ESF EUROCleftNet meeting in Bonn: 3rd July 2012

In attendance:
Carine Carels (Netherlands), Hans van Bokhoven (Netherlands), Bill Shaw (Manchester, UK), Anne Molloy (Ireland), Maarten Koudstaal (Netherlands), Gareth Davies (ECO, Netherlands), Peter Mossey (Dundee, UK), Mike Dixon (Manchester, UK), Sarah Jones (Dundee, UK), Concha Martinez (Spain), Michele Rubini (Italy), Elisabeth Mangold (Germany), Markus Noethen (Germany), Heiko Peters (Newcastle, UK), Stephan Sonntag (Polygene, Switzerland), Jayne Wright (Syngenta, UK) and Chris Lane (3dMD, UK).

The following provides a report on presentations by EUROCleftNet scientists on ongoing research and future opportunities – with a view to illustrating where academia and industry could usefully work together.

Part A: Report of presentations

1. Peter Mossey: Outline of the aims and objectives of this EUROCleftNet meeting with potential industrial collaborators.

Peter Mossey outlined the rationale for involvement of industry in research as follows:

1. **Involvement of industry,**
   - SMEs and user organisations as an emerging theme in European research;
   - Academia and industry can share research skills and professional experience for mutual benefit.
   - Development of new therapies for treatment and prevention of disease.
   - Emphasis on the training of the future generation of European scientists.

2. **Why cleft lip and palate?**
   - Apart from this being the most common craniofacial birth defect, clefts and craniofacial anomalies combined affect up to 1 in 1000 births with geographic and ethnic variation and some of the highest rates are among the poorest and most populous and countries in the world.
   - Clefts are regarded as a classical example of health inequality where mortality and morbidity rates are greater in poorer countries.

3. **Raising the awareness of orofacial clefts;**
   - At Beijing in 2005, the International community at the 3rd International meeting for Birth Defects and disabilities in the developing world that stated:

- Mortality due to birth defects and genetic / congenital conditions account for an increasing proportion of infant mortality.
- 30-50% of peri-natal deaths and 20-30% of global infant mortality are due to birth defects.
- Almost 50% of these children who die in the peri-natal period have craniofacial anomalies and this amounts to approximately 250,000 clefts per year globally.
- The scandal of invisibility – in many of the poorer parts of the world there is no infrastructure to collect birth defects data.
- There are substantial health consequences for survivors, families and society.

4. The scandal of health inequality

- There is a belief that effective health care and prevention will require costly high technology interventions i.e. a very significant burden of care.
- This misperception of costly care prompts governments to ignore these defects in the belief this may draw funding from other high priority maternal and child health efforts.
- The fact is that prevention would be cheap and highly cost effective.

5. Aetiology of non-syndromic cleft lip and palate

- In an outline of aetiology both genetic and environmental factors were discussed. Amongst environmental factors were smoking, alcohol, hyperthermia, hypoxia and a range of maternal medications and drugs while preventive factors might include folic acid, vitamin B6, B12 and trace elements such as zinc may also play a role.
- The genetic aetiology has been informed in recent years by GWAS and along with other gene discovery efforts such as linkage and association studies, animal models of palatogenesis, human monogenic syndromes with clefting as part of the phenotype and biological plausibility surrounding known factors in developmental and nutritional pathways.
- The jigsaw representing genetic aetiology and genetic predisposition to environmental factors is becoming elucidated.
- The geographic variation evident in the results of GWAS was highlighted and emphasised the need for sample collection in countries throughout the world. It was also clear that there were very significant subphenotypic difference and typical clefts which also require further analysis as we seek further clarification on genetic aetiology.

6. Role of the World Health Organisation

- The procedure from acceptance of craniofacial anomalies as a major issue in terms of global health was outlined and it was noted that orofacial clefts now have a relatively high profile among non-communicable diseases in the eyes of the WHO.
- This culminated in the inclusion of clefts in the recommendations of the 63rd World Health Assembly in April 2010 and we should build on this to ensure that member states are aware of this new emphasis on birth defects and craniofacial anomalies.

7. Research priorities

The aims and objectives of EUROCleftNet included a list of genetics research priorities as follows:

i. Ongoing identification of non-syndromic orofacial cleft gene variance through GWAS and strategic gene sequencing;
ii. Test gene-gene and gene-environment interactions through animal models and perhaps population based studies;
iii. Identify functional gene variants through functional genomics studies;
iv. Measure tissue specific epigenetic profiles – perhaps using tissues discarded at cleft surgery;
v. Develop a panel of gene variants that might be screened for NSOFC risk assessment in the clinical setting;
vi. Develop integrated models to assess risk of occurrence and recurrence of clefts;

8. Where does industry fit?

- In the Salzburg workshop report where the liaison with industry was discussed was outlined providing some information on where industrial partners could be contributors to future research.
- This was divided into three themes: diagnostic, clinical management and preventive.

9. The UK Cleft Collective

- The recent press release from the UK announcing support from the Healing Foundation for research in cleft lip and palate in the UK was mentioned.

- In the cleft collective press release it was stated that the three most important questions that a parent of a child with a cleft might ask are;

- What has caused my child's cleft?
- Will my child be okay?
- What are the best treatments for my child?

- Up to 5000 children and their families are being recruited to a birth cohort study in the UK co-ordinated by the Universities of Manchester and Bristol.

Recent correspondence with the Healing Foundation and the co-ordinators of the Manchester and Bristol cleft research efforts has indicated that links with EUROCleftNet to encourage a European perspective on the Healing Foundation supported initiative would be very well received.

2. Bill Shaw: "European research in OFC: ScandCleft, EUROCleft, EUROCRAN, TOPS and now EUROCleftNet**"**

Bill Shaw provided a history of cleft research in Europe dating back to 1986 when the first attempts at bringing together a multi-disciplinary inter-centre study of cleft outcomes was initiated between centres in Scandinavian countries, the Netherlands and the UK. Preliminary outcomes revealed very significant differences in outcomes and among the conclusions were that cleft care was generally disorganised and uncoordinated with too many centres and too many surgeons resulting in inability to

carry out audit (quality assurance) and the need for research initiatives such as inter-centre trials was highlighted.

ScandCleft: in 1997 following consensus agreement that there were too many variations on primary cleft surgical protocols, a randomised surgical trial was designed led by Professors Gunvor Semb and Bill Shaw and the research was facilitated by an EU Biomed grant which also led to the development of the first large inter-centre network across Europe who developed between 1996 and 2000 a consensus document entitled "Standards of care for cleft lip and palate in Europe". This led to improved communication, assessment of infrastructure and a co-ordinated approach to research. This initiative also enabled participation for countries in the newly associated states in the EU.

WHO consensus meetings: in 2000, an NIH grant facilitated the setting up of a series of WHO consensus meetings and the EUROCleft recommendations on strategies for inter-centre comparison, collection of standardised data and initiating inter-centre research were adopted by the WHO.

EUROCRAN application: two essentially separate initiatives were emerging in cleft research in Europe – one being the EUROCleft research efforts to improve quality of care, but in parallel a European Science Foundation (ESF) funded network of scientists involved in cleft research was established in a network entitled "Gene – environment interaction in an early human development: a demonstration project in orofacial clefts". It was agreed that these two separate research groups should joint forces to apply for an EU Framework 5 programme entitled EUROCRAN and this application was successful.

EUROCRAN output: The enabled the continuation of the quality of cleft care research and alongside this, DNA samples were taken from trios to establish an EU biobank with over 1039 trios from 11 countries collected. The EUROCRAN network research resulted in significant advances in developing cleft care protocols and reorganisation of cleft services in a number of countries (including the UK which reduced the number of centres from 57 to 12 in 2002) and the ongoing dialogue has continued to include the WHO with two WHO Collaborating Centres established – one at the University of Manchester and one in the University of Dundee.

Dissemination of the EUROCRAN message: new inter-centre comparisons were initiated in countries such as Brazil, Japan, India and the USA – all collecting standardised outcome information according to WHO protocols.

Timing of palatal surgery for CP (TOPS trial): in 2010 an NIH grant enabled the establishment of a further randomised trial of palate surgery at aged 6 months versus aged 12 months. Centres in Scandinavia, Brazil and the UK will attempt to recruit 650 infants with non-syndromic isolated cleft palate followed up until 5 years with measurement of outcome such as facial growth and speech.

Future research: a few ideas were presented about possibilities for future research:

- Improving naso-labial assessment, use of 3D imaging in future outcome assessment and 4D imaging to assess facial animation is also required.

- Following a preliminary EUROCRAN study on distraction osteogenesis, there may be value in a randomised trial on distraction osteogenesis versus osteotomy for children with maxillary growth deficiency as a result of cleft repair.
- Tissue engineering including scar free wound healing and improved or alternative methods for alveolar bone grafting in unilateral or bilateral clefts where there is a residual bony cleft deficiency are ripe areas for future research and these present opportunities for the involvement of industrial partners.

3. Elisabeth Mangold / Markus Noethen: GWAS and related OFC research in Bonn.

Markus Noethen gave a brief background of the origins and remit of the Life and Brain Centre which is a "spin-out" SME of the University of Bonn. It provides an excellent example of a multi-disciplinary approach to research with industrial involvement as combined expertise in genomics, transgenics cellomics and cognitive neuroscience has resulted in a research Centre of Excellence.

This environment has provided the genomics platform for Elisabeth Mangold's very successful cleft lip and palate GWAS studies (in collaboration with EUROCRAN and ITALCLEFT); and the exemplary liaison between academia and industry was an inspiration to our discussions at this EUROCleftNet forum.

Elisabeth Mangold gave an update on the progress of her CL/P genomics research as follows

nsCL/P - prevalence in different ethnicities
Asians ~ 1 : 500
Europeans ~ 1 : 700
Africans ~ 1 : 2500

nsCL/P – prevalence in Europe
Prevalence among live births in Central Europe ~ 1 : 1.000
male : female = 1.7 : 1
Risk for sibs of affected 1 : 20 - 1 : 25
Concordance rate monozygotic twins ~ 60%, dizygotic ~ 10%
Heritability > 90%
→ complex genetic background with several interacting causative genes and environmental risk factors

Underlying risk factors - hypothesis
Rare high penetrance mutations
Common genetic variants → genome-wide association studies
Environmental risk factors

Table 1: nsCL/P susceptibility genes/loci since GWAS

Locus/Gene	Support from
1p36	**GWAS meta-analysis**
1p22	**1 GWAS + GWAS meta-analysis**
IRF6	**Candidate gene study + 1 GWAS + Meta-analysis of linkage studies + many replication studies**

2p21	**GWAS meta-analysis**
3p11	**GWAS meta-analysis**
8q21	**GWAS meta-analysis**
8q24	**4 GWAS + several replication studies + GWAS meta-analysis**
FOXE1	**Meta-analysis of linkage studies, 1 replication**
10q25	**1 GWAS + GWAS meta-analysis + 2 replication studies**
13q31	**GWAS meta-analysis**
15q22	**GWAS meta-analysis**
17q22	**1 GWAS + GWAS meta-analysis**
20q12	**1 GWAS + GWAS meta-analysis**

Note: Those studies highlighted in red were conducted using CLP samples derived from collaboration with other EU countries.

nsCL/P – current etiological hypothesis
single genes / genetic factors of major effect

potentially modified by
- a polygenic background and/or
- environmental factors

Next steps, outlook
Causative variants at the nsCL/P susceptibility loci?

Further nsCL/P susceptibility loci/genes
- in Europeans?
- in other ethnicities?

Genes/loci underlying
- sub-phenotypes (cleft lip only, etc.)
- cleft palate only?

Any gene-gene and gene-environment interactions?

Do clefting risk alleles influence measures of the "normal" face?

Publications referred to:

- **Christensen** K, **Fogh-Andersen** P. Cleft lip (+/- cleft palate) in Danish twins, 1970-1990. Am J Med Genet. 1993 Nov 1;47(6):910-6.
- **Sivertsen A, Wilcox AJ, Skjaerven R, Vindenes HA, Abyholm F, Harville E, Lie RT.** Familial risk of oral clefts by morphological type and severity: population based cohort study of first degree relatives. BMJ. 2008 Feb 23;336(7641):432-4. Epub 2008 Feb 4
- **Grosen D, Bille C, Pedersen JK, Skytthe A, Murray JC, Christensen K.** Recurrence risk for offspring of twins discordant for oral cleft: a population-

based cohort study of the Danish 1936-2004 cleft twin cohort. Am J Med Genet A. 2010 Oct;152A(10):2468-74.

- **Birnbaum, S., et** al (2009). Key susceptibility locus for nonsyndromic cleft lip with or without cleft palate on chromosome 8q24. *Nature Genetics* 41 (4), pp. 473-477.

- **Mangold, E., et al** (2010) Genome-wide association study identifies two susceptibility loci for nonsyndromic cleft lip with or without cleft palate. *Nature Genetics* 42 (1), pp. 24-26

- **Beaty et al., (2010)** A genome-wide association study of cleft lip with and without cleft palate identifies risk variants near *MAFB* and *ABCA4*
- *Nat Genet; 42 (6): 525-529*

- **Ludwig et al.,** (2012) GWAS meta-analysis paper. Nat Genet, in press

4. Michele Rubini: Post-GWAS Functional Genomics Research

GWAS: The amazing results of the recent genome-wide association studies are indicating the gene whose common variants mainly contribute to the pathogenesis of non-syndromic orofacial clefts (nsOFCs), and providing information on cleft aetiology that was just unthinkable a decade ago.
However, the identification of the actual functional gene variants underlying the increased risk of nsOFCs remains unaccomplished.

Functional genomics: is aimed to provide information to fill the gap between genotype and phenotype, and to identify the molecular endo-phenotype at the base of complex diseases, as nsOFCs. Unlike genomics, that focuses on the static aspects of the genomic information such as DNA sequence or DNA structures, functional genomics mainly focuses on dynamic aspects such as gene transcription, translation, protein-DNA and protein-protein interactions.

Technologies: The identification of the functional effects of mutations and polymorphisms is top-priority for understanding nsOFC aetiology, and technologies as EMSA (electrophoretic mobility shift assays), ChIP (chromatin Immunoprecipitation), or reporter gene expression in transgenic embryos are promising tools for the identification of functional components of the interaction network at the base of nsOFC pathogenesis.

However, so far few studies have been carried out to identify functional gene variants that contribute to increased risk of nsOFC, and to discover the molecular endo-phenotypes at the basis of failure in lip and/or palate fusion during embryogenesis.

Interplay between Academia and Industry could contribute much the scientific advances in this research area, and possibly provide knowledge for improved risk assessment, and measures for better primary prevention. In particular, Industry could significantly contribute in the fields of sub-phenotyping (3D photography,

cephalometrics, sonography), prevention of occurrence or recurrence (supplementation with micronutrients), or risk assessment (diagnostic microchips).

5. Concha Martinez: Animal models research in genetics / gene environment interaction and quality of care.

Research area 1: Genetics / genomics / transgenics

Animal models research in genetics and gene environment interaction with a view to advancing knowledge in the field both in quality of care and aetiology / prevention.

- Group formed by biologists, dentists, physicians, surgeons.
- Collaboration with nutritionists (Gregorio Varela`s group) and vets.

Techniques used include: Organ cultures, PCR, in situ hybridization and immunohistochemistry

Results obtained in this research area:

The cleft palate presented by the *Tgf-b3* null mutant mouse is caused by:

- Decreased cell proliferation in the mesenchyme and increased in the medial edge epithelium of the pre-adhesion palatal shelves.
- Decreased adhesion of the opposing medial edge epithelia produced by the altered presence of some cell adhesion and extracellular cell matrix molecules
- Altered cell intercalation, cell death and epithelial-mesenchymal transformation in the midline epithelial seam
- This may occur in part because of the unbalanced presence of several growth factors due to the absence of Tgf-b3.

Publications referred to:

- **Martínez-Álvarez et al., 2000, Dev. Biol**., 220: 343-357.
- **Martínez-Álvarez et al., 2000, Int. J. Dev. Biol**., 44: 331-335.
- **Tudela et al**., **2002 Int. J. Dev. Biol.** 46: 333-336.
- **Gato et al., 2002, Dev. Biol.** 250: 393-405.
- **Martínez-Álvarez et al**., **2004, Dev. Biol.** 265: 207-218.
- **Martínez-Sanz et al., 2008, Differentiation.** 76: 417-430.
- **Murillo et al., 2009, Differentiation.** 77: 209-220**.**
- **Del Río et al., 2011, Cells, Tissues and Organs**. 193(3): 135-150.

Research area 2: Effects of folic acid on the cleft palate phenotype

Experimental model:

- Wild type mouse on a folic acid **deficient** diet (FAD)
- *Tgf-b3* -/- mouse on a folic acid **supplemented** diet (FAS)

Results obtained in this research area:

- Cleft palate appearance in the progeny of mouse females under a FAD diet for 8 weeks or longer
- Alteration of all the mechanisms leading to palatal fusion in mice with only 2 weeks of FAD.
- Reduced *Tgf-b3* expression in the FAD mice (2 and 8 weeks).
- Addition of TGF-b3 to 2 week FAD mouse palatal shelves normalises all the altered palatal mechanisms.

- 20x FAS *Tgf-b3* null mutant mice of both MF1 and C57 strains show less severe CP, improved mesenchymal cell proliferation and palatal shelf adhesion.

Publications referred to:

- **Maldonado et al**, **2011,** Cells, Tissues & Organs, 194(5), 406-420.

- However since several malformations of other organs appear with such high doses of folic acid, the following is planned.
- Reduced FA dosage (8mg/Kg BW) and use L-5-methyltetrahidrofolate (supplied by Merck), analysing its effects in the CP appearance and possible consequences in other organs (eye, heart, bone).
- We have applied for funds at a national level in collaboration with Gregorio Varela's team (U. San Pablo CEU. Madrid - study of biochemical markers).
- This team will also analyse the nutritional status of the families of children with cleft lip and palate in Spain (collaboration with associations).

Research area 3: Looking for new alternatives for cleft palate repair

Experimental model: Old Spanish pointer dog with congenital cleft palate

Publications referred to:

- **Martínez-Sanz et al., 2011**, Laboratory Animals, 45: 70-80.

Preliminary results obtained in this research area:

- Reduced transversal palatal measures in dogs with congenital cleft palate regarding the controls.
- Longitudinal palatal measures less altered.
- Success with the injection of a hyaluronic acid based hydrogel (AuxiGel®, TERMIRA) with BMP2 in the cleft palate edges, causing their approach to the midline and bone formation. This is followed by removal of a strip of mucosa at each side and suture.
- This is a promising alternative to palatoplasty.

Still to be analyzed: Craniofacial growth of the Injection/Adhesion and Palatoplasty groups in comparison with that from cleft control and normal palate groups.

Future research plans:

- **To trial this technique in humans:** oro-nasal fistulae, alveolar clefts and (possibly) primary repair of certain secondary clefts. This will be done in the HOSPITAL U. GREGORIO MARAÑÓN by surgeons: Beatriz González-Meli and Beatriz Berenguer (approval for the use of the AuxiGel in children still pending).

- **To avoid the morbidity of bone grafts form the iliac crest ABG,** we (UCM) will analyse the possibility of obtaining sufficient new bone at a local level by

injecting a fixed amount of hydrogel + BMP2 under the vestibular periosteum of our already operated on dogs.

6. Carine Carels / Jo Huiqing Zhou / Hans van Bokhoven: Research in cleft orthodontics and alveolar bone grafting.

Genetic and epigenetic disease mechanisms of CL/P: Using state-of-the-art functional genomics techniques, previous work on the identification of **transcription factor p63 binding sites** has shown that p63 binding sites of which many are localized in the non-coding regions function as regulatory elements to control expression of genes important for ectodermal development including IRF6 relevant to CL/P. Disruption of these binding sites is relevant to the pathogenesis of CL/P.

In our current project, we have collected a number of CL/P families for genetic studies, which increases the chance to identify genetic variations with Mendelian inheritance. We will first perform **exome sequencing** to identify mutations in coding regions, followed by sequencing of mutations in the non-coding regulatory elements using p63 and IRF6 binding sites. We are currently developing such enrichment **arrays** that can capture these regulatory elements. Once established, this technology can be shared with other partners in the consortium. Furthermore, the identification of **non-coding regulatory elements** will assist to interpret Genome-wide association studies (GWAS) of non-syndromic CL/P.

Alveolar bone grafting: the expertise in EUROCleftNet provides a unique opportunity to carry out research into alternatives to autogenous bone grafts. The dog model provides in vivo model for clinical trials of alternative bone graft, the tissue engineering companies such as XPand Biotechnology (Netherlands) who have developed osteo-inductive calcium phosphates particularly for use in dental and cranio-maxillo facial applications, would have an opportunity to have trial their products and the TERMIRA group are also interested in regenerative medicine applications with a particular interest in bone and cartilage defects. One of their marketable products in hydrogel scaffolds which could also be used as ABG substitutes in animal experiments in phase 1 or phase 2 clinical trials. This would compliment the clinical and research expertise within the EUROCleftNet group.

In addition to this the technology for volumetric measurement has been developed in the University of Dundee and it will also be important to do 3D and 4D imaging of craniofacial and naso-labial morphology in relation to alveolar bone grafting.

7. Mike Dixon: New tools for analyzing cleft lip/palate

Genetics of non-syndromic clefting
Inheritance patterns are not well-defined
Most cases are sporadic
Reduced penetrance
Heterogeneous disorder, multiple genes
Evidence for a major susceptibility gene
Influenced by environmental factors

Overview

Multiple genes now implicated
Superb animal models
Environmental epidemiology convincing
Genome-wide approach successful and expanding
Resources for collaboration critical
Good phenotypes needed
Integrated approach essential

Next Generation Sequencing

Transcriptomics

- mRNA expression profiling
- De novo transcriptome assembly
- Small RNA discovery

Functional Genomics

- Chromatin immunoprecipitation
- DNAse I hypersensitivty

Genomics

- Exome sequencing
- Genome sequencing
- Targeted re-sequencing

Van der Woude syndrome (VWS)

- VWS is an autosomal dominant orofacial clefting disorder
- VWS is the most common form of syndromic clefting
- VWS locus mapped to human chromosome 1q32-q41 and shown to be due to mutation of *IRF6*
- PPS is allelic with VWS

Treacher Collins syndrome (TCS)

- Down-slanting palpebral fissures
- Colobomas
- Hypoplasia of the zygomatic complex
- Mandibular hypoplasia
- Abnormalities of the external and middle ear
- Conductive hearing loss
- Cleft palate

8. Heiko Peters: In vivo modelling of gene-environment interactions

- Heiko emphasised the unique advantages of genetically amenable mouse model systems to test specific gene-environment interactions believed to be involved in craniofacial clefting.
- Several risk factors, in particular anti-convulsant drugs, induce transient phases of hypoxia by causing arrhythmia of the embryonic heart. Direct evidence for cleft lip and palate being caused by hypoxic stress in the lip or palate-forming embryonic structures is however missing.

- Possible ways of approaching this important gap of knowledge by generating novel mouse models were discussed at the meeting. It became clear that expertise available at Polygene (Switzerland) would not only be necessary to carry out these projects but may also generate mouse models that could be of interest for the wider research community.
- Specifically, it is envisaged to develop a mouse model expressing Cre recombinase in the embryonic structures forming the lip and palate. This can, for example, be used to inactivate Hif1 (acting as a master regulator controlling the cellular response to hypoxic stress) only in the developing lip or palate. This may be complemented by developing another mouse model allowing labeling of hypoxic cells in vivo.
- Together, these model systems will greatly facilitate systematic analyses of gene-gene and gene-environment interactions and provide an in vivo platform to test therapeutic interventions aimed at preventing orofacial clefting.
- As the project will involve substantial non-staff related costs it may not be suitable for funding through the Marie Curie Funding schemes and seeking alternative routes of funding might be more successful.

9. Gareth Davies: ECO perspective: Research impact and translation to the "consumer".

The European Cleft Organisation is an SME in the context of any application. They are a non-profit organisation promoting the advancement of medical expertise and standards of care in the treatment of cleft lip and palate in Europe. Core beliefs are:

- ☐ equality of access to care
- ☐ multidisciplinary approach
- ☐ involvement of local health professionals
- ☐ user input in decisions around management of care
- ☐ promotion of family-to-family support
- ☐ support for collaborative research into the aetiology of clefts and prevention thereof

Programme areas

- ☐ **Country Projects (to date, Bulgaria and Romania)**
  Work with identified local cleft teams and help put in place informed referral networks to ensure that every baby born with a cleft, and their family, receives timely support and treatment by a specialist multidisciplinary team. We promote the involvement of patient groups in delivery of best practice care

- ☐ **Development of an agreed set of protocols across Europe** management of babies born with clefts. The aim is to provide agreed models of care that can be used as guidelines in countries where currently no protocols exist. In Bulgaria of parents of babies born with clefts are advised to abandon them because no post natal protocols exist. Working with CEN in Brussels and BDS in Bulgaria

- ☐ **Education /Training/Audit and Research**
  Promotion of good practice, exchange of ideas and furtherance of knowledge amongst health professionals and self help patient support groups across the

whole of Europe. ECO is on steering committee of the European Science Foundation *EUROCleftNet* project

European Cleft Gateway

- European Cleft Organisation to host new cleft resource Gateway

- The European Cleft Gateway will be a new portal on our website which will
  - list all the cleft teams and patient support groups in Europe,
  - record all ongoing research projects into the causes and treatment of clefts
  - in time, provide a full online library resource for users and clinicians alike.

- The project is being done on behalf of EUROCleftNet an ESF-funded Europe-wide network of researchers and clinicians working together to support investigations into the causes of cleft lip and palate and improve treatment outcomes.

SME partner – the benefits

- Collectively we can make a real difference
- We can influence research priorities
- Our own profile is increased
- Opportunities for future partnerships though networking

Research – a user focus

- Users not subjects of research but participants, involved at every level
- Prioritising what are the needs of the patient
- Dissemination of results
- What are the practical applications of the outcome of research that will benefit user

Industry – a driver

- Industrial partners can be the key in ensuring research outcomes bring tangible benefits to the user
- Promotion of lifestyle choices (e.g. via SMS messaging)
- Marketing of dietary supplements (e.g. multivitamins)
- New medical products can help lessen the burden of care on the patient

Some statistics:

- Within 27 EU states, population just under 500 million
- Estimated total number of people living with a cleft in the EU: 715,000
- Equivalent to a town nearly 4 times bigger than Geneva

Part B: Funding opportunities

Peter Mossey: EUROCleftNet funding opportunities for European collaborative research

A brief overview of funding opportunities in Europe was discussed under 3 headings:

1. **The European Research Council (ERC)**
2. **FP7 Innovation Programme (2013)**
3. **Marie Curie opportunities – The "People" and "Co-operation" Programmes**

1. ERC opportunities

It was felt that the only ERC opportunity of interest would be the "synergy" grant which aims to bring together a group of between 2 and 4 excellent principal investigators to pursue a large scale frontier research project of their choice and requires that partners within an inter-disciplinary team would spend significant core time together at the same physical location.

2. FP7 Innovation Programme (2013)

The accompanying PowerPoint presentation gives a flavour of some of the items in the "Health" calls and the deadlines, most of which are in October / November 2012.

It is noteworthy that one of the major objectives of FP7 2013 was as follows ***"improving the health of European citizens and increasing the competitiveness and boosting the innovative capacity of European health related industries and businesses while addressing global health issues".***

"emphasis will be put on translational research, the development and validation of new therapies, methods for health promotion and prevention".

"including promotion of child health, healthy ageing, diagnostic tools and medical technologies as well as sustainable and efficient health care systems".

Involvement of SMEs:
The following statement also confirms that industrial involvement is an essential component… ***"with its many broad, bottom up topics suited for SMEs, this work programme will contribute very significantly to the European renewal – and over 20% of the budget is ring fenced for SMEs and industry".***

The calls for FP7 that may be of interest to EUROCleftNet are outlined in the attached PowerPoint presentation (**EUROCleftNet funding.ppt**).

3. Marie Curie opportunities

Overview: Marie Curie actions

Host actions	Individual Actions

Initial Training Networks (ITN) *Including:* *Innovative Doctoral Programmes (new)* *European Industrial Doctorates (new)* Industry Academia Partnerships and Pathways (IAPP) International Research Staff Exchange Scheme (IRSES)	Intra-European Fellowships (IEF) International Incoming Fellowships (IIF) International Outgoing Fellowships (IOF)

Funding model that was highlighted as a possibility for submission of a research grant application with the inclusion of industrial partners was the Marie Curie People Programme Initial Training Networks (ITN Scheme). It was felt that the main advantages of this scheme are that:

1. It is a bottom up approach and therefore we have the opportunity to design and engineer an application that includes our academic and industrial partners.
2. We have submitted an application before and have had favourable feedback (with the exception of insufficient industrial involvement).
3. It is flexible with regard to the mobility of the trainees
4. It provides PhD studentships for 36 months plus 10% overheads
5. There are consumable costs at the rate of 1800 Euros per researcher month
6. With the assistance of our previous application, it should be possible to meet the November 2012 deadline

It was agreed at the meeting that we should aim for a submission to the Marie Curie ITN but first to find out more about the nature of the mobility of students and to optimise involvement of industry in the work packages that we select.

Note: Appendix II outlines the currently interested industrial collaborators

Part C: Breakout Workshops

WORKSHOP I:

1. **Opportunities for Collaborations in CLP Genetics / Genomics:**

Delegates present: Carine Carels, Mike Dixon, Sarah Jones, Elisabeth Mangold (+PhD student Anne), Markus Noethen, Heiko Peters, Michele Rubini, Stephan Sonntag, Jayne Wright, Jo Zhou.

Summary:
The group started with a discussion on the current status and future directions post GWAS. There was some concern that the number of implicated DNA loci made it difficult to know where to start. However, it was concluded that:

- **Number of loci for CLP likely to increase rapidly**
- **Move from association to causation**
- **Identification of regulatory elements important – computational and experimental**
- **Environmental aspects are difficult to define**
- **Define what the information will be used for**
- **Is it possible to inform people of risk depending upon their genetic predisposition?**

Mike Dixon summarised the importance and validity of the current multidisciplinary and range of different experimental approaches, identifying targets and then carrying out functional analyses was providing significant insight. A good recent example is that the effect of mutations in the VAX1 transcription factor has an immediate impact on genetic counselling.

1. **To understand developmental biology & pathways**
2. **Resultant data could impact on risk analyses but probably not for pre-natal diagnosis**
3. **Eventually will result in a greater understanding of gene-environment interactions**
4. **The prevalence of risk alleles conserved in the population is important from evolutionary perspective**.
5. **Genotype / phenotype correlation (in GWAS), tissue samples (lip and palate) to look for regulatory elements and reporter gene assays were discussed.**
6. **Should epigenetics, and should copy number variation (CNV) analysis (associated with susceptibility or resistance to disease) be investigated?**

Academia / industry liaison
There was some discussion about how attractive collaboration with academia was to industrial partners. It was thought that smaller companies could benefit from placements of PhD students and post-docs who would gain industrial experience making them suitable future employees.

Companies may be interested in some of the transgenic mouse models for CLP if this had business benefits and if they had potential additional applications e.g. hypoxia model and cancer biology.

Jayne took a more holistic approach and her point of view was that understanding pathways of disease and the involvement of environmental factors would be of interest to her company (Syngenta) even if they were not directly involved.

Intellectual property rights collaborating outside academia is not perceived as a problem in that Universities were now 'switched on' regarding IP, and biotech companies such as Agilent or new companies such as DTID may be interested in collaboration on designing arrays but it is important to consider that bespoke arrays can also be obtained.

Jayne thought that being involved in a training network would be a positive thing for a company and Jo added that exchange of personnel would be good for experience, training etc, especially for PhD students.

- **Synergies (and differences) between academia and industry are clear but may require more general applicability (mouse models) or extension from existing work. Motivation for industry are varied**
- **IP issues may arise but should be handled on a case-by-case basis**
- **Possibilities for training are there e.g. GWAS to function, general training, exome sequencing**

Grant application:
It was noted that the closing date for the Marie Curie applications (November 2012), was rapidly approaching and a joint application should be prioritised. Mike Dixon added that some of the ideas discussed may also be applicable to for the EU framework programme for research and innovation – Horizon 2020.

WORKSHOP II:

2. Opportunities for Collaborations in CLP Treatment:

Delegates: Bill Shaw (UK), Gareth Davies (ECO), Concha Martinez (Spain), Maarten Koudstaal (Netherlands), Gareth Davies (ECO, Netherlands), Peter Mossey (Dundee, UK),

Workshop Report Treatment Issues

The Workshop discussed aspects of orofacial clefting treatment whereby academia and industry could usefully interact in issues concerning cleft lip and palate research with the prospect of deriving mutual benefit. These were:

(1) three dimensional imaging
(2) bone substitutes particularly in relation to ABG
(3) randomised clinical trials

(1) Three dimensional imaging
3DMD are aware that stereophotogrammetry 3D imaging technology has been disseminated throughout the UK, Europe and many parts of the world yet there is a

great diversity of purpose in the usage and lack of standards or research governance and therefore comparability between units for audit or research purposes would be difficult if not impossible. The use of 3D imaging ranges from outcome measurement in cleft lip and palate to syndrome recognition to the study of facial morphology in different ethnic groups, longitudinal assessment of facial growth and sexual dimorphism.

(2) Bone substitutes particularly in relation to ABG

There would be significant patient benefit to the development of bone substitutes avoiding the need for iliac crest bone for ABG. It is felt that many teams are "experimenting" with bone morphonogenetic proteins and other scaffolds and using stem cell technology to generate bone substitutes and in addition to human there are an increasing number of studies carried out in animal models for example looking at results with or without scaffolds and results with or without BMP2. An example is Xpand's osteoinductive calcium phosphate bone graft which could serve as an excellent carrier for the BMP as well as a scaffold for bone formation (in addition to using the hydrogel).

Complimentary studies such as subperiosteal bone generation to harvest additional bone might also be worthy of further research. It is however necessary that research in this field is more co-ordinated and this might begin with a survey of all studies published to date and perhaps the relative merits of different animal models (e.g. dogs versus sheep), the measurement of outcome e.g. 3D volumetric measurement versus 2D images and success of canine eruption. Are there other methods of tissue engineering such as stem cells that could be applied?

(3) Expertise in clinical trials

One of the objectives of the Marie Curie is to train the next generation of scientists in the field and it is important that the most robust methodologies are used when seeking best evidence to guide evidence based practice.

The industrial companies who are likely to be interested in partnering with academia for the purposes of a Marie Curie application would be (a) 3DMD and Dimensional Imaging in 3D and 4D stereophotogrammetery; (b) tissue engineering companies such as Xpand Biotechnology and Termira for the development of bone substitutes and trial management companies would become involved in the training programmes or a new generation of scientists skilled in trial management.

If we aspire to use this as a research tool we need to have standards and also quantification and statistical analysis should be developed. This would be of benefit both to industry and academia. Some currently used treatment techniques such as NAM and PSO are controversial and quantitative statistics on 3D images in measuring outcome could be used to compliment more subjective methods for measurement of outcome and this might have implications for burden of care, particularly in the Developing World. An example of a technology that would be of significant use is computer based automated point recognition and indeed liaison also with forensics might be useful in developing facial morphometric measurement.

Part D: Appendices

1. Agenda for Bonn meeting – presentations & workshops

2. Summary of opportunities for Industrial collaboration (DRAFT)

3. PowerPoint on Grant Funding opportunities

4. Industrial partner profiles

5. Report of Bonn steering group meeting

APPENDIX i

Agenda for meeting in Bonn, 3rd July 2012

Dear Colleagues

The overall objective of this meeting is to set out our stall in relation to orofacial clefting research with a particular emphasis on the fundability of our research in craniofacial anomalies.

Suggested format: Our presentations should be short, focussed approximately **12 minute** presentations and should mention any past successes as well as projecting to the future. Of those who have confirmed attendance so far, the line up of presentations and speakers might be as follows:

1. **Elisabeth Mangold**: Welcome and Introduction.
2. **Peter Mossey**: Outline of the aims and objectives of this EUROCleftNet meeting with potential industrial collaborators.
3. **Bill Shaw**: European research in OFC: EUROCleft, EUROCRAN, TOPS and now EUROCleftNet:
4. **Elisabeth Mangold / Marcus Noethen**: GWAS and related OFC research in Bonn.
5. **Michele Rubini**: Post-GWAS functional genomics research.
6. **Concha Martinez**: Animal models research in genetics / gene environment interaction and quality of care.
7. **Carine Carels**: Research in cleft orthodontics and alveolar bone grafting.
8. **Mike Dixon**: Genomics and GEI in Cleft research.
9. **Heiko Peters**: In vivo modelling of gene-environment interactions
10. **Peter Mossey**: Funding opportunities for European collaborative research.
11. **Gareth Davies**: Research impact and translation to the "consumer".

There will be opportunity for a few minutes discussion after each paper presented

Arrival: The meeting (preceded by a light buffet lunch) will commence @ 12.30 pm, these presentations (with tea / coffee breaks) would be timetabled until 3.30pm.

From 3.45 to 5.00pm there would then be 2 (or more) parallel 1 hour workshops designed to **optimise participation of industrial partners**, followed by a plenary feedback (45 minutes) and a structured discussion session.

6 – 6.30pm The last half hour of the meeting would be to brainstorm the **actual projects** / **grant funding opportunities** that would be designed to enhance our future research and strengthen our future academia / industry links.

AGENDA with timings

11.30 – 12.30: Tea / Coffee, light pre-meeting savoury snacks / fruit

12.30 – **12-35: Elisabeth Mangold**: Welcome / Introductions / Housekeeping.
12.40 – 12.50: Peter Mossey: Aims and objectives of EUROCleftNet meeting with potential industrial collaborators.
12.55 – 13.05: Bill Shaw: European research in OFC: EUROCleft, EUROCRAN, TOPS and now EUROCleftNet:
13.05 – 13.15: Elisabeth Mangold / Marcus Noethen: GWAS and related OFC research in Bonn.
13.20 – 13.30: Michele Rubini: Post-GWAS functional genomics research.
13.35 – 13.45: Concha Martinez: Animal models research in genetics / gene environment interaction and quality of care.
13.50 – 14.00: Carine Carels: Research in cleft orthodontics and alveolar bone grafting.
14.05 – 14.15: Mike Dixon: Genomics and GEI in Cleft research
14.20 – 14.30: Heiko Peters: In vivo modelling of gene-environment interactions - tissue-specific induction and monitoring of hypoxia in cleft lip formation

14.35 – 15.15: Discussion and break for Tea / coffee & refreshments

15.15 – 15.45: Peter Mossey: Funding opportunities for European collaborative research – and collaboration with industry

WORKSHOPS: **16.00 to 17.00** (One or more break out groups – see below)

17.00 – 17.15: Comfort break

17.15 – 18.00: Workshop Feedback (Plenary)

18.00 – 18.30: Future planning of application and future Academia / Industry liaison

DINNER: 20.00 – local Italian restaurant

Workshop (s) for dialogue / interaction
It would possibly be best to confine this to 2 workshops, one clinical treatment and outcomes orientated, and the other focussed on diagnostics and genetics. This would be followed by a plenary and the discussion thereafter on issues regarding collaborative research would also be plenary.

NOTE: the workshops will be preceded by a 10 minute presentation by **Gareth Davies**, CEO of the European Cleft Organisation: **Research impact and translation to the "consumer".**

Workshop questions might include issues such as:
(a) In the context of CLP what aspects of research are eligible? (discuss under Diagnosis, Treatment and Prevention)
(b) What are the synergies between academia and industry wrt OFC research?
(c) What are the differences between academia and industry wrt OFC research?
(d) What are the mutual benefits, drawbacks and risks of collaboration?
(e) What are the IP issues in Academia / Industry research discovery?
(f) In the context of grant funding, training of future researchers is considered important - what aspects of research lends itself to training programmes?

APPENDIX ii
Summary of Industry / Academia opportunities

Diagnostic:

3D imaging / facial morphology and quantification of facial dysmorphology; develop volumetric analysis.
(e.g. 3DMd, Dimensional imaging)

Genetics / genomics / transgenics / micro-arrays aimed at gene discovery; (Polygene, Syngenta)

Diagnostic bio-markers and DNA / tissue collection (Skuldtech and DNDi – pharmacogenomics)

Characterisation of selected candidate genes: expression pattern during development e.g. through mouse, dog and zebrafish model
(Polygene, DTID)

Preventive:

Environmental factors / GEI research in the quest for clues on exposures and modifiable risk factors such as folic acid, alcohol, smoking, medications and recreational drugs; with a view to personalised medicine applications.
(e.g. Syngenta, Zambon)

Drug target identification: through the assembly of protein networks for complex diseases may stimulate research into discovery of drugable targets for these conditions; (e.g. Agilent, DTID)

Pharmaceutical firms interested in the development of nutritional e.g. multivitamin supplements used in conjunction with peri-conceptional care; (e.g. Zambon, Merck, DNDi – drugs for rare diseases model)

Management / clinical care / pre-conceptional care:

At Salzburg there was a call for RCTs and one RCT to study the efficacy of distraction osteogenesis v osteotomy for midface protrusion in adolescents with OFC (3dMD, Dimensional Imaging)

The use of bone substitute to replace lost alveolar ridge, perhaps trialling use of osteoinductive calcium phosphate bone graft as a carrier for the BMP, for ABG or fistula repair without iliac crest bone. Trials could be conducted with or without stem cell technology; (Xpand, TERMIRA)

The possibility of closure of secondary palatal cleft by bone substitution as opposed to lateral releasing incisions (e.g. Hydrogel, TERMIRA) - and maybe scar free wound healing / tissue regeneration ?

Pre-conception counselling using SMS messaging (Voxiva, Slimmer Zwanger)