common craniofacial birth defects in humans. OFCs are also noted for the variability of prevalence rates observed around the world. The highest rates of OFCs are found in those of Asian and Amerindian ancestry; individuals with European ancestry have intermediate prevalence rates and individuals with African ancestry are reported to have the lowest rates. As a disorder with complex etiology, multiple genome-wide association studies (GWAS) have been completed in the pursuit of genetic risk factors for OFCs. Although initially performed in cohorts of European ancestry, the last five years have witnessed application of GWAS to diverse populations. One intriguing finding has been the identification of apparently population-specific association results. GWASs of orofacial clefts will be summarized with a particular emphasis on signals with differences in strength between populations. Explanations for the difference in strength of associations will be discussed.

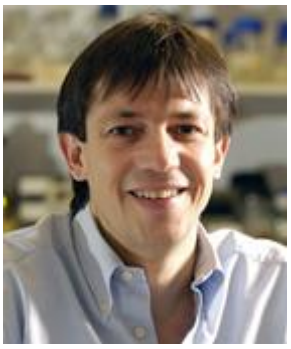
Elisabeth Mangold
MD, Principal Investigator "Genetics of Orofacial Clefting" project, Genetic Counselor, Institute of Human Genetics, University of Bonn, Germany

Abstract: ***Genetic background of nonsyndromic orofacial clefting – Where are we today?*** Nonsyndromic orofacial clefts have a multifactorial etiology, involving both genetic and environmental factors. The first molecular genetic attempts to elucidate the genetic background date back to the late 1980s, and in the early years many candidate genes and loci were studied, however, results of the most of these studies remained inconclusive and had limited overlap. Only in 2008 a candidate gene approach led to a first convincing result: the identification of a causative variant for nonsyndromic cleft lip with/without cleft palate (nsCL/) in the IRF6 promotor region. In 2009 a meta-analysis of linkage data and subsequent fine-mapping gave convincing statistical evidence for the FOXE1 region. The introduction of high-throughput genotyping technologies has enabled genome-wide association studies (GWAS), which have been extremely successful in that they identified multiple common causative loci. The first GWAS was published in 2009 and discovered the 8q24 region as a key susceptibility locus for nsCL/P. Subsequent GWAS in larger samples, meta-analyses of GWAS data, combinations with replication studies and imputation of GWAS data led to identification of many more causative loci for nsCL/P. At the time of writing only 21 susceptibility loci for nsCL/P and one for nonsyndromic cleft palate have been published, and more are yet to come. For some of these loci a sub-phenotype specific effect has been found. Functional studies have identified a causative variant at the NOG locus, and will detect more of them at other loci. In the near future whole-exome and whole-genome sequencing approaches will detect further causative variants, among them rare mutations. A better understanding of the genetic background will pave the way for a better understanding of the underlying biology.

Adrianna Mostowska
Department of Biochemistry and Molecular Biology, Poznan University of Medical Sciences, Poland

Abstract 1: ***GWAS analysis identifies three novel loci for NSCL/P in the Polish population.*** Non-syndromic cleft lip with or without cleft palate (nsCL/P) is one of the most common congenital anomalies, with a complex and not yet fully elucidated aetiology. Therefore, we conducted a genome-wide association study (GWAS) for nsCL/P in a Polish population-based cohort consisting of 288 oral cleft cases and 576 controls, with a further replication in an additional independent sample. Genome-wide genotyping was performed using Illumina HumanOmniExpressExome-8v1 array enriched for functional exonic markers. We identified three novel loci associated with nsCL/P at 3q29 (rs338217, $p_{comb} = 2.56 \times 10^{-10}$), 6p22.3 (rs9356746, $p_{comb} = 6.31 \times 10^{-6}$) and 12q14.1 (rs7305482, $p_{comb} = 3.61 \times 10^{-9}$). Promising candidate genes at these chromosomal regions include *DLG1* (discs, large homolog 1) and *CDKAL1* (CDK5 regulatory subunit associated protein 1-like 1). In addition, we confirmed the association between the previously reported loci at 1p21.3 (*ARHGAP29*), 8q24.21, 14q22.2 (*GCH1*) and 15q13.3 (*GREM1*) and the risk of nsCL/P. The most significant nucleotide variant in this study was rs17242358 (8q24.21 gene desert) with pcomb = 4.87×10^{-17}. To confirm our GWAS findings further, larger sample size studies in different populations are needed.

Abstract 2: ***Is there a genetic link between psychological factors such as stress and the risk of OFC?*** It has been shown that heightened levels of maternal psychological stress in the periconceptional period may be implicated in the aetiology of non-syndromic cleft lip with or without cleft palate (nsCL/P). However, the differences in stress measurements and a lack of consensus on the definition of maternal stress are reasons that only a small number of researchers are considering the presence of stressful life events during pregnancy as a risk factor in nsCL/P occurrence. In addition, the influence of environmental and genetic factors on each individual's variability in the stress response create additional challenges when investigating the mental/emotional stress as an nsCL/P causal factor. One mechanism by which maternal stressors may cause birth defects is dysregulation of the major stress response system, the hypothalamus-pituitary-adrenal (HPA) axis, leading to increased glucocorticoid production at the adrenals. The emotional state of the mother may also alter the function of the placenta, affecting uterine blood supply and nutrient transfer. In one of our projects, we investigated the association between nucleotide variants of stress related genes and the risk of nsCL/P affected pregnancies. We found that polymorphisms in *SLC6A4* (serotonin transporter) and *TPH2* (tryptophan hyroxylase 2) might be factors increasing the risk of having a baby with this developmental anomaly. The implication of maternal *TPH2* and *SLC6A4* variants in the risk of nsCL/P might be associated not only with the function of serotonin in the brain and regulation of HPA axis reactivity but also with the crucial role of serotonin during foetal development.



Michael Dixon
Professor of Dental Genetics, University of Manchester, England

Abstract: ***The syndromic / non-syndromic interface in the quest for causative genetic variants.*** Clefts of the lip and/or palate (CLP) are common birth defects of complex aetiology. CLP affects approximately 1/700 live births, with wide variability across geographic origin, racial and ethnic groups, as well as environmental exposures and socioeconomic status. Historically, CLP has been divided into cleft palate only (CPO) and cleft lip with or without cleft palate (CL/P); this broad sub-division of anatomical defects is consistent with the distinct developmental origins of the lip/primary palate and the secondary palate.

CLP can occur in isolation or, together with additional non-cleft anomalies, as part of a syndrome. Approximately 70% of all cases of CL/P and 50% of cases of CPO are considered to be non-syndromic. The remaining cases are composed of a wide range of malformation syndromes, including over 500 Mendelian syndromes, as well as those arising secondary to chromosomal or teratogenic effects. As syndromic forms of CLP are somewhat more tractable to genetic analysis than their non-syndromic counterparts, extrapolation from syndromic CLP has proven to be useful in the study of non-syndromic CLP.

Azeez Butali
Assistant Professor of Oral Pathology, Radiology and Medicine. University of Iowa, United States of America

Abstract: ***Optimising the value of GWAS data by data cleaning and diagnosing more homogeneous subsets.*** For us to address OFC comprehensively, it is important to understand the causes and to explore strategies for prevention. OFCs are complex traits with genes, environment and stochastic factors contributing to the phenotypic expression in any given individual. To date 6 genome wide association studies (GWAS) for cleft lip with or without cleft palate (CL/P) have been conducted and 18 risk loci identified. All these studies have either been conducted in European populations, Asian populations or both. There is currently no published GWAS for clefts in African only populations. This presentation will discuss findings during data cleaning from the first African only cleft GWAS. The characterization of a homogenous subset is an essential step towards the identification of significant risk loci.

Heiko Peters
Reader in Mammalian Genetics, Institute of Genetic Medicine, Newcastle University, Newcastle upon Tyne, England

Abstract: ***Functional validation of OFC risk loci using mouse models.*** The number of newly identified genetic risk loci involved in OFC is rapidly increasing but how they are contributing to OFC in the developing embryo is often unknown. In principle, genetic variants in non-coding regions may point to adverse changes in the timing, the levels, or the region of expression of nearby genes. Genetically modified mice and organ culture experiments can help to distinguish between these possibilities, thereby allowing to study the pathogenic mechanism of de-regulated gene expression affecting craniofacial development. Focusing on specific gene-gene and gene-environment interactions ongoing work will be described that is aimed at defining the pathogenic mechanism of cleft lip and cleft palate formation involving de-regulated expression of Grem1 and Msx1.

Mary L. Marazita
Director, Center for Craniofacial and Dental Genetics; Professor and Vice Chair, Department of Oral Biology, School of Dental Medicine; Professor, Department of Human Genetics, Graduate School of Public Health; Professor, Clinical and Translational Science; Professor, Department of Psychiatry, School of Medicine. University of Pittsburgh, United States of America

Abstract: ***Subclinical phenotypic features for genetics research within OFC families.*** Orofacial cleft (OFC) birth defects exhibit a wide range of phenotypic variability due to the multiple oral and facial structures involved, and the potential for other complexity such as complete versus incomplete defects, unilateral versus bilateral cleft lips, clefts of the hard and/or soft palate. There are also visible microforms such as bifid uvula and notches in the upper lip that are considered very minor manifestations along the spectrum of OFC defects. We hypothesize that there is additional subclinical phenotypic variation within apparently unaffected family members that reflects carrying genetic risk factors, that can help explain reduced penetrance in OFC families, and that might be useful in dissecting genetic heterogeneity. We will present results from GWAS of OFC families and controls, incorporating such subclinical phenotypes.

David R. FitzPatrick
Professor, MRC Human Genetics Unit, MRC Institute of Genetics & Molecular Medicine, University of Edinburgh Western General Hospital, Edinburgh, Scotland

Abstract: ***The clinical and molecular features associated with de novo mutations of SATB2.*** We originally identified SATB2 as a gene on 2q33 that caused cleft palate. The advent of new sequencing technologies have allowed many individuals to be screened for causative variants in all genes. I will present clinical, genetic and molecular features associated with de novo mutations (DNM) affecting SATB2 function in 19 unreported cases ascertained on the basis of intellectual disability (ID). Cleft palate was present in less than half of the cases (9/19) although other oral features were common including absent/near absent speech (16/19), drooling (12/19) and dental anomalies (8/19). 11/19 of the DNM were clear loss of function. Eight missense variants were identified and shown to cluster in the first CUT DNA binding domain (6/8). Sibling recurrence due to gonadal mosaicism was seen in one family. A stop-gained mutation in the last exon resulted in production of a truncated protein retaining all three DNA binding domains. The nuclear mobility of the tagged proteins differed significantly between the wild-type with p.Arg389Cys in CUT1 showing increased mobility and both p. Gly515Ser in CUT2 and p.Gln566Lys between CUT2 and HOX showing reduced mobility. The clinical features in the individuals with missense or stop-gain in the last exon were indistinguishable from each other or from cases with LOF mutation. De novo, heterozygous mutations in SATB2 represent one of the commonest genetic causes of syndromic ID. Haploinsufficiency appears to the universal mechanism and where mutant SATB2 protein is produced disturbance of the normal pattern of chromatin association is observed.

Faisal Khan
University of Ferrara, Italy

Abstract: ***Optimising the EUROCRAN DNA trios bio-bank with western EU biobank, and carry on some gene-environment studies.*** Our repository of genomic DNA from cleft cases, or Cleft-bio-bank, is a collection of European Case-parent trios DNA samples/data which is often derived from blood and/or saliva specimens. The maintenance of Cleft-bio-bank preserves the opportunity for future research or genetic testing in order to benefit the case-parent trios and cases community as a whole. Our Cleft-bio-bank includes samples from the EUROCRAN and ITALCLEFT/PENTACLEFT projects, and is an open access research DNA bio-bank in order to supplement EUROCleftNet members. Being a EUROCleftNet membered group, we took advantage of the Cleft-bio-bank and in order, we examined the pool of European trios for frequency of transmission between two TGFA insertion/deletion and susceptibility of developing nsCL/P. Additionally, the test of gene-environment interactions between the two insertion/deletion markers in TGFA and two common maternal exposures (smoking and folic acid supplementation) during pregnancy are factored.

Karen A. Pisani
University of Ferrara, Italy

Abstract: ***Biobank of lip tissue and genomic DNA from nsCL/P cases and pilot epigenetic study.*** Epigenetic modifications could play a role in the interaction between environmental factors and genotypes, and some evidence in mice model suggest that altered methylation could impair the correct fusion of lip prominences during embryogenesis, causing cleft lip. To date no studies have been carried out in humans to explore the role of epigenetics in clefting. A collection of tissue samples obtained at time of surgery, along with genomic DNA from case- parents trios and clinical data has been established in Ferrara from 2015. Preliminary results obtained by assaying a global DNA methylation biomarker (LINE-1) are presented.

Rita Bassi Andreasi

Medical Genetics Unit – Dept. Of Biomedical and Specialty Surgical Sciences, University of Ferrara, Italy

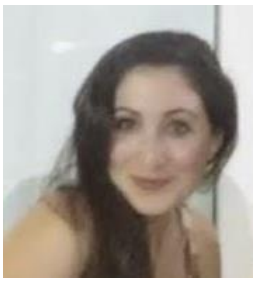
Abstract: ***Replication of CNVs studies in patients with syndromic cleft palate.*** Evidence suggests that Copy Number Variations (CNVs) may play a role in the development of cleft palate. A number of deletions or duplications have been detected in patients with syndromic cleft palate, among which those encompassing the 22q11 region are of particular interest. In previous studies, Comparative Genomic Hybridisation array (aCGH) has been used to identify association between CNVs and syndromic cleft palate. In order to confirm findings, we replicated results using a different technique: CytoScan® 750K Array Kit, Affymetrix. We report the results obtained after screening of a group of syndromic cleft palate cases from Glasgow, NHS Greater Glasgow and Clyde.

Anne C. Böhmer
Postdoctoral scientist, Institute of Human Genetics, University of Bonn, Germany

Abstract: ***Unravelling the complex genetic basis of non-syndromic cleft lip with or without cleft palate – functional follow-up studies at the 13q31 susceptibility locus.*** Genome-wide association studies (GWAS) have largely contributed to a better understanding of genetic risk regions for complex disorders. The 13q31 locus has been identified as risk locus for non-syndromic cleft lip and cleft palate (nsCLP) in a meta-analysis of single GWAS (Ludwig et al, 2012). Similar to numerous GWAS findings in other traits, the top-associated genetic variants at 13q31 map to a non-coding region. Functional data suggest that regulatory elements at the locus might play a role in the development of the murine face. During my ESF-funded research visit in Michael Dixon's lab I (i) studied regulatory regions at the 13q31 locus and (ii) performed expression analyses of the Sprouty2 gene (SPRY2). The data revealed that (i) the top-associated variants mapped to enhancer elements involved in craniofacial development and (ii) that SPRY2 is expressed in palate forming structures in mouse embryos at stages E11.5 and E13.5. The integration of functional data generated during my research visit and our genetic data from the meta-analysis suggest functional mechanisms underlying the genetic association at 13q31 and generate new hypotheses for further follow-up studies.

Shaho Al Talabani
University of Dundee, Scotland

Abstract: ***Identification of phenotypic and genotypic markers in the predisposition to OFC through parental microforms.*** Facial shape, lip prints, orbicularis oral muscle (OOM) status, dental features and the genotype of 81 parents of children with NSOFC were compared to a control group of 73 non-cleft participants of Celtic background. The facial shape and dental features statistically differed between the groups and the OOM defect rate was higher in the parental group in comparison to the controls, however no lip print differences were detected. A candidate gene approach analysis detected some genetic association with case-control status but there were no genotype-phenotype correlations using selected candidate gene polymorphisms.



Paola Franceschelli
University of Ferrara, Italy

Abstract: ***High density genome wide arrays applied to parents of children born with non-syndromic cleft lip and/or palate.*** The complex aetiology of Non-syndromic Orofacial Cleft is still not completely understood, and there is little information about how the genetic risk carried by unaffected relatives can be inherited by children with overt oral clefts. In the present work, aimed to investigate the possible role of cleft candidate genes in unaffected parents, we conducted a case-control association study, using 80 unaffected parents of children with overt orofacial cleft (recruited through clinics in Scotland) and 70 controls (recruited at the University of Dundee, Scotland). Samples were genotyped using high-density genome-wide human arrays (Cytoscan 750K _array, Affymetrix), collecting data from 200,000 SNPs. Considering the small sample set, the association analysis has been restricted to known associated loci and specific candidate regions, to avoid some of the difficulties presented by multiple testing: the Odds Ratio was calculated for 122 SNPs across 30 candidate loci. Among this, 3 SNPs showed a significant inverse association: rs6657063 C>G, an intron variant in ARHGAP29 gene (ORCCC/GG = 0.21, 95%CI: 0.05-0.83, P=0.02), rs3901678 C>A, an intron variant in THADA gene (ORCA-/CC = 0.36, 95%CI: 0.14-0.89, P=0.025), and rs10956463 A>C, located in 8q21.13 (ORAA/CC = 0.31, 95%CI: 0.109-0.902, P=0.02). These preliminary results demonstrate how unaffected relatives present variations in genes thought to be involved in cleft lip and palate development and suggest the possibility of a phenotype-genotype correlation in presence of facial cleft-related subclinical features.

Kerstin U. Ludwig
Emmy-Nöther Junior Research group leader, Institute of Human Genetics, University of Bonn, Germany

Abstract: **Novel insights in orofacial clefting: the power of integrative approaches using clinical and genotype information as well as data from animal models.** Despite considerable advances in our understanding of genetic risk loci contributing to nonsyndromic cleft lip with or without cleft palate (nsCL/P), little is known about etiological factors that discriminate between its most common types cleft lip only (nsCLO) and cleft lip and palate (nsCLP). We suggest that the integration of detailed clinical phenotype data, high-resolution genotypes and information obtained from animal models can help to identify novel risk factors for nsCL/P in general, but also provide new insights into nsCL/P subphenotypes. Along these lines, in a recent study, we performed a meta-analysis on three large nsCL/P cohorts and identified strong association between a region on chromosome 15q13, close to the Gremlin-1 (GREM1) gene. In the nsCLP group, relative risks were even higher, and no association was observed in nsCLO. Based on analyses of the murine Grem1 expression pattern during embryonic craniofacial development, the nsCLP patient cohort was further subdivided according to precise clinical data on affected structures. We observed a more than two-fold increase in risk for patients displaying concurrent clefts of both the lip and soft palate but with an intact hard palate. This study identified the first genetic contribution to a rare clinical nsCLP entity which specifically involves clefts of the lip and the soft palate, which develop at different embryological time points.

Julian Little
Professor and Director, School of Epidemiology, Public Health and Preventive Medicine, University of Ottawa, Canada

Abstract 1: ***Epidemiology of folic acid in mitigating orofacial clefts.*** Two key randomized controlled trials in the 1990's clearly demonstrated that folic acid supplementation in the periconceptional period prevented the first occurrence and recurrence of NTDs by approximately 70%. In Canada, where folic acid fortification of white flour, pasta and some other cereal-based products has been mandatory since November 1998, the prevalence at birth of NTDs has declined by approximately 45%. Fortification has also been implemented in the United States, Argentina, Brazil and Chile. For all types of orofacial clefts (OFC) combined, there was a decrease in prevalence at birth after the introduction of fortification in the United States, but not in the other jurisdictions where fortification was mandated.

Multivitamin supplements are associated with a reduced risk for CL±CP and perhaps CP. In a meta-analysis published in 2008, combined effect estimates indicated risk reductions of 25% and 12% for CL±CP and CP respectively. It is not possible to determine from these studies which of the nutrients in the multivitamins are protective and whether or not other healthy behaviours of multivitamin users confound these results. Similarly, the effect of dietary or supplemental intake of folic acid on OFCs is uncertain, with one observational study even suggesting an increased risk associated with supplemental intake. The uncertainty was affirmed in a recently updated Cochrane review.

Possible mechanisms involved in putative effects of folic acid supplementation on OFCs have been investigated. These include whether variants of genes thought to be involved in folate metabolism present in the mother or the infant, such as MTHFR, contribute to the risk of OFC. Recent studies also suggest that folic acid may influence the pathogenesis of congenital anomalies through other pathways, including epigenetic mechanisms with possible trans-generational effects. A possible implication of epigenetic mechanisms is that a possible beneficial effect of folic acid fortification in reducing the prevalence at birth of congenital anomalies other than NTDs may take more than one generation to become fully apparent.

Abstract 2: ***Environment-specific maternal genetic effects in orofacial clefts.*** More than two thirds of CL±CP cases and about half of CP cases occur are non-syndromic, while the other cases are associated with known teratogens, chromosomal anomalies or single-gene syndromes. Non-syndromic OFCs are thought to be caused by genetic and environmental factors and their joint effects. Although the heritability of OFC has been estimated to be high, as yet few genes or loci involved in the etiology of OFC have been identified, either from GWA or candidate gene studies.

The mother can influence her offspring's risk of disease (1) as a genetic donor; (2) through the effect of her genotype on the intrauterine environment directly; (3) indirectly through feto-maternal gene-gene interactions; or (4) through her exposures and associated interactions with her genotype or that of the developing fetus. Several candidate gene studies of OFCs have suggested maternal genetic effects, and in a recent GWAS, several signals of maternal effects almost reached genome-wide significance. Evidence of maternal genetic effects, and of environmental specificity will be discussed, together with challenges in identifying these.

George Wehby
Associate Professor, Health Management and Policy Doctoral Program Director, University of Iowa, United States of America

Abstract: ***Genetic Instrumental Variables (Mendelian Randomization): Using Genes to Identify Causal Effects of Environmental Factors.*** Behavioral risk factors for oral clefts such as smoking, excessive alcohol, and obesity have been suggested in several studies, but previous studies have focused on association estimates and did not provide causal evidence. We examine the effects of maternal smoking, pre-pregnancy weight, and alcohol consumption on the risk of having a child with oral clefts using genetic instrumental variables (Mendelian Randomization). We employ data on a large sample from an international consortium of several population-based case-control and case-cohort studies from the United States and Europe. The presentation will cover study methods and preliminary results.

Amanda J. Neville

EUROCAT registry leader and JRC EUROCAT joint management committee member; IMER Emilia Romagna birth defects registry; Centre for Epidemiological and Clinical Research, University of Ferrara, Italy - Azienda ospedaliero universitario di Ferrara

Abstract: ***The EUROCAT approach to primary prevention.*** Congenital anomalies (CA) are the paradigm example of rare diseases liable to primary prevention actions due to the multifactorial etiology of many of them, involving a number of environmental factors together with genetic predispositions. Yet despite the preventive potential, lack of attention to an integrated preventive strategy has led to the prevalence of CA remaining relatively stable in recent decades. The 2 European projects, EUROCAT and EUROPLAN, have joined efforts to provide the first science-based and comprehensive set of recommendations for the primary prevention of CA in the European Union. The resulting EUROCAT-EUROPLAN 'Recommendations on Policies to Be Considered for the Primary Prevention of Congenital Anomalies in National Plans and Strategies on Rare Diseases' were issued in 2012 and endorsed by EUCERD (European Union Committee of Experts on Rare Diseases) in 2013. The recommendations exploit interdisciplinary expertise encompassing drugs, diet, lifestyles, maternal health status, and the environment. The recommendations include evidence-based actions aimed at reducing risk factors and at increasing protective factors and behaviours at both individual and population level. Moreover, consideration is given to topics specifically related to CA (e.g. folate status, teratogens) as well as of broad public health impact (e.g. obesity, smoking) which call for specific attention to their relevance in the pre- and periconceptional period.

Ester Garne

Paediatric Department, Hospital Lillebaelt, Kolding, Denmark

Abstract: ***Orofacial clefts and fetal medication exposure in the first trimester of pregnancy: what is the risk?*** In 2006 FDA sent out an alert of increased risk for cleft palate after fetal exposure to lamotrigine in pregnancy. EUROCAT explored this in a large European dataset and was not able to confirm the increased risk. The initial EUROCAT study has now been updated with more years and more exposed pregnancies. The 4-year EUROmediCAT project aimed to build a system for reproductive safety evaluation, to enable the systematic and comprehensive identification of possible adverse effects in pregnancy of medication in humans at the earliest stage post marketing. Four medication groups for chronic maternal diseases were the main focus. Risk of orofacial clefts were evaluated for medications used in asthma, depression, diabetes and epilepsy. Orofacial clefts often came up as a risk from the literature reviews performed in the EUROmediCAT study. Results and impact of the findings will be presented.

Régine P.M. Steegers-Theunissen
Professor in Periconception Epidemiology, Erasmus MC, the Netherlands

Abstract: ***Novel opportunities for primary and tertiary prevention of (non)syndromic clefting using evidence-based mHealth coaching programs.*** The (non)syndromic clefts (NSC) originate during the first trimester of pregnancy being a largely missed period in antenatal care. Derangements in growth, development and epigenetic programming are involved in the causation of NSC and are largely determined by gene-environmental interactions. The environmental factors also include parental conditions and behaviors such as folic acid supplement use, nutrition, smoking, obesity and non-prescribed medication use. Although most of these personal environmental factors are modifiable, they are extremely difficult to change. Parents–to-be however are most motivated to change poor behaviors when they are aware of the detrimental effects on their (unborn) child. Therefore, these couples should be empowered to use evidence-based and personalized effective tools to improve poor conditions and behaviors. Moreover, these tools should be implemented as instruments to support healthcare professionals in delivering 'nutrition and lifestyle care' in routine patient care. From this background the usability and effectiveness of the mHealth coaching programs (www.SmarterPregnancy.org.uk, www.SlimmerZwanger.nl and www.SlimmerEtenMetJeKind.nl) aiming to change poor behaviors and to improve pregnancy outcome and support healthy eating during the first year of life of the child will be presented as opportunity to customize these tools for the target group of families with enhanced risks of NSC offspring or a child with NSC as well as for their healthcare professionals.

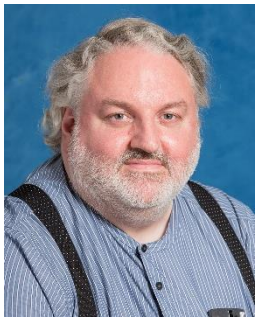
Lina Moreno
Assistant Professor, Department of Orthodontics, College of Dentistry & Dental Clinics, University of Iowa, United States of America

Abstract: ***A Population-Based Assessment of Effects of Top Candidate Loci for Nonsyndromic Clefting.*** Recent GWAS have reported several genes and loci to be associated with non-syndromic oral clefts. However, the generalizability of effect estimates is unclear as the evidence is heavily based on clinic-based samples that may suffer from ascertainment bias. Small samples have also limited the investigations of effects by cleft type and non-syndromic versus syndromic status as well as underlying genetic mechanisms involving maternal gene and parent-of-origin effects. We estimate the effects of main previously reported loci on cleft types in both non-syndromic and syndromic forms using data from a large consortium of population-based case-control and case-cohort studies from the United States and Europe. We examine fetal, maternal gene, and parent-of-origin effects. The presentation will cover study methods and preliminary results.

Ron Munger
Professor, Department of Nutrition, Dietetics, and Food Sciences, Utah State University, United States of America

Abstract 1: ***Biomarkers of maternal exposures and metabolic abnormalities and risk of orofacial clefts.*** Biomarkers may be useful additions to self-reported data to estimate the magnitude of maternal environmental exposures and to classify mothers according to the type and level of metabolic abnormalities that may be relevant to the risk of having a child with an orofacial cleft (OFC). Prospective collection of biological specimens very early in pregnancy in large cohorts would be helpful, but few ideal studies exist with adequate sample collections. A less ideal but more feasible approach is to conduct case-control studies with careful collection and processing of biological specimens; this approach may be useful when there is relative stability in diets and environmental exposures, when genetic and epigenetic determinants of biomarker levels are important, and for metabolic conditions such as diabetes that tend to be chronic and worsen over time. Two metabolic themes with evidence linking to risk of OFCs include folate-related one-carbon metabolism (1CM) and the cluster of metabolic abnormalities that are precursors to diabetes, known collectively as metabolic syndrome. Our biomarkers studies from the U.S., Philippines, and India provide evidence of the importance of several nutrients involved in 1CM including folate, vitamins B6 and B12, and zinc; the nutritional backgrounds of these populations are very different, hence there are very different patterns of biomarker associations with OFCs. Our studies in Utah indicate that mothers of affected children are less responsive to folic acid intake than controls, that multivitamin supplements are only protective when combined with healthy dietary patterns, and that several genes known to be involved in 1CM may interact with maternal nutrient status to alter risk of OFCs. Obesity and diabetes have been linked to risk of OFCs clefts. Gestational diabetes is far more common than pre-existing diabetes among women of reproductive age and is associated with OFC risk but is tested for and diagnosed in later pregnancy and the mechanisms for OFC formation early in pregnancy are unknown. Our ongoing studies of metabolic syndrome biomarkers in case and control mothers provide evidence that systemic inflammation and altered adipokine levels may be promising biomarkers for mechanistic studies early in pregnancy. Biomarker studies will allow greater understanding of the mechanisms of OFC formation in gene-environment studies and may be important in public health approaches to screen high-risk individuals and populations for targeted nutritional and lifestyle interventions for health promotion and OFC prevention.

Professor Munger's second presentation was on the theme of ***International Development Goals*** during Session 8 (the final Session on Friday 1st July). This discussed the way forward globally in the funding of clefts in parallel with other disorders.



Matthew Darlison
Informatics Lead, WHO Collaborating Centre for Community Genetics, UCL Centre for Health Informatics & Multiprofessional Education (CHIME)

Abstract: ***Orofacial Clefts in the Global Context of Congenital Disorders.*** Focussing initially on haemoglobin disorders, work has been ongoing in conjunction with the human genetics programme of the World Health Organisation since the early 1980s to make estimates of the prevalence of a variety of congenital disorders and the associated health burdens.

The most recent round of estimation was initiated by an invitation to the March of Dimes to facilitate inputs to the Global Burden of Disease project, and has now led to the formation of an international working group of experts. The group is led from London by Professors Bernadette Modell (UCL) and Joy Lawn (LSHTM), and aims to estimate baseline birth prevalences, effects of interventions, plus survival and disability to 5 years and beyond, for congenital disorders at the country, WHO region and global levels. Work is ongoing to enable comprehensive publication of data from the Modell Global Database of Constitutional Congenital Disorders (MGDb) online, and estimation methods in a special edition of the Journal of Community Genetics.

Collaborative work is also in progress with the Eastern Mediterranean Regional Office of WHO to support the development of a regional strategy for congenital disorders covering both environmental and "constitutional" causes.

Databases like MGDb help to locate individual disorders, such as orofacial clefts, in a global context in which the priorities are broader: transparency and consensus around epidemiological science as a foundation for sound, evidence-based health policy advocacy and policymaking, leading ultimately to provision of effective, responsive services by knowledgeable and well-supported practitioners. To achieve this, however, we need community-wide consensus on what we are counting, and how, and on how we attribute various levels of disability.

Since they are easily diagnosed and can be treated surgically with high clinical effectiveness, orofacial clefts represent a point of entry for assessing the effect of paediatric surgery and may thus be in a position to lead the way in defining and developing models for capturing epidemiological data, measuring cost effectiveness and delivering care.

Speaker abstracts

Gareth Davies
European Cleft Organisation

Abstract: ***The role of the European Cleft Organisation (ECO) in supporting best practice and prevention agendas through advocacy, education, and research networking.*** ECO has adopted a multi-faceted approach in highlighting the challenges of cleft care and promoting prevention. This presentation will explore strategies at various levels. Firstly, as a political lobbying body at European level we aim to engage with, and influence, health agendas at national level. Secondly, we have a track record of using EU instruments to turn knowledge into action by producing and disseminating evidence-based best practice guidelines for health professionals and support groups. Finally we, aim to impact upon the research and prevention agenda through a new role as an accredited European Patient Advocacy Group (ePAG) within the European Reference Network (ERN) for Rare Craniofacial Anomalies and ENT Disorders.

Neil Stewart
University of Dundee, Scotland / Kite Innovation (Europe) Ltd

Abstract: ***Horizon 2020 Funding Opportunities: Health, Demographic Change and Wellbeing.*** Horizon 2020 is the financial instrument implementing the Innovation Union, a Europe 2020 flagship initiative aimed at securing Europe's global competitiveness. By coupling research and innovation, Horizon 2020 is helping to achieve this with its emphasis on excellent science, industrial leadership and tackling societal challenges. The goal is to ensure Europe produces world-class science, removes barriers to innovation and makes it easier for the public and private sectors to work together in delivering innovation. The focus of this presentation is on forthcoming funding opportunities in Societal Challenge 1 Health, Demographic Change and Wellbeing.

Breakout group 1: "Funding priorities and opportunities for collaborative genetics research"

In the previous session of the conference a presentation on H2020, an opportunity under **"Societal Challenge 1: Health, Demographic Change and Wellbeing"** and specifically under: "Diagnostic characterisation of rare diseases" is presented below under **APPENDIX I.**

Political environment
Unsurprisingly we discussed the EU situation with some concerns that this would limit funding opportunities - although the overall view seems to be that this shouldn't influence a decision to apply.
The NIH is also a key funder, and it may make sense to have an international programme led from the USA to leverage this. NIH funding comes with some advantages, including the 'block booking' of sequencing facilities that makes sequencing available at no additional cost.

Application Details
There was a lot of discussion around (1) is cleft rare enough – sub-phenotyping can make it so, but would this be transparent to reviewers (2) if there are very few EU grants on rare disease - cleft may not be enough in itself - and would be better as a part of a bigger craniofacial programme. This would also need to address the issue of Mendelian versus complex (polygenic) craniofacial disorders – so syndromic / non-syndromic as EUROCRAN did but possibly collaboration with colleagues in other craniofacial disorders and / or Robin sequence would be worthy of consideration.
Any H2020 application has to tick ALL of the boxes with lots of international collaborators - and some of the boxes would be hard to tick e.g. changing the landscape of management i.e. "development of new therapies and for a better treatment outcome". Could we do this?

Looking at areas to develop:

1. Sub phenotyping is essential and developing an international database and general ontology is really important (see **APPENDIX II**). **ACTION: Jonathan Berg / Michele Rubini / Mary Marazita / Peter Mossey**
2. Scoping low level environmental interventions to prevent cleft would also be a strong point in management. **ACTION: to be discussed in Chennai in Feb 2017**
3. Building bigger and better GWAS sample sets with new patients (rather than rehashing the old ones) and better phenotypes. **ACTION: Engage colleagues from Poland, Estonia and Czech Republic**
4. Focussing on less well mapped forms such as isolated cleft palate (CP). **ACTION? Does this mean in terms of human population studies?**

Other sources of funding
The EUROCleftNet is an impressive and extant collaboration. It would be unfortunate if it couldn't be held together to develop things further. Other sources of support discussed included the NIH, but also Marie Curie Fellowship funding, which although person and skill focused would allow continued effective communication between labs.

Chairs: **Jonathan Berg (rapporteur)**, Michele Rubini and Mary Marazita

Breakout group 2: "Future of OFC clinical and phenotype research networks from a global perspective"

Sub-phenotyping: The European approach in the past has, in an effort to emphasise the importance and magnitude of the problem of OFC, combined all types of cleft to provide an overall figure of approximately one in 700 live births. This has also resulted in many studies combining all clefts or merely some dividing into two major sub-types CP and CLP. It is increasingly evident from recent genomics research that this lumping approach is hampering progress, and what is emerging is a clear need for sub-phenotyping. **ACTION: Jonathan Berg / Michele Rubini / Mary Marazita / Peter Mossey**

Coding / classification: Combined with this a discriminating and user friendly classification system is necessary …with collaboration to ensure sufficient numbers. The classification system should be capable of sub-dividing clefts into complete and incomplete and the LAHSHAL system can be utilised for this. This would also have beneficial effects on treatment so that protocols could be better tailored to specific clef types. This approach would also be very valuable in the light of the move towards European Reference Networks (ERN) which combine clinical care with building capacity and networks that will also facilitate research. **ACTION: Peter Mossey and team (paper already written / action through ICD-11 beta version).**

Linking databases: EUROCAT representatives were very supportive of this and the potential for linkages across Europe in terms of not only health but also education maternal illnesses & medications (prescribed) and even social and psychological issues. A recent initiative described as Eurolinkcat has been proposed where existing databases are linked to provide better information across a range of birth defects. A good example of successful Networking is that used by DDD / Discern in the identification of similar phenotypes in different countries leading to discoveries of genetic cause (e.g. SATB2). **ACTION: RD-CONNECT initiative as part of EUROCAT / ERN via Amanda Neville / Ester Garne / Peter Mossey**

Future research: Beyond clefts was also discussed as a future objective with apparent overlaps across different congenital conditions, and therefore different ERN s. Beyond Europe collaborations with transatlantic collaboration makes sense with synergies in expertise and resources as is the importance of thinking of clefts as a global problem.

While Europe has a track record in the past of leading in the field of cleft lip and palate treatment research, the ultimate objective is to translate this to improve standards of care across the world, and to prevent clefts if possible. There are instances where this has already begun with the TOPS trial (Timing of Palatal Surgery) NIH funded and led by Bill Shaw. EUROCLEFT being rolled out to Americleft, and EUROCRAN being translated to AFRICRAN - by just adopting the same standardised protocols and methods. An element of this approach has also resulted in a combination of clinical researchers collaborating with laboratory-based research using genetics / genomics with epidemiology, nutrition, psychology etc. An emphasis on mapping of expertise and sharing the work accordingly would be a future objective. **ACTION: this will be on the CHENNAI 2017 agenda**

Funding agencies: Within Europe collaborations with COST in psychology and ERASMUS in education and beyond. it is important to think beyond the usual funders of cleft and Craniofacial

research such as NIH and ESF, and innovative funding strategies might include adoption of the Common risk factor approach, clefts as an example or a sentinel birth defect, part of the WHO SDGs, address clefts in the context of inequalities, and **seek to address the problem of mortality** as well as morbidity in parts of the world where clefts are a cause of infant mortality. Engaging with global agencies such as CDC and MoD, and action the GBD statements such as the Beijing manifesto and the Dar es Salaam declaration from the September 2015 ICBDDDW. (**APPENDIX III**)

Clefts alone may not be sufficient and there should be concerted efforts to address other reproductive health issues simultaneously and other health issues. Among the global priorities in contemporary health relates to possibilities for improving cost effectiveness and cost benefits of healthcare, and the principle of "Healthier people cheaper" addressing health AND WELLBEING, and simultaneously addressing health inequalities. **ACTION: Part of the WHO CC Agenda with global stakeholders as collaborators**

Chairs: **Peter Mossey (rapporteur)**, Bill Shaw and Matthew Darlison

Breakout group 3: "Global priorities for research in Prevention of OFC"

Although the ultimate goal is primary prevention, the group noted that very solid evidence is needed before undertaking trials of efficacy and, if efficacy determined, effectiveness and cost-effectiveness. Such trials are very expensive, e.g. The Brazilian trial of alternative doses of folic acid in prevention of recurrence of CL/P cost more than $10 million. Further, trials cannot be done to assess the effect of potentially harmful environmental risks.
The group initially discussed further aetiological research, and noted that a limitation of much such work on chronic diseases in general was that the majority was done in populations with similar confounding structures. This led the group to consider how research might be established in less studied populations, and this led to the following priority order of discussion.

Clearly intervention trials to prevent the occurrence or recurrence of clefts are not feasible at this time. An alternative approach would be a kind of experimental approach in investigating the responsiveness of case-mothers vs. control mothers to nutritional supplements or challenges, such as oral glucose tolerance tests. The general hypothesis is that case-mothers have inherited and acquired metabolic characteristics that persist (hence increasing the risk of recurrence) and that these adverse metabolic characteristics may be "unmasked" either in the non-pregnant state (easier to do) or in a study following case and control mothers through their next pregnancy (harder to do). I feel this could be an innovative approach to move the field forward. **ACTION: Identify those collaborators with pregnancy cohorts, work up protocols and apply for funding Ron Munger / Julian Little / Michele Rubini / Régine Steegers-Theunissen / Peter Mossey**

Global health aspects-tertiary prevention. The issue of the "missing children with clefts" indicated by the survival curve analyses should be prominently publicized. The group identified **tertiary prevention** as a key focus from a global health perspective, because of the enormous difference in survival curves between LMIC and other countries. This essentially requires prioritising research on treatment and treatment outcomes, and **developing registries** to support such an initiative is necessary. This prioritisation would be expected to maximise buy in from clinicians and other health professionals, and such buy in is key for sustainability. Thus, the group **prioritised establishing registries where there are gaps**, and for setting baseline data in advance of any attempt at a preventive intervention. This should maximise use of existing resources and requires government prioritisation in the developing world. In addition, using photographs for data collection was discussed, taking account of high levels of Smartphone usage in LMIC. This could be seen as an area of novelty and hence potential commercial investment. **ACTION: Thanks to SmileTrain robust and reliable data are available and should be used to convince the respective government ministers, public health bodies, WHO regional offices in different LMICs to take the appropriate actions. Julian Little / Ron Munger / Peter Mossey will undertake.**

The major cleft surgical organizations, Smile Train and Operation Smile, have developed methods to organize community health workers to locate affected infants using smartphones. Smile Train and Operation Smile have strong fund-raising connections with major corporations that might see an interest in this such as telecom companies, Microsoft (promoting smartphone banking via Cloud computing in LMICs) and others. **ACTION: This could be the focus of a workshop at the Chennai 2017 Cleft Congress.**

***Principles for registries in LMIC*.** Potential to build on EUROCAT model, in which some registries initially were hospital based and later moved to being population based. EUROCAT has a World affiliate member program, e.g. Riyadh, New Zealand and Iran [Ester, please correct if necessary]. Of note, 15 of the EUROCAT registries are participating in a record linkage project to investigate treatment and survival over 5-10 years. A high priority is to engage EUROCAT in discussions about developing more affiliate member hospital-based registries. **ACTION: This could be done in Chennai where Peter Mossey and Ron Munger are currently working. Others with an interest may be Azeez Butali (Nigeria and other African countries) and Carmen J. Buxó-Martínez, (working in Puerto Rico; but who could not attend the Dundee meeting).**

Aetiological research. It was suggested that existing, and in LMIC new, case- control and case-parent trio studies could be used to detect initial signals on gene-environment joint effects, including epigenetics. Many such studies were developed with a primary focus on detecting and replicating genetic association – they may have been enriched for the purpose of detecting genetic effects, which would result in selection bias that could distort magnitude of effects (N.B. In certain circumstances, marginal effects may be biased but effect of interaction on multiplicative scale not biased). From a classical epidemiological perspective, they may be based on convenience samples with a substantial proportion of missing information that may be differential between cases and controls. Measures of exposure are likely to be different between studies (including between centres within single GWA studies) but methods have been developed to harmonise exposure variables across studies (e.g., for GWA studies through the PhenX initiative). Nevertheless, unless nested within cohort studies, the case control design is vulnerable to non-differential misclassification for exposure information obtained by recall, and in theory to differential misclassification between cases and controls (usually parents in the situation under consideration) although empirical evidence suggests that this is a major concern in general only for highly publicised exposures.

The topic of air pollution, specifically household air pollution in LMICs, passive smoking, and urban air pollution should be emphasized more. Given the very consistent findings from published studies on direct and passive tobacco smoke exposure and the fact that the toxic effects of partially combusted biomass—whether tobacco, wood, charcoal, grass, or animal dung—are likely to be similar (Kirk Smith of Berkeley stressed this point in our Chennai meeting). Given the consistency of association of OFC with tobacco smoke, it is an imperative that the relationship between air pollution and reproductive health is explored thoroughly. **ACTION: Ron Munger / Julian Little / Peter Mossey / Azeez Butali (Nigeria)**

Collaboration: The initial signals then could be tested and characterised in cohort studies – there are existing birth cohorts in the Nordic countries, Canada, Europe (CHICOS – framework 7 child cohort research strategy for Europe) and the US. To have adequate statistical power (and to replicate findings), it would be necessary to combine data across studies. Again, heterogeneity between studies in exposure measurement is a concern but methods for harmonisation have been proposed within the framework of the Public Population Project on Genomics and Society (P3G) – DataShaper - and latterly through the Maelstrom project (we understand that work on exposures with a paediatric focus is ongoing).

We should mention the importance of pre-conception health education, interventions, and behaviour change. The approaches of Régine Steegers-Theunissen and colleagues in Rotterdam are impressive and comprehensive—though it is unclear how well similar programs would be taken up in less education and lower SES populations. Further work in this area should be encouraged. **ACTION: Continue ongoing collaboration between Régine Steegers-Theunissen / Julian Little / Peter Mossey / Michele Rubini and others**

***Funding*.** The possibility of funding from the Canadian Institute for Health Research (CIHR) that was mentioned at the Workshop has come to fruition (**see APPENDIX IV**) and international development agencies such as IDRC. NIDCR has workshop on gene-environment interaction in September 2016. This is yet another example of the effectiveness and legacy of EUROCleftNet and should serve as a stimulus for ongoing collaboration. **ACTION: CIHR grant co-applicants and encouragement to ALL!**

Chairs: **Julian Little (rapporteur)**, Ron Munger and Ester Garne

APPENDIX I

HORIZON 2020 - Work Programme 2016 - 2017 Health, demographic change and well-being

SC1-PM-03-2017: Diagnostic characterisation of rare diseases

Specific Challenge: Rare diseases are diseases which affect not more than 5 per 10 000 persons in the European Union. It is estimated that rare diseases encompass between 6 000 and 8 000 different entities which affect altogether more than 30 million people in the EU. However, patient populations for individual rare diseases are small and dispersed, which makes international collaboration crucial. Despite the recent advances in understanding the molecular pathogenesis of these diseases, today many rare diseases still lack means of molecular diagnosis. An accurate molecular diagnosis is an essential starting point for the understanding of mechanisms leading to diseases as well as for adequate patient management and family counselling and it paves the way for therapy development.

Scope: The aim of this research should be to apply genomics and/or other –omics and/or other high-throughput approaches for the molecular characterisation of rare diseases in view of developing molecular diagnoses for a large number of undiagnosed rare diseases. Undiagnosed rare diseases may range from a group of unnamed disorders with common characteristics to a phenotypically well described disease or group of diseases with an unknown molecular basis. Genetic variability due to geographical distribution and/or different ethnicity should be taken into account as well as genotype-phenotype correlation whenever applicable. In addition, age, sex and gender aspects should be included where appropriate. This large-scale proposal should promote common standards and terminologies for rare disease classification and support appropriate bioinformatics tools and incentives to facilitate data sharing. Existing resources should be used for depositing data generated by this proposal. Molecular and/or functional characterisation may be part of the proposal to confirm diagnosis. The proposal should enable and foster scientific exchange between stakeholders from countries and regions with different practices and strategies of rare disease diagnostics.
The selected proposal shall contribute to the objectives of, and follow the guidelines and policies of the International Rare Diseases Research Consortium IRDiRC (www.irdirc.org).
The Commission considers that requesting a contribution from the EU of around EUR 15 million would allow this specific challenge to be addressed appropriately. Nonetheless, this does not preclude submission and selection of a proposal requesting other amounts.

Expected Impact: Providing better and faster means of high quality and clinical utility for the correct diagnosis of undiagnosed rare diseases for which there is no or unsatisfactory diagnosis available.
• Contribute towards the IRDiRC objectives.
• Foster dissemination of scientific results and knowledge exchange between stakeholders.
• Develop knowledge management strategies, with the view of facilitating models of care and access to the data gathered.
• Providing better knowledge for improved family counselling as well as to improve follow-up for patients and research initiatives.
• Gather a big number of patients with similar phenotypes to facilitate match making, to avoid duplication and to unravel a considerable number of diagnoses.
• Pave the way to the development of new therapies and for a better treatment outcome in rare disease patients.

Type of Action: Research and Innovation action

APPENDIX II

The need for cleft sub phenotyping in future studies

The role of specific genes and/or environmental factors in causation of nonsyndromic common oral clefts remains obscure, but a few have been implicated over the years such as IRF6, MSX1 and smoking (van den Boogaard et al., 2000;van den Boogaard et al., 2008; Jugessur et al., 2009;Mossey et al., 2009; Dixon et al., 2011). Within this multifactorial group, huge variations in cleft subphenotypes exist. These various cleft types originate from different developmental time periods (Vermeij-Keers, 1990; Vermeij-Keers, unpublished data) and therefore have different exposures to genes and environmental factors (Krapels et al., 2006).

It is accepted that there are fundamental differences between CP and CLP in anatomy, developmental chronology, epidemiology, heritability, sex predilection and in terms of aetiology most epidemiological and biological studies suggest that CL/P and CP have a differing genetic aetiology (Bernheim et al., 2006; Carinci et al., 2007; Rahimov et al., 2008; Grosen et al., 2010; Brito et al., 2012; Carroll and Mossey, 2012; Ludwig et al., 2012). Recent debate has been around the etiological separation of cleft of the lip with or without cleft palate (CL/P) into cleft lip only (CL) and CLP (Sivertsen et al., 2008; Birnbaum et al., 2009), or whether they should be regarded as a single phenotype being exhibited by varying severity (Marazita, 2012). The problem is however that if patients with different cleft subphenotypes are treated as a single group, and if aetiological differences do exist, linkage studies with genes and/or environmental factors may not be as fruitful as hoped (Dixon et al., 2011).

Despite considerable advances in our understanding of genetic risk loci contributing to CLP little is known about the aetiological factors that discriminate between sub-phenotypes, such as clefts of the hard and soft palate, CL and CLP, complete clefts and incomplete, left and right side, etc.. This is almost certainly hampering progress on investigations into aetiology, and the integration of detailed clinical phenotype, high resolution genotypes and information obtained from animal models will help identify novel genetic risk factors for CLP and new insights into CLP sub phenotypes.

The case of Van der Woude syndrome

One of the most common syndromic forms of cleft lip and palate is the Van Der Woude syndrome which is still the single most interesting locus for not only syndromic but also non-syndromic clefts and in a meta-analysis carried out by Jeff Murray and colleagues in 2006, this accounted for 12% attributable risk in cohorts of non-syndromic CLP patients. Mike Dixon described Van der Woude as one of the peridermopathies because of its effect on the peridermal layer and there is evidence that the pathogenesis is via adhesion molecules with epithelium being not "adhesion competent" in the presence of IRF6 mutations. Further investigation of this pathway has also identified that P63 down-regulates IRF6 and also *IRF6* was identified as a direct target of AP2alpha. It is interesting that AP2alpha is much more strongly associated with the CL subset (P=5×10−11) than in the CLP subset (P=0.0004), and not associated in the PALATE subset (P=0.79) (Rahimov et al., 2008).

Luijsterburg and Vermeij Keers argued for three basic OFC categories: cleft lip/alveolus (CL/A), cleft lip/alveolus and palate (CL/AP), and cleft palate (CP), and that it was logical to further sub-divide the common oral clefts into fusion defects, differentiation defects, or a combination of fusion and differentiation defects. However, elucidating pathways in their development is extremely difficult because of the multigenetic influences and their interaction with environmental factors (Jiang et al., 2006; Gritli-Linde, 2007; Juriloff and Harris, 2008; Dixon et al., 2011). If one could relate groups of cleft types to specific time periods, identification of specific known and unknown genes that are expressed during these periods may follow.

An interesting observation is that a genetic marker at the 15q31 region close to the GREM1 gene is associated with a cleft of the lip and cleft of the soft palate but with the hard palate intact - and it is an example of the possibility that there are other genotype - phenotype associations, but these have not been explored. Furthermore Heiko Peters has noted that in a mouse model with the GREM1 protein not expressed the palatal shelves do not develop and a cleft of the secondary palate results.

APPENDIX II

Another important issue in delineation of VDW into syndromic and non-syndromic IRF6 relates to the phenotypic feature of lower lip pits, and to clarify this delineation it is important to investigate further the association with genotype. Lina Moreno also noted that 15q22 and NOG1 signal stronger for CL while ABCA4, ARHGAP29, THADA and FOXE1 are more strongly associated with the CLP. She emphasised the need for a sub-phenotype meta analyses to further explore.

The study and significance of cleft microforms

Also, submucous and microform clefts (including orbicularis oris muscle defects) are often not registered in other classifications. However, these subclinical forms may be just as important for further delineating the pathogenesis, clinical genetics, and understanding of the epidemiology (Jugessur et al., 2009; Dixon et al., 2011). As shown by our findings, the pathoembryological sequelae can be described in any individual case.

The work of Mary Marazita in linking phenomics to genomics through parental microforms is an interesting area of enquiry with a significant public health benefit in that subclinical phenotypes may in future assist with the delineation of genetic predisposition and therefore assist the personalised medicine agenda. There may be variable expression of cleft risk genes and phenotypic manifestation in sub clinical phenotypes such as orbicularis oris muscle fibre discontinuity, non-cleft VPI, pleiotropy with additional expression of genes causing hypodontia or lip print patterns (whorls), developmental instability due to asymmetry (e.g. through SATB2) or increase in facial width. A recent candidate gene approach to the parents of children with clefts in Scotland looked at 122 SNPs across 30 candidate loci and ARHGAP29, THADA and 8q21.13 were all inversely associated (as yet unpublished data).

It is clear that a better understanding of the genetic background will pave the way for better understanding of the underlying biology, and to date GWAS has been a more valuable tool for biology than for clinical medicine. It is intriguing to note that not only are there differences in SNP signals between cleft sub-phenotypes, but also between populations and ethnic groups, and allelic dose response for various loci. It will be an imperative to replicate GWAS findings with larger samples and sub-phenotyping. There still remains a significant imbalance in the genetic and GWA studies carried out on the more prevalent CLP compared with the less prevalent CP. Elizabeth Mangold reported that 21 susceptibility loci for CLP and only one for CP have been published.

Considering further the secondary palate, the distinction of a microform is more obvious; the aetiology of CP and submucous cleft palate (SMCP) may share a common genetic aetiology with SMCP representing a mild manifestation of CP (Friedman et al., 2011). Alternatively, Reiter et al. (2015) indicated that CP and SMCP may represent differing genetic entities. Submucous cleft palate tends to be diagnosed later in childhood rather than at birth because of the presence of hypernasal speech: this diagnosis is confirmed by the identification of Calnan's triad (the presence of soft palate muscle separation in the midline with an intact mucosal surface, bifid uvula, and a notching defect of the bone at the posterior aspect of the hard palate). Genetic research has identified two susceptibility genes for SMCP (TGFB3 andMN1 [Meningeoma 1 gene]; Reiter et al., 2012). TGFB3 has also been associated with CL/P (Dixon et al., 2011), while MN1 appears to be unique to SMCP and CP (Davidson et al., 2012; Reiter et al., 2012).

UK epidemiological study of the variation in cleft severity (Complete v incomplete clefts)

A study analyzing data across four U.K. cleft registers found regional variation in terms of the relative proportions of cleft type (Carroll and Mossey, 2012). The results indicated a similarity in the proportions of cleft types in Scotland, Belfast, and Merseyside, where most cases were CP. This was in contrast to East England, where the major cleft type was CLP.

This study demonstrated many similarities in the distribution of different types of OFC but also some significant differences. Patients with CP and CLP in Scotland were more likely to have a cleft involving only a portion of the hard palate in comparison with those from the East of England, where patients with CLP were more likely to have a full-length cleft of the hard palate.

APPENDIX II

There was also an association between sex and the subphenotype severity of cleft in clefts involving the lip (i.e., CL and CLP). Females were significantly more likely to have a cleft involving the full height of the lip in isolated unilateral CL compared with males. In subjects with isolated CL, a complete cleft of the lip occurred in 39% of females and 25% of males. Logistic regression analysis of the data revealed that the differences in proportion of complete and incomplete clefts between males and females for these two groups of patients (CL and CLP) were significant (P= 0.0003). This further adds to previous research in which complete clefting was more likely in females with isolated CL or CLP (Meskin et al., 1968). In cases of unilateral CLP, males were significantly more likely to have a cleft involving the full height of the lip as compared with females (Carroll and Mossey, 2012).

Furthermore, a predominance for isolated cleft palate among females was noted by Freitas et al. (2004), whereas there was a male predominance for all other cleft subphenotypes. Further to this, the authors recorded that complete CLP was the most common subphenotype (37.1%), with isolated cleft lip being the least common (28.4%; Freitas et al., 2004).

In conclusion detailed clinical phenotype, high resolution genotypes and information obtained from animal models are now facilities almost universally available throughout the world. We therefore have the tools that will enable sub-phenotyping and this will help identify novel genetic risk factors for CLP and new insights into CLP sub phenotypes thus enabling ongoing progress on investigations into aetiology of all types of clefts of the lip and palate.

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APPENDIX III

The Seventh International Conference on Birth Defects and Disabilities in the Developing World (ICBDDDW) @ Dar es Salaam, September 2015

ABSTRACT

ABSTRACT | INTRODUCTION | CALL TO ACTION | CONCLUSIONS | ARTICLE INFORMATION | REFERENCES

As the Sustainable Development Goals are adopted by United Nations member states, children with congenital disorders remain left behind in policies, programs, research, and funding. Although this finding was recognized by the creation and endorsement of the 63rd World Health Assembly Resolution in 2010 calling on United Nations member states to strengthen prevention of congenital disorders and the improvement of care of those affected, there has been little to no action since then. The Sustainable Development Goals call for the global health and development community to focus first and foremost on the most vulnerable and those left behind in the Millennium Development Goal era. To maximize the opportunity for every woman and couple to have a healthy child and to reduce the mortality and severe disability associated with potentially avoidable congenital disorders and their consequences for the children affected, their families and communities, and national health care systems, we propose priority measures that should be taken urgently to address this issue.

CALL TO ACTION

ABSTRACT | INTRODUCTION | CALL TO ACTION | CONCLUSIONS | ARTICLE INFORMATION | REFERENCES

To maximize the opportunity for every woman and couple to have a healthy child; to reduce the consequences of potentially avoidable congenital disorders for those affected, their families, the health care system, and the wider society; and to promote the well-being of children who have a congenital disorder, there are many measures that should be taken urgently to address this issue. In this context and in order that no child is left behind, we pledge an initial focus that supports the following:

- Improving data quality:
    1. Building consensus on and widespread use of a standardized definition of congenital disorders, such as “abnormalities of structure or function, which are present from birth,”[3(p2)] to facilitate data comparison and ensure that the contribution of congenital disorders to the burden of disease is comprehensively represented.
    2. Establishing registries and surveillance systems and their integration, where possible, into existing data platforms to monitor the toll and risks of congenital disorders and evaluating the outcome of interventions for prevention and care. Consideration should also be given to the collection of pertinent data available from existing registries and surveillance systems in other countries.
- Reducing risk:
    1. Promoting family planning, allowing women and couples to choose when they have their first child, space their pregnancies, plan family size, define the ages at which they wish to complete their family, and reduce the proportion of unintended pregnancies.

2. Ensuring a healthy, balanced diet for girls and during a woman's reproductive years through an adequate intake of macronutrients (protein, carbohydrates, and fats) and a broad range of micronutrients. Special attention should be given to adding 400 µg of synthetic folic acid daily to the diet through fortification and supplementation while also promoting a diet rich in food folates, correcting iodine deficiency through fortification, and ensuring iron sufficiency through fortification, supplementation, and therapy for those with deficiencies.
3. Removing teratogenic substances from the diet, the most important of which is alcohol, and minimizing environmental contaminants in foods.
4. Controlling infections in women of reproductive age, including rubella and syphilis, and optimizing maternal health through detection and management of chronic illnesses associated with an increased risk of congenital disorders, such as type 2 diabetes mellitus and epilepsy, which require teratogenic medications.

- Improving care:
  1. Training physicians, nurses, allied health care professionals, and workers in the fundamentals of the recognition, causes, and care of children with congenital disorders and ensuring physical examinations of all newborns by trained health care professionals before discharge from the hospital or clinic.
  2. Aligning medical and social services to provide timely treatments for congenital disorders, including surgery, medications, dietary modifications, and rehabilitation services when needed.
  3. Providing emotional and practical support for parents to enable them to understand and manage their risk of congenital disorders and to help families in supporting the growth and development of children with congenital disorders.
- Empowering the public and civil society:
  1. Educating the public about congenital disorders and the steps mothers and fathers can take with their health care professionals to maximize the chances of a healthy pregnancy.
  2. Strengthening civil society—including patient and parent support groups, faith-based groups, and nongovernmental organizations—to advocate for improved prevention of congenital disorders and access to high-quality, family-centered patient care, including facilitating community and professional awareness and education and advocating for increased funding for research on the causes of congenital disorders.
- The following additional actions should be taken by countries as priorities and circumstances allow:
  1. Training physicians, nurses, and allied health care professionals in the essentials of medical genetics. This training should include diagnosis of common congenital disorders before and at birth; means of treatment where possible in the primary health care setting; knowing when to refer a patient for more specialized treatment; basic genetic counseling, including best practices in communicating unfavorable health information to parents; and support for families who have a child or are at risk of having a child with a congenital disorder. Genetic counseling aims to empower those who are counseled to make autonomous decisions regarding their health in ways that are consonant with their religious and ethical beliefs and circumstances and to support them in their decisions.

APPENDIX III

2. Establishing periconception medical services to assist women and their partners in attaining optimal physical and mental health and well-being and to facilitate a healthy pregnancy and delivery of a healthy infant. These services include rubella immunization; screening for the risk of genetic, partially genetic, and teratogenic congenital disorders; and mental health counseling, including identification and support for depression.
3. Implementing preconception or prenatal medical screening to identify women and couples at risk of having a baby with hemoglobin disorders; Down syndrome; blood type incompatibility; congenital sexually transmitted infections such as syphilis, human immunodeficiency virus, and herpes simplex virus; and structural malformations, particularly neural tube defects.
4. Establishing newborn screening to identify congenital hypothyroidism, phenylketonuria, galactosemia, sickle cell disease, glucose-6-phosphate dehydrogenase deficiency, and other metabolic disorders.
5. Supporting research into the diagnosis, prevention, etiologic factors, and treatment of congenital disorders to enable improved outcomes for children into the future.

The full report can be accessed as follows:
http://archpedi.jamanetwork.com/article.aspx?articleid=2529986

APPENDIX IV

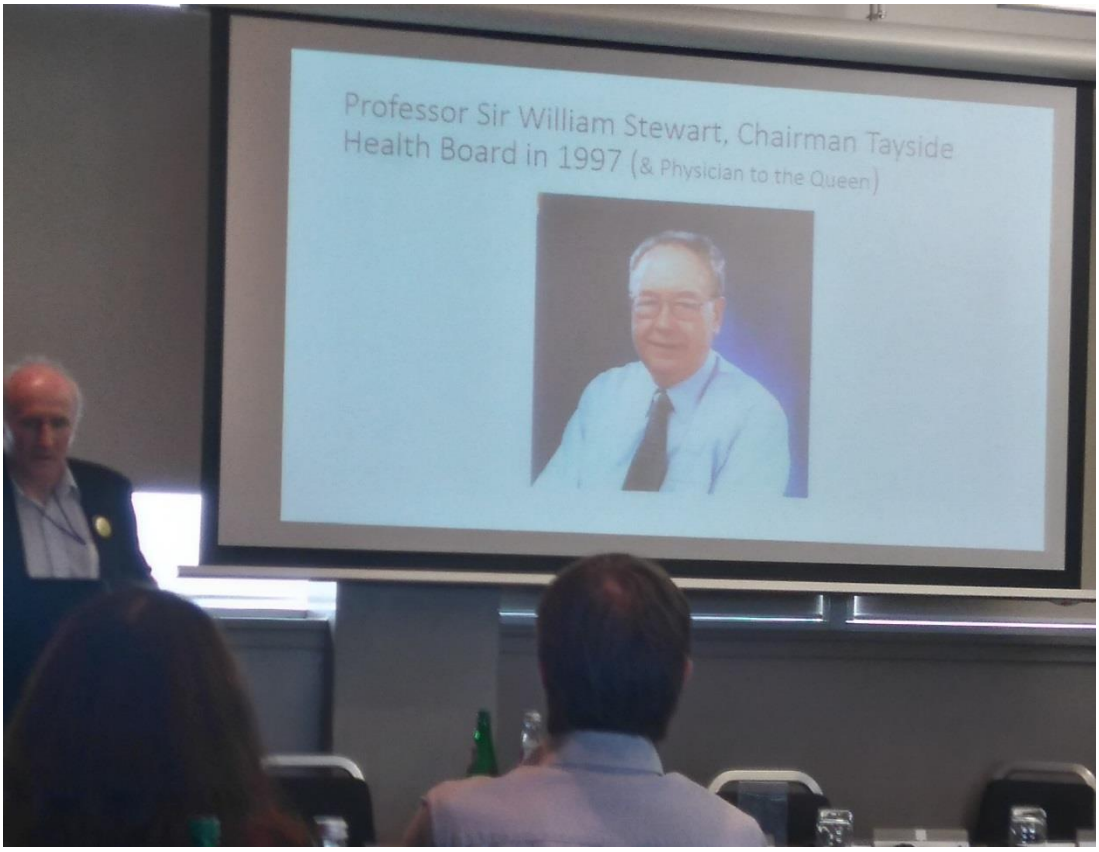
"Environment-specific maternal genetic effects in orofacial clefts"

The above grant led by Professor Marie-Helene Roy-Gagnon, genetic epidemiology and biostatistics at the University of Ottawa along with co-applicant and colleague Professor Julian Little, a world leader in Human Genome Epidemiology is in collaboration with Prof Drs. Michele Rubini (University of Ferrara) Peter Mossey (University of Dundee) and Régine Steegers-Theunissen (Erasmus MC, Netherlands), all experts on OFCs and PIs of the European studies.

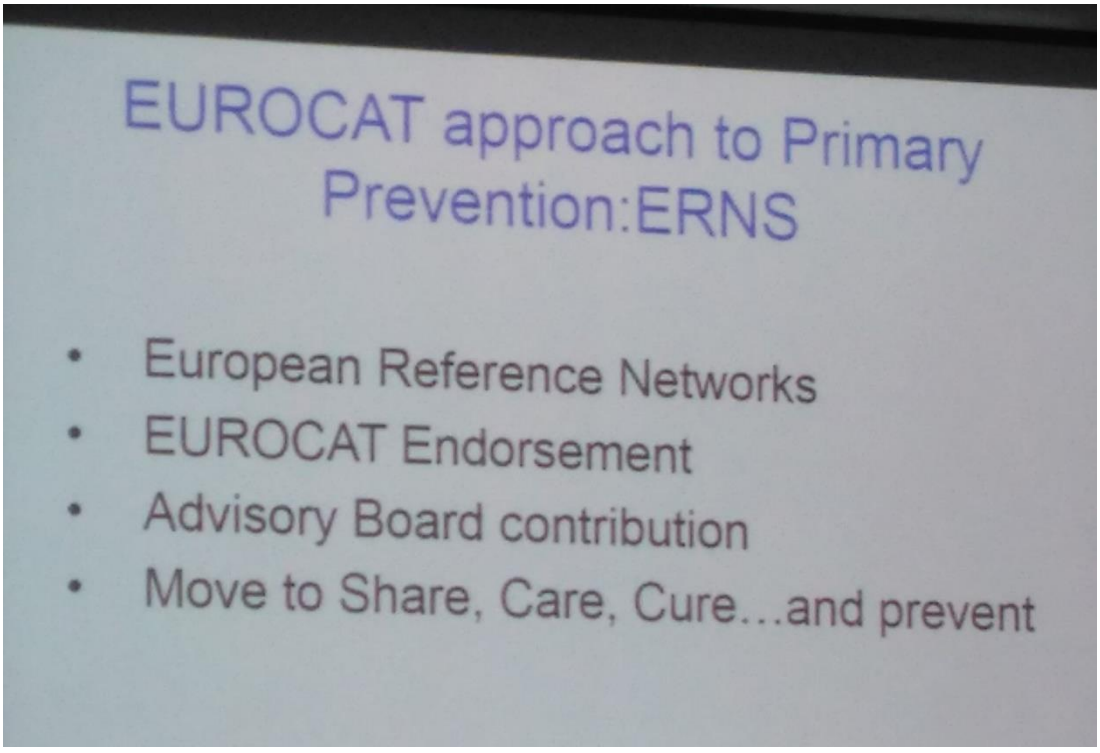
Orofacial clefts (OFCs) have been associated with increased risk of death from birth to age 55. OFCs clearly bring a substantial burden on affected individuals and their families and substantial costs to the health care system. Despite research efforts, the causes of OFCs are still largely unknown. Maternal environmental exposures in combination with maternal genetic effects can disrupt important processes involved in facial development. ***Hence, maternal genetic effects involved in OFC aetiology could act only, or more strongly, under specific environments***.

Our project will be the first to investigate maternal gene-environment interactions at the genome-wide level. The environment is more easily modifiable compared to genetic factors. Our study could thus lead to important public health applications in terms of designing effective, targeted prenatal interventions in Canada and elsewhere.

This fits extremely well with the discussions in Dundee with a very significant focus on interactions between genetic and environmental factors in relation to maternal exposures and maternal metabolics in relation to identifying risk of having an infant born with a cleft.



Opening remarks



EUROCAT presentation

Day 1, Session 3: Early career researchers take centre stage

Professor Peter Mossey addresses delegates at the civic reception

Delegate group photo, Dundee City Quay

Professors Rubini, Steegers-Theunissen and Mossey

Dundee icon: "Oor Wullie"

"Oor Wullie" and friends