ECFS-Diagnostic Network Working Group meeting
Stockholm, February 11th and 12th 2011

On Friday **Michael Wilschanski** opened our meeting by showing all current DNWG members on a world map. As a group we would like to expand even more and have more people and different countries represented in our working group. Our present participants are from Australia, Austria, Belgium, Canada, England, France, Germany, Greece, Ireland, Israel, Italy, Netherlands, Poland, Portugal, Sweden, Switzerland, and Ukraine.

This year there was a lot of competition between the abstracts of the different young investigators. The 4 young investigators that won the travel award are: Michelle Ettorre (Italy), Andrea van Barneveld (Germany), Aleksandra Norek (Poland) and Emilie Saussereau (France).

Kevin Southern gave an overview of the literature regarding CF diagnosis as a background for the Delphi consensus we want to perform in the DNWG on CF diagnosis. He showed summaries of Rosenstein et al 1998, De Boeck et al 2006, Gobeau et al 2009, Farrell et al 2008, and Borowitz et al 2009. Within the DNWG we aim to proceed with the Delphi consensus on the diagnosis of CF. KS will initiate this with assistance of: Doctors Bronsveld, De Boeck, Derichs, Munck, Sermet.

Michael Wilschanski showed a new approach to combine NPD and sweat test results to differentiate CF versus non-CF. The statistician came up with a Z-score with the NPD and ST results included. Dependent on how much weight you assign to either the NPD or ST (e.g. 50%/50% or 60%/40% etc) you will get a different cut-off point for CF vs non-CF. Before using this Z-score one has to decide whether both tests are equally important. These calculations were made using only PI patients. We will proceed within the working group to do these calculations with also PS patients included that will be collected from the different centres. **Action: Yasmin/Jerusalem.**

Young investigator Emilie Saussereau presented new data in CF mice. She performed nasal PDs in CF mice to obtain reference values in order to have a CF murine model to test possible CF therapies. She obtained cut-off points for the baseline PD, delta amiloride and delta low chloride values.

Isabelle Fajac explained the method of electrochemical skin conductance that is published in JCF 2011 (Hubert et al). The test person places his hands on the cathode and his feet on the anode. First a voltage of 1V during 1 sec. is applied where after the voltage is increased in steps up to 3.8V. A difference in the electrochemical skin conductance between the value at 1.6V and 3.6V was calculated and cut-off values were established for CF and non-CF.

Nico Derichs gave an update of the progression on the European SOP for ICM. He also showed some of the ICM data from the Thorax paper 2010. The plan for the coming year is to validate the new protocol that is in the SOP. Validation will be done in collaboration between Rotterdam/Utrecht, Berlin, Jerusalem, Verona and possibly Paris. **Action: Nico/Berlin.**
If possible, we can aim for concurrent validation of the new NPD SOP. **Action: Inez/Utrecht.**

Young investigator Michelle Ettorre told about his work on CFTR function in monocytes between CF and non-CF. In parallel he measured nasal potential difference in these same subjects. CFTR is expressed in human monocytes and they calculated an index capable of discriminating between CF and wild type monocytes.

Jeannette Dankert showed the results from the novel strategies in newborn screening for CF in The Netherlands. From 2008 to 2010 3 different strategies were tested:
1. IRT-PAP test, sensitivity 87-95 %
2. IRT-DNA (only F508del screened), sensitivity 86-94%
3. IRT-DNA (with multiple mutations screened), sensitivity 95-99%
The Dutch ministry of health still thought this gave too many false positive results. Therefore, the strategy that will be implemented starting in May 2011 will be: IRT-PAP-DNA-sequencing. Uptil now this has not resulted in any false positive results.

Garry Cutting, our special guest, reported on the CFTR-2 project. The number of patients included is 39614 with 1099 CFTR mutations. In addition there is clinical information on sweat test, pancreatic status, infections, lung function, age, age and reason for diagnosis and anthropomorphic data.
Of the 1099 mutations entered 57 had errors in entry so 1042 were analysed.
Functional studies in FRT cells will be performed by F. van Goor; CFTR processing by P. Thomas (HeLa cells); CFTR splicing by M. Amaral; CFTR trafficking by G. Cutting.
Dr Cutting also highlighted a few CFTR alterations like R117H, M470V and I148T.
A mutation is classified as disease causing when the mutation is clinically consistent AND functionally consistent.
When CFTR function (in log scale for I_{SC}) was plotted against the sweat chloride value there was a linear correlation. The CFTR function (in log scale for I_{SC}) and lung functions (FEV1 percent predicted) gave a very small correlation.

Harry Cuppens explained us about 3 different in silico tools for interpreting consequences of DNA mutations. The PolyPhen2 programme makes a prediction based on sequence, phylogenetics and structural information. It gives 3 possible results on a certain DNA profile: benign, probably damaging and possibly damaging. Certain known CF causing mutations are scored benign by this programme.
The SIFT programme looks only at the phylogenetic level. It results in a score between 0 and 1.
The Panther programme only looks at phylogenetics. It scores from 0 to -12 and the more negative the score the higher the probability that the mutations are damaging at the functional level. With this programme there is great overlap between the CF and non-CF mutations. Obviously, these in silico tools cannot be used in clinical testing.

Michael Wilschanski presented a case report about a patient he knows from 9 months of age who is now 1.5 years. The patient has elevated liver enzymes, no cholangitis, normal gallbladder, no classical CF symptoms. The liver disease was classified as possible with CF but not classical. ICM was normal but the sweat test was 55. With genetic analysis F508del/5T was found. Is this atypical CF or CFTR related disease? There was no concluding answer in the DNWG. The advice is to regularly see the patient at the CF-clinic. Furthermore, to determine the TG repeat on the 5T allele as we know that this influences the amount of CFTR protein.

Young investigator Andrea van Barneveld showed her results of protein expression in rectal biopsies. In 10 out of 13 F508del homozygous patients not only the expected mutant mannose-rich B-band of CFTR was detected but also the mutant complex-glycosylated C-band, which is different from findings in cell culture experiments. Because of these results they initiated a study on lung, to clarify the distribution and abundance of isoforms of normal and mutant CFTR.
In contrast to their findings in intestine neither the homozygous F508del CF subjects nor the other CF subjects with different mutation genotypes showed the mature complex-glycosylated C-band of CFTR in lung tissue at the stage of terminal respiratory insufficiency. The mannose-rich B-band was visible in most mutant lungs. In non-CF lungs both B-and C-bands were clearly visible.

Godfried Roomans showed results on the composition of the airway surface liquid (ASL) after sleeping in a mist tent for 8 hours. The concentration of Cl^- and Na^+ in ASL are higher in CF patients. When sleeping in a mist tent Cl^- and Na^+ concentrations decrease in CF and controls to equal values. However, when individuals leave the mist tent the Cl^- and Na^+ concentrations increase to original levels, i.e. CF patients regain their initially higher concentrations. Within 4 hours after leaving the mist tent levels are back to their original values in both control and CF.

Isabelle de Monestrol presented a case report on a third child in a family without a history of CF with failure to thrive at 17 months old. The patient had clinically recurrent URTI, no pneumonias, normal stools. Sweat test 54 and 64. Further investigations showed bronchial thickening, normal lung function, normal elastase. With genetic analysis her mother tested positive for a R117C mutation and when the child was tested it was homozygous for R117C. It was diagnosed with Silver-Russell Syndrome with iso-uniparental disomy of chromosome 7.

Young investigator Alexandra Norek reported on a novel de novo mutation in a Polish patient. It concerned a patient at 3 months old with an elevated sweat test, respiratory tract infections, Pseudomonas colonisation, a low BMI and pancreatic sufficiency. By genetic analysis and sequencing only an F508del mutation was found. However, by further MLPA a deletion of an R domain was detected. The hypothesis is that non-homologous end joining (NHEJ) occurred. This has been described in literature before.

To end the Friday programme, **Kris De Boeck** discussed whether we as the DNWG want to be an official working group of the ECFS. On Saturday we voted and the majority prefers to become an official working group.

On Saturday February 12th **Dr Garry Cutting** showed the clinical data from the CFTR-2 project. Clinical features expected of a disease causing mutation are an elevated sweat test, exocrine pancreatic insufficiency, a reduced FEV1 and infections with Pseudomonas. The sweat test (ST) showed a normal distribution with a mean around 100. In addition, it showed a continuum: it was high for 2 severe mutations, lower with 1 mild mutation and even lower when 2 mild mutations are present.
R117H is a useful example that in case of a skewed distribution in a clinical feature, we should look at complex alleles (5T versus 7T) as a cause for the non-normal distribution.
The S1455X mutation in the last exon of CFTR showed an uncoupling of clinical features: the sweat test was above 70 in all these patients, however, the FEV1 was normal.
In the data set the lung function was plotted against ST and had a very slight correlation. This correlation became more robust when it was plotted separately by mutation.

The available websites for the CFTR2 project are:
www.cftr2.com/index.php , and a site still under development:
http://cftr2dev.igm.jhmi.edu/index.php (user cftr2 password: hopkins).

François Vermeulen highlighted an interesting topic on who should be included in the ECFS CF Registry. He evaluated the data that are in the registry now. At the moment the CF diagnosis is done by the CF centre supplying the patient data. However, maybe we should review the CF diagnosis with the data from CFTR1, CFTR2, and expert opinions on difficult DNA mutations. When the present registry data were analysed for a CF diagnosis by the diagnostic criteria from the algorithm Thorax 2006 there was 5,9 % without a confirmed CF diagnosis. With the criteria from Castellani et al, JCF 2008 10% was without a CF diagnosis and with the CFTR2 criteria 9% did not have a confirmed CF diagnosis. This urges us to look more carefully to the entries into the registry. Another example: from the 20000 patients in the ECFS Registry there were 6737 with sweat chloride results. Therefore, a few possible projects were suggested.

Suggested projects for the DNWG (Action: whole DNWG):

Increase quality of ECFS data
Case definition of CF
Case definition of CFRD
Cut-off points for different tests

Peter Middleton gave a summary of his NPD data published in the AJRCCM, 2003. In his experiments calcium-free perfusion solutions were used resulting in low chloride responses in CF towards a more normal value. The authors hypothesized that perfusion of the CF airway with nominally calcium-free solutions reduces tonic inhibition of chloride secretion.

Inez Bronsveld demonstrated the new facilities on the ECFS-DNWG website. The intention is to use this website as an aid for diagnostic cases and also for the discussion of difficult cases. Microsoft Office and LabChart files can be posted. In addition a forum can be used to discuss these cases. We will discuss what legal issues are attached to the advice/comments that we will be giving and will incorporate a disclaimer on the website. An e-mail will be sent out to the DNWG for volunteers consulting in genetics, sweat test, electrophysiology, clinical features and diagnosis.

As concluding talk **Isabelle Sermet and Inez** commented on the final version of the NPD SOP. The differences were highlighted between the US SOP and this European SOP. Summarised the differences are: floor measurement instead of turbinate; solutions at room temperature; no AT measurements or multiple basal measurements along the turbinate.
Isabelle showed the results from A.G.E.P.S (Agence Générale Electronique -Alarmes et surveillance) that investigated the possibility to provide the NPD solutions commercially. Stock solution A (Ringer's) and B (low Cl^-) would then be provided. We will further investigate the constituents and costs for these solutions and compare them to the present TDN solutions.

The DNWG will meet again at the ECFS meeting in Hamburg on:
Friday June 10th, 13:00 to 15:00.

The next 2 day meeting will be held in Verona, Italy hosted by Carlo Castellani