Meeting report
10th ECFS Diagnostic Network Working Group Meeting
Jerusalem, February 14-15th 2013

February 14th, 2013

On this special occasion Michael Wilschanski opened the 10th ECFS-DNWG meeting. Dr Osnat Lev-Zion, Director of the Hadassah hospital, welcomed the group. Dr Inbar Ori, chairman of the Israel CF Foundation, gave a word of welcome, and Dr Eitan Kerem welcomed us by wishing us a nice and interesting meeting.

Dr **Batsheva Kerem, Israel,** gave the keynote lecture. She reported on the review written by Tsui and Estivill (2013) on the cloning of the *CFTR* gene.
She also reported on the article by Carlo Castellani, who showed that in Northeastern Italy the decline in live births with CF from 4/10000 to 1.5/10000 was caused by carrier testing. She talked about incidence and prevalence and thereafter on new therapeutic approaches. She mentioned VX-770 which gave 10% increase on FEV1 already at 24 weeks in G551D patients, while there was only 1.7% increase in FEV1 in homozygous F508del patients. However, VX-770 and VX-809 together gave 8.5% increase in FEV1. Ataluren in CF patients that did not use inhaled aminoglycosides gave 5.7% increase in FEV1 while patients that inhaled aminoglycosides had a small decline in FEV1.
Another new therapeutic approach is the antisense oligonucleotides approach: small synthetic nucleic acid molecules can bind to intronic or exonic sites of pre-mRNA and modify expression of a targeted gene. This can restore CFTR function in cells with splicing mutations as the 3849+10kbC>T mutation.

The first young investigator **Bente Aalbers** from the **Netherlands** talked about NPD results in suspected CF patients with 5T polymorphism. She showed the incidence of CBAVD and symptoms, NPD results and sweat tests. There is a selection bias because 5T carriers that have a high sweat chloride or the ones with symptoms get referred to a hospital. Most geneticists suggest to leave out 5T in carrier testing because it is such a heterogeneous group. Bente invites other centers to participate in this study.

Carlo Castellani, Italy, talked about CFTR2. New mutations that are entered in the database are: mutations that were found in trans with a mutation that is already known to cause CF in patients that have a sweat test above 60mmol/l. Clinical data that were entered were pancreatic status, sweat test, FEV1, pseudomonas status and other items (CBAVD, MI). He reminds us that the data in the CFTR2 database should be applied to population groups and not be used for the individual patient. Actions forward are: coverage of more mutations, beware of overlapping old and new data, and the need for longitudinal data.

Kevin Southern, UK, gave an update on the CF newborn screening. He also pointed out health economic features (Sims et al, 2007). In the article from Castellani (Am J Med Genet 2005) you can read that IRT is very sensitive and even picks up carriers in newborn life. The NBS program results in 1 out of 10 patients with an equivocal diagnosis and patients with unknown mutations. In the US there are now 1 in 10 infants

registered in the US database that do not have CF but CFTR related metabolic syndrome (CRMS). Kevin showed an illustrating table on balancing positive and negative issues of the IRT newborn screening program: high IRT cut-offs, a second IRT at day 21 of life, a safety net in case of high IRT but no mutations, extended DNA analysis, the consequences of a PAP+IRT program which reduces sensitivity. The D1152H mutation was discussed, whether at all it should be tested in the NBS.

The second young investigator was **Patrick Staffler** from **Israel** who reported on the impact of CF population carrier screening. He showed the origin of the NBS program in CF that is based on the trial from Farrell (1997). In Israel the NBS screens for 14 mutations + 3 extra mutations for the Arabic population. Patrick showed a study of 87 patients who underwent antenatal screening and investigated whether these patients would have been identified by the NBS program.

The 3rd young investigator **Sara Caldrer** from Italy presented on a subject with the CFTR186-8T/C allele in intron 1, which is reported as possibly influencing the splicing and skipping of exon 2, and the subject was diagnosed with CF in the past. However, the sweat test and NPD were not consistent with CF diagnosis and also the membrane polarization test was not consistent with CF.

Dorota Sands, from Poland showed a case report on a boy that had a negative NBS, with the protocol IRT/IRT. At 12 yrs old he still had a negative sweat test. Since 2006 Poland changed to the IRT/DNA (CFTR sequencing) schedule. The boy had a sister tested by this protocol and she was found with R117H-7T/R553X. Dr Sands is haunted by lawyers whether she missed the diagnosis in the boy. The difference in the IRT result could be the result of the alternative splicing that results from the R117H-7T mutation.

We started the afternoon with a case by **Alexandra Norek** from Poland, a girl of 3 yrs old with a 2183AA>G/unknown CFTR genotype. Because there was no second CFTR mutation found after extensive analysis, the genes endcoding 3 ENaC subunits were screened and found a H280R mutation in the SCNN1gamma gene. The sweat test and nasal PD were consistent with CF disease. It was concluded that CFTR/ENaC transheterozygosity might lead to CF-like clinical disease.

Aurelie Hatton from France reported on a patient with the A357T mutation who had a positive sweat test and a normal forskolin response with the new ECFS ICM SOP, but a reversed carbachol response, pointing out an atypical patient.

Lutz Naehrlich from Germany reported on a survey he did among the ECFS NPD centers by asking them to evaluate 5 tracings he sent around. The intra-observer variation was not that high, however, the difference between the right and left nostril was the most important variable factor.

Isabelle Sermet from France presented the project for the ECFS CTN NPD certification process. There will be a certification for new sites (5 non-CF and 5 CF). For already operating sites we ask 2 non-CF and 2 CF tracings.

Inez Bronsveld from The Netherlands showed the newest update on the ECFS NPD SOP which we will now start using for the DNWG diagnostic validation study. Plan: 1. Get IRB

approval to perform NPD at your centre; 2. Check whether you can access all the needed documents on the ECFS website; 3. Get your certification; 4. Start diagnostic validation in 10 non-CF; 10 CF-PI (at least 5 F508del homozygous); 5 CF-PS (at least 2 CF causing CFTR mutations, or with 2 CFTR mutations with one known CF causing mutation and a sweat chloride >60mmol/L).

Nico Derichs, **Germany,** showed the data for the multicentre diagnostic ICM validation with the ECFS ICM SOP. Each centre performs this protocol in 10 non-CF, 10 CF-PI patients and 5 CF-PS patients. At the moment data from Berlin and Giessen are included, one other centre is expected to be ready within the deadline.

Young investigator **Sheila Scheinert, Germany** reported on the variability and the reliability of ICM measurements. Controls, CF-PI and CF-PS patients were tested on day 1, day 8 and day 14. The variability and reproducibility were tested and it was demonstrated that ICM can be reliably performed e.g. in repetitive measurements as needed for clinical trials.

Hugo de Jonge, The Netherlands talked on the organoid research in 2013. Organoids are convenient models for testing and correcting CFTR dysfunction. Till now the golden standard for CFTR corrector validation was in bronchial epithelial cells from CF patients. However, in the individual patient you have to wait till the lungs are transplanted and it takes a few weeks to culture the ALI cells. Secondly, we have the ICM which is still a very good method for CFTR corrector testing. It is native tissue, CFTR is highly expressed in the distal colon, and the test is highly sensitive for CFTR potentiators and correctors. The con side is that it can only test one drug at the time. The organoids is an inexhaustible source of epithelium from the individual CF patient. Organoid genotype and phenotype stays very stable even after >50 generations.
The organoids showed to express all the cell types: villus type, goblet, Paneth and EC crypt cells. It was shown that in the organoids you can inhibit the total forskolin dependent swelling when you add GlyH101 and CFTRinh-172.

Paola Melotti, **Italy,** talked about the diagnostic aspects of CFTR research in Italy. She reported on the current projects being performed in Verona. One of the projects is the testing of CFTR function in monocytes (Sorio, PlosOne 2011) in different genotypes, which was also tested with different drugs for instance Ataluren and VX-325. She also suggested collaborate projects like searching for site specific cut-off points for NPD; organoid testing in rare genetic variants and combined approaches for a CF diagnosis by creating a possible score from different diagnostic test results.

Kris de Boeck, Belgium, talked about an educational project for CF caregivers, funded by an unrestricted grant by Vertex and NanoCHIP developing a new educational tool for CF caregivers and paramedical personnel. The accompanying website is www.cftr.info. It gives info on how to talk to patients about special subjects and gives links to CF databases.

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Eitan Kerem from Israel gave a nice overview of the history on the building of new hospitals around Israel and the middle east. And then he started a tale of 3 swords: the sweat test, the genetic mutation analysis and the NPD. How was CF diagnosed when ST is negative: by family history. He talked about the difficulties encountered with the R117H mutation and a family with 10 children with the W1182X and D1152H mutations. In earlier days he performed a survey in D1152H patients and 50% had a ST <40mmol/l and 70% had a ST <60mmol/l. Four out of 19 patients had normal NPD. The swords are sweat test, genetic mutation analysis and NPD.

Then **Aleksandra Norek, Poland,** presented a study on 51 Polish patients with CF-like symptoms where no CFTR mutations were found and the ENaC mutations were determined. Symptoms did not distinguish patients between CFTR or ENaC mutations. All patients underwent NPD, where ENaC-related disorder was defined as a new CF-like clinical entity, independent of CFTR mutations.

Isabelle Heesen, Germany, presented an interesting family: 2 children with borderline sweat test. The girl had pancreatitis and the NPD gave a Wilschanski index of 1. The old protocol of the ICM a carbachol response of 33 microA/cm2. The girl appeared to be heterozygous for the 1717G>A and R117H-7T mutations. We had a discussion whether this patient should be classified as CF or not. One group said yes because there is abberant chloride transport in at least 2 organ systems (sweat gland and respiratory tissue), another group said no because a lot of patients with the R117H mutation never get symptoms.

Kris de Boeck and Kevin Southern had a very interesting and animated pro/con debate on the usefulness of CF diagnostic algorithms.

Thereafter**, Kris De Boeck** reported on the DNWG CF registry projects: workpackage 1: defining the needs for diagnostic documentation and terminology. She showed registry data from Belgium, France, Germany and The Netherlands. France has a lower mean age, because of the newborn screening and a slightly higher proportion of pancreatic sufficient patients, most likely because of the R117H mutation group. The missing data in the registry are mostly sweat test and CFTR mutations that are not reported completely.
Also the other workpackages were mentioned. In workpackage 2 the question is which data measures should be added to improve documentation of CF diagnosis in CF registries? Workpackage 3 is concerned with issues like: are national registries with and without neonatal screening comparable? Is the CF cohort of patients younger than 8 years different in national registries with and without newborn screening?

Michael Wilschanski, our leaving coordinator, elaborated on the past, present and future projects of the ECFS-DNWG. He showed highlights of 10 years of ECFS Diagnostic Network Working Group and reflected on the past 10 years.
The future should bring standardization throughout Europe, many more countries getting involved, intensifying the use of the website, more public relations and promoting of our network, a recognized working group within the ECFS with projects,

an algorithm update, and involvement in the multinational studies like the PTC and Vertex trials.

Nico Derichs as the new DNWG coordinator thanked Michael for his leadership and awarded him with the unique NPD catheter in gold, together with the title "ECFS master of ion transport". Nico concluded with the DNWG perspectives for the future and summarized current activities and new plans. The ongoing projects are included in the business plan for 2012-2014, the registry projects and workpackages 1 to 4, the ICM validation, the NPD validation, and further extension of the webforum.
New upcoming projects include (i) the 5T project, (ii) a new project on functional characterization of rare CFTR mutations by ST, NPD and ICM (CFTR3), and (iii) an update for the CF diagnostic guidelines/algorithms planned for 2014/2015. For the network we will intensify the use of the website, create a newsletter, involve more countries and new young people.

Michael Wilschanski closed the meeting for the last time as DNWG coordinator. Nico Derichs will take over his function, with the help of Inez Bronsveld.

May I end by thanking Michael Wilschanski for his charismatic leadership of the past years. Thank you, Michael!

Our next meeting is at the ECFS meeting in Lisbon, Friday June 14th, 12.30-14.30.

Report by Inez Bronsveld & Nico Derichs