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REPORT OF A JOINT WHO/ICF(M)A TASK FORCE ON CYSTIC FIBROSIS

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## 1. INTRODUCTION

The geographical distribution and control of cystic fibrosis (CF) has been the subject of four previous joint meetings between WHO and the International Cystic Fibrosis (Mucoviscidosis) Association (ICF(M)A)<sup>1-4</sup>. This disease is caused exclusively by mutation of a single gene, is inherited in autosomal recessive fashion, and is the commonest such disorder in populations of Caucasian origin. Although very considerable progress has been made during the last 50 years in its clinical management, with a corresponding improvement in the mean life expectancy in developed countries from a few months to a few decades, it remains incurable, and a complete understanding of its biochemical basis is still being sought. Consequently, attention has been given to the possibility of screening for carriers of the defective gene - who represent up to 5% in some populations - so that they may be given appropriate genetic counselling, including the possibility of termination of affected pregnancies. Whereas it was previously possible to identify carriers only when they became parents of affected children, in recent years those carriers who were more distantly related to CF patients could often be identified by means of genetic linkage techniques.

The joint WHO/ICF(M)A meeting held in London in 1989 discussed the potential advantages and disadvantages of carrier detection, and concluded that different strategies for CF control would have to be developed for countries with different incidence of the disease, different stages of economic, educational and health service development, different religious, cultural and legal backgrounds, and different priorities in health care. At that time, while awaiting precise identification of the responsible gene and its alteration in CF, no general recommendations could be made concerning the most appropriate technologies. However, there was agreement that educational programmes, and the development of structures for the delivery of health care and genetic support services targeted on CF would be necessary.

The discovery of the CF gene and its related protein, later in the same year<sup>5-7</sup>, provided not only a means of further investigating the biochemical defect but also a powerful new means of population screening. The most frequent gene mutation was present in approximately 70% of the CF mutant chromosomes. Within weeks of publication of the gene discovery, calls were being made for population screening.

The WHO Advisory Meeting on Hereditary Diseases, at its meeting in Geneva in November 1989, considered this new knowledge and proposed that a Task Force be convened to develop guidelines for the development of screening programmes for CF<sup>8</sup>. The Task Force should examine different possibilities, both technical and organizational, drawing on experience gained from other genetic disorders. It should also consider epidemiological, ethical, economic, educational and legal issues, and include contributions from experts in the relevant fields who would not necessarily have previous knowledge of CF. This proposal was endorsed and the first meeting of the Task Force was convened in Leningrad.

## 2. THE GENETIC NATURE OF CF

### 2.1 CF gene

The gene contains 27 exons and spans about 250,000 base pairs. Expression of the gene has been demonstrated in a variety of tissues affected in CF patients, including lung, pancreas, liver, sweat gland and nasal epithelium. Based on its resemblance to other proteins of known function, its predicted product includes regions thought to span the plasma membrane, with two associated nucleotide-binding folds (NBF). This symmetry is also found in previously known proteins which have transport functions, including the P-glycoprotein which is responsible for removing anticancer drugs from mammalian cells. To reflect its likely involvement in regulating the conduction of ions across cell membranes, the putative protein has been named "Cystic Fibrosis Transmembrane Conductance Regulator" (CFTR).

### 2.2 Major mutation

As there were no functional assays for the CF gene product, the only way to ensure that the gene involved was the CF gene was to identify a mutation in it. Upon sequence comparison between cDNA clones from normal and CF individuals, a 3 bp deletion was discovered among the CF cDNA clones. This mutation would result in the deletion of a single amino acid residue at amino acid position 508, within the first NBF of the predicted CFTR, and was thus named delta F508. Population studies showed that  $\Delta F508$  could account for approximately 70% of the CF mutant chromosomes and it had a strict correlation with the disease (i.e., the sequence alteration was only found in the mutant chromosomes but not in any of the normal ones), providing a strong argument for the gene being responsible for CF. Studies with DNA markers (haplotypes) flanking the site of the mutation excluded the possibility of a sequence variation and further suggested a common origin for all the CF chromosomes carrying  $\Delta F508$ .

### 2.3 Other mutations

To facilitate identification of the CF mutations in the remaining 30% of CF chromosomes and to coordinate a worldwide effort in estimating the population frequencies for each of the CF mutations, a consortium has been formed; current members of the consortium include over 80 groups of CF researchers from over 20 countries around the world. The first report from the consortium showed that the frequency of  $\Delta F508$  varies greatly among different geographic locations, from as low as 30% to as high as 85% of the CF chromosomes studied. In addition, more than 60 different mutations have since been reported to the consortium.

### 2.4 Genotype and phenotype

The varied symptoms among different CF patients suggest that disease severity is at least in part related to the mutations in the CF gene. Such association is expected to be concordant among patients within the same family, as they should have the same genotype at the CF locus. Approximately 85% of CF patients are severely deficient in pancreatic enzyme secretion, and thus described as "pancreatic insufficient" (PI), while the other 15% have sufficient enzyme, and are thus "pancreatic sufficient" (PS). Patients homozygous for the  $\Delta F508$  mutation were found to be almost exclusively PI<sup>9</sup>.

Some genotype association is also detected for certain other clinical manifestations although the correlation is not absolute. For example, PI patients appear to be more susceptible to development of meconium ileus which is observed in about 15% of CF patients<sup>9</sup>. On the other hand, patients with homozygous nonsense mutant alleles can have a mild lung involvement<sup>10</sup>.

## 2.5 Complementation of CF defect in vitro

Using viruses, full-length cDNA clones of the normal gene for CFTR have been introduced into cell cultures derived from CF patients. Ion flux and electrophysiological studies showed that the defect in regulation of chloride transport, characteristic of CF, had been corrected<sup>11,12</sup> thereby confirming that the reconstructed 'gene' is biologically functional.

## 2.6 Prospects for gene therapy

Gene therapy is simple in concept but likely to be difficult to execute. An active trial of gene therapy, using autologous lymphocytes transfected with a cloned gene for adenosine deaminase (ADA), is being conducted in the USA in patients deficient in this enzyme and therefore suffering from an otherwise fatal bone marrow disorder. Delivery of a cloned gene to organs affected in CF will be much more difficult. It may be possible to reach the lungs by means of inhaled aerosols, but there is no information on the likely permanence of any effects, or the frequency with which inhaled doses may have to be repeated. Organs such as the pancreas are irreversibly damaged before birth in most CF patients, and therefore no beneficial effects of gene therapy on digestive function could be expected, nor can it prevent meconium ileus unless, as seems very unlikely at present, it can be delivered to the CF fetus. However, chronic lung disease is by far the most important determinant of death and disability in CF, and the fact that the lungs are "normal" at birth makes control of pulmonary disease the priority in research.

Most importantly, little is known about the safety of gene therapy. Viruses used to deliver ('transfect') synthetic genes to cells in culture are engineered to be harmless to the host cells, but whether they remain innocuous in the long term remains to be seen. Exhaustive tests in tissue culture and animal models will be necessary before gene therapy in CF patients can be contemplated. Nevertheless, there is optimism that these difficulties can be overcome.

## 2.7 New prospects for conventional therapy

As a result of the gene and CFTR discovery, cell physiologists and pharmacologists have new tools to investigate control of the disordered ion transport in CF. Knowledge of the function of proteins structurally related to CFTR will be of assistance. There are already some attempts to correct the basic functional abnormality: the diuretic amiloride partly rectifies the ion transport abnormality in CF epithelia, and early trials of regular inhalations of amiloride have claimed some success<sup>13</sup>. One drawback is the short duration of effect, and the inhalations must be repeated several times daily. It is hoped that longer acting analogous drugs may be developed, or that new pharmacological approaches can be designed.

Both gene therapy and a pharmacological 'cure' may be objectives which cannot be reached. A worldwide improvement in the standards of care to those achieved in the best CF centres, using currently available treatments, would make a greater impact than any foreseeable developments and would in some cases produce a ten-fold enhancement of mean survival.

### 3. EPIDEMIOLOGY OF CF

The incidence of CF varies widely between the populations where it has been identified, but it is nevertheless widespread. It appears to be very rare in Chinese people, wherever they are domiciled. Small numbers of well-authenticated cases have occurred in Japan. Although relatively rare in India and Pakistan, interested physicians in those countries have found significant numbers of patients, while among Pakistani children living in England the incidence may be almost as high as that in the local European population. This suggests that CF may be misdiagnosed or overlooked unless the community, and in particular the medical profession, are acquainted with its diverse clinical features. It also indicates that some form of population screening may be the only effective way of determining the true incidence.

The major CF mutation  $\Delta F508$  is present in about 70% of mutant chromosomes in North America and Northern Europe. This proportion also varies considerably according to the population studied. It may account for up to 90% of CF chromosomes in Denmark<sup>14</sup>, but in Southern Europe generally the expected frequency of  $\Delta F508$  is only about 48%, with a range of 33% (Yugoslavia) to 54% (Greece)<sup>15</sup>. Within the USSR the range is from 70% in Kiev down to 23% in Moldavia, and the mean for the various ethnic groups tested is about 46%<sup>16</sup>.

At least 60 other mutations of the CF gene have been identified, none of them accounting for more than a small percentage of the total and most of them might appear to be rare or unique. The population incidence of CF seems to be highest in those places where the  $\Delta F508$  incidence is also high, suggesting that this mutation alone may largely account for the varying incidence of CF, and that the milder or atypical disease often associated with other mutations is more likely to be undetected by clinicians. Nevertheless, other mutations as well as  $\Delta F508$  could still account for a significant number of childhood deaths, probably from pneumonia. There is a clear need for more epidemiological studies, utilizing the new genetic technology, to determine both the overall incidence and the relative contributions of different mutations, in populations where the recognition and diagnosis of CF are believed to be deficient.

### 4. APPLICATIONS OF CURRENT KNOWLEDGE TO POPULATION CONTROL OF CF

Despite the progress made in clinical management of CF and the resulting amelioration or postponement of complications, it remains a serious and ultimately fatal disease. It places a great burden on the individual and the family, and makes demands on health care services which are out of proportion to its prevalence. For these reasons, prevention of CF may be regarded as a worthwhile objective. Effective prevention requires a programme of carrier detection, followed by genetic counselling to those carriers identified and their voluntary decision not to have affected children. In turn, this usually implies the availability of services for prenatal diagnosis and termination of pregnancies where the fetus is affected.

Control of the disease in the individual with CF will be reflected by improvements in the quality of life and longer survival. Patients may benefit from screening programmes for CF mutations applied either to the adult population or to neonates, if these programmes lead to early diagnosis and introduction of current or anticipated forms of effective treatment.

#### 4.1 Carrier screening

This may be offered to the whole population or to extended families of CF patients. There is potential for its application at different times in life, with concomitant advantages and disadvantages.

At birth: Advantages include the relative ease with which it could be included in existing laboratory and counselling programmes, its cost-effectiveness if the families of carriers so identified are followed up, and the fact that CF infants (homozygotes) will also be identified and can be offered treatment.

Disadvantages include the difficulties of consent and pre-test counselling, the inevitable uncovering of non-paternities, the lack of immediate impact on reproductive choice for the individual and the possible stigmatization of healthy carriers. Records would need to be stored for a generation and subjects would need access to their own data when they reach reproductive age.

In school: Advantages include the natural association with education about genetics and human reproduction, the ease of collecting samples (by mouthwash) and the timing, before reproduction, allows affected carriers maximum choice of reproductive options. Disadvantages include uncertainty over whether consent should be obtained from the child or from the parents, and the child's vulnerability to stigma and group pressure during early teenage years.

At adult pre-pregnancy, premarital or family planning clinics: Advantages are that it could be incorporated into services provided by the general practitioner in countries such as the UK. This approach emphasizes the couple's joint responsibility and also allows individuals considerable choice. However, there may be an educational or social bias in the population reached, and in many countries there is a high illegitimacy rate and/or couples have already started a pregnancy before marriage. Experience with thalassaemia suggests that this approach works well in countries with strong church influences and low illegitimacy rates (e.g., Cyprus).

At pregnancy clinics: This has the advantage that it is easy to organize, and education and counselling would be easy to deliver, being accepted as a logical extension of care to a motivated group. A high uptake could be expected. A major disadvantage is the delay in registration of many women until pregnancy is far advanced, and the limited autonomy and reproductive choice available when a carrier is identified.

All of these considerations apply to an 'ideal' screening test, i.e., one with 100% sensitivity and specificity. While DNA-based testing is completely specific for the mutation(s) sought, the relatively low proportion of  $\Delta F508$  in some populations of CF carriers, and the large

number of other mutations so far discovered, make precise calculations of sensitivity essential before the likely benefit of carrier screening for a given population can be assessed. Assuming that a battery of tests which identify more than 90% of carriers can be developed<sup>17</sup> the impact of its application to a population will vary according to the uptake of selective abortion.

Through extended CF families: This type of testing is very cost-effective and is applied to a sub-population with high motivation and knowledge of CF. It is however limited in its impact, because it would detect less than 10% of carriers. Both specificity and sensitivity would be high, the precise mutation(s) affecting the family being known in most cases. It could give rise to family tension if some individuals refuse the test. However, genotype testing of extended families is already a reality in some genetic centres.

#### 4.2 Neonatal screening

For CF homozygotes. Programmes of neonatal screening for CF have been used in various countries with the primary objective of identifying affected newborns in order to offer them treatment. They are based on (i) the detection of pancreatic damage, either by reduced breakdown of protein in meconium, which is a very cheap, simple test with relatively low sensitivity and specificity, or (ii) the detection of raised levels of immunoreactive trypsin (IRT) in dried blood spots. The latter method has the advantage that it can be performed on the blood samples collected for other forms of neonatal screening (hypothyroidism and phenylketonuria) and has greater sensitivity and specificity, but it is far from being a perfect tool. Both forms of neonatal screening are relatively cheap and acceptable.

The benefits of neonatal screening programmes for homozygous, affected CF infants are:

- early diagnosis and the implementation of active treatment, which can be expected to improve both life expectancy and the quality of life
- diagnosis and treatment of those children with CF which might otherwise have been fatal before diagnosis (thereby reducing the apparent, perceived incidence of CF in the population)
- elimination of the parental feelings of guilt and anger at delayed diagnosis
- identification of families at risk, who will be given appropriate counselling and can be offered carrier detection tests
- demonstration to the public, medical and allied professions, and health service administrators of the need to develop and improve services for CF patients. In countries where the diagnosis of CF is often missed or delayed, introduction of a national neonatal screening programme has a marked beneficial effect on the standard of care offered to CF patients<sup>18</sup>.

If or when effective genetic or pharmacological therapy for CF becomes available, some form of neonatal identification will be essential if affected patients are to derive maximum benefit.

For CF gene mutations. The possibility now arises that a test based on DNA analysis could also be performed on neonatal blood spots, and that such a test would be completely specific for the mutation(s) being sought. If it were able to detect 90% or more of the CF mutations present in a given community it would be as good as the IRT test in sensitivity and, of course, completely specific. It would carry the additional advantage that heterozygotes would also be identified, so that if appropriate long-term records were kept it would, after a generation, only need to be applied to the offspring of known carriers. The possibility of screening neonates as a primary means of identifying carriers has been mentioned above.

## 5. PREREQUISITES FOR A CARRIER SCREENING PROGRAMME

### 5.1 Epidemiological

Before a carrier screening programme can be contemplated in any country, CF must be perceived as a significant health burden. It is therefore essential to have accurate information not only about the apparent incidence of the disease, i.e., the number of diagnosed CF patients, but also about the gene frequency, from which the true incidence of diagnosed and undiagnosed patients can be calculated.

Independently of these data, the relative importance of CF prevention in the priorities of health care funding will vary according to the country's economic status and the stage of health service development.

### 5.2 Economic and technical

Screening tests must be safe, reliable, acceptable and cost-effective.

At present, no single test is available which will detect all CF mutations, and the relative proportion represented by the major mutation  $\Delta F508$  varies between populations. A test for this mutation alone would detect up to 90% of CF carriers in Denmark but only 33% in Yugoslavia, and would clearly be less cost-effective in the latter. Careful calculations on marginal costs are required to find out how many other mutations would need to be screened for in different populations in order to identify 90% of carriers. This is the level at which US geneticists consider that CF testing would be regarded as standard care for that country<sup>17</sup>. Such a test would identify 81% of pregnancies at risk, with a corresponding theoretical reduction in births of affected infants.

The cost estimates for the technical aspects of DNA screening tests vary at present from US\$2-100 per individual. It is expected that centres with existing infrastructure and experience may be able to provide tests at about US\$10 or less for the combined screening of up to 10 frequent mutations, which may reach or exceed the arbitrary 90% limit. Should it be decided to screen neonates rather than adults, the existence of an established neonatal screening programme to which CF could be added would reduce the investment otherwise required.

In some countries in which CF is infrequent or in which other priorities in health care must be met, the cost of neonatal screening with the additional high costs of care for infants detected may make its implementation unrealistic. In such places carrier screening, with its general application and potential for an immediate impact in reducing the births of CF children, may be a more appropriate choice.

### 5.3 Ethical, religious and cultural

Screening must not be imposed on an unwilling and uninformed public and can only be carried out with the informed consent of the individual. Informed consent requires basic general knowledge of human heredity, including understanding of heterozygosity - at least in terms of its immediate implications for the family. This basic knowledge should preferably be given as part of school education.

Specific information on the clinical features of CF and its likely effects on the lungs and digestive system, resulting in reduced quality and duration of life, should be given in the context of new possibilities for therapy which may result from discovery of the CF gene. The scope and limitations of any screening test offered must also be clearly stated when informed consent for testing is offered.

Couples have a right of choice, which must be clearly understood by subjects and counsellors, and which must be without penalty, sanctions, pressure or discrimination whatever choice they make. Information obtained from screening programmes may not be used by employers or insurance agencies.

Provision of information obtained from the screening programme is personal and confidential to the persons concerned and may not be disclosed to any other person without the expressed permission of the subjects screened.

Any voluntary or legal framework in which carrier screening or prenatal diagnosis programmes operate should conform with the principles agreed by the Council of Europe's Ad Hoc Committee of Experts on Bioethics (CAMBI), 1990<sup>19</sup>.

Screening programmes must be compatible with population customs. In countries with a legal ban on termination of pregnancy, screening and control programmes will stop short of prenatal diagnosis. This may also be the case in other countries or communities where, even if legal, there is a cultural or religious objection to abortion and uptake is therefore likely to be low.

Gene therapy may be developed in the future. It should not involve altering inheritance, and in this context raises no particular new ethical issues. Fetal therapy could be applied to prevent pancreatic disease, but if so it should only be in the context of improved treatment and not given as germ line therapy.

## 6. COMPONENTS OF A CARRIER SCREENING PROGRAMME

### 6.1 Educational

#### 6.1.1 Public

The objective of an educational campaign directed towards the general public should be two-fold: to enable individuals to make informed personal choices about CF and to prepare them to participate in collective decisions, such as determining the nature and scope of CF-related activities in schools. To do so, education must inform individuals of both the benefits and the possible disadvantages of CF-related preventive interventions. Education must also be designed to reduce undue fears and correct misunderstandings about CF. It is essential that stigmatization of carriers is avoided. One approach is to create general awareness that all individuals carry mutant genes. If such programmes are successful, individuals and communities will make informed decisions about CF that are consistent with both their health needs, and their ethical, religious or cultural values.

The potential roles of the school have been identified above. The educational role is clearly important because individuals must have a basic understanding of heredity to adequately understand the causes of CF and the opportunities for preventing it. Teaching about heredity should be part of a comprehensive school health education programme. Additionally, school personnel must be prepared to educate students about CF without creating stigma about carriers or those who are patients. Finally, the school can play an important role in educating the community about CF, especially in areas where school-based screening programmes are considered for implementation.

The mass media should be used to educate the public about CF and to create the necessary support and resources for the patients and those involved in the many dimensions of CF. Use of the media should create a public demand for more information, and should concentrate on the benefits expected to result from the application of enhanced public knowledge about CF.

The health care professions and national cystic fibrosis organizations should participate in the planning with public health authorities and prepare and provide detailed literature and information to follow up the mass media campaigns.

#### 6.1.2 Health professionals

The professions involved may differ slightly between countries but will probably include general practitioners, physiotherapists, obstetricians, paediatricians, medical geneticists, public health doctors, nurses, genetic counsellors, social workers and health care administrators.

They should be provided with sufficient scientific knowledge about the nature and clinical course of CF, its treatment and prognosis. It is essential that they are informed and regularly updated on the methodology and efficacy of screening. The principles

of screening programmes should be incorporated into the curricula and textbooks of medical students and nurses.

Information about planned screening programmes should also be given prominence in medical journals, newsletters and professional communications.

## 6.2 Technical

### 6.2.1 Clinical services

Heterozygote screening for CF should be developed in conjunction with adequate treatment, counselling and support services for CF patients (homozygotes) who may be born as a result of a couple exercising their right to have such children. Counselling and support services for those who opt to terminate affected pregnancies must also be provided, as well as the gynaecological services to perform such terminations. The clinical services must be directed by physicians.

### 6.2.2 Laboratory services

Facilities for blood and/or buccal cell collection must be organised. Tests can be performed in a standard biochemical laboratory with close links to medical genetics. The laboratory must be equipped to use radioactive material and include equipment and trained staff for polymerase chain reaction (PCR), electrophoresis, and work with oligonucleotides and enzymes. The laboratory must be under the direction of a scientist with knowledge of medical genetics and molecular biology. Laboratory staff must be updated whenever necessary on developments in mutation analysis.

### 6.2.3 Organization and records

Quality control systems must be arranged within and between laboratories participating in a national programme.

An adequate records system must be set up which will ensure prompt feedback to screened persons via the clinical services, and must ensure confidentiality. Entering a screening programme should always be through a physician and not directly via the laboratory, but access of individuals to their own genetic data must be ensured.

## 6.3 Training programmes

Screening for CF should be based on dedicated genetic centres, or on larger well-equipped CF centres, which will include representatives of the health professions listed under 6.1.2. These staff will all have their own general professional training, to which must be added the specific knowledge and skills required for a CF screening programme.

In order to offer a service to the public, professional training programmes will therefore be required in order to:

- expand the number and/or extend the work of centres.

- manage larger and more responsive, but confidential, databases.
- further develop communication and counselling skills.
- provide information and updating for paediatricians, obstetricians, primary care physicians and respiratory physicians caring for CF patients, even if they are not directly involved with running the service.

#### 6.4 Research

Preliminary research into the incidence of CF and its mutational variations within the country or region is a prerequisite.

If it is decided to implement a screening programme, research will be needed into:

- the needs for information, education and support of the population served.
- qualitative and technical improvement in laboratory testing including the development of automation, and the cost/efficiency ratios of various test combinations.
- pilot studies to evaluate:
  - . the most effective ways of delivering counselling.
  - . the most effective entry points into the screening system (e.g., neonates, pregnant women, school-children).
  - . organizational arrangements and their cost/benefit ratio.

WHO should encourage, compare and evaluate different modes of delivery.

Neonatal screening programmes, in addition to identifying patients, will also provide useful research information. They facilitate comparison between different approaches to treatment, whose outcomes can be measured in clinical and actuarial terms. Coordination between neonatal screening laboratories and international quality control should be a responsibility of WHO. There is an urgent need to set recognized international standards for the IRT test.

#### 7. THE ROLE OF WHO AND ICF(M)A IN THE DEVELOPMENT OF SCREENING PROGRAMMES

The following principles indicate ways in which the two organizations may collaborate towards the common objective of reducing the burden of cystic fibrosis:

- the main responsibility of WHO would be to monitor and evaluate programmes on CF heterozygote and neonatal screening: in particular, useful information could be shared through arranged international workshops on progress on screening programmes, and by distribution of information on individual experiences and new technical developments.

- links between the existing mutation analysis consortiums and WHO to help identify and distribute the relevant research findings for use in screening programmes should be established.
- the main responsibilities of ICF(M)A are: to ensure that adequate detailed information on CF is available for health legislators, administrators and professionals within member countries; to identify sources of authoritative advice which national and international bodies may consult; and, to give practical support to the genetic counselling and follow-up services through their national associations.
- WHO and ICF(M)A should jointly support and coordinate the exchange of information on heterozygote and neonatal screening at international level, and ensure it is disseminated to health professionals in all appropriate countries. This can be achieved by preparation of literature, sponsorship of special meetings and the introduction of items on screening into national and international professional conferences and assemblies.

## 8. CONCLUSIONS

Discovery of the gene for CF has opened up new possibilities for control of this disease, including that of population screening for heterozygotes and prenatal diagnosis. Such possibilities may substantially reduce the burden of CF on families and public health services if it is linked with genetic counselling and voluntary avoidance or selective termination of pregnancy. Any programmes of population screening for CF carriers must operate within the principle of individual choice. Such programmes must not be seen as an alternative to provision of proper health care for CF patients. The ethical and legal principles enunciated by the Council of Europe for genetic screening provide an appropriate framework for such screening programmes.

Before a population carrier screening programme is set up, consideration must be given to the epidemiological features of CF and its various mutational forms in the local population, its likely cost-effectiveness, its acceptability within the local cultural and religious context, the level of public awareness of CF and the availability of counselling, prenatal diagnosis and follow-up medical services. The nature and extent of optimal carrier screening programmes will vary according to economic, cultural and medical factors in different countries and populations.

Neonatal screening programmes for CF homozygotes have an important role in the control and management of CF in some countries, and will be essential when gene therapy or specific pharmacological treatment for CF becomes available.

Further research is needed into: the distribution of CF and its mutations; improved technical methods of screening for carriers of CF mutations and prenatal diagnosis; and, the acceptability and effectiveness of pilot screening programmes in different types of population.

## 9. RECOMMENDATIONS

- (a) WHO and ICF(M)A should collaborate in the preparation and international dissemination of information on cystic fibrosis and possible strategies for its control. Such information must be regularly updated and revised in the light of research developments and experience.
- (b) WHO and ICF(M)A should stimulate and/or help to generate funding for research projects targeted on reducing the burden of CF in countries with different needs, customs and resources.
- (c) WHO should coordinate, compare and evaluate the outcomes of such projects.
- (d) WHO should undertake responsibility for international standardization and quality control of the neonatal IRT screening test.
- (e) WHO and ICF(M)A should assist in setting up training programmes for technical and health care workers involved in CF control programmes.
- (f) WHO should support the collaborative studies on the distribution and analysis of mutations of the CF gene which are currently organized by an unofficial consortium.
- (g) All carrier screening projects should conform to the ethical and economic principles listed under 5.3, and enunciated in greater detail elsewhere<sup>19</sup>.
- (h) Knowledge of CF, its genetic variations, epidemiology, natural history and treatment is increasing rapidly. WHO and ICF(M)A should arrange periodic reviews and be prepared to revise the above recommendations in the light of new knowledge and possibilities.

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## 12. REFERENCES

1. Report of a Joint WHO/ICF(M)A Meeting on Cystic Fibrosis, Vienna, 6-8 October 1983 (HMG/ICF(M)A/83.8)
2. Report of a Joint WHO/ICF(M)A Meeting on the Distribution of Cystic Fibrosis, Nicosia, Cyprus, 30 April - 2 May 1985 (HMG/ICF(M)A/85.2)
3. Report of a Joint WHO/ICF(M)A Meeting on Prevention and Control of Cystic Fibrosis, Oslo, 19 June 1987 (HDP/ICF(M)A/WG/87.3)
4. Report of a Joint WHO/ICF(M)A Meeting on the Feasibility Study on Hereditary Disease Community Control Programmes - Cystic Fibrosis (WHO/HDP/ICF(M)A/WG/89.2)
5. Rommens, J.M., Iannuzzi, M.C., Kerem, B., Melmer, G., Drumm, M.L., Melmer, G., Dean, M., Rozmahel, R., Cole, J.L., Kennedy, D., Hidaka, N., Zsiga, M., Buchwald, M., Riordan, J.R., Tsui, L.-C., and Collins, F.S. Identification of the cystic fibrosis gene: chromosome walking and jumping. *Science* 245, 1059-1065. 1989.

6. Riordan, J.R., Rommens, J.M., Kerem, B., Alon, N., Rozmahel, R., Grzelchak, Z., Zielenski, J., Lok, S., Plavsic, N., Chou, J.-L., Drumm, M.L., Innuzzi, M.C., Collins, F.S., and Tsui, L.-C. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* 245, 1066-1073. 1989.
7. Kerem, B., Rommens, J.M., Buchanan, J.A., Markiewicz, D., Cox, T.K., Chakravarti, A., Buchwald, M., and Tsui, L.-C. Identification of the cystic fibrosis gene: genetic analysis. *Science* 245, 1073-1080. 1989.
8. Report of a WHO Advisory Meeting on Hereditary Diseases, Geneva, November 1989. WHO/HDP/AG/89.4.
9. Kerem, E., Corey, M., Kerem, B., Rommens, J., Markiewicz, D., Levison, H., Tsui, L.-C., and Durie, P. The relationship between genotype and phenotype in cystic fibrosis - analysis of the most common mutation ( $\Delta F508$ ). *New Eng. J. Med.* 1990. vol. 323:1517-22.
10. Tsui, L.C. 1990 (unpublished) - Annex 1
11. Drumm, M.L., Pope, H.A., Cliff, W.H., Rommens, J.M., Marvin, S.A., Tsui, L.-C., Collins, F.S., Frizzell, R.A., and Wilson, J.M. Correction of the cystic fibrosis defect in vitro by retrovirus-mediated gene transfer. *Cell* 62, 1227-1233. 1990.
12. Rich, D.P., Anderson, M.P., Gregory, R.J., Cheng, S.H., Paul, S., Jefferson, D.M., McCann, J.D., Klinger, K.W., Smith, A.E., and Welsh, M.J. Expression of cystic fibrosis transmembrane conductance regulator corrects defective chloride channel regulation in cystic fibrosis airway epithelial cells. *Nature* 347, 358-363. 1990.
13. Knowles, M.R., Church, M.L., Waltner, W.E., Yankaskas, J.R., Gilligan, P., King, M., Edwoods, L.H., Helms, R.W., and Boucher, R.C. A pilot study of aerosolised amiloride for the treatment of lung disease in cystic fibrosis. *New Eng. J. Med.* 1990. vol. 322:1189-94.
14. The Cystic Fibrosis Analysis Consortium: Worldwide survey of the  $\Delta F508$  mutation. *Am. J. Human Genetics* 47: 354-359. 1990.
15. Mastella, G. (1990). Present possibilities in the prevention of Cystic Fibrosis in the Southern European area.
16. Baranov, V.S. et al (1990). Please see list of working papers.
17. Roberts, L. Cystic Fibrosis pilot projects go begging. *Science* 250: 1076-1077. 1990.
18. Elliott, R.B. Newborn screening for cystic fibrosis: an historical perspective. *Pediatric Pulmonology* 1991 (in press).
19. Kokkonen, P. Summary. Council of Europe Recommendation No. R(90)13 of the Committee of Ministers to the Member States on Prenatal Genetic Screening, Prenatal Genetic Diagnosis and Associated Genetic Counselling (1990).

13. LIST OF WORKING PAPERS AVAILABLE ON REQUEST

BARANOV, V. (WP.1)

DNA markers in CF locus. A first analysis of the European population in the USSR.

CASSIMAN, J.-J. (WP.2)

Knowledge about CF and other genetic diseases in affected families and in the general population.

COUELLE, C. (WP.3)

Applicability of molecular genetic approaches to CF-control programmes.

DORING, G. (WP.4)

Microbial lung infections: prevention by epidemiological investigations and new treatment strategies.

KOKKONEN, P. (WP.5)

Summary of comments on Appendix III of Recommendation No R (90) of the Committee of Ministers to the Member States on Prenatal Genetic Screening, Prenatal Genetic Diagnosis and Associated Genetic Counselling.

MASTELLA, G. (WP.6)

Present possibilities in the prevention of CF in southern Europe.

NORMAND, C. (WP.7)

The economics of health care and the cost-effectiveness of prevention.

O'BYRNE, D. (WP.8)

Education for health improvement.

THOMPSON, I. (WP.9)

The perspective of parent/patient associations.

WILLIAMSON, B. (WP.10)

What's new in gene therapy? Gene therapy and cystic fibrosis.

\* \* \* \* \*

ANNEX 1

MOLECULAR GENETICS OF CYSTIC FIBROSIS  
AND POSSIBLE MECHANISMS OF PROTEIN FUNCTION

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1. INTRODUCTION

While it is generally believed that the heavy mucus found in the respiratory tracts and the blockage of exocrine secretion from the pancreas are related to the abnormal regulation of ion transport in the epithelial cells, the basic biochemical defect in CF is unknown. With the development of gene mapping and recombinant DNA cloning techniques in the past decade, it became possible to tackle the CF problem through a molecular genetic approach, bypassing the requirement of knowledge about the biochemical lesion in CF patients or their epithelial cells. The precise localization of the CF locus on the long arm of chromosome 7, region q31, facilitated various molecular strategies to isolate the gene. In 1989, the gene responsible for CF was identified (Rommens et al. 1989; Riordan et al. 1989; Kerem et al. 1989).

## 2. THE CF GENE

The CF gene contains 27 exons and spans 250 kb of DNA (Zielenski et al. 1991a). A schematic diagram of the gene is shown in Figure 1. The encoded mRNA is about 6,500 nucleotides in length. Expression of this gene could be observed in a variety of tissues that are affected in CF patients, for example, lung, pancreas, liver, sweat gland and nasal epithelia. An open reading frame consisting of 1,480 amino acids could be deduced from the reconstructed cDNA sequence. Based on its alignment with other proteins of known functions, this predicted polypeptide shows 2 regions thought to be spanning the plasma membrane and each followed by a nucleotide-binding fold (NBF) (Figure 1); this internal sequence symmetry between the first and second half of the protein resembles some of the prokaryotic and eukaryotic proteins in various transport systems, most notably, the P-glycoprotein which is responsible for removing anticancer drugs from mammalian cells. To reflect the possible role of the CF gene product in epithelial cells, the putative protein has been named "Cystic Fibrosis Transmembrane Conductance Regulator" (CFTR). The predicted molecular mass of CFTR is about 170,000.

## 3. THE MAJOR MUTATION

As there were no functional assays for the CF gene product, the only way to ensure that the gene involved was the CF gene was to identify a mutation in it. Upon sequence comparison between cDNA clones from normal and CF individuals, a 3 bp deletion was discovered among the CF cDNA clones. This mutation would result in the deletion of a single amino acid residue at amino acid position 508, within the first NBF of the predicted CFTR, thus named  $\Delta F508$ . Population studies showed that  $\Delta F508$  could account for approximately 70% of the CF mutant chromosomes and it had a strict correlation with the disease (i.e., the sequence alteration was only found in the "mutant chromosomes" but not in any of the normal ones), providing a strong argument for the gene being responsible for CF. Studies with DNA markers (haplotypes) flanking the site of the mutation excluded the possibility of a sequence variation and further suggested a common origin for all the CF chromosomes carrying  $\Delta F508$ .

## 4. OTHER MUTATIONS

To facilitate identification of the CF mutations in the remaining 30% of CF chromosomes and to coordinate a worldwide effort in estimating the population frequencies for each of the CF mutations, a consortium has been formed; current members of the consortium include over 80 groups of CF researchers from over 20 countries around the world. The first report from the consortium showed that the frequency of  $\Delta F508$  varies greatly among different geographic locations, from as low as 30% to as high as 85% of the CF chromosomes studied. In addition, more than 60 different CFTR mutations have been reported to the consortium; many of them have been published or submitted for publication (see Table 1). The locations of these mutations are illustrated in Figure 2.

Different types of mutations, including missense, nonsense, frameshift and mRNA splicing mutations, have been detected throughout the entire length of the coding region. While the distribution of frameshift and non-sense mutations is approximately the same between the two halves of the gene, there appear to be more missense mutations in the first half than the second half, with most found in the first NBF, particularly within exon 11. This uneven distribution may suggest that the two halves of CFTR have different functions.

Preliminary reports to the consortium indicate that most of the mutant alleles are rare, with some represented by only single examples. The more frequent ones are G551D (Cutting et al. 1990a), R553X (Cutting et al. 1990a), G542X (Kerem et al. 1990) and Y1303N (Osborne et al. 1990), each of which constitute as high as 5% of the CF chromosomes in some populations (data from the CF Genetic Analysis Consortium).

The mutation screening study confirms that the ATP-binding domains detected by sequence alignment is important for CFTR function as multiple, different mutations have been found for many of the highly conserved amino acid residues in these regions. The locations of the various mutations also identified other functionally important regions in CFTR. There is, for example, a second 3 bp deletion resulting in the omission of an isoleucine residue at position 506 or 507 of the putative protein while amino acid substitutions at these position are apparently not disease-causing; this observation argues that the length of the peptide is more critical than the actual amino acid residue in the 506-508 region. Further, the existence of a large number of nonsense, frameshift as well as mRNA splicing mutations in the CF gene implies that absence of CFTR is not incompatible with life. It is also of interest to note that only two mutations have been found in the R-domain, a region of the protein thought to have an regulatory function.

## 5. GENOTYPE AND PHENOTYPE

The varied symptoms among different CF patients suggest that disease severity is at least in part related to the mutations in the CF gene. Such association is expected to be concordant among patients within the same family, as they should have the same genotype at the CF locus. A careful review of a large number of clinical records showed that only the pancreatic involvement appeared to have a direct consequence of the CF genotype (Corey et al. 1989). Approximately 85% of CF patients are severely deficient in pancreatic enzyme secretion, thus diagnosed as pancreatic insufficient (PI), and the other 15% have sufficient enzyme, thus pancreatic sufficient (PS). Subsequent studies showed that patients homozygous for the  $\Delta F508$  mutation were almost exclusively PI.

There are other mutations that would also be classified in the same group as  $\Delta F508$ , the so-called severe mutant alleles with respect to pancreatic function. In contrast, patients with one or two copies of another class (i.e., mild) of alleles are expected to be PS. The proportion of mild alleles is estimated to account for about 10% of all CF chromosomes; examples of mild alleles are A455E, Y563N and P574H.

Some genotype association is also detected for some other clinical manifestations although the correlation is not absolute. For example, PI patients appears to be more susceptible to development of meconium ileus which is observed in about 30% of CF patients (Kerem et al. 1989). Other the other hand, patients with homozygous nonsense mutant alleles can have a mild lung involvement (Cutting et al. 1990b).

## 6. COMPLEMENTATION OF CF DEFECT IN VITRO

To construct a recombinant clone capable of producing large quantities of mature CFTR protein for biochemical and physiological analysis, appropriate portions of cDNA fragments and genomic DNA segments were joined through multiple

steps of ligation. The assembly of the entire coding sequence for the 1480 amino acids of the predicted protein proved difficult due to frequent rearrangements of the resulting plasmids in *E. coli* hosts. With several minor sequence modifications, the plasmids appeared to be stable and able to propagate efficiently (J.R. Rommens and L.-C. Tsui, unpublished result). The use of a low-copy number plasmid also seemed to circumvent the problem of this sequence instability (Gregory et al. 1990).

Protein products of the appropriate size have been detected in the cytoplasmic membranes of heterologous cell systems expressing the full-length cDNA clones. Glycosylation is also observed in the processed CFTR (Gregory et al. 1990). In one study (Drumm et al 1990), the full-length clone was repackaged into retrovirus vector and introduced into a pancreatic adenocarcinoma cell line from a CF patient; the result of ion flux and electrophysiological measurements showed that the defect in the regulation of the cyclic AMP-mediated chloride channel (see below) could be restored in cells that received the full length cDNA. In another study (Rich et al. 1990), full-length transcripts were introduced into airway epithelial cells through the vaccinia virus transfection system and a positive complementation result was also obtained. These results therefore provided the formal proof that the reconstructed, full length CFTR cDNA is biological functional.

## 7. POSSIBLE FUNCTION OF CFTR

Results of electrophysiological studies indicate that the basic defect in CF epithelial cells is associated with the cAMP-mediated regulation of chloride ion transport (Schoumacher et al. 1987; Li et al. 1988). In the normal epithelia, chloride channels are activated (opened) by an increase in intracellular levels of cAMP. It is generally believed that the pathway involves cAMP-dependent protein kinase which phosphorylates either the chloride channel itself or an associated regulatory protein. In CF cells, chloride channels are present but they cannot be activated by cAMP or cAMP-dependent protein kinase. The immediate question is, of course, how CFTR can fit in this regulatory pathway. On the basis of recent DNA transfection experiments (Anderson et al. 1991; Kartner et al. 1991), it appears that CFTR is a cAMP-regulated chloride channel itself. How CFTR acts as a channel remains to be explained, however, especially since the primary structure of CFTR is highly consistent of being an active transporter (Hyde et al. 1990). Further, it is unclear how the regulatory defect can lead to abnormalities in sodium transport in CF epithelial cells (Boucher et al. 1986). These and other questions obviously become the focus of studies to understand the function of CFTR.

## 8. CONCLUSIONS

With the identification of the CF gene, progress is being made in understanding of the basic defect and pathophysiology of the disease. In addition to the detection of additional CF mutations and correlation of genotype and phenotype, advances are also being made in studies of the regulatory mechanisms governing the expression of this gene, and of the biosynthesis and function of the protein. Suitable animal models, such as those generated by transgenic mice, and other lower organisms are being explored as experimental systems. While all these above studies point towards the development of improved treatments for CF patients, another area of application is the use of the mutation information in genetic diagnosis. It is anticipated that significant progress will continue to be made in CF research for the next decade.

9. REFERENCES

- Anderson, M.P., Rich, D.P., Gregory, R.J., Smith, A.E., and Welsh, M.J.: Generation of cAMP-activated chloride currents by expression of CFTR. *Science* 251:679-682, 1991.
- Boucher, R.C., Stutts, M.J., Knowles, M.R., Cantley, L., and Gatzky, J.T.: Na<sup>+</sup> transport in cystic fibrosis respiratory epithelia. Abnormal basal rate and response to adenylate cyclase activation. *J. Clin. Invest.* 78:1245-1252, 1986.
- Corey, M., Durie, P., Moore, D., Forstner, G.G., and Levison, H.: Familial concordance of pancreatic function in cystic fibrosis. *J. Pediatr.* 115:274-279, 1989.
- Cuppens, H., Marynen, P., De Boeck, C., and Cassiman, J.J.: Study of the G542X and G458V mutations in a sample of Belgian patients. *Pediatr. Pulmonol. Suppl.* 5:203.
- Cutting, G.R., Kasch, L.M., Rosenstein, B.J., Zielenski, J., Tsui, L.-C., Antonarakis, S.E., and Kazazian, H.H.Jr.: A cluster of cystic fibrosis mutations in the first nucleotide-binding fold of the cystic fibrosis conductance regulator protein. *Nature* 346:366-369, 1990a.
- Cutting, G.R., Kasch, L.M., Rosenstein, B.J., Tsui, L.-C., Kazazian, H.H. Jr., and Antonarakis, S.E.: Two patients with cystic fibrosis, nonsense mutations in each cystic fibrosis gene, and mild pulmonary disease. *New Engl. J. Med.* 323: 1685-1689, 1990b.
- Cystic Fibrosis Genetic Analysis Consortium: Worldwide survey of the  $\Delta F508$  mutation - Report from the Cystic Fibrosis Genetic Analysis Consortium. *Am. J. Hum. Genet.* 47:354-359, 1990.
- Dean, M., White, M., Amos, J., Gerrard, B., Stewart, C., Khaw, K.-T., and Leppert, M.: Multiple mutations in highly conserved residues are found in mildly affected cystic fibrosis patients. *Cell* 61:863-870, 1990.
- Drumm, M.L., Pope, H.A., Cliff, W.H., Rommens, J.M., Marvin, S.A., Tsui, L.-C., Collins, F.S., Frizzell, R.A., and Wilson, J.M.: Correction of the cystic fibrosis defect in vitro by retrovirus-mediated gene transfer. *Cell* 62:1227-1233, 1990.
- Gasparini, P., Nunes, V., Savoia, A., Dognini, M., Morral, N., Gaona, A., Bonizzato, A., Chillon, M., Sangiuolo, F., Novelli, G., Dallapiccola, B., Pignatti, P.F., and Estivill, X.: Identification of 6 new mutations in the cystic fibrosis gene by direct sequencing. *Genomics*, in press, 1991.
- Gregory, R.J., Cheng, S.H., Rich, D.P., Marshall, J., Paul, S., Hehir, K., Ostedgaard, L., Klinger, K.W., Welsh, M.J., and Smith, A.E.: Expression and characterization of the cystic fibrosis transmembrane conductance regulator. *Nature* 347:382-386, 1990.
- Guillermit, H., Fanem, P., and Ferec, C.: A 3' splice site consensus sequence mutation in the cystic fibrosis gene. *Hum. Genet.* 85:450-453, 1990.

Highsmith, W.E., Jr., Strong, T., Burch, N., Smith, T., Silverman, L.M., Collins, F.S., Boucher, R., and Knowles, M.R.: Identification of a splicing error of exon 14b giving rise to a frameshift mutation in a consanguineous family with mild cystic fibrosis. *Pediatr. Pulmonol. Suppl.* 5:11A, 1990.

Hyde, S.C., Emsley, P., Hartshorn, M.J., Mimmack, M.M., Gileadi, U., Pearce, S.R., Gallagher, M.P., Gill, D.R., Hubbard, R.E., and Higgins, C.F.: Structural model of ATP-binding proteins associated with cystic fibrosis, multidrug resistance and bacterial transport. *Nature* 346:362-365, 1990.

Ivaschenko, E., White, M.B., Dean, M., and Baranov, V.S.: A deletion of two nucleotides in exon 10: A new termination mutation of the cystic fibrosis gene in Soviet patients. *Genomics*, in press, 1991.

Kartner, N., Hanrahan, J.W., Jensen, T.J., Naismith, A.L., Sun, S., Ackenley, C.A., Reyes, E.F., Tsui, L.-C., Rommens, J.R., Bear, C.E., and Riordan, J.R.: Expression of the cystic fibrosis gene in non-epithelial invertebrate cells produces a regulated anion conductance. *Cell* 64:681-691, 1991.

Kerem, B., Rommens, J.M., Buchanan, J.A., Markiewicz, D., Cox, T.K., Chakravarti, A., Buchwald, M., and Tsui, L.-C.: Identification of the cystic fibrosis gene: genetic analysis. *Science* 245:1073-1080, 1989.

Kerem, B., Zielenski, J., Markiewicz, D., Bozon, D., Gazit, E., Yahav, J., Kennedy, D., Riordan, J.R., Collins, F.S., Rommens, J.M., and Tsui, L.-C.: Identification of mutations in regions corresponding to the 2 putative nucleotide(ATP)-binding folds of the cystic fibrosis gene. *Proc. Natl. Acad. Sci. USA* 87:8447-8451, 1990.

Kerem, E., Corey, M., Kerem, B., Rommens, J., Markiewicz, D., Levison, H., Tsui, L.-C., and Durie, P.: Association between the DF508 mutation and phenotypes in cystic fibrosis. *New Engl. J. Med.* 323:1517-1522, 1990.

Kobayashi, K., Knowles, M., O'Brien, W.E., and Beaudet, A.L.: Benign missense variations in the cystic fibrosis gene. *Am. J. Hum. Genet.* 47:611-615, 1990.

Lemna, W.K., Feldman, G.L., Kerem, B., Fernbach, S.D., Zevkovich, E.P., O'Brien, W.E., Collins, F.S., Tsui, L.-C., and Beaudet, A.L. 1990. Mutation analysis for heterozygote detection and prenatal diagnosis of cystic fibrosis. *New Engl. J. Med.* 322:291-296.

Li, M., McCann, J.D., Liedtke, C.M., Nairn, A.C., Greengard, P., and Welsh, M.J. cAMP-dependent protein kinase opens chloride channels in normal but not cystic fibrosis airway epithelium. *Nature* 331:358-360, 1988.

Osborne, L., Knight, R.A., Santis, G., and Hodson, M.: A mutation in the second nucleotide binding fold of the cystic fibrosis gene. *Am. J. Hum. Genet.* 48:608-612, 1991.

Rich, D.P., Anderson, M.P., Gregroy, R.J., Cheng, S.H., Paul, S., Jefferson, D.M., McCann, J.D., Klinger, K.W., Smith, A.E., and Welsh, M.J.: Expression of cystic fibrosis transmembrane conductance regulator corrects defective chloride channel regulation in cystic fibrosis airway epithelial cells. *Nature* 347:358-363, 1990.

Riordan, J.R., Rommens, J.M., Kerem, B., Alon, N., Rozmahel, R., Grzelchak, Z., Zielenski, J., Lok, S., Plavsic, N., Chou, J.-L., Drumm, M.L., Iannuzzi, M.C., Collin, F.S., and Tsui, L.-C.: Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* 245:1066-1073, 1989.

Rommens, J.M., Iannuzzi, M.C., Kerem, B., Melmer, G., Drumm, M.L., Melmer, G., Dean, M., Rozmahel, R., Cole, J.L., Kennedy, D., Hidaka, N., Zsiga, M., Buchwald, M., Riordan, J.R., Tsui, L.-C., and Collins, F.S.: Identification of the cystic fibrosis gene: chromosome walking and jumping. *Science* 245:1059-1065, 1989.

Sanguuolo, F., Novelli, G., Murru, S., and Dallapiccola, B. Detection of a missense mutation (A to C) in the exon 11 of the cystic fibrosis gene. Submitted.

Schoumacher, R.A., Shoemaker, R.L., Halm, D.R., Tallant, E.A., Wallace, R.W., and Frizzell, R.A.: Phosphorylation fails to activate chloride channels from cystic fibrosis airway cells. *Nature* 330:752-754, 1987.

The Cystic Fibrosis Genetic Analysis Consortium. Worldwide survey of the  $\Delta F508$  mutation - Report from the Cystic Fibrosis Genetic Analysis Consortium. *Am. J. Hum. Genet.* 47:354-359, 1990.

Vidaud, M., Fanen, P., Martin, J., Ghanem, N., Nicolas, S., and Goossens, M.: Three mutations in the CFTR gene in French Cystic Fibrosis Patients: identification by denaturing gradient gel electrophoresis. *Human Genetics* 85: 446-449, 1990.

White, M.B., Amos, J., Hsu, J.M.C., Gerrard, B., Finn, P., and Dean, M.: A frame shift mutation in the cystic fibrosis gene. *Nature* 344:665-667, 1990.

White, M.B., Krueger, L.J., Holsclaw, D.S., Jr., Gerrard, B., Stewart, C., Quittell, L., Dolganov, G., Baranov, V., Ivaschenko, T., Kapronov, N.I., Sebastio, G., Castiglione, O., and Dean, M.: Rare and widely distributed frame-shift mutations in the cystic fibrosis gene. 1991a (submitted).

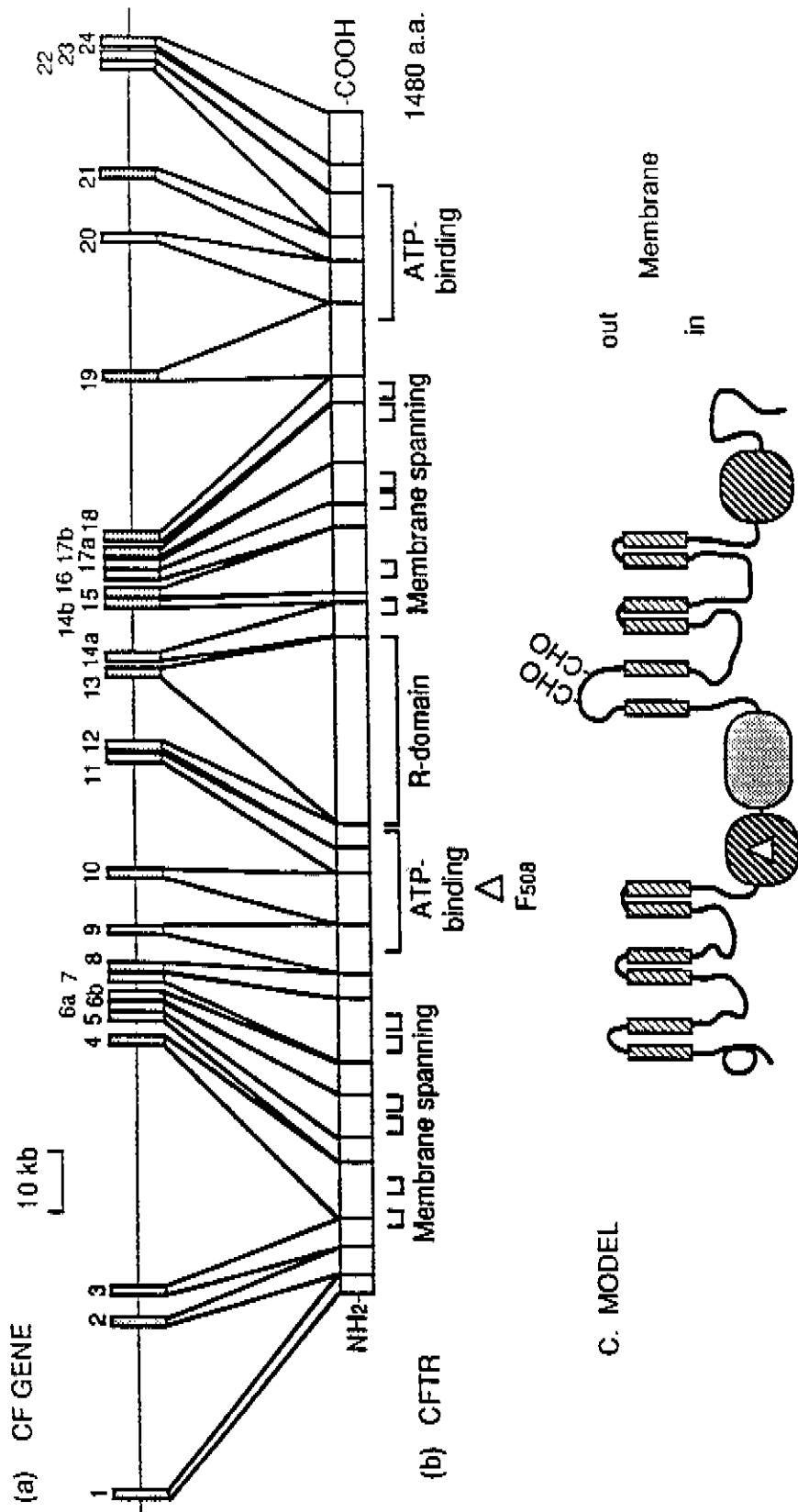
White, M.B., Leppert, M., Nielson, D., Zielenski, J., Gerrard, B., Stewart, C., and Dean, M.: A de novo cystic fibrosis mutation. 1991b (submitted).

Zielenski, J., Rozmahel, R., Bozon, D., Kerem, B., Grzelczak, Z., Riordan, J.R., Rommens, J.M., and Tsui, L.-C.: Genomic DNA sequence of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. *Genomics* 10, in press, 1991a.

Zielenski, J., Bozon, D., Kerem, B., Markiewicz, D., Durie, P., Rommens, J.M., and Tsui, L.-C.: Identification of mutations in exons 1 through 8 of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. *Genomics* 10, in press, 1991b.

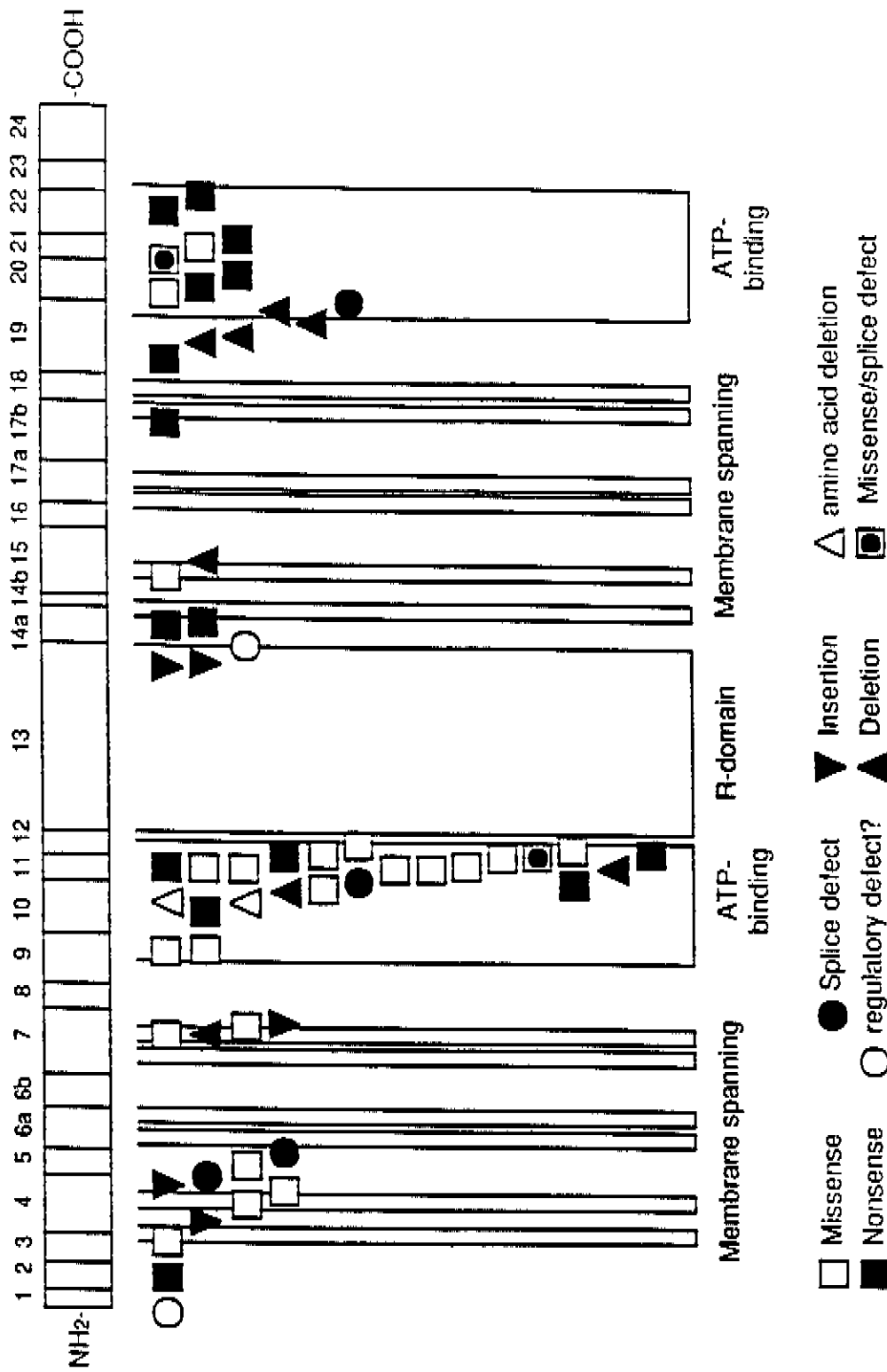
TABLE 1 - List of CF Mutations (published or submitted as of 20 October 1990)

Name	Amino acid change	Nucleotide change	Exon	Reference
124G→C	none; regulation?	G→C at 124	1	Zielenski et al. submitted
G85E	Gly→Glu at 85	G→A at 386	3	Zielenski et al. submitted
444delA	frameshift	deletion of A at 444	4	White et al. submitted
D110H	Asp→His at 110	G→C at 460	4	Dean et al. 1990
R117H	Arg→His at 117	G→A at 482	4	Dean et al. 1990
556delA	frameshift	deletion of A at 556	4	Zielenski et al. submitted
621+1G→T	splice mutation	G→T at 621+1	intron 4	Zielenski et al. submitted
G179R	Gly→Arg at 179	G→A at 664	5	Zielenski et al. submitted
711+1G→T	splice mutation	G→T at 711+1	intron 5	Zielenski et al. submitted
R334W	Arg→Trp at 334	C→T at 1132	7	Gasparini et al. submitted
R347P	Arg→Pro at 347	C→G at 1173	7	Dean et al. 1990
A455E	Ala→Glu at 455	C→A at 1496	9	Kerem et al. 1990
G458V	Gly→Val at 458	G→T at 1505	9	Cuppens et al. 1990
Q493X	Gln→Stop at 493	C→T at 1609	10	Kerem et al. 1990
ΔI507	deletion of Ile at 506/7	3 bp deletion	10	Kerem et al. 1990 Schwarz et al. per.comm.
ΔF508	deletion of Phe at 508	3 bp deletion	10	Kerem et al. 1989
1677delTA	frameshift	deletion of TA at 1677	10	Ivaschenko et al. submitted
1717-1G→A	splice mutation	G→A at 1717-1	intron 10	Kerem et al. 1990 Guillemet et al. 1990
G542X	Gly→Stop at 542	G→T at 1756	11	Kerem et al. 1990
S549R(A→C)	Ser→Arg at 549	A→C at 1777	11	Sangiulio et al. 1990
S549N	Ser→Asn at 549	G→A at 1778	11	Cutting et al. 1990b
S549I	Ser→Ile at 549	G→T at 1778	11	Kerem et al. 1990
S549R(T→G)	Ser→Arg at 549	T→G at 1779	11	Kerem et al. 1990
G551D	Gly→Asp at 551	G→A at 1784	11	Cutting et al. 1990b
R553X	Arg→Stop at 553	C→T at 1789	11	Cutting et al. 1990b
A559T	Ala→Thr at 559	G→A at 1807	11	Cutting et al. 1990b
R560T	Arg→Thr at 560 splice mutation (?)	G→C at 1811	11	Kerem et al. 1990
Y563N	Tyr→Asn at 563	T→A at 1819	12	Kerem et al. 1990
P574H	Pro→His at 574	C→A at 1853	12	Kerem et al. 1990
2522insC	frameshift	C insertion after 2522	13	White et al. submitted
2566insAT	frameshift	AT insertion after 2566	13	White et al. 1990
W846X	Trp→Stop at 846	G→A at 2670	14a	Vidaud et al. 1990
R851X	Arg→Stop at 851	C→T at 2683	14a	White et al. submitted(a)
2789+5G→A	splicing mutation	G→A at 2789	intron 14b	Highsmith et al. 1990
Y913C	Tyr→Cys at 913	A→G at 2870	15	Vidaud et al. 1990
R1162X	Arg→Stop at 1162	C→T at 3616	19	Gasparini et al. submitted
3659delC	frameshift	deletion of C at 3659	19	Kerem et al. 1990
3821delT	frameshift	deletion of T at 3821	19	White et al. submitted
S1255X	Ser→Stop at 1255	C→A at 3896	20	Cutting et al. 1990a
W1282X	Trp→Stop at 1282	G→A at 3978	20	Vidaud et al. 1990
N1303K	Asn→Lys at 1303	C→G at 4041	21	Osborne et al. 1990



**FIGURE 1**  
 Model of the putative CFTR gene product - cystic fibrosis transmembrane conductance regulator, showing possible relationship with cellular membrane and potential glycosylation sites (-CHO).

Cystic fibrosis mutations



**FIGURE 2** Schematic diagram showing the location of mutations in the CFTR gene. Open squares = missense; filled squares = nonsense; open triangle = amino acid deletion; closed triangle = nucleotide deletion leading to frameshift; inverted triangle = nucleotide insertion leading to frameshift; circle = splicing mutation.

ANNEX 2

MANAGEMENT OF CYSTIC FIBROSIS

by

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1. INTRODUCTION

Current clinical treatment of cystic fibrosis is directed towards the prevention or control of the secondary and tertiary manifestations of the disease. At the time of writing, the fundamental physiological defect is still unknown and the prospect of its pharmacological correction is a matter of speculation. Knowledge of the biochemical consequences of the genetic defect

would explain why affected individuals are prone to respiratory infection, produce excessive mucus in respiratory and alimentary tract epithelium, lose excessive salt in their sweat, develop pancreatic fibrosis in utero and hepatic fibrosis in later life, and are at risk of intestinal obstruction. Some or all of these consequences might be preventable by intervention at one or more points in the biochemical pathway. Until now, treatment has been directed at the consequences of these pathophysiological abnormalities, including, as far as possible, prevention of the lung infection which is the most important tertiary result. Just as basic research has yielded partial answers to enquiries into the nature of CF, with many disappointing false leads, clinical research has resulted in considerable improvement in the life span of cystic fibrosis patients, despite the fact that from time to time various forms of treatment have been shown to be of no value or even potentially harmful. It should not be forgotten that clinical research must be carried out within strict ethical parameters, the potential benefits of any experimental treatment being carefully weighed against its possible adverse effects. The fact that most cystic fibrosis patients are children who are unable to give legally valid consent for experimental treatments increases the ethical problems associated with research, while at the same time clinicians are aware of the need to start potentially helpful treatment as early as possible, before serious lung damage has occurred. The fact that the mean survival of patients is now measured in decades rather than the few months or years of the early reports is a tribute to the dedication and opportunism of the clinicians who have made a special study of this disease, and ensured that their patients received the benefit of medical advances as they have occurred.

Some of the standard approaches to treatment of the various clinical aspects of cystic fibrosis are discussed in this annex. Only general principles will be discussed: practical details may be found elsewhere (e.g., Goodchild and Dodge, 1985).

## 2. THE SWEAT ABNORMALITY

Increased sodium chloride content of the sweat is one of the most consistent biochemical features of cystic fibrosis, and has been the basis of the standard diagnostic test for many years. The salt loss itself is usually of no great importance to the patient, and in temperate climates there is no need for routine supplementation of the diet with extra salt. However, in heat waves or in the hot dry summers of southern Europe children with cystic fibrosis sometimes collapse with heat stroke. This can be prevented by a generous salt intake with meals.

The salt content of the sweat normally rises with age so that levels which might be considered diagnostic of cystic fibrosis in a young child would be of uncertain significance in an adult. A short course of dexamethasone may clarify the situation, because it significantly lowers the sweat chloride in normal individuals but has little or no effect in those with cystic fibrosis.

## 3. PANCREATIC INSUFFICIENCY

Although pancreatic function is reasonably well preserved in some affected infants, sooner or later the majority develop steatorrhoea as a result of lipase deficiency. There is also malabsorption of protein but carbohydrate absorption is generally satisfactory. Dried extracts of animal pancreas in capsule or

tablet form may be taken at meals to compensate for the absence of the patient's own pancreatic enzymes. The various products available differ considerably in their enzyme content and bioavailability. The most satisfactory formulation is one in which individual granules of pancreatin are enteric coated to prevent their inactivation by acid in the stomach. The enteric coating is lost in the duodenum, where the pancreatin is released. Pancreatic enzymes work best in an alkaline medium, but secretion of bicarbonate by the pancreas is nearly always very poor in cystic fibrosis so that the pH may never rise to an optimal level in the small intestine. Sodium bicarbonate can be given with meals or a histamine receptor antagonist can be used to suppress gastric acid secretion. However, these measures are rarely necessary if the dose of pancreatin is adequate, and fat absorption can be improved to nearly normal levels by pancreatin alone.

As life expectancy has increased, so has the incidence of diabetes mellitus. This is presumed to be a complication of increasing pancreatic fibrosis which results in impaired blood supply to the islets of Langerhans and their eventual demise. The diabetes is treated in the standard way, with insulin given according to individual requirements.

#### 4. NUTRITIONAL PROBLEMS

Malnutrition was formerly one of the major clinical features of cystic fibrosis in early childhood but modern management has considerably improved growth and development. When adequate replacement pancreatin is given normal growth and weight gain can be expected. Later, some faltering of growth or loss of weight usually develops. This is generally associated with deteriorating respiratory function and increasing pulmonary infection, but the relationships between various contributory factors are complex. It has been shown that the ratio of weight to height in CF is more closely related to the degree of respiratory disease than to the adequacy of pancreatic function (Kraemer 1978), although the severity of these two major features of CF tends to be similar (Gaskin et al 1982).

There is a complex interaction of digestive, metabolic, physiological and adaptive factors which combine to increase the overall caloric requirements to as much as 120-150% of the recommended daily allowance, and even when this level of intake is achieved severe impairment of lung function increases the work of breathing to a point where weight loss or growth failure may be inevitable (Dodge, 1987). If body weight or growth are to be maintained in this situation, intensive nutritional rehabilitation using enteral or parenteral methods become necessary. Ideally, the child with CF should be growing at a normal velocity along his own genetically determined height and weight centiles, while adults should be maintaining their body weight.

Before efficient pancreatin preparations were available, patients were often advised to take a low-fat diet. The high caloric density of fat meant that they were often depriving themselves of a large proportion of the calories in a normal diet, and this deficiency was not usually adequately compensated for by increases in carbohydrate and protein intake. Moreover, low fat diets carry a theoretical and probably clinically important risk of producing essential fatty acid deficiency. It has even been proposed that essential fatty acid deficiency can explain some of the pathological features of CF (Carlstedt-Duke et al, 1987). Current nutritional advice to patients includes a recommendation that fat intake should be at least normal, and some centres actually increase

the fat intake to boost total calories, while others advice specific supplements of essential fatty acids. Of course, pancreatin supplements must also be increased proportionately.

Fat malabsorption is likely to lead to deficiency of fat-soluble vitamins, such as A, D, E and K. Case reports of clinical deficiency of all these vitamins have been published, particularly in infancy. Babies with CF detected by neonatal screening methods before clinical symptoms have occurred have been found to already have biochemical deficiencies of vitamins A and E. Standard practice is to advise supplementation with a multi vitamin product in twice the "normal" daily dosage, and to give additional supplements of vitamin E. Neurological complications may occur in the long term in vitamin E-deficient individuals.

Deficiencies of trace elements are also sometimes observed. Frank iron-deficiency anaemia is uncommon, although serum iron and ferritin levels are quite often low. It should be remembered that patients with advanced respiratory disease from any cause tend to have a raised haemoglobin, and a level towards the lower end of the normal range may represent true anaemia for that group of patients. Low levels of serum or plasma zinc are not uncommon, while biochemical evidence of selenium deficiency has also been recorded in CF. Long term selenium deficiency has also been speculatively associated with an increased risk of carcinoma in patients who survive to adult life (Stead et al 1985). However, there is absolutely no clinical or other evidence to support the view that cystic fibrosis itself is due to either deficiency or disordered metabolism of selenium, and routine selenium supplementation is not recommended (Dworkin et al, 1987).

## 5. BOWEL COMPLICATIONS

Between 10 and 20% of cases of CF present in the newborn period with meconium ileus. In this condition, the lumen of the distal small intestine is obstructed by tenacious plugs of meconium, and in some cases the distended bowel becomes twisted before birth (volvulus), compromising the blood supply to the bowel or even causing gangrene and perforation. Meconium ileus is an emergency which requires transfer of the baby to a neonatal surgical unit. Operative treatment is often required but in some uncomplicated cases the obstruction can be relieved by an enema of "gastrografin", a water soluble iodinated contrast medium. This preparation has high osmolality and has the effect of drawing fluid into the intestinal lumen. Clearly, large amounts of intravenous fluid are required to prevent dehydration, and the procedure requires skill and experience. It should not be undertaken in peripheral centres away from the surgical skills which may be required if the procedure is unsuccessful. Under radiological control, gastrografin is introduced per rectum and it passes through the small, unused colon to the point of obstruction. If the procedure is successful, the retained meconium is dislodged and passed within a few hours along with large amounts of fluid and gastrografin. It may be necessary to repeat the procedure.

In those cases where surgical intervention is necessary, i.e., where the diagnosis is in doubt, or there is evidence of volvulus or perforation, the standard operation is known as the Bishop-Koop procedure in which the small bowel is divided, any gangrenous or non-viable gut excised, and the lumen irrigated to remove as much of the inspissated material as possible. The proximal small gut is joined to the distal portion in an end-to-side fashion,

and the distal portion brought to the surface as an ileostomy. Continuity is thus retained, and when stools are being passed normally via the colon the ileostomy is closed.

Many older patients complain of abdominal pain, particularly when pancreatic supplementation is inadequate. In some, a mass can be felt in the right lower quadrant of the abdomen, which is sometimes tender. Occasionally, subacute or acute intestinal obstruction occurs and these patients sometimes come to operation when the cause of the problem is not recognised. They are found to have intraluminal obstruction by a thick porridge-like mass in the caecum, ascending colon and terminal ileum. This condition is sometimes known as "Meconium Ileus Equivalent", but as there is no meconium or ileus it is more properly referred to as the Distal Intestinal Obstruction Syndrome. Like meconium ileus, it can be managed with gastrografin either as an enema or given through a naso-gastric tube. Other agents such as n-acetylcysteine have also been used.

Intussusception sometimes occurs in children with CF, and is probably precipitated by inspissated bowel contents blocking the ileo-caecal valve. It may require operative treatment.

Rectal prolapse is an occasional problem of the pre-school child, often associated with bulky stools and malnutrition. Although distressing, it is not serious in itself and the extruded rectal mucosa can be returned through the anus with the child lying down. Prevention is by increasing pancreatic supplements to an effective level.

## 6. LIVER DISEASE

Although histological evidence of increased hepatic fibrosis is almost universal in older patients with CF, it is an important clinical problem in less than 10%. With improved survival, the incidence of clinically significant liver disease is expected to rise. Its pathogenesis is uncertain, and prevention therefore impossible. When cirrhosis is established, alcohol and aspirin should be avoided, the former because it may further increase the damage and the latter because it may cause erosion of the gastro-oesophageal mucosa, where varices may be present. Haemetemesis may occur spontaneously from bleeding varices, and is treated conservatively with sedation, intramuscular vitamin K and fresh blood transfusion, followed if necessary by intravenous pitressin. Gastric acid secretion should be suppressed by a histamine-receptor antagonist such as cimetidine or ranitidine. Following an episode of haemetemesis, the varices may be injected under direct vision through an endoscope with a sclerosing agent. Several treatments are usually necessary. Rarely, the first haemetemesis is so severe that it does not respond to conservative measures and emergency surgical treatment is required, with oesophageal or gastric transection. As patients may have varices for many years before bleeding occurs, there is no indication for routine endoscopy or radiography to determine their presence, even when portal hypertension is suspected because of splenic enlargement.

In a small proportion of patients liver failure may be the major clinical problem. This is treated along conventional lines, with a high protein diet and diuretics. Gall stones are not uncommon, but rarely become symptomatic.

## 7. RESPIRATORY DISEASE

Pathological changes are found throughout the respiratory tract and present by far the most serious problem in most cases.

### Obstruction

Within weeks of birth, mucus glands in the lungs are seen to be distended, and mucus often blocks the small airways, where it stagnates. Infection with bacteria is a common sequel. Later in childhood, and in adult life, many CF patients show increased sensitivity of the bronchi to a variety of challenges ranging from exercise to inhaled proteins, in other words they behave like true asthmatics. This component of airways obstruction responds to bronchodilators such as isoprenaline, most effectively given as an aerosol.

Physiotherapy is the cornerstone of the treatment of respiratory disease, and is directed at removing mucus, whether infected or not, from the lungs. Various techniques are employed. They may involve manual percussion of the chest by a physiotherapist, parent or other helper; deep breathing and forced expiration by the patient himself (Forced Expiration Technique, FET); expiration against positive pressure administered by a mask (PEP mask); chest compression by the patient himself or by an assistant; and, postural drainage, in which one or more of these manoeuvres is applied while the patient adopts various body positions in order to drain different parts of the lungs. Most patients need to carry out their physiotherapy two or three times a day, each session taking about 20-30 minutes. Patients of all ages find this irksome and increasingly are looking to alternatives. Regular programmes of exercise including swimming, running, tennis and dancing may make the patient cough and bring up retained secretions but if exercise is to be used as a substitute for physiotherapy it must be carried out daily.

Nasal obstruction from polyps is common in CF and is occasionally the presenting symptom of the disease. If removed, the polyps nearly always return and moreover the anaesthetic required for surgery is itself a hazard, often being followed in many cases by a deterioration in lung function (Price, 1986). The polyps often become smaller when treated with corticosteroid sprays or drops, and unless both nostrils are completely blocked, which is unusual, surgery is best avoided.

### Infection

Sooner or later, mucus retained in the lungs becomes infected, and infection, once established, is extremely difficult to eradicate. A wide variety of pathogens may be responsible, but those which are encountered most frequently include Staphylococcus aureus, Haemophilus influenzae, Pseudomonas aeruginosa, Pseudomonas cepacia, and certain fungi such as Aspergillus Fumigatus and Candida albicans. Viruses such as influenza may also affect the lungs, just as in other people, and predispose to secondary infection with bacteria. There is no evidence that the individual with CF is excessively prone to such infections but the consequences are likely to be more serious.

It is obviously desirable to prevent infection occurring in the first place, and this is one of the strongest arguments for instituting physiotherapy, even in young infants, as soon as the diagnosis is made in order to keep the lungs drained of mucus. Because staphylococcal infection is particularly damaging,

and most likely to occur in early life, many physicians treat their young children with continuous narrow spectrum anti-staphylococcal antibiotics such as flucloxacillin, while others use antibiotics in high dosage at the first sign of a respiratory infection. Immunisation against whooping cough and measles is an important part of the care of young children with CF, but immunisation against Pseudomonas infection has been unsuccessful.

The majority of patients eventually become infected with Pseudomonas aeruginosa (Pitt, 1986). This organism has relatively low pathogenicity but is almost impossible to eradicate. It gives a characteristic green colour to the sputum, which is often produced copiously. Laboratory cultures often show that several strains of Pseudomonas are present at the same time. A highly characteristic feature is the so-called mucoid change, whereby Pseudomonas strains isolated from CF patients frequently produce very large amounts of a mucoid exopolysaccharide or alginate when cultured in the laboratory. Pseudomonas strains found in CF may be highly sensitive to antibiotics in vitro but nevertheless continue to be isolated during and after a full course of appropriate therapy. Treatment of Pseudomonas infection is therefore directed towards containment rather than elimination, and there are practical reasons for giving anti-pseudomonal drugs in courses of limited duration. The antibiotics employed are mostly  $\beta$ -lactam drugs such as certain penicillins (carbenicillin, azlocillin) or aminoglycosides (gentamicin, tobramycin, netilmicin), often given in combination. The aminoglycosides have toxic effects on the kidneys and the inner ear, and courses are usually limited to about 14 days. All of these drugs must be given by injection several times a day, and this is most conveniently given through an intravenous line. Recently, another class of drugs, quinolone derivatives such as ciprofloxacin and norfloxacin, have been shown to have powerful anti-pseudomonal activity, and they have the advantage that they can be given by mouth. Unfortunately resistance usually develops within a few weeks, but when treatment is stopped sensitivity usually returns so that further courses can be given.

Some centres have adopted a policy of admitting patients on a regular basis, every few months, for a course of anti-pseudomonal therapy regardless of symptoms. Others treat only acute exacerbations characterised by weight loss, malaise, deteriorating lung function and x-ray changes, increased cough and sputum and sometimes pyrexia. The choice of antibiotics is often dictated by in vitro laboratory sensitivities, and occasionally limited by allergy to certain drugs. Antibiotics are also widely used by inhalation as aerosols, and have been shown to reduce the frequency of admission for exacerbations of infection when given on a regular basis (Hodson et al, 1981)

Secondary infection with fungi is not uncommon, and some patients develop allergic aspergillosis in which severe bronchospasm plays a major role. It usually responds to corticosteroids with or without anti-fungal therapy. Vigorous and frequent physiotherapy is an essential component of any course of treatment.

#### Other complications

Streaks of blood in the sputum are not uncommon but occasionally a large blood vessel in the bronchial tree will become eroded and massive haemoptysis occurs. This is a life threatening emergency: the patient requires blood transfusion and urgent transfer to a large regional centre. Sophisticated radiological techniques or bronchoscopy are used to identify the source of

bleeding, and the vessel can be embolised with gelatin foam or a tiny metal coil introduced through an arterial catheter. Rupture of a thin-walled air sac produced by distortion of lung architecture sometimes occurs, particularly in older patients, causing leakage of air into the pleural cavity which builds up to produce tension and compresses the underlying lung (pneumothorax). Some episodes resolve spontaneously but others require insertion of a drainage tube into the pleural cavity. Recurrent pneumothorax may require surgical treatment in which an irritant substance is introduced to produce adhesions between the two layers of pleura.

#### Lung transplantation

Heart-lung transplants have been given to a number of young CF adults with advanced lung disease. Early experience was not encouraging, the first British and US recipients dying within 2 months of surgery. Subsequent results have been much better. Although clearly this cannot be a standard approach to treatment of advanced CF, it will probably continue to have a role in well-selected cases. It is interesting - and scientifically important - to note that the characteristic ion transport defect does not develop in the donor lungs although of course it remains in the nasal mucosa, and in tracheal mucosa above the site of anastomosis (Alton et al, 1987).

#### 8. PSYCHOLOGICAL PROBLEMS

The whole family is involved when the diagnosis of cystic fibrosis is made in a child (Burton, 1975). At first, the burden falls particularly on the parents. They must come to terms with the fact that their child has an inherited disorder which they as unwitting carriers have passed on. It is important to explain to them that feelings of guilt are inappropriate. At the same time they have to adjust their expectations for the child to the possibility that his life will be shortened by this disease, for which we have no cure. Nevertheless, a very demanding regimen of treatment will be prescribed, which will interfere with their own lives as well as that of the child. They must also realise that there is a 25% risk that any further child of the marriage will be affected. Sometimes this genetic advice comes when another pregnancy has already been started, while occasionally there is the added blow that an older sib of a newly diagnosed patient, previously thought to have asthma or bronchitis, is another CF victim. The fact that the child has inherited CF from both sides of the family sometimes brings parents together in adversity, but where the parental relationship was already under strain the added burden of a child with CF not uncommonly precipitates further marital problems leading to separation or divorce. Parents go through the familiar sequence of emotions which follow bereavement, in this case loss of the healthy child they had hoped for - and may experience shock, denial, anger and depression before they can make a realistic adjustment. Anger is directed at themselves and also at medical staff who may have overlooked the diagnosis for some time, particularly when their anxieties may have been dismissed or when lung damage is found to be already present.

Other family members also become involved. Grandparents, particularly when the parents themselves are young, may wish to take over an unreasonable amount of the child's care on the grounds that the parents are unable to cope with this added burden. On the other hand, they may deny the diagnosis or fail to understand the bilateral nature of the inheritance and blame it all on the

spouse. Other relatives may wish to know whether they are carriers, and the risk of their future offspring having CF: something which could only be answered empirically until the recent discovery of the gene locus and adjacent polymorphisms.

When he reaches school age, the child himself may be teased by other children because of persistent cough, poor stature or offensive flatus. If he has significant lung disease he may be unable to participate fully in games and other activities, and is usually very self conscious about the need for physiotherapy and medicines. There is a strong tendency for parents to over-protect the affected child, with resultant delay in emotional development and achievement of independence.

Adolescents often experience the same feelings of anger, denial and depression as their parents had when the diagnosis was made. By that time they are aware of the nature of their disability, and their reduced life expectancy. They may experience difficulty in making close relationships, particularly with the opposite sex. The discovery that they are probably infertile often comes as a shock to young men. Some occupations will be closed to them and unemployment may add its own burden of depression. They may have encountered other young people with cystic fibrosis whose health is worse than their own, and the death of such friends and acquaintances is a further challenge to emotional well being.

Adults who marry may wish for children, but the great majority of males are infertile as a result of congenital blockage of the vas deferens. Fertility in females is also reduced, although many have successfully borne children. The likely effects of pregnancy and the physical demands of raising young children on the mother's health must be kept in mind, as well as the likely duration and extent of her ability to look after them. Finally, there comes the realisation that the intervals between treatment are becoming shorter and the courses of treatment themselves less effective. The support of family, friends and professional staff is then critical, up to and including the stage of terminal care.

It is clear that clinical management of these patients requires an understanding of their problems and those of the family. The help of an experienced social worker is invaluable. Some patients find it easier to discuss their problems with a nurse or physiotherapist than with the doctor. Whatever their clinical condition, patients must be encouraged to express their feelings and helped to develop a positive attitude to treatment.

## 9. DELIVERY OF HEALTH CARE

Because cystic fibrosis is such a complex condition and its treatment so protracted, health care is most effectively given by a team familiar with the many problems which can occur and who have at their disposal a variety of therapeutic interventions. It has been convincingly shown from Denmark and elsewhere that life expectancy is better when treatment is given at such specialised clinics. (Nielson & Schiøtz, 1982; Warwick, 1982). Even when patients live at a long distance from such a centre and their routine care must be provided locally, responsibility can be shared. Annual assessments at the cystic fibrosis clinic may detect early evidence of infection, nutritional deficiencies or other complications, and give the opportunity for discussion

with physiotherapists, dietitians and social workers with particular experience of CF. Regional clinics should also be able to provide authoritative genetic counselling, heterozygote identification and prenatal diagnosis.

Neonatal screening programmes are in operation in various parts of the world, based on measurement of pancreatic enzymes in blood spots. Blockage of small ducts in the pancreas by thick secretions occurs before birth and in early neonatal life, resulting in increased absorption of retained pancreatic enzymes such as trypsin and lipase into the circulation. More than 90% of infants with CF have raised levels of serum immuno-reactive trypsin (IRT), which can be measured by radio-immunoassay or enzyme-linked assays. It has not yet been proved that such early diagnosis substantially affects morbidity or eventual life expectancy, but the cost of screening can be more than offset by the savings from reduced hospital admissions of affected babies during the first two years of life (Wilcken & Chalmers, 1985). Apart from any actuarial benefits, neonatal screening allows parents of affected babies to receive early genetic counselling, and prevents the distress and recriminations which result from diagnostic delay. It is likely that the IRT test, which is not completely sensitive or specific, will be replaced by more effective methods resulting from discovery of the basic genetic abnormality. Of course, screening is of no benefit unless it is followed by close surveillance and an active treatment programme. If and when more fundamental treatment of the CF abnormality becomes available, it should obviously be started as early in life as possible.

#### 10. REFERENCES

- Alton, E.W.F.W., Batten, J., Hodson, M., Wallwork, J., Higgenbottom, T. and Geddes, D.: Absence of electrochemical defect of cystic fibrosis in transplanted lung. *Lancet* i, 1137-1139, 1987.
- Burton, L.: The family life of sick children. Routledge & Kegan Paul, London, 1975.
- Carlstedt-Duke, J., Bronnegard, M. and Strandvik, B.: Pathological regulation of arachidonic acid release in cystic fibrosis: the putative basic defect. Proceedings of the National Academy of Science USA, 83, 9202-9206, 1986.
- Dodge, J.A.: Nutritional requirements in cystic fibrosis. *Journal of Paediatric Gastroenterology and Nutrition*, 1987, (in press).
- Dworkin, B., Newman, L.J., Berezin, S., Rosenthal, W.S., Schwarz, S.M. and Weiss, L. Low blood selenium levels in patients with cystic fibrosis compared to controls and healthy adults. *Journal of Parenteral and Enteral Nutrition*, 11, 38-41, 1987.
- Gaskin, K., Gurwitz, D., Durie, P., Corey, M., Levison, H. and Forstner, G.: Improved respiratory prognosis in CF patients with normal fat absorption. *Pediatrics*, 100, 857-862.
- Goodchild, M.C. and Dodge, J.A.: Cystic Fibrosis. Manual of Diagnosis and Management. 2nd edition Bailliere Tindall, London, 1985.
- Hodson, M.E., Penketh, A.R.L. and Batten, J.C.: Treatment of chronic *Pseudomonas aeruginosa* infection in patients with cystic fibrosis. *Lancet* ii, 1137-1139, 1981.

Kraemer, R., Rudeberg, A., Hadorn, B. and Rossi, E.: Relative underweight in cystic fibrosis and its prognostic value. *Acta Paediatrica Scandinavica* 67, 33-37, 1978.

Nielson, O.H. and Schitz, P.O.: Cystic fibrosis in Denmark in the period 1945-1981. Evaluation of centralised treatment. *Acta Paediatrica Scandinavica* Suppl. 301, 107-119, 1982.

Price, J.F.: The need to avoid general anaesthesia in cystic fibrosis. *Journal of the Royal Society of Medicine Supplement No. 12*, 79, 10-12, 1986.

Stead, R.J., Redington, A.N., Hinks, L., Clayton, B., Hodson, M. and Batten, J.C.: Selenium deficiency and possible increased risk of carcinoma in adults with cystic fibrosis. *Lancet*, ii, 862-863, 1985.

Warwick, W.J.: Prognosis for survival with cystic fibrosis: the effects of early diagnosis and cystic fibrosis center care. *Acta Paediatrica Scandinavica* Suppl. 301, 27-31, 1981.

Wilcken, B. and Chalmers, C.: Reduced morbidity in patients with cystic fibrosis detected by neonatal screening. *Lancet* ii, 1319-1321, 1985.

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