ECFS-DIAGNOSTIC NETWORK WORKING GROUP

Year of report: 2021	
Name of Working Group: ECFS-Diagnostic Network Working Group	Date of initial approval of working group: 2004 (M Sinaasappel)

Contact details of coordinator, vice coordinator and assistant (if applicable) including ECFS membership numbers:

Coordinator: Elke De Wachter
Vice coordinator name: Nick Simmonds
Assistant name: Marlies Destoop

Long term aims of the working group (maximum 100 words) including estimated achievement date:

1/ To achieve pan-European cooperation on definitions of CF and clinical entities of the CF-spectrum.
2/ Standardization of existing electrophysiological techniques. Development, validation and standardization of new diagnostic technologies.
3/ Interpretation of disease liability of CFTR-mutations and discussion of difficult cases within the group
4/ Improvement/support for sweat test facilities in LMIC
5/ Cooperation with other networks:
- ECFS-CTN and CFF TDN: diagnostic techniques serve also as biomarkers in clinical trials for CF.
- ECFS NSWG: newborn screening and diagnosis are closely related
- European CF-Registry: Inclusion criteria remain: fulfilling the diagnostic criteria of CF

Outcomes already achieved (maximum 100 words):

1/ CFF-ECFS recommendations for CF Diagnosis - Farrell 2017: a close link between CFF experts in diagnosis and ECFS-DNWG is maintained and will be used in future projects
2/ Sweat test Project:
- Sweat test survey (Cirilli 2017): highlighted important deficiencies/barriers in sweat testing in real life in Europe.
- Hands-on Training Workshops to improve sweat test performance in Europe. (Belgrade 2018, Liverpool 2019)

3/ CFTR biomarkers: standardization and diagnostic reference values
- NPD and ICM validation studies have been performed in HC and CF in different European centres. Summary of the results, further statistical analysis is needed

Report for this year (max 1000 words)

- short term goals for the year
- current number of members
- measures taken to encourage ECFS membership
- outcomes/achievements (e.g. meetings, activities, website development, awards, publications etc).

1/ Short term goals for the year

- Despite the Covid pandemic we organised our **meetings** in a virtual way (see further)
- The next step in the **sweat test project** is the development of a manuscript:

"A quality improvement tool for sweat testing". The aim of this work is to develop a tool to guide CF centres/labs in Europe to improve the performance of the sweat test. Authors: N. Cirilli, K.W. Southern, J. Barben, F. Vermeulen, A. Munck, M. Wilschanski, Thao Nguyen-Khoa, M. Aralica, N Simmonds, E. De Wachter. The group had 7 online meetings between July 2020 and April 2021.

- Development of new guidelines on CFTR-related disorders (CFTR-RD):

The Bombieri paper (JCF 2011) is 10 years old and describes recommendations on how to establish the diagnosis of CFTR-RD. New insights in CFTR-disease liability led to the need of new recommendations on how to diagnose, treat and follow up people with a CFTR-RD. A pilot project (M Destoop, E De Wachter) showed that knowledge to exclude CF in CBAVD men (described by Bombieri) is variable in CF doctors and below the expected rate in the target group of fertility doctors and geneticists. This stresses the need for unambiguous recommendations. This project started in March 2020. CFTR-RD core group: Carlo Castellani, Kevin W Southern, Elke De Wachter, Nick Simmonds, Kris De Boeck. The group had monthly (apart from August) virtual meetings since March 2020- April 2021.

2/ Current number of members: 95 members, however, despite repeated reminders to renew ECFS membership still 34 did not pay their ECFS membership. We should discuss with the board how to solve this issue and if members should be withdrawn after repeated reminders

3/ Measures to encourage ECFS membership

- During the introduction of the webinar of the ECFS-DNWG session (23rd sept 2020) it was stressed that being part of the European CF Society is mandatory to become/remain membership of the DNWG.
- Several emails (before each registration for a meeting AND before the registration date to renew ECFS-membership) were sent to all DNWG members to remind them that ECFS-membership is mandatory to remain part of ECFS-DNWG.
- For the upcoming virtual DNWG-meeting (29th and 30th April) only registered ECFS-members will receive a gift (postcard + Belgian Chocolate) as a token of appreciation.
- For future initiatives and annual meetings we will only fund CONFIRMED ECFS members for their housing/meals

4/ Meetings

- Mini 43rd ECFS - Virtual Conference: 23rd September DNWG session 11.00-13.00 CET
- Annual ECFS-DNWG meeting in February 2021 (planned to be held in Montpellier), was postponed to 2022 and replaced by a Virtual Meeting on: 29th April 2021 (14.00-17.00) and 30th April (9.00-12.00).

5/ Website development

News regarding DNWG activities was posted on the website. Webinar, filmed during the September meeting is available on the website.

A new logo was developed, to increase visibility AND to stress the link with ECFS - this may improve ECFS membership (?)

6/ Publications from the DNWG as a group:

Aalbers BL, Yaakov Y, Derichs N, Simmonds NJ, De Wachter E, Melotti P, De Boeck K, Leal T, Tümmler B, Wilschanski M, Bronsveld I. Nasal potential difference in suspected cystic fibrosis patients with 5T polymorphism. J Cyst Fibros. 2020 Jul;19(4):627-631. doi: 10.1016/j.jcf.2019.07.001. Epub 2019 Jul 19. PMID: 31331863.

6.1 Publications in the **field of diagnosis/biomarkers from DNWG members** are listed below:

- Aalbers BL, Hofland RW, Bronsveld I, de Winter-de Groot KM, Arets HGM, de Kiviet AC, van Oirschot-van de Ven MMM, Kruijswijk MA, Schotman S, Michel S, van der Ent CK, Heijerman HGM. Females with cystic fibrosis have a larger decrease in sweat chloride in response to lumacaftor/ivacaftor compared to males. J Cyst Fibros. 2021 Jan;20(1):e7-e11. doi: 10.1016/j.jcf.2020.05.004. Epub 2020 May 21. PMID: 32448708.

- Cuyx S, Ramalho AS, Corthout N, Fieuws S, Fürstová E, Arnauts K, Ferrante M, Verfaillie C, Munck S, Boon M, Proesmans M, Dupont L, De Boeck K, Vermeulen F; Belgian Organoid Project. Rectal organoid morphology analysis (ROMA) as a promising diagnostic tool in cystic fibrosis. Thorax. 2021 Apr 15:thoraxjnl-2020-216368. doi: 10.1136/thoraxjnl-2020-216368. Epub ahead of print. PMID: 33859053.

- Prenzel F, Ceglarek U, Adams I, Hammermann J, Issa U, Lohse G, Mainz JG, Meister J, Spittel D, Thoss K, Vogel M, Duckstein F, Henn C, Hentschel J. Audit of sweat chloride testing reveals analytical errors. Clin Chem Lab Med. 2021 Apr 7. doi: 10.1515/cclm-2020-1661. Epub ahead of print. PMID: 33826811.

- Kyrilli S, Henry T, Wilschanski M, Fajac I, Davies JC, Jais JP, Sermet-Gaudelus I. Insights into the variability of nasal potential difference, a biomarker of CFTR activity. J Cyst Fibros. 2020 Jul;19(4):620-626. doi: 10.1016/j.jcf.2019.09.015. Epub 2019 Nov 4. PMID: 31699569.

- Salvatore M, Amato A, Floridia G, Censi F, Ferrari G, Tosto F, Padoan R, Raia V, Cirilli N, Castaldo G, Capoluongo E, Caruso U, Corbetta C, Taruscio D. The Italian External Quality Assessment Program for Cystic Fibrosis Sweat Chloride Test: Does Active Participation Improve the Quality? Int J Environ Res Public Health. 2020 May 4;17(9):3196. doi: 10.3390/ijerph17093196. PMID: 32375358; PMCID: PMC7246827.

- Treggiari D, Tridello G, Menin L, Borruso A, Pintani E, Iansa P, Cipolli M, Melotti P. Role of sweat ion ratios in diagnosing cystic fibrosis. Pediatr Pulmonol. 2021 Apr 6. doi: 10.1002/ppul.25395. Epub ahead of print. PMID:

33822490.

- Treggiari D, Kleinfelder K, Bertini M, Tridello G, Fedrigo A, Pintani E, Iansa P, Casiraghi A, Minghetti P, Cipolli M, Sorio C, Melotti P. Optical Measurements of Sweat for in Vivo Quantification of CFTR Function in Individual Sweat Glands. J Cyst Fibros. 2021 Apr 1:S1569-1993(21)00052-7. doi: 10.1016/j.jcf.2021.03.003. Epub ahead of print. PMID: 33814321.

- Graeber SY, van Mourik P, Vonk AM, Kruisselbrink E, Hirtz S, van der Ent CK, Mall MA, Beekman JM. Comparison of Organoid Swelling and "In Vivo" Biomarkers of CFTR Function to Determine Effects of Lumacaftor-Ivacaftor in Patients with Cystic Fibrosis Homozygous for the F508del Mutation. Am J Respir Crit Care Med. 2020 Dec 1;202(11):1589-1592. doi: 10.1164/rccm.202004-1200LE. PMID: 32687398.

- Ramalho AS, Fürstová E, Vonk AM, Ferrante M, Verfaillie C, Dupont L, Boon M, Proesmans M, Beekman JM, Sarouk I, Vazquez Cordero C, Vermeulen F, De Boeck K; Belgian Organoid Project. Correction of CFTR function in intestinal organoids to guide treatment of cystic fibrosis. Eur Respir J. 2021 Jan 5;57(1):1902426. doi: 10.1183/13993003.02426-2019. PMID: 32747394.

- Vonk AM, van Mourik P, Ramalho AS, Silva IAL, Statia M, Kruisselbrink E, Suen SWF, Dekkers JF, Vleggaar FP, Houwen RHJ, Mullenders J, Boj SF, Vries R, Amaral MD, de Boeck K, van der Ent CK, Beekman JM. Protocol for Application, Standardization and Validation of the Forskolin-Induced Swelling Assay in Cystic Fibrosis Human Colon Organoids. STAR Protoc. 2020 Jun 3;1(1):100019. doi: 10.1016/j.xpro.2020.100019. PMID: 33111074; PMCID: PMC7580120.

- de Winter-de Groot KM, Berkers G, Marck-van der Wilt REP, van der Meer R, Vonk A, Dekkers JF, Geerdink M, Michel S, Kruisselbrink E, Vries R, Clevers H, Vleggaar FP, Elias SG, Heijerman HGM, van der Ent CK, Beekman JM. Forskolin-induced swelling of intestinal organoids correlates with disease severity in adults with cystic fibrosis and homozygous F508del mutations. J Cyst Fibros. 2020 Jul;19(4):614-619. doi: 10.1016/j.jcf.2019.10.022. Epub 2019 Nov 15. PMID: 31735562.

- Cohen-Cymberknoh M, Ben Meir E, Gartner S, Reiter J, Spangenberg A, Garriga L, Eisenstadt I, Israeli T, Tsabari R, Shoseyov D, Gileles-Hillel A, Breuer O, Simanovsky N, Kerem E. How abnormal is the normal? Clinical characteristics of CF patients with normal FEV_1. Pediatr Pulmonol. 2021 Mar 11. doi: 10.1002/ppul.25371. Epub ahead of print. PMID: 33704929.

- Minso R, Schulz A, Dopfer C, Alfeis N, Barneveld AV, Makartian-Gyulumyan L, Hansen G, Junge S, Müller C, Ringshausen FCC, Sauer-Heilborn A, Stanke F, Stolpe C, Tamm S, Welte T, Dittrich AM, Tümmler B. Intestinal current measurement and nasal potential difference to make a diagnosis of cases with inconclusive CFTR genetics and sweat test. BMJ Open Respir Res. 2020 Oct;7(1):e000736. doi: 10.1136/bmjresp-2020-000736. PMID: 33020115; PMCID: PMC7537139.

6.2 Publications in the **field of CFTR mutations and their disease liability from DNWG members:**

- Fidler MC, Buckley A, Sullivan JC, Statia M, Boj SF, Vries RGJ, Munck A, Higgins M, Moretto Zita M, Negulescu P, van Goor F, De Boeck K. G970R-CFTR Mutation (c.2908G>C) Results Predominantly in a Splicing Defect. Clin Transl Sci. 2021 Mar;14(2):656-663. doi: 10.1111/cts.12927. Epub 2020 Dec 6. PMID: 33278322; PMCID: PMC7993255.

-Boussaroque A, Audrézet MP, Raynal C, Sermet-Gaudelus I, Bienvenu T, Férec C, Bergougnoux A, Lopez M, Scotet V, Munck A, Girodon E. Penetrance is a critical parameter for assessing the disease liability of CFTR variants. J Cyst Fibros. 2020 Nov;19(6):949-954. doi: 10.1016/j.jcf.2020.03.019. Epub 2020 Apr 20. PMID: 32327388.

- Martin N, Bergougnoux A, Baatallah N, Chevalier B, Varilh J, Baux D, Costes B, Fanen P, Raynal C, Sermet-Gaudelus I, Girodon E, Taulan-Cadars M, Hinzpeter A. Exon identity influences splicing induced by exonic variants and in silico prediction efficacy. J Cyst Fibros. 2020 Dec 16:S1569-1993(20)30935-8. doi: 10.1016/j.jcf.2020.12.003. Epub ahead of print. PMID: 33341408.

- Bienvenu T, Lopez M, Girodon E. Molecular Diagnosis and Genetic Counseling of Cystic Fibrosis and Related Disorders: New Challenges. Genes (Basel). 2020 Jun 4;11(6):619. doi: 10.3390/genes11060619. PMID: 32512765; PMCID: PMC7349214.

- Girodon E, Rebours V, Chen JM, Pagin A, Levy P, Ferec C, Bienvenu T. Clinical interpretation of SPINK1 and CTRC variants in pancreatitis. Pancreatology. 2020 Oct;20(7):1354-1367. doi: 10.1016/j.pan.2020.09.001. Epub 2020 Sep 7. PMID: 32948427.

- Pagin A, Bergougnoux A, Girodon E, Reboul MP, Willoquaux C, Kesteloot M, Raynal C, Bienvenu T, Humbert M, Lalau G, Bieth E. Novel ADGRG2 truncating variants in patients with X-linked congenital absence of vas deferens. Andrology. 2020 May;8(3):618-624. doi: 10.1111/andr.12744. Epub 2019 Dec 26. PMID: 31845523.

- Laudus N, Audrézet MP, Girodon E, Morris MA, Radojkovic D, Raynal C, Seia M, Štambergová A, Torkler H, Yamamoto R, Dequeker EMC. Laboratory reporting on the clinical spectrum of CFTR p.Arg117His: Still room for improvement. J Cyst Fibros. 2020 Nov;19(6):969-974. doi: 10.1016/j.jcf.2020.05.005. Epub 2020 Jun 3. PMID: 32505523.

- Gauthier S, Pranke I, Jung V, Martignetti L, Stoven V, Nguyen-Khoa T, Semeraro M, Hinzpeter A, Edelman A, Guerrera IC, Sermet-Gaudelus I. Urinary Exosomes of Patients with Cystic Fibrosis Unravel CFTR-Related Renal Disease. Int J Mol Sci. 2020 Sep 10;21(18):6625. doi: 10.3390/ijms21186625. PMID: 32927759; PMCID: PMC7554933.

- Zybert K, Wozniacki L, Tomaszewska-Sobczyńska A, Wertheim-Tysarowska K, Czerska K, Ołtarzewski M, Sands D. Clinical characteristics of rare CFTR

mutations causing cystic fibrosis in Polish population. Pediatr Pulmonol. 2020 Aug;55(8):2097-2107. doi: 10.1002/ppul.24823. Epub 2020 May 22. PMID: 32442342.

- Petrova NV, Kashirskaya NY, Vasilyeva TA, Kondratyeva EI, Zhekaite EK, Voronkova AY, Sherman VD, Galkina VA, Ginter EK, Kutsev SI, Marakhonov AV, Zinchenko RA. Analysis of "CFTR" Mutation Spectrum in Ethnic Russian Cystic Fibrosis Patients. Genes (Basel). 2020 May 15;11(5):554. doi: 10.3390/genes11050554. PMID: 32429104; PMCID: PMC7288340.

- Kondratyeva E, Efremova A, Melyanovskaya Y, Petrova N, Satsuk N, Bulatenko N, Bukharova T, Zodbinova A, Sherman V, Kashirskaya N, Zinchenko R, Kutsev S, Goldshtein D. Clinical and genetic characterization of patients with cystic fibrosis and functional assessment of the chloride channel with the pathogenic variant c.831G>A (p.Trp277*), described for the first time. Gene. 2020 Nov 30;761:145023. doi: 10.1016/j.gene.2020.145023. Epub 2020 Aug 3. PMID: 32758581.

- Kotnik Pirš A, Krivec U, Trebušak Podkrajšek K. The c.3140-26A>G Variant of the CFTR Gene in Homozygous State Causes Mild Cystic Fibrosis - Overview of Longitudinal Clinical Data of the Patient Managed in our CF Center and Review of the Literature. Acta Chim Slov. 2020 Jun;67(2):666-673. PMID: 33855558

6.3 Publications in the **field of neonatal screening/CFSPID from DNWG members:**

- Barben J, Southern KW. Why Do We Screen Newborn Infants for Cystic Fibrosis? Int J Neonatal Screen. 2020 Jul 8;6(3):56. doi: 10.3390/ijns6030056. PMID: 33123637; PMCID: PMC7570329.

- Barben J, Castellani C, Munck A, Davies JC, de Winter-de Groot KM, Gartner S, Kashirskaya N, Linnane B, Mayell SJ, McColley S, Ooi CY, Proesmans M, Ren CL, Salinas D, Sands D, Sermet-Gaudelus I, Sommerburg O, Southern KW; European CF Society Neonatal Screening Working Group (ECFS NSWG). Updated guidance on the management of children with cystic fibrosis transmembrane conductance regulator-related metabolic syndrome/cystic fibrosis screen positive, inconclusive diagnosis (CRMS/CFSPID). J Cyst Fibros. 2020 Nov 27:S1569-1993(20)30909-7. doi: 10.1016/j.jcf.2020.11.006. Epub ahead of print. PMID: 33257262.

- Munck A, Southern KW, Castellani C, de Winter-de Groot KM, Gartner S, Kashirskaya N, Linnane B, Mayell SJ, Proesmans M, Sands D, Sommerburg O, Barben J; European CF Society Neonatal Screening Working Group (ECFS NSWG). Defining key outcomes to evaluate performance of newborn screening programmes for cystic fibrosis. J Cyst Fibros. 2021 Feb 23:S1569-1993(21)00041-2. doi: 10.1016/j.jcf.2021.02.006. Epub ahead of print. PMID: 33637432.

- Castellani C. Newborn Screening for Cystic Fibrosis: Over the Hump, Still Need to Fine-Tune It. Int J Neonatal Screen. 2020 Jul 9;6(3):57. doi: 10.3390/ijns6030057. PMID: 33117904; PMCID: PMC7569808.

- Terlizzi V, Claut L, Tosco A, Colombo C, Raia V, Fabrizzi B, Lucarelli M, Angeloni A, Cimino G, Castaldo A, Marsiglio L, Timpano S, Cirilli N, Moroni L, Festini F, Piccinini P, Zavataro L, Bonomi P, Taccetti G, Southern KW, Padoan R. A survey of the prevalence, management and outcome of infants with an inconclusive diagnosis following newborn bloodspot screening for cystic fibrosis (CRMS/CFSPID) in six Italian centres. J Cyst Fibros. 2021 Apr 18:S1569-1993(21)00097-7. doi: 10.1016/j.jcf.2021.03.015. Epub ahead of print. PMID: 33883100.

- Armstrong RE, Frith L, Ulph FM, Southern KW. Constructing a Bioethical Framework to Evaluate and Optimise Newborn Bloodspot Screening for Cystic Fibrosis. Int J Neonatal Screen. 2020 May 26;6(2):40. doi: 10.3390/ijns6020040. PMID: 33073032; PMCID: PMC7422997.

Aims for the coming year (please state year) (max 50 words):
Period June 2021-2022
1/ Virtual DNWG meeting during ECFC (11/06)
2/ Closed DNWG-brainstorming meeting during NACFC 2021
3/ Finalize and publish sweat test paper (end 2021)
4/ Finalize and publish CFTR RD paper (June 2022)
5/ Improve link with ECFS education committee to create an online sweat test educational tool

Summary (maximum 100 words):

DNWG is an active, heterogeneous group of enthusiastic CF specialists with special interest in diagnostic issues. It consists of CF physicians (adult and paediatric), geneticists, clinical biochemists, basic scientists, making them very complementary. This enables us not to focus only on one topic of diagnosis, but to have in depth expertise. We are strongly convinced that our work has not finished yet. The interpretation of CFTR mutations, their disease liability, the CF spectrum and the interpretation of biomarkers that measure CFTR activity and its translation to the individual patient are topics that need further investigations and a platform supporting this.

Breakdown of expenses (please include total amount received as well as expenditure and, if applicable, the outstanding balance (Euros)):

Apart from expenses used for the annual DNWG meeting, the DNWG had no other expenses between June 2020-June 221

1/ Expenses related to the annual DNWG meeting in Utrecht:

Diagnostic Network WG	EUR		Year Result
	40,984	Opening Balance	
Income	4,500	Sponsorship	14,500
	10,000	ECFS Support	
Expenses	- 15,902	DNWG Utrecht	
			- 15,902
	39,582		- 1,402

2/ Expenses related to annual DNWG meeting 221 (virtual): postcards and Chocolates only sent to registered ECFS-members: around 5000 euros' - more detailed overview follows soon (meeting is held on 29th -30th April 2021)

Budget amount requested for next year (please give the amount in Euros and the year):

- We would like to receive the annual 10.000 euro to support the annual DNWG-meeting in 2022 - Montpellier.
- We plan to set up an educational tool for sweat testing, in line with the recommendations published. A link with the educational platform will be made. We assume that ECFS has provided funding to the education committee to make this happen.

Appendix

There are currently 93 current members