Meeting of the ECFS-DNWG / EuroCareCF WP3 diagnosis June 10th, during the ECFS conference in Brest, France

The meeting was opened by Michael Wilschanski.

Inez Bronsveld – Finalisation of the development of a common European SOP.
The collection and evaluation of NPD data from the Diagnostic Network for CF and non-CF individuals comes now to a finale in which we have to decide which techniques and procedures are accepted to implement in the common European SOP. We are glad to announce that recently we have been able to add data from the Brompton hospital to the total data set. By evaluating the differences in results and seeing all the different techniques, we identified the problems on which we have to reach consensus. Some points cannot be decided upon at the moment because of lack of evidence for superiority of one of either methods. For the undecided items we have formed subgroups that will set-up small studies in order to compare two different techniques, before a decision is made. Subgroups were formed to investigate warming of the solutions, testing skin bridges by abrasion versus subcutaneous needle, and low flow rate during basal PDs versus high flow rate for the rest of the measurement. Meanwhile, a draft SOP with the protocol agreed upon up till now will be distributed between the members for review.

Nico Derichs – Development of a European SOP for ICM.
Nico started off by showing the differences between the JCF protocol from H. de Jonge et al, 2004 (the 'Rotterdam' method) versus the 'Freiburg' method.
European ICM centres were identified and these centres handed in photo's of ICM set-up, methodology and protocols to be compared. As with the NPD protocol evaluation, we collected 5 CF and 5 non-CF measurements. The centres of which we collected data are Rotterdam (with old and new sequence of solutions), Hannover, Freiburg, Lisboa and Jerusalem.
The main differences between the Rotterdam method and the Freiburg method are:
The Rotterdam method is using chambers in which the buffer is recirculated through the chambers. In the Freiburg method the tissue is superfused with fresh buffer continuously and we call it a continuous perfusion method. The advantage of continuous perfusion is that you can easily wash out pharmaca. However, Dr de Jonge showed recently with a new protocol that in the reperfusion chamber you can wash out pharmacological agents as well. Another difference is that Rotterdam measures short-circuit currents (Isc) while Freiburg measures open circuit (voltage, Vte). In the Freiburg method they use forceps biopsies taken during sigmoidoscopy, while Rotterdam is using rectal suction biopsies which can be done without sigmoidoscopy.
Rotterdam is currently using a different sequence of secretagogues for diagnostic purposes with a wash out period. We will both include the old and new Rotterdam data for analysis.
When results were compared, mean Carbachol responses were very different for the 5 centres, however each centre could identify CF from non-CF. Like in NPD protocols there are still too many differences between the 5 different centres to be able to compare data.
Items that have to be decided upon: diagnostic protocol versus outcome measure, forceps versus suction biopsy, buffer solution, recirculation vs perfusion, Isc measurement versus voltage, sequence of secretagogues.
The great advantage of ICM in general is that it can be used in newborns. Upon a question in the audience it was said that a biopsy can be kept in cold PBS for a couple of hours before it is not viable. Furthermore, for diagnostic purposes try to take 5 biopsies per patient. The plan is

to continue working on a common SOP, and to define a SOP for diagnosis versus a protocol used for ICM as outcome measure.

P. Paterlini-Brechot – Circulating fetal cells: lymphoid, myeloid, erythroid, epithelial cells.

Dr Paterlini reported on a method for isolation by size of epithelial tumor/trophoblastic cells (Am J Pathol 2000 by Vona et al). This is a report on a fairly easy way to isolate cells, collect them on a filter, and can then be used for FISH, DNA mutation tests or other measurements. Dr Paterlini showed how you can use the method to diagnose DNA mutations prenatally on amniocenteses material or chorion villa biopsies. By using ISET on this material one can segregate fetal and maternal DNA. By comparison of the fetal material to the maternal DNA it could be identified whether the fetus is carrying a certain disease or not. She demonstrated how this technique was used in spinal muscular atrophy that is caused by mutations in the SMN1 gene.

This method can also be used to segregate the diseased genotype when the mutation of a certain disease is not known, provided you have 1. cells genotyped with 2 markers that segregate with the diseased mutation, and 2. DNA from the index case.

In CF the use of ISET method was published in Saker et al, Prenatal Diagnosis 2006. First, a protocol was defined by developing the F508del mutation analysis and addressing it to single trophoblastic cells, isolated by ISET and identified by short tandem repeats (STR) genotyping. It was thereafter validated in leucocytes from F508del carriers and subsequently applied in 12 pregnant women with a ¼ risk of CF in their offspring. Ten of these couples were F508del carriers, 2 had unknown *CFTR* mutations. The method identified one mother with an affected fetus and 7 with a carrier fetus, and 4 non-CF homozygotes. These findings were consistent with those obtained by chorionic villus sampling (CVS).

Trophoblastic cells start circulating at the 5^{th} week of gestation. Thus, ISET can be an easy and non-invasive method for prenatal diagnosis by genetic analysis on isolated circulating fetal trophoblastic cells. The early circulation of trophoblastic cells will allow an early and non-invasive method for prenatal diagnosis. In case this test is inconclusive, the family still has the opportunity to perform invasive methods at 10 to 12 weeks of gestation.

The current perspective is that there will start a phase IV study for risk-benefit analysis. Upon a question from the audience it was clear that for each CFTR mutation you need to develop a new method with its own marker set. To develop different markers for each mutation you need about 2 months per mutation. This methodology may be a significant advance in prenatal diagnosis of CF

Kris De Boeck and Inez Bronsveld – The ECFS-DNWG website.

At the moment our website of the DNWG contains reports and photos of the different meetings. In the future, we like to use it for guidelines and also as a forum for communication and consultancy between the different members. We have now the opportunity to develop a file repository area on our website with access for designated persons. If we also want to use this file repository area for patient information, we will need a separate server that will only host our network. This costs 300 euro's/month. If our DNWG would like this, the ECFS will provide this for us. We discussed in the group whether this is useful and decided democratically to ask the ECFS for a test period of one year to see whether we as a group will use this facility. Nico Derichs and Inez Bronsveld will communicate this with the NCFS (already in progress with Sarah Young).

We will hold a short meeting of the DN at the North American CF Conference in Minneapolis in October ; details will be announced. The next formal meeting is set for February 12 -13,

2010 Paris. By e-mail this date will be checked with the members for convenience. Possible subjects for presentation will be: Delphi protocol for atypical cases, the ICM SOP, reports from the NPD subcommittees. Also the website will be launched in Paris. We will also inform young investigators to submit their work for presentation.

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Pictures from the June meeting in Brest, France