**European CF Society – Diagnostic Network Working Group**
**Annual meeting in Paris, France on February 12th and 13th 2010**

Organised by Isabelle Sermet and Anne Munck

The audience was welcomed by our chair
Michael Wilschanski.

The day was started by Inez Bronsveld who gave a summary of the European Guidelines for NPD. This guideline is a result of the discussions on NPD during the ECFS-DNWG meetings of the past several years. The controversial points that are still open and not decided upon were outlined by Jane Davies.

Hugo de Jonge gave an overview of the progression of the ICM survey in which all European ICM methods have been collected and compared (started last year and presented by Nico Derichs at the Liverpool meeting). The CFF-TDN is now standardising ICM measurements for participation in international trials and asked Hugo de Jonge and Martin Hug to join from Europe.

Martin Hug presented the controversial points between the two main protocols, the Rotterdam protocol being the recirculation chambers with mounting plates and short circuit current measurements. The Freiburg protocol with continuous perfusion chambers, an insert to mount the tissue and voltage measurements, i.e. an open circuit measurement.

Kevin Southern reported on the development of a guideline for the sweat test. Recent documents written on the sweat test are an update of the CFF-TDN SOP, a CLSI (Clinical and Laboratory Standards Institute) sweat test guideline, and a draft of a UK guideline.

Michele Rota showed the results of a multicentre study comparing different sweat test protocols among which the Gibson and Cooke, coulometry and conductivity measurements, to outline the differences between the used methods.

Two Young Investigators presented their recent work. The first was Eric Caudron working at AGEPS, a public pharmacy in Paris, France. He reported on the problems that are experienced by them when they prepare the new (CFF-TDN) solutions. The former solutions that were used for NPD in France (Middleton et al) had a pH around 7.4 and stability at room temperature of more than 2 years: stability in pH, ion concentration and no precipitation. The new solutions now tested gave precipitations because of the calcium and magnesium phosphate concentrations, and this happened within 2 months after preparation. AGEPS means that when you ask a pharmacy to prepare solutions for the hospital, they will want to sterilise it and the stability should be around 1 to 2 years to be profitable for a company to prepare.

The second Young Investigator was Roel de Nooijer, working at the UMC Utrecht, The Netherlands. He showed results on the genotype-phenotype relationship in R117H homozygosity of a male and a female patient. Sweat test, ICM, NPD and faecal elastase were compared. It shows the different penetrance of the R117H allele of which the cause is still uncertain.

Friday afternoon there were working group meetings on standardisation of different diagnostic procedures.

In the NPD workgroup it was decided to standardise:

The measurement on the nasal floor instead of underneath the turbinate, since this is an easier procedure. Consequently, the Marquat (Sermet) catheter will have holes on the side instead of at the tip. We will ask for cm coding on the upper side of the catheter and for a size as short as possible (now 45cm). For the solutions being either room temperature versus warmed solutions there will be a pilot study comparing these. There is only one report available at the moment. There was a discussion with the pharmaceutical company about centralised solutions, they prefer stability of solutions for around 2 years, instead of the present stability data on the CFF-TDN solutions (3 months at 4 degrees). We will discuss in the ECFS-CTN whether centralised solutions is possible around Europe.

The ICM workgroup had the following consensus: standardise the Ussing chambers to those of Physiological Instruments Inc; this is a recirculating chamber; suction or forceps biopsies are both permitted; the bleeding time is checked before the measurement in the ear lobe; tissue is collected in phosphate buffered saline; keep the tissue at 0 degrees as short as possible; apply microscopic control for orientation; Meyler buffer for volume reservoirs; voltage and current electrodes of Ag/AgCl; amplifier type is flexible, present WPI VC devices can be used; software type flexible; registration mode: since mostly VC has been performed in recirculating chambers this will be used for diagnostic procedures; sequence of pharmacon additions will be as follows: preincubate tissue with indomethacine for 30 min, after that wash out, add amiloride, cAMP/forskolin/IBMX, carbachol; for interpretation calculate the mean of all biopsies.

In the sweat test working group there was agreement on the stimulation apparatus that is commercially available; use the standard SOP for pilocarpine concentration, time and current; collection will be with the microduct for 30 minutes; storage at room temperature for 24 hours, at 4 degrees for 72 hours or up to 3 months when frozen. Sweat analysis will be performed in each sample and no combination of results.

There was still debate on the standardised technique for stimulation; the collection either with micropore or filter paper; for sweat analysis the conductivity can be used as a screening test after which sweat chloride should be measured by a validated technique with a CV <5%. The nanoduct measurement is still being assessed.

The working group on genetics discussed the newborn screening methods and the penetrance of R117H in different European countries. Epidemiological data from different countries and the prevalence in different countries should be collected and analysed. There was a discussion on how to increase the number of disease causing mutations in NBS panels. Momentarily, the *CFTR* mutations mentioned by Farrell et al, J Pediatr 2008 are being used as reference which suggests only 28 disease causing mutations.

In the afternoon Harry Cuppens demonstrated new developments in sequencing by the GS-FLX next generation sequencing system and the Hiseq2000. This machine can sequence 2 human genomes for 10.000 dollars/each. With all new generation sequencing one should not test and report mutations with unknown clinical consequences.

Emmanuelle Girodon reported on genetic studies and showed the limitations and difficulties of discovered mutations by the DGGE, SSCP, DHPLC, hrMCA procedures. All these tests have false positive and false negative results. When 2 mutations are found results have to be validated by testing the parents to identify compound heterozygosity or homozygosity. A few case reports on family consulting were demonstrated for the R751C, V754M, and cryptic exon in intron 6b. Epidemiological data and continuous exchange of findings is necessary for the genotype – phenotype correlations.

On Saturday, Aleksandra Norek, a Young Investigator from Warsaw, Poland, reported on the newborn screening in Poland. There were 147 CF patients detected in 814966 newborns. This resulted in a CF frequency of 1:4268 and CF carrier frequency of 1:32. There were 7 new *CFTR* variants detected. The F508del mutation was only detected in 57%.

The fourth Young Investigator was Dr Kusova from Moscow, Russia, on the analysis of the results of the Russian NBS test. There were 364 patients diagnosed with CF in 3.669.283 persons testes. This gave an incidence of CF of 1:10.080 in Russia.

Hugo de Jonge gave an overview of recent Ussing chamber experiments. He demonstrated that about 20% of wt-cftr is sufficient to normalize transepithelial anion secretion and that in non-cf mice cftr is not rate-limiting for the Isc. Below 20% of wt-cftr the icm becomes very sensitive to identify abnormal anion secretions. Experiments in the 3272-26A>G splice mutation showed that residual anion secretion is due to a low percentage of normally spliced WT-CFTR. There was residual anion secretory function of 1% in F508del homozygosity, 20% in 3272A>G/F508del, and 43% in 3272A>G homozygosity.

Hereafter, Kevin Southern led us through the document "Guidelines on the diagnosis of cystic fibrosis". The questions were discussed and comments should be forwarded by mail. We voted that there should be a level of agreement of 80% on the outcome.

Aleksander Edelman presented a very interesting talk on proteomics in nasal epithelium. A few of his findings were for instance a decrease in annexin1 in human nasal cells with CF stop mutations and decreased mitochondrion GSH in CF cells. On the basis of his results he suggested possible targets for CF therapy, like the reticulum-stress proteins and mitochondrion-targeted antioxidants.

Saturday afternoon there was a satellite meeting sponsored by PTC Therapeutics Inc.

The first speaker was Tanja Goska who presented the new concept for diagnosis and assessment on CFTR function in sweat glands that was recently published in Thorax: Sweat gland bioelectrics differ in cystic fibrosis and normals.

Isabelle Sermet showed her study on NPD measurements in a group of hypertrypsinemic children. She showed that NPD can be useful to identify children at risk to develop a CFTR related lung disease.

Finally, Manfred Ballmann showed the combined data from Hannover and Rotterdam on ICM experiments that were performed on children between 1,5 and 2 months old (Rotterdam) and between 1 and 6 years old (Hannover).

On our ECFS-DNWG website it can be reported that everybody that is an ECFS and DNWG member can soon get access to a separate ECFS-DNWG directory where we can exchange files (non-patient information). We will send out an e-mail message as soon as this feature is running.

The next ECFS-DNWG annual meeting will be held in Stockholm, Sweden in 2011.

Michael Wilschanski and Inez Bronsveld