9th ECFS-Diagnostic Network Working Group meeting, Verona February 10-11th 2012

Carlo Castellani and Michael Wilschanski opened the meeting:

Session 1

Drucy Borowitz from the US started with a talk on CFTR-related metabolic syndrome (CRMS), a diagnosis that was chosen because of the new patients identified by the newborn screening. Patients with CRMS are healthy hypertrypsinogenemic patients with intermediate sweat Cl- levels on at least 2 occasions and < 2 CF causing mutations; or a normal sweat Cl- and 2 *CFTR* mutations of which no more than 1 is known to be CF causing. CRMS can be classified as ICD-9: 277.9: unspecified disorder of metabolism or ICD-10: E88.9: metabolic disorder unspecified. This diagnosis does not imply that the infant has CF and it includes patients with mutations that are of unproven or uncertain clinical consequence. She indicated the problems that the CRMS label creates: on one side there are physicians that are reluctant to give a diagnosis to patients that will never have symptoms. On the other hand there are physicians that want to follow-up these CRMS patients to prevent delayed diagnoses when these patients would develop symptoms later in live. She pointed out the CFTR-2 project that will we available for everybody via the world wide web around half 2012. In the US there are recommendations for follow-up of patients with CRMS. In California there is a trial in which all children that are detected by the NBS program will get extended gene analysis. After a positive NBS test patients can fall into one of the following 3 groups but can also get a different diagnosis later in life by which they will fall into another group: CRMS (without symptoms), CFTR-related disorders (with symptoms) and CF. In the US the CRMS patients are asked if they agree to have their data entered in the CF registry. For this purpose they have an informed consent that is separate from the Registry form, because these patients have to be aware that they are not considered to have CF. In addition, when these patients do not visit the CF centre regularly, patients are not contacted, in contrast to CF patients that are contacted and child protection will even be involved when staying away from the clinic too long. They have a US guideline for the follow-up for these difficult diagnostic cases.

Sarah Mayell from the UK presented on the European Consensus for the Newborn Screening. They used the Delphi process to come to 19 consensus statements. She also suggested having all infants with an equivocal diagnosis entered into the UK CF registry, which does raise some problems because the registry is developed for CF patients and the non-CF diagnosed patients do not fit into the database structure. In addition the criteria and money for CF centres are based on the CF registries that should not include the undiagnosed patients. The guideline can be found on www.ecfs.eu/publications/consensus_reports.

In France a cohort of F508del/R117H has been followed-up with ST and NPD. Part of these patients had an abnormal NPD and developed pulmonary symptoms during follow-up.

Session 2

Kevin Southern from the UK talked about the challenges of measuring immune-reactive trypsinogen (IRT) as a screening tool. There are several tests available, what are we measuring in the IRT assay:

1. Antibodies against trypsinogen; fluorescence (Perkin Elmer)
2. Immune-reactive trypsinogen; 2 isoforms: IRT I is positively charged and IRT II is negatively charged.

The IRT cut-off point is the most important element in the NBS program that determines specificity and sensitivity. With a too low cut-off you will be increasing the number of sweat testing that has to be done in IRT positive patients; with a too high cut-off level you will get a lot of false negative results.

Kevin highlighted the factors that can have an impact on the result:

1. temperature, humidity
2. from the baby: age, gestation: pre-term infants have higher IRT values, ethnicity, meconium ileus infants can have low/normal results

New developments in assays are a new multiplex immunoassay, measuring both isoforms of IRT, and you can combine IRT with the pancreatitis-associated protein (PAP) (Thorax 2012).

Our young investigator Aleksandra Norek from Poland presented results from the NBS program in Poland. With the available INNO lipa tests there is only a detection rate of 77%. Recently 7 new mutations have been found in the Poland population representing 17% of the mutations. It appears that the population is very heterogeneous.

Session 3

Nico Derichs from Germany started with an update of the validation of the ICM SOP. He showed tracings of the new protocol in non-CF, CF pancreatic sufficient patients and CF pancreatic insufficient patients. The new protocol shows the typical large responses to cAMP-agonists compared to the old SOP. In addition, he can register residual chloride secretion in pancreatic sufficient patients.

Inez Bronsveld showed recent data that compared the old and new SOP in R117H/F508del patients.

Hugo de Jonge showed the influence of the TMEM16/anoctamin-mediated chloride secretion in ICM rectal biopsies. He showed that in human rectal tissue there was actually no effect of the TMEM16-A01 inhibitor indicating there is no TMEM16 function in distal human colon in control persons. In addition, he assessed the effect of tannic acid in this tissue and it also did not give any inhibition.

Young investigator Francois Vermeulen from Belgium showed new data of NPD measurements using a catheter with a larger opening creating a large contact area. He compared the catheter with a side hole, to the new catheter first looking for the most negative value and with results by just placing the new catheter at a fixed position. With the new catheter he lost some difference in the basal PD and also the chloride secretory response was a little smaller. However, repeatability and the needed number to treat were better with the new catheter.

Young investigator Bente Aalbers from the Netherlands presented data comparing the CFTR function tests: sweat test, ICM and NPD. There were significant differences in the mean results between PS and PI groups and between mutation classes in all three CFTR function tests. This implicates that the level of residual chloride transport is correlated with pancreatic function and mutation class in these three different CF affected epithelial tissues. The overlap found in 95% CI between the PS and PI groups, and between the different mutation classes implicates that isolated CFTR function tests do not differentiate between PS/PI or mutation classes. Thus, she concluded that there is a correlation between mutation class and outcome of CFTR function tests.

Session 4

Natalia Cirilli (Italy) gave a Young Investigator talk on sweat testing and gave recommendations for the performance and interpretation of results, of the Italian Cystic Fibrosis society. She mentioned indications for sweat testing with pulmonary, gastrointestinal and metabolic symptoms. She collected data from different CF and screening centres using questionnaires in 2007 and in 2009. She reported on the methods of ST, the information given to the patients, what is tested with the ST, reference data in each clinic and quality control procedures. It was especially noted that sweat tests are also performed in pediatric clinics that are not CF centres.

In the second part of the talk she showed some of her NPD data. She showed repeatability data, and the comparisons of measurements for the left and right nostril. She is used salbutamol as cAMP-agonist instead of isoproterenol.

Paola Melotti and Claudio Sorio gave an update of their project of CFTR expression and function in human leukocytes. The question is if we can use something else compared to NPD/ICM and other tests, to register CFTR function.

They looked at the expression of CFTR in Western blot and by confocal microscopy. They showed the membrane localisation of CFTR in WT monocytes and compared this with R1162X/R1162X homozygous cells. They also compared the single-cell fluorescence imaging with NPD measurements on the same day. Moreover, they tested fluorescence values in CF monocytes with or without addition of PTC124. They could divide the patients in responsive, partially responsive and non-responsive. The groups could not be discriminated by genotype, the same genotype could be found in either one of the three groups.

This test will be further developed and most hopefully it can be a functional assay to aid in the differentiation between CF and normal cells.

Saturday February 11th

Session 5

Michael Wilschanski and Isabelle Sermet presented the future projects of the DNWG.

The projects that have started or will start soon are the validation of the new ICM and NPD SOPs (Nico Derichs and Inez Bronsveld).

The large project of the next years are the Workpackages:

Workpackage 1: Analysis of CF Registries, such as the Belgian, German, French and Dutch registries. To produce definitions for individual data fields and evaluate definitions for the diagnosis of CF.

Workpackage 2: Measures to improve documentation in CF registries: e.g. missing test results; diagnostic criteria, hierarchy in tests.

Workpackage 3: Are national registries with and without New Born Screening comparable. For instance, there are more patients with borderline STs in the French registry. Concept workplan: compare cohort of < 8 yrs in France vs other countries: in age, PI/PS, sweat test, FEV1.
Workpackage 4: Who should be analysed in CF patient registries; proposal for stratified analysis by subject characteristics. For instance, only F508del patients; cut-off values for diagnostic tests: for sweat test, NPD, ICM; create an expanded *CFTR* mutation list.

Carlo Castellani talked about the trends of CF diagnosis around Europe. CF birth rates go down by a) a partial failing of detection systems; b) less carriers in the population, caused by a more ethnically mixed population and a decreased pressure from heterozygote advantage; c) a reduction in rate of consanguinity; d) heterozygosity awareness: extended family testing and carrier screening. Factors that raise CF birth rates are for instance the improved detection systems. Carlo also showed the current CF neonatal screening protocol operative in Verona. It was started in the 70s. In the 80s the IRT analysis was included and in the 90s the DNA analysis was included.
In 16 years there were 8 false negatives because of normal IRT levels and 1 with a positive IRT but an error in genetic testing.
He showed the annual incidence of CF in northeastern Italy from 1990 to 2005 in which you see a few trends going up and down but the long-term trend was downward. He showed a prediction that in 2025 the amount of children with CF will go down; and 80% of the patients will be above 18 yrs.

Session 6
Started with a talk from R Camero (Spain) who showed a multicentre clinical evaluation of a new device (ISEsweat) for the direct measurement of sweat chloride concentration for the diagnosis of cystic fibrosis.
Peter Middleton gave an update on his data from Australia. He reported on an evaluation of pin-prick IRT values in adults to establish new reference values. Questions were raised as: is IRT affected by alcohol, meals or the time of day. In the non-CF group there were IRT levels in males around 16.4 and females around 18; ranging from 10 to 24 microg/L. The IRT levels increased with age. There was no significant influence by alcohol, meals or time of day.
He also showed a comparison between PS vs PI CF patients. PI was defined clinically as requiring pancreatic enzymes. The PS group had a mean IRT of 18.1. Patients with pancreatitis had levels increased by 3 times. The PI group had IRT levels of 1.7. They were asked if they could do without pancreatic enzymes. The patients that would get abdominal cramps without enzymes had a slightly elevated level compared to the others. The age range of these CF patients was 18 to 70 yrs.
Cristina Bombieri from Italy concluded our very interesting ECFS-DNWG meeting. She presented the results of the Eurocare CF working group on the Recommendations for the classification of diseases as CFTR-related disorders (JCF 2011). Clinicians, geneticist and basic scientists from Europe and North America came to a consensus report. This is an important issue because the diagnosis CF has a lot of clinical and other consequences.
The definition of CFTR-related disorder was made as: a clinical entity associated with CFTR dysfunction that does not fulfill the diagnostic criteria for CF. For example, 3 main clinical entities that illustrate these phenotypes are: CBAVD, recurrent or chronic pancreatitis or disseminated bronchiectasis

The next ECFS-DNWG meeting will be during the ECFS conference, on June 8th 12.30-14.30 in Dublin.

The 10th Anniversary DNWG meeting will be in Jerusalem (hosted by Michael Wilschanski):
Arrival: Feb 6, 2013
Meeting: Feb 7-8, 2013
Departure: Feb 9, 2013

Pictures from the meeting – please see next page