Cystic Fibrosis European Network

NEWSLETTER

APRIL - MAY 2003 – N° 10-11

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NEWSLETTER CONTRIBUTIONS

The closing date for the next issue of the CF Thematic Network newsletter is July 2003.
Please forward your data and contributions for the newsletter to:

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EDIOTRIAL

Dear friends and colleagues,

While the Directorate Research of the EU commission is apparently considering giving research on Cystic Fibrosis and networking around different aspects of CF management back its well-deserved place in the 6th framework program in the future, the activities in the field have nevertheless continued with an ever-increasing intensity. In addition to the efforts made by many national CF societies and the ECFS to fund research, our Thematic Network has continued its activities as if its future was not in doubt. The 2002 EQA, again larger than in the past, was evaluated and the results will be sent to the participants in June; the group around Margarida Amaral met in Lisbon to finalize a large number of entries in the virtual repository. This is becoming a quite unique collection of methods and procedures for CF diagnosis and research that will also be published in the near future in the Journal of Cystic Fibrosis. Additional regional training sessions for diagnostic laboratories have been organized, a successful meeting with representatives of the diagnostic industry took place in Brussels, new guidelines for CF testing are being finalized, to mention just a few of the activities.
It would be a shame to loose the momentum, which during the past 12 years has established a unique relationship between hundreds of excellent scientists and diagnostic services in Europe, with the financial help of the EU in a series of concerted actions, culminating in the present thematic Network. Together we have come a long way in the attempt to better understand CF and the related diseases, to make CF better known in the community and to significantly improve the diagnostic services. For all these reasons, not in the least for all the work that remains to be done, and in an attempt not to loose the momentum, we are looking for temporary financial support, which should allow us to continue some core activities of the network, while at the same time preparing for the day new opportunities will be created in the 6th framework program. We have already taken some initiatives, which might be successful in the coming months, but we are of course open for any suggestion that might help us in achieving this goal.
In the mean time keep working hard on your projects and on your services. The CF community needs our best efforts.

Jean-Jacques Cassiman
Coordinator of the CF-network
Leuven, May 2003

3RD INDUSTRY MEETING

BRUSSELS, BELGIUM, NOVEMBER 26, 2002

Present:
Industrial partners:

Stephan Schrooten - Transgenomic Ltd., United Kingdom
Kevin Daish - MWG Biotech, United Kingdom
Conny Van Loon - Innogenetics N.V., Belgium
Annemie Vergote - Innogenetics N.V., Belgium
Gerd Michel - Abbott GmbH & Co.KG, Germany
Hedwig Petry - Abbott GmbH & Co.KG, Germany
Edwin Roovers - Abbott GmbH & Co.KG, Germany
Sven Thamm - Abbott GmbH & Co.KG, German
Guido De Sadeleer - Perkin Elmer Life Sciences, Belgium
Nicolas François - Serial Genetics, France
Christophe Valat - Serial Genetics, France
Christine Heylen - Orchid Diagnostics Europe, Belgium
Vince Tevere - Third Wave Technologies, USA
Wolfgang Trautwein - Nanogen Europe B.V., Germany
Roderik Van den Bogaard - Nanogen Europe B.V., The Netherlands

University Participants:

Jean-Jacques Cassiman - Department Human Genetics, KUL, Belgium
Harry Cuppens - Department Human Genetics, KUL, Belgium
Els Dequeker - Department Human Genetics, KUL, Belgium
Dragica Radojkovic - Department Human Genetics, KUL, Belgium
Claudia Vits - Department Human Genetics, KUL, Belgium
Imgard Vinck - Centre for Biomedical Ethics and Law, KUL, Belgium
Geertrui Van Overwalle - Centre for Intellectual Property Rights, KUL, Belgium
Pierre Saelen - Centre for Intellectual Property Rights, KUL, Belgium
Milan Macek Jr. - CF Centre and Institute of Biology and Medical Genetics, Czech Republic

The third Industry meeting in the frame of CF network was traditionally held in Sheraton Brussels Airport on November 26, 2002. It was organized in the form of a round table discussion based on 5 oral presentations, and was attended by 19 industrial partners representing 9 different pharmaceutical companies and 10 university participants.

The first presentation given by JJ Cassiman, the coordinator of the European Cystic Fibrosis Thematic Network, and was devoted to the overview of the activities of the network during the past two years. The principle of the network was represented in the form of Eastern egg and it is based on the close interactions between different working groups: research projects, working group on the genetic diagnostic laboratories, the medical profession and the patient organisations, resources, legal and ethical group, intellectual property group and industrial partners.

Activities of the CF Network include quaternary issued newsletters, leaflet of the CF Network, a manual for CF patients and their families in 12 different languages, steering committee meetings, industry meetings, meetings with CF associations as well as meetings regarding guidelines for genetic testing. The activities also include: annual satellite CF network symposia, annual Eastern and Central European CF network International symposia and annual meeting of CFTR expression working group, as well as joint meetings with WHO-ICF(M) meetings and ECFS Board meetings. There is also a number of European fundamental projects such as: CF-CHIP, Sensitive Proteomic, PNA CHIPS, GENE THERAPY, CF PRONET, CRMGEN, EMQN and ESTO-STRATA in the frame of CF thematic network. Information regarding CF thematic network are available on the web site: http:/www.cfnetwork.be.

In the second presentation, E. Dequeker, coordinator of CF network Working group diagnostic laboratories, presented the main objective of the working group, and reported on the activities in 2002, involvement in other projects, and planned activities in 2003. The main objective of the working group is to improve the quality of genetic testing laboratories, trough organisation of external quality assessment schemes, regional training sessions, short fellowships, implementation of the recommendations for molecular diagnostic laboratories and validation of new testing methods. This working group also closely interacts with ACMG, CDC, CRMGEN and ESTO. So far, 7 annual EQA schemes were successfully accomplished, from 1996 with 136 till 2002 with 210 participating molecular diagnostic laboratories. Aims of EQA-CF schemes are to evaluate accurate genotyping and written reports. The annual EQA schemes are planned to be continued in the future.

The third presentation was a joint presentation by H. Cuppens and M. Macek Jr. on report of the Genoa WHO meeting " World Wide Molecular Genetic Epidemiology of Cystic Fibrosis". Harry Cuppens gave the summary of the Genoa meeting, which was attended by 22 scientists from all over the world, involved in the genetic epidemiology of cystic fibrosis. CFTR gene is very polymorphic, and so far, it has been registered 1203 mutations, with the different frequencies and regional distribution. M. Macek Jr. presented results based on 15605 cystic fibrosis patients data obtained from 121 Consortium members from European and Mediterranean countries. The mean mutation detection rate is 87%. Results regarding North Africa, Middle East, USA and Asia-Pacific were also briefly presented. The data obtained in this worldwide mutation distribution are of great importance, and can serve as a guideline for targeted and economical cystic fibrosis mutation screening.

The fourth presentation "Rethinking Gene Patents", was given by G. Van Overwalle, coordinator of intellectual property group, within CF Network. In 2002, the gene patent debate, especially regarding BRCA gene and Myriad patents is revived. There are

mainly two sorts of criticism: one regarding legitimacy of gene patents (initiated by civil society, national parliaments, scientific community and social and legal research institutes), and the other regarding the scope of gene patents initiated by European Commission. Keeping in mind that gene patent discussion is back to stay, cooperation of industry is highly desirable, both in the legitimacy and scope debate. A copy of the paper "Gene Patents: A Different Approach" (P. Jacobs and G. Van Overwalle) was distributed to the participants.

The fifth presentation "Guidelines for genetic testing" by I. Vinck, member of the legal and ethical group within CF network, addressed the importance and need for guidelines for the application of new diagnostic and therapeutic.

A very extensive survey of the literature on the ethical, legal and social aspects of the application of diagnostic and therapeutic, national and international guidelines and reports related to genetic testing and screening in general, and relevant literature on related issues as genetic and employment and genetics and insurance, was performed.

Different issues were addressed such as: free and informed consent, general information prior to genetic testing, different kinds of tests/screening, way of communication after the testing, test information and storage, and different societal aspects. A part of the study is published by the European Commission as " Genetic testing- patients rights, insurance and employment; a survey of regulation in the EU" (H. Nys, I. Dreezen, I. Vinck, K. Dierickx, E. Dequeker, J.J. Cassiman). A sample copy of the book circulated among the participants, and a wide interest was expressed.

Dragica Radojkovic, Leuven, Belgium

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DEVELOPMENT OF ULTRA-SENSITIVE METHODS FOR PROTEOME: APPLICATION TO CYSTIC FIBROSIS (QLG2-CT-2001-01335, COORDINATOR ALEKSANDER EDELMAN)

Area: 8.2 Functional genomics and proteomics, Research into Genome and diseases of genetic origin

Objectives of the research

The objectives are to **find and characterise differentially displayed proteins** and mRNAs whose expression patterns are specific to a particular disease e.g. **cystic fibrosis, CF. Several high performance proteome techniques** which will allow the identification of **proteins with high and also low abundance** will be improved and developed. We will **establish a database** correlating the identified differentially displayed proteins (dd-PROT) and mRNAs. A better understanding of the mechanisms underlying cystic fibrosis should be gained in order to improve diagnosis and/or therapy.

Analysis is performed on proteins prepared from cells of CF patients and from non-affected members of their families, as well as from cultured cells. Several high performance techniques will be used:

1. Multi Photon Detection (MPD) - enhanced differential display of proteins (dd-PROT/MPD), *i.e.* the supersensitive analysis of 2D electrophoresis gels by the MPD technique,
2. Protein sequence determination by mass spectroscopy techniques using new, high sensitivity methods;
3. High performance microarray technique for mRNA analysis.
4. Innovative bio-informatics tools, eg. new software for 2D electrophoresis gel analysis and data base development.

We hope

1. To develop an integrated proteomic/genomic system for the detection of low abundance proteins.
2. To adapt the analysis of 2D gels by MPD to proteins prepared from cells of cystic fibrosis patients bearing the same mutation but presenting different phenotypes.
3. To optimise protein analysis on 2D gels by developing fractionation methodology based on the MPD technique and capillary electrophoresis.
4. To determine the peptide sequences of differentially displayed proteins.
5. To analyse post-translational modifications of differentially displayed proteins.
6. To analyse the mRNAs and proteins from the same biological samples using a high performance microarray technique.
7. To establish a data base correlating differentially expressed proteins and mRNAs in CF cells.

The partners of the projects are from France (M. Goossens INSERM U.468, Créteil, Aleksander Edelman (coordinator) INSERM U.467, Paris), Germany (A. Schrattenholtz, Proteosys AG, Mainz), England (J. Godovacs-Zimermann, Royal Free and University College Medical School University College London), Denmark (Bog-Hansen, University of Copenhagen, The Protein Laboratory, Institut Molecular Pathology), Poland (Piotr Zielenkiewicz, Polish Academy of Science Institut of Biochemistry and Biophysics). Starting from February 2003 a new Polish partner jointed the project (M. Dadlez, Polish Academy of Science Institut of Biochemistry and Biophysics).

Principle investigator (coordinator):
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CF AGING STUDY

Three reports of aging in CF have been presented at CF meetings in the last two years. They all emphasised the need to study the risks for CF-associated diseases of aging before CF centres are filled with geriatric patients who have new or different responses to diseases of aging.

All stated the need for a prospective international study of all patients age 40 and older concerning their health problems, plus an annual review of nutrition, body weight; lifestyle; treatments including physiotherapy and alternative treatments; pulmonary function, lung and other infections; hospitalisations; surgery; gastrointestinal or renal problems; joint and skeletal problems; medication including self-medication and alternative medicines; quality of life; new diagnoses; and new symptoms even if no diagnosis has been made. Because the numbers of such patients in individual clinics are few, there is a need to collaborate and share our experience so that we may more quickly build up a knowledge base which will be appropriate for older patients' needs

A comprehensive survey of these senior patients will be repeated once a year. All included patients will give written, informed consent. For confidentiality, individual data will only require birth date, sex and the first two letters of the first name. Data will be kept in a secure, off-line computer with access only to an international study committee including CF physicians, gerontologists, database experts, and epidemiologists. Special groups who will decide on the data to be collected are: Coordination, Database, Epidemiology, Stakeholders, Caregivers, and Sponsors. The Coordination Group will include a WHO observer, CF patients, epidemiologists, database specialists, experienced CF physicians, and representatives of the ECFS and the NACFF.

Progress will be reported at the ECFS and NACFF conferences, and published in journals related to CF and to aging. Other physicians may ask questions of the database with the approval of the Coordinating Group and database safeguards for confidentiality.

Support for the study and for future projects will be sought from governmental, foundation and charitable sources, and from pharmaceutical organisations. For success, CF Worldwide will be an essential partner, in supporting the recruitment of CF patients.

Warren J. Warwick, Minneapolis, USA, email: warwi001@umn.edu

John A. Dodge, Swansea, UK, email: j.a.dodge@btinternet.com

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OECD SURVEY: ENABLING QUALITY ASSURANCE OF GENETIC TESTS

Genetic testing is a fast moving and exciting new technology. Genetic mutations responsible for almost 100 monogenetic inherited disorders have been identified, and it is now possible to find out whether you carry a genetic disease, or even have a predisposition to one - for example, breast cancer. The proliferation and increasing availability of these genetic tests has led to calls for international regulation and quality assurance, especially since samples are often sent abroad for analysis. In Europe, there are no common requirements or standards to guarantee that the tests are reliable and are carried out by reputable organisations, although voluntary accreditation schemes do exist in many countries. The European Commission is funding research by the OECD (Organisation for Economic Co-operation and Development) to survey hundreds of genetic testing laboratories across its member countries. The aim is to find out who they are, what tests they are doing and whether they are currently using quality standards - such basic information is an absolute prerequisite when devising international regulations for genetic testing.

The research is being funded under the Fifth Framework Programme's Quality of Life Programme to promote international synergy. A pilot study has already been carried out in laboratories in nine OECD countries, and a full-scale survey involving at least 18 countries will begin at the end of January 2003 and last for two years.

Standard practice

The need for an international regulation of genetic testing has been repeatedly highlighted around the world. In the late 1990s, a Concerted Action on Genetic Services in Europe, and an EC-sponsored international symposium both called for guidelines and internationally agreed principles, whilst a Task Force on Genetic Testing in America reported concern about the overall quality of genetic tests.

At an OECD workshop in February 2000, many countries reported that proficiency testing is not an obligatory part of the certification procedure, so can operate without any independent check on their performance or standards. In America, an External Quality Assessment scheme is now mandatory, but this is only respected in a few OECD countries. Elsewhere, quality assessment schemes are voluntary, which is mainly due to the rapid pace of development in molecular testing technology. The best and most reliable tests to be employed are decided by consensus amongst practising professionals so that changes in technology can be incorporated easily. The problem is that this approach involves no enforcement and provides no insurance against badly run laboratories.

At the moment, there is no compiled information on what tests are being done and what quality assurance schemes are operating in Europe. Researchers plan to survey practising laboratories anonymously to establish this. To further gauge the need for international regulation, the survey will include questions about the qualifications and experience of the practitioners and whether or not laboratories receive samples from abroad. Labs will

also be asked whether they require written consent or promise confidentiality, since there are pressing ethical issues surrounding the ownership of genetic information.

Information on-line
The survey will be mainly carried out on the web, using up-to-date technology to maintain the privacy of respondents. Results will be published in peer-reviewed journals and by the OECD. The ultimate goal of the project is to identify areas for international co-operation in the development of standards, proficiency testing and guidelines. Even if these are not mandatory, at least members of the public will have a way of checking that the laboratory handling their genes adheres to an internationally recognised set of standards.

E. Ronchi, OECD, Biotechnologie Unit, Paris, France

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REPORT TRAINING

VISIT CYSTIC FIBROSIS LABORATORY, VERONA, ITALY

Aleksandra Divac, research scientist in Laboratory for molecular biology, headed by Prof. Dr. Ana Savic, at the Institute of Molecular Genetics and Genetic Engineering, Belgrade, Yugoslavia, visited Cystic Fibrosis Laboratory, headed by Prof. Dr. Pier Franco Pignatti, Section of Biology and Genetics, Department of mother and child, and Biology - Genetics, University of Verona, Verona, Italy, from 28th October to 12th November 2002.
The purpose of the visit was to gain experience in sequencing, using the ABI Prism 377 automated sequencer. Additionally, the use of statistical methods in calculating genetic risks and population genetics was to be studied.

The samples that were sequenced have been previously analyzed using denaturing gradient gel electrophoresis, in Belgrade, and found to be carriers of some change in one of the exons of CFTR gene. The Big Dye Terminator v 2.0 protocol for ABI Prism 377 was used for sequencing reactions.

The exchange and comparison of protocols for denaturing gradient gel electrophoresis and PSM and digestion analysis of the CFTR gene also took place.

I had the opportunity to learn more about the statistical analysis of data, especially about calculations of genetic risks of having an affected child depending the parent's carrier status. Also, I was introduced to the fundamentals of genetic epidemiology.

The expertise gained from this visit was very valuable, and the contacts that were made make a good basis for future collaboration.

Aleksandra Divac, Institute of molecular genetics and genetic engineering, Belgrade, Yugoslavia.

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REPORT TRAINING

VISIT CYSTIC FIBROSIS LABORATORY, LISBOA, PORTUGAL

Stephanie Hirtz, research technician in the Cystic Fibrosis Group, headed by Dr Marcus Mall, in the Department of Pediatrics and Adolescent Medicin, University of Freiburg, visited the Cystic Fibrosis Research Laboratory, headed by Prof Margarida Amaral and Dr Deborah Penque, Centro de Genetica Humana, Instituto Nacional de Saude Dr Ricardo Jorge (INSA), Lisboa, Portugal, between 18-22 February 2003.

The purpose of the visit was to contribute with expertise from the Freiburg group to train members of the INSA Cystic Fibrosis research group on Ussing chamber measurements to evaluate Cl^- secretion in human rectal biopsies from individuals suspected to have CF similar to that done by Mall et al (Am J Physiol Gastrointest Liver Physiol 2000, 278, G617-24). Considerable expertise was gained in the method and in the interpretation of results. In some of the experiments, it was also possible to test the effects of some compounds on CFTR channel properties. Dr. David Sheppard, from the Department of Physiology, University of Bristol was also visiting the group at the same time and contributed to the success of the study.

The studies so far initiated will form the basis of further experiments to be carried out in Lisboa to test more compounds. The expertise gained from the visit was essential for the establishment of a protocol for in vivo validation activators / blockers of CFTR.

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ANNOUNCEMENTS

CF EXTERNAL QUALITY ASSESSMENT SCHEME 2002

Since 1996, annual external quality assessment schemes for CF have been organized. Two hundred and six laboratories participated in the EQA scheme of 2002. The aim of the QA scheme was to evaluate both practical analysis, reporting and interpretation of the data. It was therefore requested that each laboratory used its routine testing procedure and sent written reports in the manner routinely used.

Laboratories that gave correct genotype data and no serious interpretation errors in the reports will receive a certificate of successful participation. Those that sent only correct genotypes on the data sheet, but did not send reports, will receive a letter to

confirm their technical successful (error-free) genotyping.

The DNA samples of the EQA scheme of 2002 were obtained from individuals carrying the following genotypes/

Case 1
Janssen Robert CF02-1: 1717-1 G to A / wild type
Sue Cate CF02-2: wild type / wild type

Case 2
Morley John CF02-03: F508del / 621+1 G to T

Case 3
Meyer Kristen CF02-4: F508del / G551D

Case 4
Heutink Jenthe CF02-5: W1282X / S1251N

Case 5
Barreto H. CF02-6: G542X / F508del

Some of the laboratories will be surprised on the genotype of sample CF02-5. One of the mutation detection methods gave another genotype. At present we are looking what went wrong with this kit in different laboratories. As soon as the problem is solved, we will contact immediately all the laboratories.

The EQA scheme also included a second part that was optional. The expected genotype for sample CF02-7 was 1717-1 G to A / 3272-26 A to G.

The general report, the individual reports for the first and the second part of the EQA scheme 2002, and a certificate of those who participated successfully, will be sent in June 2003. The report of the second part of EQA scheme of last year (2001 - evaluate the quality of sequencing technology) will also be sent in June 2003.

The next EQA scheme for CF is foreseen for autumn 2003.

Els Dequeker, Leuven, Belgium

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MANUAL FOR CF PATIENTS AND FAMILY

A manual for CF patients and family is available in English, French, German, Italian, Spanish, Czech and Slovak, Croatian, Ukrainian, Latvian, Serbian and Russian. Recently the manual has also been made available in Hungarian, Lithuanian, Polish and Macedonian. All languages can be downloaded as a pdf file from the CF network website (http://www.cfnetwork.be/Informationdocuments.htm)

The manual includes the following topics:

- what is cystic fibrosis
- what happens in the lungs
- what happens in the pancreas
- when to suspect cystic fibrosis
- how is cystic fibrosis inherited from the parents
- to have a child with CF … and to accept a child with CF
- treatment of cystic fibrosis
- hospital
- relatives and friends
- you are not alone

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CFTR-GENE PRIMERS FOR DGGE

Ingeny International offers a special price for the CF network members ordering CFTR-gene primers for DGGE. For more information see Newsletter July 2000 or contact:

Ingeny International BV
Amundsenweg 71,4462 GP Goes
The Netherlands
Tel. +31 222 920 - Fax +31 222 923
http://www.ingeny.com - e-mail: info@ingeny.com

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CONGRESSES AND MEETINGS

Under this heading we would like to inform you on congresses and meetings of interest to members of the CF network. If you organise a meeting open for the public, please inform us. We will be happy to announce your meeting via the CF network newsletter and website. Please inform us also about other interesting meetings not yet mentioned in this list.

European Human Genetics Conference
Birmingham, U.K., 3-6 May 2003

26th European Cystic Fibrosis Conference
Belfast, Northern Ireland, - 4-7 June 2003

Workshop Diagnostic Laboratories for Nordic / Baltic countries
Lund, Sweden, - 11-12 September 2003
Contact: Els.Dequeker@med.kuleuven.ac.be
ulf.kristoffersson@klingen.lu.se

7th European CF Network International Symposium 2003 organised for Eastern and Central Europe
Bratislava, Slovakia, - 20-21 September 2003 (tentative date)

4th Meeting with Industrial Partners
Brussels, Belgium, 27 November 2003

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