Update/Le point

New possibilities for population control of cystic fibrosis*

J.A. Dodge[1] & V. Boulyjenkov[2]

Cystic fibrosis (CF), which 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 much 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. Whereas previously carriers were identified only when they became parents of affected children, in recent years carriers who were more distantly related to CF patients have often been identified by means of genetic linkage techniques. A new strategy for the control of CF at the population level is now proposed. It is based on the report of a joint WHO/ICF(M)A (International Cystic Fibrosis (Mucoviscidosis) Association) Task Force on CF which met in November 1990.

Introduction

At a joint WHO/International Cystic Fibrosis (Mucoviscidosis) Association (ICF(M)A) meeting in London in 1989 it was concluded that different strategies for cystic fibrosis (CF) control would have to be developed for countries, taking into account the differences in disease incidence, in stages of economic, educational and health service development, in religious, cultural and legal backgrounds, and in health care priorities.[a] As the responsible gene and its alteration in CF had still not been precisely identified 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 in 1989 provided not only a means of further investigating the biochemical defect but also a powerful new means of population screening (*1–3*). The most frequent gene mutation was present in approximately 70% of the CF mutant chromosomes. Considering this new knowledge, it was proposed that a Task Force should make recommendations for the development of screening programmes for CF—examining different possibilities (technical and

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[1] Professor of Child Health Nuffield Department of Child Health, Institute of Clinical Science, Queen's University of Belfast, Belfast, United Kingdom.

[2] Responsible Officer, Hereditary Diseases Programme, Division of Noncommunicable Diseases and Health Technology, World Health Organization, 1211 Geneva 27, Switzerland. Requests for reprints should be sent to this address.

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[a] *Report of a Joint WHO/ICF(M)A Meeting on the Feasibility Study on Hereditary Disease Community Control Programmes—Cystic Fibrosis, London, 12–14 June 1989.* Unpublished document WHO/HDP/ICF(M)A/WG/89.2, 1989.

organizational), based on experience gained from other genetic disorders, as well as epidemiological, ethical, economic, educational and legal issues. This paper summarizes the main ideas in that Task Force's report.[b]

The genetic nature of CF

The CF gene contains 27 exons and spans about 250 000 base pairs (bp). 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. As there were no functional assays for the CF gene products, 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 nucleotide-binding fold (NBF) of the predicted cystic fibrosis transmembrane conductance regulator (CFTR), and was thus named delta F508. Population studies showed that ΔF508 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 found only in the mutant chromosomes and not in any of the normal ones), providing a strong argument for the gene being responsible for CF.

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 ΔF508 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.

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 have been described as "pancreatic insufficient" (PI), while the other 15% have sufficient enzyme and are thus "pancreatic sufficient" (PS). Patients homozygous for the ΔF508 mutation were found to be almost exclusively PI (*4*).

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 (*4*).

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 among the Chinese, wherever they are domiciled. Small numbers of well-authenticated cases have occurred in Japan. Although relatively rare in India and Pakistan, interested physicians in these 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 ΔF508 is present in about 70% of mutant chromosomes in North America and northern Europe. This proportion also varies considerably according to the population studied (*5*).[b] It may account for up to 90% of CF chromosomes in Denmark, but in southern Europe generally the expected frequency of ΔF508 is only about 48%, with a range from 33% (Yugoslavia) to 54% (Greece). 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%.

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 ΔF508 incidence is also high, which suggests 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 ΔF508 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

[b] *Report of a Joint WHO/ICF(M)A Task Force on Cystic Fibrosis, Leningrad/Moscow, 26–29 November 1990.* Unpublished document WHO/HDP/ICF(M)A/TF/90.4, 1990.

the overall incidence and the relative contributions of different mutations, in populations where the recognition and diagnosis of CF are believed to be deficient.

Applications of current knowledge

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.

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.

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, as described below.

- *At birth.* Advantages include the relative ease with which it could be included in existing laboratory and counselling programmes 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.

- *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), which allows affected carriers maximum choice of reproductive options. Disadvantages include 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 like the United Kingdom. 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 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 ΔF508 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. On the assumption that a battery of tests which identify more than 90% of carriers can be developed (*6*), 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 subpopulation 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.

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 (hypothyrodism 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 concerned with an early diagnosis and the implementation of active treatment, which can be expected to improve both life expectancy and the quality of life; the elimination of parental feelings of guilt and anger at delayed diagnosis; and identification of families at risk, who will be given appropriate counselling and can be offered carrier detection tests.

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 (*7*).

New prospects for 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 (*8*). 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.

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.

Prerequisites for a carrier screening programme

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.

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 ΔF508 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 geneticists in the USA consider that CF testing would be regarded as standard care for that country (*6*). 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 to $ 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.

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 of Bioethics.[c]

Components of a carrier screening programme

Educational. The objective of an educational campaign directed towards the *general public* should be twofold: 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. It is essential that stigmatization of carriers is avoided. One approach is to create general awareness that all individuals carry mutant genes.

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.

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.

Technical. The *clinical services* must be directed by physicians. 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.

Facilities for blood and/or buccal-cell collection must be organized. 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.

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.

Training programmes. In order to offer a service to the public, professional training programmes will be required in order to expand the number and/or extend the work of CF centres; to manage larger and confidential databases; to further develop communication and counselling skills; and to provide information and updating for health professionals caring for CF patients, even if they are not directly involved with running the service.

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

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

[c] **Kokkonen, P.** *Health Legislation and genetics: summary.* Describes the Council of Europe's Recommendation No. R(90)13 on Prenatal Genetic Screening, Prenatal Genetic Diagnosis and Associated Genetic Counselling, which was adopted in a resolution by the Committee of Ministers to the Member States of the Council of Europe on 21 June 1990. Unpublished document WHO/HDP/ICF(M)A/TF/90.4/WP.5, 1990.

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 the 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 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.

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