Cystic Fibrosis European Network

NEWSLETTER

FEBRUARY 2001 – N° 4

CONTENTS

NEWSLETTER CONTRIBUTIONS

The closing date for the next issue of the CF Thematic Network newsletter is June 15, 2001.
Please forward your data and contributions for the newsletter to:

Dr. Els Dequeker
Tel: 32-16-34.58.81
E-mail: Els.Dequeker@med.kuleuven.ac.be

Renée Haesendonck
Tel: 32-16-34.58.72
E-mail: Renee.Haesendonck@med.kuleuven.ac.be

Center for Human Genetics
University of Leuven - Campus Gasthuisberg
Herestraat 49 - B-3000 LEUVEN
BELGIUM
Fax: 32-16-34.59.97

EDITORIAL

Dear colleagues and friends,

The Network is already in its second year and so many activities remain to be planned and executed. Not that the network is lingering around. You will find proof in this newsletter that many aspects are being covered and this well within the planned time frame.

During the recent board meeting of the ECFS the relationship between the Network and the society was discussed. Apparently some members of the CF community see the network as a potential competitor. I have explained to the board members that if the president of the European CF society is a member of our steering committee, it is precisely to make the relations with the society as close as possible. The Network organises each year a session at the European CF congress, again to formalise its genuine interest in the society. The network regroups mainly scientists and other professionals, who at present are fairly peripheral to the mainstream member of the society. Bringing all these people together can only benefit CF research and treatment in Europe and thus the society. In other words, the Network has specific aims and activities open to all interested people. As the society grows it could in time take over these activities creating a real platform for all those suffering from or working one way or another on CF.

On another matter, the Network has grown in the last few days with another research project. The EU has approved our CF-Pronet fundamental research proposal for a period of 3 years. More details on this later in the year. It was our initial aim to fill the Network egg (cf. the figure on the web) with research projects. We now have two: the one coordinated by Michel Goossens on New Diagnostic Tools and one coordinated by me on the CF Protein network. If other groups, whom we do not yet know, want to join us they are of course welcome to do so.

Plenty of work and activities before us. Keep at it and keep using the facilities of the Network; they are there for you.

Jean-Jacques Cassiman
Coordinator of the CF-network
Leuven, February 2001.

■■■■■■■■■

STEERING COMMITTEE MEETING REPORT
Verona, Italy, January 27 - 28, 2001

Present: Jean-Jacques Cassiman (Leuven, Belgium), Els Dequeker (Leuven, Belgium), John Dodge (Swansea, U.K.), Margarida Amaral (Lisboa, Portugal), Michel Goossens (Créteil, France), Pier Franco Pignatti (Verona, Italy), Cristina Bombieri (Verona, Italy), Dirk Schindelhauer (Munich, Germany), Geertrui Van Overwalle (Leuven, Belgium), Kris Dierickx (Leuven, Belgium), Pierre Saelen (Leuven, Belgium)

Apologies: Gerd Doering (Tuebingen, Germany), Roland Kozlowski (Bristol, U.K.), Herman Nys (Leuven, Belgium)

The second steering committee of the CF network took place the last weekend of January in Verona.

In the first part of the meeting we reviewed the workpackages described in the project. The Workpackage information consisted of Newsletters sent in March, July and October 2000 electronically and by regular mail for the participants without e-mail (all together to 800 participants of the network). The webpage of the CF network contains information on the CF network and the different working groups. A database with all the coordinates of participants is linked to this website.

Different meetings were organised by the network, including the steering committee meeting, the annual symposium of the CF network during the International CF congress in Stockholm, a meeting with representatives of diagnostic and therapeutic industries, and a CF symposium for Eastern and Central European countries. The representatives of the CF network steering committee participated in a working meeting of the WHO on "Classification of Cystic Fibrosis and related disorders" and in the Board meeting of the European Cystic Fibrosis Society.
The different working groups coordinated by M. Amaral, G. Van Overwalle, H. Nys and E. Dequeker presented their activities and work of the last year. A report of their activities can be found elsewhere in this newsletter. D. Schindelhauer, P.F. Pignatti and M. Goossens, all representing CF research projects, gave a short overview of their activities. J. Dodge summarised the report of the working group meeting of the WHO organisation on "Classification of CF and related disorders".
In the second part of the meeting the proposal of informed consent form to take, preserve and use tissue samples was discussed. The discussion is not finished yet and a working group of international legal, medical and ethical experts will try to finalise this work in the next few months.

Els Dequeker, Leuven, Belgium

■■■■■■■■■

INDUSTRY MEETING REPORT
Brussels, Belgium, November 28, 2001

A meeting with representatives of the diagnostic and therapeutic industry (Ingeny International, Innogenetics, Applied Biosystems, Roche Diagnostic Molecular Biochemicals, Roche Molecular Systems) was held in November in Brussels. With 10 persons from the industry and the steering committee of the CF network, a roundtable discussion was held on the tools and aims of the CF network, which information channels the CF network can use, the possible interaction with the industry, the need for further translation of the manual for CF patients and their family. Furthermore the participants agreed to be involved in drafting the consensus guidelines for the application of diagnostic tools for CF.

This information meeting with the industry will be organised annually; the next meeting will be in the autumn 2001. If you are aware of someone in the industry who would be interested in our CF network, or you are a representative of a diagnostic or therapeutic industry yourself, please contact
Prof. J.-J. Cassiman and/or Dr. E. Dequeker,
tel. + 32 16 34 58 60, fax + 32 16 34 59 97,
or e-mail CF.network@med.kuleuven.ac.be

Els Dequeker, Leuven, Belgium

■■■■■■■■■

4TH INTERNATIONAL SYMPOSIUM FOR CYSTIC FIBROSIS: SCIENTIFIC AND PERSONAL POINT OF VIEW

4th International Symposium for Cystic fibrosis, organised under umbrella of CF EU Network was held at the Semmelweis University, Faculty of Medicine in Budapest, Hungary. This two days meeting aimed at being an opportunity to bring together clinicians and researchers involved in diagnostics, treatment and research of cystic fibrosis and related diseases. The programme included 4 thematic panels (Clinical, Epidemiology, Research and Development and Testing) covered by 17 oral presentations and 16 posters. The Symposium was attended by almost 50 participants, mainly from Central and Eastern Europe and Balkan Countries. Both scientific and clinical presentations were of high quality, reflecting a steady progress in diagnosis, treatment and research in cystic fibrosis and related diseases. All sessions were followed with great attention, and there were a lot of productive discussions. That was really a good opportunity to bring clinicians and laboratory people close together, so they were able to understand better the problems and needs of both sides.

As a sort of a « veteran » of the meetings started in 1997 under the umbrella of INCO-BIOMED, I was really glad to see the obvious progress that we made from Prague 97' till nowadays. In my opinion, that

progress was very well highlighted in the Milan Macek JR oral presentation on « Distribution and ethnic origin of 151 CF mutations in Balkan, Central and Eastern European populations ».

During the past 4 years, in Central and Eastern Europe and in the Balkan countries, despite of a lot of difficulties, both clinicians and diagnostic services improved their standards. It was really encouraging and promising to see new young faces, especially in the research field. Also, with recent political changes in this part of Europe, I am pretty sure that new possibilities will arise for further improvement of our collaboration. What we have now is a solid ground for the future tasks.

Dragica Radojkovic, Belgrade, Yugoslavia

■■■■■■■■■

THE EUROPEAN QUALITY ASSESSMENT SCHEME FOR CF 2000

Since 1996, the European Quality assessment (QA) scheme for Cystic fibrosis was organised on a large scale. 195 laboratories participated in the QA scheme of 2000, which is an increase of 23 laboratories compared with last year. This quality assessment scheme was a joint QA scheme of the European CF network and the EMQN project (European Molecular Genetic Quality Network). The scheme was designed to evaluate both the practical analysis (genotype results) as well as the interpretation of the data (the written reports with the test results sent to the physician who asked for the tests).

The evaluation of the QA scheme was done in two parts: first the genotype results (data sheet and the raw data), secondly the interpretation of the written reports.

The correct genotype results for the six DNA samples were as follows:

Case 1

Cecile Fribourg, CF00-1, F508del/wild

Pierre Hudson, CF00-2, G542X/wild

David Hudson, CF00-3, G542X/F508del

Case 2a

Peter Bach, CF00-4, G551D/R553X

Case 2b

Elisabeth Ducan, CF00-5, F508del/R117H

Case 3

Teresa Mitchell, CF00-6, N1303K/wild

The final evaluation meeting, with three quality assessors and the scheme organiser took place in Leuven, in the beginning of February. Ten percent of the laboratories (total of 191 laboratories) incorrectly typed one or more alleles (on a total of 12 alleles). As in previous quality assessments, the error types ranged from administrative errors, erroneous technical results to misinterpretation of the (technical correct) data.

174 laboratories (91%) sent written reports together with the genotype results. In general, the quality of the reports has improved; many laboratories have incorporated suggestions made after last year's scheme. In particular, sample and patient information has been improved, as well as the technical information and interpretation of results.

Unfortunately, 39% of the laboratories which submitted reports made in one or in more reports mistakes. These reports contained administrative errors or a wrong interpretation of the results. 3 laboratories mixed the genotype results or samples from patients and carriers.

The general report, the individual reports, and a certificate for those who participated successfully will be sent at the end of March 2001.

The next CF QA scheme is foreseen for autumn 2001.

Els Dequeker, Leuven, Belgium

■■■■■■■■■

ADVANCES IN THE ACTIVITIES OF THE WORKING GROUP ON CFTR EXPRESSION (CFTR-EWG) - RESOURCES

The CFTR Expression Working Group (CFTR-EWG) – Resources, in collaboration with the Coordinating Centre Leuven, has now its **webpage** available on-line:
http://www.med.kuleuven.ac.be/cme/cf/CFTRexpression.htm

The CFTR-EWG includes **six thematic areas** and for each a Rapporteur (i.e., a Scientific Coordinator) has been assigned. These are:

A. Transcript analysis (Ann Harris, Oxford, UK)
B. Cell Biology and Histology (Edith Puchelle, Reims, France)
C. Protein Biochemistry / Biophysics (Aleksander Edelman, Paris, France)
D. Cell Physiology (David Sheppard, Bristol, UK)
E. *In vivo* and *ex vivo* functional assessment (Burkhard Tümmler, Hannover, Germany)
F. Models (Bob Scholte, Rotterdam, Netherlands)

The CFTR-EWG has now **57 members**: 11 from North America (USA and Canada), 3 from non-EU Europe (Czech Republic, Switzerland and Israel) and the rest are from EU country members. Membership is totally free and open to any scientist in the CF field. Members receive the Newsletters on CFTR Expression (before it is on-line) besides additional information and news from the CFTR Expression WG. To apply for membership please consult the CFTR-EWG webpage (see below).

The **Newsletter** of the CFTR-EWG is published every 3 month (*i.e.*, March, June, September and December) to disseminate the activities of the CFTR-EWG. So far, 3 issues were published starting in June 2000. The published issues are available on the CFTR-EWG webpage (see above) and contain reviews by the Rapporteurs/members, reports, announcements, forum for discussion, etc. on the six methodological expertise areas of the CFTR-EWG.

The CFTR-EWG has created the **Virtual Repository of Methods and Resources**, *i.e.*, a catalogue on-line to be used freely by the scientific community briefly describing useful tools for CFTR expression and function studies: **reagents** (antibodies, primers, drugs, etc.) also indicating how these can be obtained (commercially, or through researchers), **models** (cell lines, animal models, etc.) and **detailed protocols** with the respective bibliographic references. This Virtual Repository includes all the above-mentioned six thematic areas and its structure already exists. It will soon be on-line (at the moment only available by downloading Newsletter no. 2). There are already a few contributions from members and many more are already scheduled for the year 2001. All scientists working in the field of Cystic Fibrosis are encouraged to contribute to this Virtual Repository (for details, please consult our webpage).

It is also an aim of the CFTR-EWG to promote **Annual Meetings** for discussion of all six thematic topics. The next annual meeting of our WG will take place in Estoril (near Lisbon), 30 March - 1 April, 2001. This year, the CFTR-EWG has decided to include also the topic 'Novel Methodologies' (including novel DNA analysis methods, RNA arrays and Proteomics). Deadline for abstracts and registration: 28 February 2001. The meeting will be open to all scientists in the CF field who are interested in discussing and reporting CFTR expression with a focus on methodological approaches. Members of the CFTR-EWG will have reduced registration costs.

The CFTR-EWG also promotes the exchange **of scientists** between labs for the acquisition or comparison of methodologies used in CFTR expression studies, by covering travel and accommodation costs (up to 750 Euro). From each stay a **contribution to the Virtual Repository** is expected (en entry such as a novel cell line, a new antibody, or a protocol), besides a small report to be published in the CFTR-EWG Newsletter. Anyone interested in applying to visit another lab for this purpose should consult our webpage (see above). During 2000 we only had one application (from Lisboa to Uppsala).

Margarida D. Amaral, Lisboa, Portugal

■■■■■■■■■

INFORMED CONSENT AND PATENT LAW: Analysis of the Key Notions of "Public Order and Morality" in Patent Law

During the last few months we undertook an in depth analysis of the legal concepts of Public Order and Morality. This analysis was divided up in four main questions.

First, what is the meaning of Public Order and of Morality in law in general? We analysed this on a national and on an international level. On the national level both concepts have very different meanings in each Member State. However a common European core is emerging. On the international level the interpretation of this twin concept seemed to be more important for our study, because treaty law mostly shapes patent law in Europe.

Second, is the informed consent requirement part of Public Order and Morality in national law?

Third, what is the meaning and function of Public Order and Morality in patent law, with regard to national patent laws, the European Patent Convention and the Agreement on Trade-related aspects of Intellectual Property rights of the WTO (TRIPs)?

Fourth, can or should the informed consent requirement be introduced in patent law through the Public Order and Morality clause?

We only have preliminary results for now. The following months will consist of the exemplifying of those results and of a follow up of the implementation of the EU-Biotechnology Directive in the EU Member States.

Geertrui Van Overwalle, Leuven, Belgium
Pierre Saelen, Leuven, Belgium

■■■■■■■■■

CONSENT FORM TO TAKE, PRESERVE AND USE TISSUE SAMPLES

After intensive discussions with J-J. Cassiman and his collaborators (Center for human genetics) and with the fruitful help of Caroline Trouet (Center for Biomedical Ethics and Law), the medico-legal and medico-ethical group of the CF Network (Herman Nys & Kris Dierickx) has elaborated a consent form to take, preserve and use human tissue samples. All comments, suggestions and questions may be sent to herman.nys@med.kuleuven.ac.be

As a general rule, a competent adult should give explicit informed consent to participation in any research. In this contribution we present an example of a consent form to take, preserve and use human tissue samples for quality assessment / research. This consent form is a consent form in the strict sense of the word. This means among others that it is part of a process of information giving and

explanation facilitating informed decision-making, rather than as a one-off act. The signed consent form, which is often seen as 'consent', is simply evidence that this process has been carried out. This implicates that the quality and clarity of the information which is given, is very important. This information, which precedes the consent to take, preserve and use human tissue samples for quality assessment and/or research is not included in this text, but presupposed. Here, only the formal consent page is presented.

"I …… (family name and first name) have read the accompanying information and I understand the nature and purposes of the scientific research and quality assessment that will be performed with the blood/tissue sample that will be taken with my consent

I have had the opportunity to ask any question regarding this research and I confirm that all my questions have been answered in a satisfactory way.

I have been informed that my identity will be kept secret and that the identification of my biological material can only be done through a unique code.

I am aware that my biological material will not be destroyed and will be preserved for research in the future unless I have requested explicitly for its destruction.

Herewith I give my explicit and free consent for <u>each</u> *of the following purposes:*

1. *the use of my biological material for quality assessment*
2. *the use of my biological material for genetic research regarding CF and general scientific research (genetic and non-genetic)*
3. *the supply of my biological material to specially organised international institutions that collect and preserve biological material and supply it against payment to researchers*
4. *the use of my biological material for the development of an international quality standard*
5. *the possible application for patents for inventions for which my biological material has been used*
6. *the possible development of commercial and industrial applications of these inventions*

I wish / I do not wish (delete what is not applicable) to be contacted when in the course of the above mentioned scientific research/ quality assessment during which my biological material has been used, new information is revealed concerning my health status or that of my relatives .

Read and approved

Signature *Place and date*"

Herman Nys, Leuven, Belgium
Kris Dierickx, Leuven, Belgium
Ingrid Dreezen, Leuven, Belgium

■■■■■■■■

REPORT INSERM MEETING

Report on a two-days meeting entitled "About good uses of genetic tests" organised in PARIS, France (Friday 26th and Saturday 27th January, 2001) by INSERM (Institut National de la Santé et de la Recherche Médicale).
Claude Griscelli (Director of INSERM), Marie-Anne Bach and Marc Fellous welcomed the lecturers (37) and the participants (more than 250) and explained the scope of the meeting i.e. to remind regulations on genetic testing and exchange information and experience between Research and Clinical labs on the questions of utility/validity of genetic tests in diverse clinical situations. The meeting was focused on four main topics.

<u>1. The prescription of genetic tests</u>
Marie-Anne Bach (from INSERM Public Relations, Paris) gave a presentation about the legal frame of genetic testing in France. The practice of prenatal diagnosis is subjected to the "Law of Bioethics" of 1994, and a specific consent delivered by the Ministry of Health is necessary. Recently, the legislation has been strengthened, as only authorised laboratories will be able to perform "the study of the genetic characteristics of an individual" for predictive or diagnostic purposes (decree of 23 June 2000). An overview on the constraints and insufficiencies of the law was given.

a) Genetic diagnosis of a declared disease (coordinator: Jean-Louis Mandel from Strasbourg).
Several speakers analysed the issues of benefits and risks of genetic testing in different clinical contexts.
Vincent des Portes, from the Neuropaediatric Department at Saint-Vincent de Paul (Paris) distinguished three different situations for genetic testing in *mental retardation* which affects more than 2% of new-borns: confirmation of the clinical diagnosis, presymptomatic or prenatal diagnosis. A major problem is that the result cannot be predictive of the future severity of the handicap.
Martine Aiach, from the Haematological Department of Hospital G. Pompidou (Paris) reported on *thrombosis*, which is associated with mutations in Factor V or Factor II in 20-30% or 8-10% of cases, respectively. Genetic investigations include at present 5 genes: antithrombin, protein C, protein S, and the two mutations in Factors V and II.
Marc Fellous from Institut Pasteur (Paris) presented the state-of-the-art of the genetic causes of *male infertility* (the frequency of this phenotype is 10% in the population), then focused on the clinical relevance of molecular diagnosis of Y-chromosomal microdeletions and best practice guidelines for genetic counselling.
Alain Bernheim from Institut Gustave Roussy (Villejuif) gave a comprehensive overview on the potential benefits in using new powerful tools (such as FISH or CGH combined with micro-arrays) to

study *somatic genetics* for the diagnosis of a variety of tumours.
He replaced Anne Janin from Hospital Saint-Louis (Paris) to present the multiple problems associated with the organisation in France of *tissues banking and cryopreservation of tumoral specimen.* Pathologists, Haematologists and Cancerologists agreed to write consensus best practice guidelines in order to maintain the level of quality of tissues, which is necessary for molecular analysis.

The clinical validity (sensitivity, specificity and predictive value) of genetic testing in ***multifactorial diseases*** was presented by François Cornelis from Hospital Lariboisière (Paris) who showed how different can be the positive predictive value according to the tested population. The conclusion was that genetic testing for common diseases has no useful predictive value for an individual who does not present clinical signs, due to the high frequency of unaffected people positive for the test, especially illustrated for HLA and arthritis.
Xavier Jeunemaître from Hôpital G. Pompidou (Paris) had a different view on the present and future of genetic tests in *hypertension* (whose frequency in the general population is 10-30%). It is estimated that 30% of the variance of blood pressure is associated with genetic factors. Because empirical treatments are not satisfactory, there is hope that new microarrays technologies will improve the study of genetic susceptibility and lead to the development of personalised medicines.
Francis Vasseur from Institut Pasteur (Lille) presented a review of all the genes that have been associated with *diabetes*, and a comprehensive strategy for testing the five forms that are well genetically characterised (MODY1-5). By now, only monogenic forms of *obesity* can be tested (Leptine Ob, Leptine receptor, Pro-opiomelanocortin, Pro-hormone convertase 1, and Melanocortin receptor MC4R). A recent study estimated that 4% of obese individuals carry MC4R mutations.

b) Presymptomatic tests (coordinator: Josué Feingold, INSERM, Paris)
Predictive testing is of two types: presymptomatic (eventual development of symptoms is certain when the gene mutation is present, e.g., Huntington disease) and predispositional (eventual development of symptoms is likely but not certain when the gene mutation is present, e.g., breast cancer).
Alexis Brice from Hôpital Pitié-Salpêtrière (Paris) presented presymptomatic tests in *late onset diseases*. Predictive testing may be medically indicated if early diagnosis allows interventions which reduce morbidity or mortality or give specific genetic counselling or better follow-up. There is a general agreement that testing protocols should include a pre-test assessment of emotional state so that post-test counselling can be further adapted to individual situations.
Dominique Stoppa-Lyonnet from Institut Curie (Paris) reported guidelines for the management of *breast cancers* and criteria for genetic testing for cancer susceptibility that were recommended by the group of experts from INSERM and FNCLCC, then presented the network of clinicians and laboratories involved in breast and *ovarian cancers* in France.
Sylviane Olschwang from Hôpital Saint-Antoine (Paris) showed that *colorectal cancers* account for 10% of death due to cancer in developed countries. She mentioned the difficulties in documenting a family history of cancer in order to provide counselling regarding familial cancer risk and options for prevention and early detection. Because genetic testing raises a host of medical, social, psychological and ethical issues for patients and their families, it is imperative that these issues are addressed both before and after genetic susceptibility testing.

The next session was devoted to the "**management of uncertainty**" in those syndromes with major variable expressivity.
Catherine Boileau from Hôpital Ambroise Paré (Paris) gave an exhaustive presentation of *Marfan syndrome*, a disorder of connective tissue with variable expressivity involving the skeletal system (abnormal proportions, scoliosis, arachnodactyly), cardiovascular system (mitral and atrial valve prolapse, aortic dilatation leading to aortic dissection) and ocular system (lens dislocation). The diagnosis has to be established by specialists and may be facilitated by testing for fibrillin-1 mutations. However, a positive test cannot be predictive of the evolution and severity of the disease.
Pierre Wolkenstein from Hôpital Henri Mondor (Créteil) explained that the diagnosis of *Neurofibromatosis type 1* is based on definite clinical criteria so that molecular study of NF1 gene is of poor interest. Mutations are difficult to find and are not predictive of the clinical expression, which complicates genetic counselling.
Hervé Le Marec from Hôpital Laennec (Nantes) reviewed the genes found to be involved in *cardiac arrhythmia*; genetic testing can be essential to prevent the risk of sudden death, especially for long QT and Brugada syndromes. Philippe Charron from Hôpital Pitié-Salpêtrière (Paris) described genotypes and phenotypes associated to familial forms of *hypertrophic cardiomyopathy;* molecular genetics allow to revisit the diagnostic criteria used so far, and a multidisciplinary approach including geneticists, cardiologists and psychologists is currently developed in France based on a national cardiomyopathy network.
This first day ended with a discussion on demands for prenatal and preimplantation genetic diagnosis in late-onset diseases by Arnold Munich from Hôpital Necker (Paris).

2. Genetic screening (coordinator: Ségolène Aymé, INSERM SC11, Villejuif)
New-born screening identifies individuals who have an increased chance of having a specific genetic disorder so that treatment can be started as soon as possible.
Jean-Pierre Farriaux, president of AFDPHE (Lille) and A. Lordier-Brault (representative of Direction of Health, Paris) gave the state-of-the-art of *neonatal*

screening in France. From 1967 to 2000, 24 millions new-borns have been tested for phenylketonuria and more than 7000 affected babies have been detected. Such efficiency will not be reached for other diseases. A new programme for cystic fibrosis is being initiated in France this year.
Ségolène Aymé from Villejuif presented the results of a collaborative investigation about the practices of *genetic screening in Europe*, which had been conducted on behalf of the European Society of Human Genetics with the aim to produce European recommendations (www.eshg.org). To date, there is no screening programme shared by all the countries (excepted PKU and hypothyroidism).
Hélène Gaumont-Prat (National Ethic Committee, Versailles) reported on *the ethic and legal aspects* of genetic screening. The laws of Bioethics of 29th July 1994 (completed by the decree of 23rd June 2000) rule very severely prenatal and pre-implantation diagnosis, but not specifically neonatal screening. They provide limited protection against discrimination.
Claude Ferec from CHU Morvan (Brest) presented the 10 years' experience of his group on *neonatal screening for Cystic Fibrosis* in Brittany and its impact on prenatal screening of subsequent pregnancies in couples with an affected child. The screening consisted of an immunoreactive trypsynogen assay from dried blood spots, plus, from 1993, mutation analysis.
Jacqueline Yaouanq (Hôpital de Pontchaillou, Rennes) gave a presentation on the screening for *Hemochromatosis* and criteria for an improved medical management of tested patients. Jean-Yves Le Gall (INSERM, Rennes) reviewed the classification of primary iron overload and the genes that have been recently associated with monogenic and multifactorial forms.

3. The practice of Genetic Testing (coordinator: Marc Delpech, Hôpital Cochin, Paris)

Michel Vidaud from Hôpital Beaujon (Paris) presented the *regulatory environment* of genetic testing in France and described the contents of laws and decrees. In the general context of national standards for clinical laboratory testing, *Internal Quality Assurance* (best practice) has to be provided. French regulations state that each diagnostic laboratory must follow preanalytical and analytical procedures for quality assessment. However, to date, there are no official specific standards for genetic testing and molecular biology methods and there is no official cost for genetic testing.
Gilles Thomas from CEPH, Fondation Jean Dausset (Paris) illustrated the necessary technology transfer from Research to Clinical labs, with a new partner coming on, Industry. A discussion was raised on *disease gene patents* and clinical molecular genetics testing. Most basic academic and medical discoveries historically have not been patented. Some argue that patents promote and reward innovation by granting exclusivity to sell or to use an invention for a limited period of time. However extensions of patent terms on genetic tests and related discoveries will interfere with the practice of medicine, which can be viewed by others as unacceptable.
Marc Delpech from Hôpital Cochin (Paris) presented the project of organising genetic testing in France though the *creation of National networks*, in order to offer better Genetic Services than the existing "anarchistic" system. Genetic tests have been developed by laboratories in the absence of national organisation, depending on regional human and financial resources of each University Hospital, in relation with initial scientific grant support from Parent's Associations such as AFM (Association Française contre les Myopathies) or VLM (Vaincre la Mucoviscidose) and others. For some diseases, the number of testing laboratories is too high, while for many other prevalent or rare diseases there is no laboratory service delivery. There is no specific and appropriate coverage of costs associated with genetic testing in France. We wish that the need for new policies concerning genetic testing is recognised by the Ministry of Health, in order to follow the main recommendations made by other countries on genetic tests:

- the introduction of new genetic tests into clinical use is based on evidence of their analytical and clinical validity and utility to those tested,
- all stages of the genetic testing process in clinical laboratories meet quality standards,
- health providers who order genetic tests have sufficient competence in genetics and genetic testing to protect the well-being of their patients,
- there be continued and expanded availability of tests for rare genetic diseases.

As a first step towards the achievement of these goals, the ANPGM (Association Nationale des Praticiens de Génétique Moléculaire) proposes an organisation of genetic diagnostic services in France at two levels: one local, testing frequent mutations and a limited number of regional or national expert laboratories testing for less frequent mutations or genes using more sophisticated technologies and providing support and training. This network should allow harmonisation of practices and equal access to genetic counselling in all parts of the country.
Mireille Claustres (from Montpellier, southern France) presented the *External quality assessment* schemes organised since 1997 by the European Molecular Genetics Quality Network (EMQN) and the unique international experience acquired through CF schemes over several years with the European Concerted Action against Cystic Fibrosis (ECACF). The web sites and specific guidelines and meetings for best practice were described. It is recognised that these two European networks can have an important role in improving the safety and efficacy of genetic testing where none exists, which is the case for France.
Florence Weber from the *Direction of Health Organisation Ministry* (Paris) was very interested by the propositions of ANPGM, whose members have produced a written document describing what is genetic testing and why it is different from other

biological tests. The idea of creating a network by professional geneticists was welcomed by the Government, which will examine very soon the needs for organising and funding genetic tests in France.

4. Genetic testing and medical care (coordinator: Didier Lacombe, Bordeaux)

Didier Lacombe described the current organisation of *genetic counselling* in France, which is delivered exclusively by physicians specialised in medical genetics. The number of genetic counsellors is so low that it is impossible to cover the needs. This existing organisation has to be deeply modified.

Jean-Pierre Grünfeld from Hôpital Necker (Paris) presented interesting views and outcomes of genetic testing in *kidney diseases*, depending on the severity and age at onset. Affected children may be discovered through unjustified systematic radiological investigations, which raises questions about this form of presymptomatic "phenotypic" test.

Didier Lacombe from Hôpital Pellegrin (Bordeaux) reported on problems caused by *genetic testing in asymptomatic children*. Predictive testing may be medically indicated if early diagnosis allows interventions which reduce morbidity or mortality. By contrast, predictive testing of asymptomatic children at risk for adult onset disorders should be strongly discouraged when no medical intervention is available, as stated by the law.

Alexandra Durr from Hôpital de la Salpêtrière (Paris) described the invaluable benefits offered to patients and families by a *multidisciplinary consultation*, with the example of Huntington's disease, for which a defined genetic counselling protocol has been applied for more than 8 years. Issues of concern include benefits, autonomy, informed consent, disclosure of results, confidentiality, family communication, equality, and the right of the "at risk" parent to *not* know.

Marcela Gargiulo from Pitié-salpêtrière (Paris) gave the point of view of *psychologist* and described the possible short and long-term consequences for tested individuals. Because genetic testing raises a host of medical, social, psychological and ethical issues for patients and their families, it is imperative that these issues be addressed both before and after testing is offered. For some diseases, it is important to include a pre-test assessment for emotional state.

In conclusion, the development of tests to predict future disease often precedes the development of interventions to prevent, ameliorate, or cure that disease in those born with genotypes that increase its risk. Consequently, the utility of some genetic tests may be questioned. There is a general agreement that before a genetic test can be usually accepted in clinical practice, data must be collected to demonstrate the benefits and risks that accrue from both positive and negative results. In many instances, DNA-based testing may serve as a useful adjunct to disease risk counselling.

Specific brochures are being distributed by INSERM, SFG (Société Française de Génétique) and SFBC (Société Française de Biologie Clinique) presenting core scientific data of use in genetic counselling or best practice guidelines.

The last lectures of this two-days meeting were given by **the Parents' Associations**. Marie-Odile Perrousseaux from Huntington's France Association (Paris), Viviane Viollet from the Orphan Diseases Alliance (Paris), illustrated the fundamental roles of the Associations, not only in supporting and encouraging continuously Researchers but also in organising a network of invaluable regional assistance for patients and their families.

Mireille Claustres, Montpellier, France

■■■■■■■■■

REPORT TRAINING

Filipa Mendes from Centro de Genética Humana, Instituto Nacional de Saude Dr. Ricardo Jorge, Lisboa, Portugal, visited the Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden headed by Prof. Godfried Roomans, September 2000.

This training visit was approved under the « Scientific Exchange and Travel Grants » program of the European CF Thematic Network.

The purpose of the visit was training in the MQAE technique for measurement of chloride fluxes. cAMP-induced chloride transport was studied in nasal epithelial cells collected by nasal brushing and in cell lines with stable expression of normal or mutated CFTR protein.

The function of CFTR measured through this chloride-sensitive fluorescent indicator was compared with the cellular localisation of the protein studied by immunocytochemistry.

In the future, this technique may allow the study of chloride secretion in easily accessible epithelial cells from respiratory tissues of patients, for various purposes, including the assessment of efficiency of treatments, e.g. gene therapy or pharmacological agents.

Filipa Mendes, Lisboa, Portugal

■■■■■■■■■

SILVER STAINING OF DGGE GELS

The continuing progress in the identification and characterisation of genes that cause human genetic diseases has greatly increased the requests for a method that can rapidly identify mutations in these genes. The introduction of PCR has made it possible to rapidly obtain a relatively large amount of single-copy genomic DNA that can be used for subsequent analysis. Direct sequencing of amplified DNA, although a standard procedure in many laboratories

is still time-consuming and laborious. Consequently, different methods have been developed to pre-identify the region of gene in which the mutation is located, which can be subsequently be amplified and sequenced. We have chosen denaturing gradient gel electrophoresis (DGGE) of PCR amplified material for screening for mutations in CFTR gene.

As far as we know from literature search and personal communications, most laboratories use ethidium bromide for staining of DGGE gels, although it is a powerful mutagen and not sensitive as silver staining. When DGGE is used for screening large genes (e.g., CFTR, FVIII), it is particularly convenient to relay on a method that is both sensitive and economical. Silver staining reduces the cost of testing by reducing the volume of the PCR mixture to 10µl, improves the sensitivity and provides a permanent record of results.

In our lab we routinely use silver staining of DGGE gels. Multiplex PCR reactions for CFTR gene under conditions given by Fanen et al. are performed in the final volume of 10µl, and a 1,5µl aliquot is used for DGGE. The gel is silverstained using the following protocol. After fixation in 150ml of a solution of absolute ethanol (100ml/l) and acetic acid (5ml/l) for 10 min at room temperature with gentle shaking, staining is performed in 150 ml of 1g/l silver nitrate aqueous solution for 10 min at room temperature with gentle shaking. The gel is rinsed twice with distilled water to remove excess silver solution, and developed in a solution containing, per litre, 15g of NaOH, 0.1g of $NaBH_4$, and 0.48g of formaldehyde. The gel is first washed in 150ml of this solution (for 30 sec) to precipitate the excess silver, and then incubated in 150ml of the same solution, with gentle shaking, until the bands are visualised (approximately 20 min). The stain is fixed with an aqueous solution of 7.5g/l $NaCO_3$ for 10 min at room temperature with gentle shaking. The gel is laid on a bench top and covered with Whatman 3MM paper. The Whatman paper is picked up and turned over, so that the gel is peeled of the bench top. The gel is covered with Saran Cling Wrap and stored at 4°C temporarily (up to 2 weeks), or dried under reduced pressure for 1.5 h at 80°C.
We believe that the described procedure is useful both for its economical cost and its sensitivity.

Fanen P, Ghanem N, Vidaud M, Besmond C, Martin J, Costes P, et al. Molecular characterisation of cystic fibrosis: 16 novel mutations identified by analysis of the whole transmembrane conductance regulator coding regions (CFTR) and splice site junctions. Genomics 1992; 13:770-6.

Dragica Radojkovic, Belgrade, Yugoslavia
Jelena Kusic, Belgrade, Yugoslavia

■■■■■■■■■

ANNOUNCEMENTS

CF associations meeting - Leuven, Belgium March 9, 2001

The Thematic Network on CF creates a unique platform for scientists involved in fundamental CF research, for the more than 160 genetic diagnostic laboratories, for CF associations of families, patients and clinicians, ethical, legal and IPR experts and representatives of the industry.

A round table discussion with different CF associations from Europe will be organised in Leuven. The agenda of this meeting includes an introduction of the European CF network, information channels of the CF network, discussion groups with scientists, geneticists and industry, informed consent for DNA sampling, guidelines for genetic testing. Time is foreseen for discussion on possibilities to create a committee which represents the European CF associations and which would be the contact for the CF network to the patient organisations. A selected group of CF associations was invited. If you or your CF association are interested to attend this meeting feel free to contact Dr Els Dequeker or Prof. J.-J. Cassiman, tel. + 32 16 34 58 60, fax + 32 16 34 59 97, or e-mail CF.network@med.kuleuven.ac.be

■■■■■■■■■

Second consensus meeting towards validation of CFTR expression and function assays – Estoril, Portugal
March 30 – April 1, 2001

More information can be found in this newsletter (p. 4) "Advances in the activities of the working group on CFTR expression (CFTR-EWG) - resource" or on the web page of the CFTR resources
http://www.med.kuleuven.ac.be/cme/cf/NextMeeting.htm

■■■■■■■■■

Workshop “Risk Calculation”

On Wednesday, 16 May 2001 during the 10th International Congress of Human genetics in Vienna, a workshop on risk calculation will be organised. Two educative lectures will be given: Prof J. Ott on (US) "Risk calculations for mendelian disorders", and C. Chapmann (UK) on "Computer assistance with risk calculation". Registration for this workshop is also necessary in order to obtain copies of the slides presented during the workshop.
For registration see web page
http://www.med.kuleuven.ac.be/cme/cf/meetings.htm
or the registration form attached at the end of this newsletter.
Conference website: http://www.ichg2001.org/

■■■■■■■■■

Artificial Chromosome round table
June 7, 2001 - 0.45 p.m. – 2.00 p.m.

Dirk Schindelhauer, member of the steering committee of our CF network, organises a round table discussion on artificial chromosomes during the 24th European Cystic Fibrosis Conference in Vienna. For more information contact
dirk@pedgen.med.uni-muenchen.de

▪▪▪▪▪▪▪▪▪

Second annual satellite symposium of the CF Network
Friday June 8, 2001 - 2.00 p.m. – 4.30 p.m.

During the 24th European Cystic Fibrosis Conference in Vienna, we will organise our second annual CF network satellite symposium. The program of this special session will be available at the end of March on the web page
http://www.med.kuleuven.ac.be/cme/cf/meetings.htm

▪▪▪▪▪▪▪▪▪

Mailing on "homozygous or compound hetero-zygous for G85E"

Dear colleagues,

At the end of December we have sent a mail in which we mentioned we wanted to study the phenotypic outcome of cystic fibrosis patients who are homozygous or compound heterozygous for the G85E mutation.

We were very pleased to see that so many people answered our letter, even if they did not have patients who could participate the study. Approximately 30 centra replied that they could provide us with clinical information of their patients. So it seems that thanks to your cooperation we'll have enough index cases to set up our European survey.

There were also a few questions concerning the screening of the G85E mutation. In our center the INNO-lipa-kit is used, but it's also possible to screen with the OLAkit (Information given by H. Cuppens).

Thank you very much for answering our letter.

Karin Decaestecker, Els Aertgeerts,
Prof. Dr. C. De Boeck

▪▪▪▪▪▪▪▪▪

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

CONGRESSES AND MEETINGS

Under this heading we would like to inform you on congresses and meetings of interest to members of our 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.

HGM 2001 - Human Genome Meeting
Edinburgh, Scotland, 19-22 April 2001
Conference website: http://hgm2001.hgu.mrc.ac.uk
Abstract receipt deadline: January 31, 2001

VI International symposium on mutations in the Human Genome
Mutation Detection 2001,
Bled, Slovenia, 3-7 May 2001
Conference website:
http://www.mutations2001.bled.si
Abstract receipt deadline: March 15, 2001

ICHG congress
International Congress of Human Genetics,
Vienna, Austria, 15-19 May 2001
Conference website: http://www.ichg2001.org
Abstract receipt deadline: December 15, 2000
CF-network: Wednesday May 16, Workshop on Risk Calculation

24th European Cystic Fibrosis Conference
Vienna, Austria, 6-9 June 2001
Conference website:
http://www.ecfsoc.org/vienna/welcome.htm
or e-mail at congress@mondial.at
Abstract receipt deadline: March 1, 2001
Parallel session of the CF-network

4th Australian Cystic Fibrosis Conference
Brisbane Queensland, Australia,
23-25 August 2001
Conference office: Cystic Fibrosis Australia,
PO Box 254, North Ryde NSW 1670, Australia
or e-mail: general@cysticfibrosisaustralia.org.au

ASHG meeting
San Diego, California, U.S. 12-16 October 2001.
Conference website:
http://www.faseb.org/genetics/ashg/meet-2001/2001meetmenu.htm

15th North American Cystic Fibrosis conference
Orlando, Florida; U.S., 25-28 October 2001

□□□□□□□□□□

CF-COORDINATION
Center for Human Genetics - University of Leuven
Campus Gasthuisberg - Herestraat 49
B-3000 Leuven - BELGIUM
Tel. 32-16-34.58.60 – Fax 32-16-34.59.97
CF.network@med.kuleuven.ac.be

Cystic Fibrosis European Network

WORKSHOP RISK CALCULATION

10th International congress of human genetics

Vienna, May 16, 2001

0.15 pm – 1.45 pm

Congress venue: Austria Center Vienna, Bruno Kreisky Platz 1, A-1220 Vienna, Austria

Registration form

Name:

Laboratory / Institute:

Address:

Country:

Tel.:

Fax:

E-mail:

☐ I will attend the Workshop risk calculation during the 10th International congress of human genetics in Vienna, May 16, 2001.

☐ I am not able to attend the Workshop risk calculation during the 10th International congress of human genetics in Vienna, May 16, 2001.

Please complete and return the registration form no later than April 30, 2001 to Dr. E. Dequeker.

If you return this registration form, you will receive a copy of the slides during the meeting.