

**Network for orofacial Clefts Research, Prevention and Treatment
(EUROCleftNet) 09-RNP-023**



Report of the 3rd EUROCleftNet Steering Group meeting

Date: 20th December 2011, Manchester
www.esf.org/eurocleftnet

Present: Professor Peter Mossey, Dr Elisabeth Mangold, Dr Heiko Peters, Mr Gareth Davies, Professor Mike Dixon, Professor Concha Martinez, Professor Anette Lohmander, Professor Bill Shaw, Professor Kevin O'Brien, Dr Ian Ellis, Professor Michele Rubini, Professor Carine Carels, Professor Hans van Bokhoven and Miss Kelly Leslie.

It was decided to hold the Steering Group meeting as a plenary with not only members of the Steering Group but the other attendees at the workshop also in attendance for the Steering Group meeting and discussions.

1. EUROCleftNet Steering Group meeting

This meeting was concerned mainly with the feedback from the September 2011 workshop in Salzburg and the follow up from the afternoon workshop reports resulted in the decision to hold an additional meeting in Manchester to discuss two major priorities (a) the EUROCleftNet website and plans for its future development and (b) application for research grant funding to facilitate the EUROCleftNet research objectives.

2. Priorities for future research

It was recognised that there is a range of research interests in EUROCleftNet ranging from developmental biology including animal models for cleft lip and palate, genomics, gene-environment interaction, epigenetics and clinical research including clinical trials. Other priorities identified particularly in the European forum at present were liaison with industry and SMEs and public involvement in deciding research priorities – both are extremely important.

3. 2012 and 2013 EUROCleftNet meetings:

In a brief update the proposals for a conference aligned to the Scottish Association for Cleft Lip and Palate 2012 conference in Glasgow on either Friday 14th or Friday 21st September 2012 was mentioned and the theme of this conference would be translational research. There would be a EUROCleftNet Steering Group meeting on the Thursday prior to this meeting. In 2013, the possibility of holding a EUROCleftNet conference in Bulgaria was discussed at the Salzburg meeting and it was agreed that the host, Dr Youri Anastassov would provide further details on venue, format, possible dates etc. Gareth Davies announced that Youri had forwarded information to him and that he in turn was forwarding this to all members of the EUROCleftNet Steering Group. (proposal enclosed). It was agreed that an attempt to bring the Eastern European partners on board would be desirable and it would be useful if the EUROCleftNet Steering Group members

could contribute to the dialogue and discussion to assist Youri with ideas on research with a view to making this a successful EUROcleftNet conference.

4. Future grant funding applications:

This is one of the ongoing areas for dialogue, debate and discussion and it is important that in the near future we apply our energies towards a focussed strategy aimed at submitting one or more competitive research grant applications. The following workshop presentations would begin to address this agenda.

5. Questionnaire and directory of resources:

One of the methods used in the EUROcran project to build up an effective collaborative body of researchers was to build a directory of resources from information obtained from all those partners interested in participating in future research. This would outline their research expertise, current research activity, grant funding, useful links to other organisations etc and it would be useful to do this in EUROcleftNet. This could be one of the priorities associated with the development of the EUROcleftNet website.

6. An ECO perspective:

For the benefit of all those present, Gareth Davies provided an update on ECO and a brief overview of the ECO perspectives in Europe and in European research. ECO is a pan-European cleft support organisation, facilitating education, research and advocacy amongst patient groups and health professionals alike. It is run by a board of directors and an advisory panel comprising representatives from 12 EU member states and a range of cleft disciplines as well as users.

7. Influencing politics:

Gareth mentioned the Standards project which has been initiated by ECO and aims to standardise standards of cleft care from the point of diagnosis to first surgery. Part of this initiative is dependant on buy-in from health services and health ministries across Europe and inevitably there will be political involvement. Peter Mossey also mentioned the opportunity that presents through the European Parliament in Brussels; and it may be possible in spring 2012 to make a presentation to MEPs in Europe outlining the opportunities for improvement in health care and eradicating inequalities through the work that we are planning to do within EUROcleftNet and ECO.

8. EUROcleftNet participants:

During the Steering Group meeting a copy of the current list of partners signed up for EUROcleftNet was circulated. Any additional partners or potentially interested contributors should be notified to Kelly Leslie (k.z.leslie@dundee.ac.uk) and a **copy of the database** is attached.

9. Structure of meeting for rest of the day:

It was noted that the programme included plenary presentations and in the afternoon, workshops. However in light of the relatively small group of delegates and in the interests of being fully inclusive in the discussions surrounding website, future EUROcleftNet priorities and pursuit of funding, it was agreed that the discussions should all be in a plenary forum.

It was therefore agreed that the first plenary presentations could commence and lunch and coffee breaks would be worked into the programme by the consensus of the delegates.

NOTE: Those interested in animal models palatogenesis research, Peter Mossey, Heiko Peters, Concha Martinez and Ian Ellis had a brief preliminary meeting in the Waterside Hotel, and the report of this meeting appears in **Appendix II** below.

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Report of the 2nd EUROCleftNet Workshop

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Presentations / workshop: as planned in the programme there was thereafter a mixture of plenary presentations and discussion. The presenters have all provided their slides for distribution as part of the workshop report and these are as follows:

Bill Shaw and Kevin O'Brien provided an overview of existing and proposed websites that overlap with EUROCleftNet's aims and objectives, including the now lapsed EUROcran website. Kevin O'Brien is the leader of the UK Clinical Research Centre at Manchester University supported by The Healing Foundation (Bill Shaw, Director and Kevin O'Brien, Associate Director) and is in the process of developing a new website designed to facilitate the projected research studies and clinical trials that aim to involve all the UK cleft research centres. Kevin advised that his preference was to have a web presence as a means of information dissemination and direction but which would be relatively low maintenance.

Gareth Davies also provided an overview of the ECO website which could also be used as a tool to further EUROCleftNet's aims. It is pan-European, multilingual and has a strong patient focus.

Anette Lohmander commented on some of the limitations of the current EUROcran website and the desire to ensure that inter-centre studies could be facilitated with the exchange of speech records and the ability to carry out inter-centre comparisons of speech quality.

After discussing various options and in an attempt to move things forwards, it was agreed that Gareth would put in a proposal for developing the ECO site as a platform for some of EUROCleftNet's work, especially relating to the updating of a European Directory of services and resources.

The expediently produced draft document from Gareth was and is attached to this report (**[Gareth Davies website.doc](#)**). The response from Peter Mossey to the proposal from Gareth Davies is also attached (**[Peter Mossey website.doc](#)**) and discussions on the best way forward are also ongoing.

Research funding opportunities: in the facilitation of a discussion on future research funding opportunities for EUROcleftNet, **Mossey, Dixon, Rubini and Mangold** all presented slides and these slides are appended as part of the workshop report. While the slides provide a good overview of the discussions, a few supplementary comments and analysis are useful:

- Firstly it is very clear that while research grant funding opportunities exist in Europe, all of these are highly competitive with relatively low success rates.
- For calls in FP7 framework programme, careful scrutiny has not succeeded in identifying any realistic opportunities for orofacial clefting research in Europe. We are therefore forced to examine carefully the “bottom up” research grant opportunities and among these are the European Research Council (ERC) grants and the FP7 Marie Curie programmes.
- The European Research Council grants are all based on absolute excellence and “frontier research”. All PIs must have outstanding track records and leadership profiles and the two types of grants that are offered are starting grants and advanced grants. They also offer synergy grants to enable a group of between 2 – 4 excellent PIs to pursue a large scale frontier research project.
- Marie Curie programmes require industrial links and a number of scientists within the group have had experience of applying in the past with industrial links being the perceived weakness.
- One of the Salzburg workshops was dedicated to brainstorming on industrial links (**PowerPoint slide appended below**) and it is this initiative which the group agreed was worthy of further consideration. Delegates were encouraged to think about possibilities with respect to encouraging industrial involvement in the orofacial clefting research and if we could secure sufficient interest, an application to a Marie Curie integrated network would be considered.
- Ysbrand Poortman, founder member of the Dutch National Federation of Patient and Consumers Association and a leading advocate of causes for patients with disabilities made a presentation at the ICBD / WHO conference in Lodz in Sept 2011. It would be useful to make personal contact and those among the group who were familiar with him and his activities agreed that he could be approached about the need for bringing industrial influence into our network.
- **Regine Steegers** presentation: due to contingency back at her home institution, Regine Steegers was unable to make it to the meeting and instead provided a pdf of a PowerPoint presentation (**Regine.pdf attached**). This deals with the issue of epigenetics in orofacial clefts and possibilities for prevention. This area of significant interest in recent years is an eligible area for further research in the field of orofacial clefts and will be one of the aspects that will be prioritised for funding.
- The other aspect that Regine is involved in is the e-health tool being used in peri-conceptional and pregnancy care and this which concentrates on nutrition and lifestyle will be a valuable contributor to prevention and aim towards orofacial clefts, NTDs and congenital heart disorders (CHD). Regine will be informed of the outcome of this meeting and our desire to include both the above epigenetics approach and primary prevention in any future research grant application.

Future research grant funding strategy: it would be ambitious to expect that all aspects of our research covered in the ESF application would be encompassed in one application and therefore we should think about the possibility of submitting a portfolio of different research applications but if an opportunity arose where these could be linked then every attempt would be made to be as inclusive as possible.

In the first instance there would be a need to have industrial co-operation and collaboration and therefore those who feel that industrial partnership can be encouraged in their field should approach their industrial contact with a view to encouraging their collaboration.

ACTION: This would require a co-ordinated approach and in a follow up message a list of information regarding the previous Marie Curie application with the work packages will be circulated and leaders of each of these will be identified – with a view to modifying the application and/or attempting to bring an industrial or commercial partner on board.

Mike Dixon outlined the progress with Facebase: Mike Dixon is involved in the NIH FaceBase initiative and there are a number of projects being prioritised through FaceBase, some of which may have either an overlap or an influence on what research we carry out in Europe. A list of the FaceBase project with a brief summary and the partners involved is attached. It was noted that all of the FaceBase projects are currently led by US partners and a number of these involve animal models so our EUROcleftNet partners involved in animal models research should study these carefully. The FaceBase project is also online and can be found at <https://www.facebase.org/project.and> a brief summary of the 8 Facebase projects is also appended (**Facebase.pdf**)

It is apparent that there are differences but also possible synergies with Facebase: Although Facebase proposes to utilize specific mouse models such as TGF beta, TGF-beta receptor, MSX1 and FGF receptor knockout strains (so this may/would overlap with the TGF-beta 3 knockouts we use (the Research on Functional Genomics, Image Analysis and Rescue of Cleft Palate project) - it would appear that no mention is made of PAX9 or EGF-R strains. This could be a possible niche to exploit. The exploitation of these niche areas does fit with the wider Facebase mouse strain repository (Genetic Tools and Resources for Orofacial Clefting Research). The other potential would be to exploit is the GWAS looking for Transcriptional elements (Genome-Wide Atlas of Craniofacial Transcriptional Enhancers). Sarah Jones in Dundee is keen to test for TGF-beta 3 specific transcriptional elements in the HAS2 promoter, and also has an interest in HASnt. This is a naturally occurring anti-sense to HAS2. It would appear no one is looking at this in the development of the palate or clefting. Other groups have looked at this in terms of kidney development. This could be a possible synergy with (Identification of miRNAs Involved in Midfacial Development and Clefting). It might be fruitful to explore these possible collaborations.

Common core protocols: Elisabeth Mangold gave a very useful presentation to follow on from the Salzburg workshop where there was debate about collecting human DNA samples and the debate centred around the use of blood or saliva for the

optimum yield of DNA for future studies. The conclusion was that if it is feasible to collect blood then this is certainly the gold standard for future projects and it would be extremely useful to revise the common core protocols that were developed in the previous European Science Foundation network grant over a decade ago – one of the outcomes of which was to develop common core protocols for collection of data for GEI studies in Europe. These protocols were designed to underpin the EUROCRan GEI project. The [common core protocols.pdf](#) document will be circulated in the near future as it will be necessary to identify a range of expertise among EUROCLeftNet partners to undertake a review.

Clinical trials in OFC: The UK Clinical Research Centre for Orofacial Clefts based at Manchester University and the support of The Healing Foundation and the Craniofacial Society of Great Britain offers a unique opportunity to involve EUROCLeftNet in the discussions surrounding initiatives such as clinical trials where a European dimension may enhance numbers and shorten the timescale. It would of course be incumbent on the European partners to obtain parallel funding but this may be facilitated through EUROCLeftNet. Some dialogue at Salzburg called for clinical trials (in **distraction osteogenesis** and **alveolar bone grafting**) in an attempt to provide a better evidence base for current clinical practice.

Genetics research associated with the TOPS trial: the TOPS initiative headed by Bill Shaw and Gunvor Semb is about to be launched with all the surgeon calibration having been carried out and recruitment having started in 2010. One opportunity that might be explored in relation to multi partner research is whether it could be aligned to some of the genetics initiatives that we are contemplating – and at our meeting in Salzburg there was a mention of involving our Brazilian partners. **Jill Clayton-Smith** leads on this for TOPS and it would be interesting to discuss this with Jill. In Salzburg a number of potential areas of mutual interest that may also be fundable through EU/Latin America schemes were discussed.

Proposal re collaboration with Greg Elgar (MRC, London)

A proposal re regulome sequencing for OFC to identify regulatory elements in the vertebrate genome that are associated with developmental transcription factors was received from Greg Elgar (details passed on by David Fitzpatrick) – and about 60 CNEs are located at the OFC1 locus on 6p24, in a 2Mb interval downstream of TFAP2A, covering orofacial clefting chromosomal breakpoint region 1.

Greg has obtained the enrichment oligo set from Illumina to start making libraries, and the oligos can potentially select from up to 1152 different libraries (i.e. samples), although it would be prudent to initially start making libraries from a smaller number of samples. He also has a PhD student who is creating a database of variation in these regulatory sequences from the HapMap and 1000 genomes projects

In our discussions in Manchester, we felt that there is sufficient evidence supporting that this transcriptional factor is a central regulator in the craniofacial development, and so the regulation of its coding gene (TFAP2A) could be a player in the lip and palate development.

Dissemination of the information from the Manchester EUROCLeftNet meeting and workshop: It would be useful to distribute the information widely among our partners and encourage their involvement in future research.

Date of next meeting: with an immediate priority being to resolve the two main issues in Manchester – the website and grant funding application, these will be progressed and it may be that one or more additional meetings will be required prior to submission of an application for grant funding. Peter Mossey will meet with the European Officer at the University of Dundee to discuss possibilities for Marie Curie or ESR submission.

Appendix I: Salzburg Workshop 2: Industrial links for EUROcleftNet research
(some suggestions)

1. Diagnostic:

- a. **3D imaging of facial morphology in cleft cases to quantify facial dysmorphology before and after surgery. e.g. 3dMD**
- b. **Genetic assessments / analyses. e.g. Illumina**
- c. **Tissue sampling / DNA collection. e.g. DNA Genotek**

2. Preventive:

- a. **The use of multi-vitamins for reduction or prevention of cleft deformities (NutrEvent, Italpharma, Theralogix etc)**
- b. **Folic acid / Folinic acid / 5-methylTHF / inositol (NTD RCT)**
- c. **Minimising scarring following surgical repair of cleft lip (Renovo)**

3. Corrective:

- a. **Prevention of keloid formation?**
- b. **The use of bone substitute to replace lost alveolar ridge (BMP)**
- c. **The use of distraction devices for correction of skeletal deformities**
- d. **Rehabilitation of the oral cavity using dental implants**

Appendix II: Animal model palatogenesis research discussion.

The Concha Martinez group in Spain are working on the two different strains of TGF-beta 3 knockout mouse and are interested in gene-environment interaction with main interests being nutritional with folic acid, experiments already underway and they were encouraged also to consider multi-vitamin trials. Spain also have a unique dog model but they were not suggesting that this would be a particularly useful model for ongoing collaborative research.

Heiko Peters mentioned the particular interests he has in gene-environment interaction (and also gene-gene interaction), and his interest is mainly in MSX1 & PAX9 and in terms of environmental agents the anticonvulsant phenytoin is one of his ongoing models. He suggested that the group might look at developmental aspects of lip and palate development and in particular:

1. patterning
2. growth
3. morphogenesis
4. fusion

He also emphasised the concentration on epithelial mesenchymal interaction and that the European group may find a unique niche different from the mainly US based FaceBase Consortium (Facebase document attached) but with collaborative links – perhaps through Mike Dixon. Heiko Peters is also interested in the concept of morphogenesis in his MSX1 deficient mice and also in the PAX9 during primary palatal fusion.

Ian Ellis mentioned the work in Dundee being done by Jennifer Galloway in respect of the hyaluronic acid syntheses (HAS1, 2 and 3) and that the group have experience in using the two different TGF-beta3 knockout mouse models. This work is an ongoing collaboration with Mike Dixon and Concha Martinez group. The Dundee group are also developing a TGF-beta 3 knock-down model in human embryonic fibroblast cells and how this affects HAS expression.

One additional comment made in relation to future plans for using the mouse models in palatogenesis research is to look carefully at the new candidate genes that are emerging from GWAS and not to lose sight of the link between those genes felt to be important in human palatogenesis and in cleft lip and palate human population studies and to establish the link between the laboratory research and its application to human clinical situations.

It is very well to understand that a mouse model gives us a handle on which genes are involved in mouse cleft palate. However, as both myself and Heiko pointed out this does not always transfer into the human. Heiko used the idea of how the mouse “face” developed differently in terms of elongation of snout etc. From my own work we have seen differences in how tumours in mice have different properties to human tumours- this is in terms of migration and HA synthesis. Therefore my argument would be that the mouse may tell you how a process works but the factors in humans may be different.

Documents to be attached to the final report:

- **EUROcleftNet partners database**

Draft document from Gareth regarding website



Microsoft Word
Document

Regine Steeger's Powerpoint presentation



Adobe Acrobat
Document

A list of the FaceBase projects and partners



Microsoft PowerPoint
Presentation

ESF "Common Core Protocols" document



Microsoft Word
Document

EUROcleftNet partners database



Microsoft Office
Excel Worksheet