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Cystic fibrosis identified by neonatal screening: incidence, genotype, and early natural history

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Abstract

The incidence of cystic fibrosis over the last 10 years in East Anglia (a region of the United Kingdom with a population of 2·1 million) has halved. This has happened during the establishment of a neonatal screening programme, which has enabled early diagnosis, genetic counselling, and latterly the option of prenatal diagnosis in subsequent pregnancies. One hundred and seven children were born with cystic fibrosis between 1981 and 1990, eight of whom were siblings. The Guthrie blood spots of 82 infants detected by neonatal immunoreactive trypsin screening between 1981 and 1990 were examined for the presence of the most common cystic fibrosis gene mutation (δF508). It was present in 135 (82%) of the 164 cystic fibrosis genes analysed with 54 (66%) cases being homozygous and 27 (33%) heterozygous. Sixty nine per cent of infants were symptomatic at the time of diagnosis regardless of genotype. No association was found between the early clinical or biochemical features of the disease and homozygosity or heterozygosity for this mutation. Screening for cystic fibrosis using the blood immunoreactive trypsin assay alone remains an effective method of identifying infants with the disease soon after birth, thereby allowing early therapeutic intervention. Genetic counselling and prenatal diagnosis have contributed to a reduction in the number of children born with cystic fibrosis, but may not entirely explain the decreasing incidence of the disease.

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Screening for cystic fibrosis by blood immunoreactive trypsin assay in the neonatal period was introduced in East Anglia in 1980 and since 1982 every infant born in the region has been screened for the disease. The introduction of screening offers a unique opportunity to study the early natural history of cystic fibrosis (before clinical presentation), and to provide genetic counselling before another child is conceived, with antenatal detection of subsequent affected fetuses.

The purpose of this paper is to report the incidence, clinical presentation, and biochemical and genotypic characteristics, and to describe the early natural history of infants with cystic fibrosis detected by immunoreactive trypsin screening, together with the outcome of subsequent pregnancies of affected couples in East Anglia. The initial results of a controlled prospective trial of the effects of continuous prophylactic antibiotic treatment on clinical progress of a cohort of these children are reported elsewhere.[1]

Infants and methods

The subjects of this study were 107 infants with cystic fibrosis born in East Anglia between 1981 and 1990 (table 1). Heel prick blood specimens obtained at 6–9 days of age for the Guthrie test were assayed for immunoreactive trypsin at the Regional Neonatal Biochemical Screening Laboratory at Peterborough District Hospital. Blood specimens were assayed in batches of not less than 250, and for each assay the immunoreactive trypsin concentration at three standard deviations from the mean was multiplied by a factor of 1·2 to determine the screening test cutoff. Infants whose blood immunoreactive trypsin concentration exceeded this value (0·5% of the screened population) underwent a second screening blood test at 3–4 weeks of age; those whose blood immunoreactive trypsin concentrations were then greater than three standard deviations above the assay mean were referred for clinical examination and sweat testing. The biochemical method for immunoreactive trypsin assay is described elsewhere.[2] Confirmation of the diagnosis of cystic fibrosis was made by measurement of sweat osmolality using pilocarpine iontophoresis with a Wescor macroduct system.[3] This was performed in the patient's home by the nurse coordinator (KN) who counselled the parents and coordinated care thereafter.[4]

Delta F508 status was determined retrospectively in all those infants in whom Guthrie card blood specimens were available and suitable for analysis. Approximately 4 mm^2 sections of the blood spot were placed in 25 μl polymerase chain reaction (PCR) mixture and heated to 95°C for 15 minutes. Taq polymerase (0·5 U) was added to each tube and the PCR performed. The PCR products were run on a 10% polyacrylamide minigel to distinguish 95 base pair (bp) (δF508) and 98 bp (normal) alleles.[5]

Neonatal data (including birth weight, gestational age, mode of presentation, and early natural history) were obtained from hospital

Table 1 Number of infants with cystic fibrosis identified by neonatal screening each year and incidence of disease in East Anglia

Year	*No screened*	*No with confirmed cystic fibrosis*	*Incidence*
1981	21 812	14 (7 retrospective)	1 in 1558
1982	22 088	12	1 in 1841
1983	22 847	11	1 in 2077
1984	22 718	14	1 in 1623
1985	23 741	10	1 in 2374
1986	23 423	9	1 in 2603
1987	25 067	11	1 in 2279
1988	25 766	8	1 in 3221
1989	25 572	10	1 in 2557
1990	25 956	8	1 in 3245
Total	238 990	107	1 in 2234

Table 2 Biochemical and clinical data of infants with cystic fibrosis detected by neonatal immunoreactive trypsin (IRT) screening according to their genotype

	δF508/F508	δF508/other	Other/other
Total percentage (No)	65·8 (54)	32·9 (27)	1·2 (1)
Sex (F=female, M=male)	F23, M31	F17, M10	M1
Mean (SD) birth weight (g)	3270 (490)	3220 (440)	3200
Mean (SD) gestation (weeks)	39·5 (1·7)	39·3 (2·9)	39
Mean (SD) IRT 7 days (μg/l)	121 (46)	130 (50)	63
Mean (SD) IRT 4 weeks (μg/l)	119 (45)	124 (36)	–
Mean (SD) IRT 6 months (μg/l)	64 (39)	74 (40)	–
Sweat tests			
Mean (SD) sweat osmolality (mmol/kg)	253 (27)	267 (60)	–
Mean (SD) sweat sodium (mmol/l)	103 (34)	94 (26)	69
Mode of presentation			
% (No) screening alone	75 (41)	56 (15)	0 (0)
% (No) meconium ileus	17 (9)	22 (6)	0 (0)
% (No) jejunal atresia	4 (2)	0 (0)	0 (0)
% (No) clinical* and screening	4 (2)	22 (6)	100 (1)
Symptoms at time of diagnostic sweat test			
% (No) respiratory	35 (19)	26 (7)	100 (1)
% (No) gastrointestinal	17 (9)	26 (7)	0 (0)
% (No) respiratory plus gastrointestinal	19 (10)	15 (4)	0 (0)
% (No) none	26 (14)	30 (8)	0 (0)
% (No) not known	3 (2)	3 (1)	0 (0)

*Clinical=respiratory or gastrointestinal symptoms or failure to thrive or a combination.

notes. Symptoms and signs at the time of confirmation of diagnosis were obtained by the nurse coordinator. Since 1985 all children with cystic fibrosis detected by neonatal screening have been the subjects of a prospective trial of the effects of continuous flucloxacillin treatment by mouth.[1] Details of the genetic counselling received by the parents of these children and of their further pregnancies were obtained.

The significance of the change in incidence of cystic fibrosis over time was assessed using linear regression analysis. Birth order was analysed by the method of Haldane and Smith.[6] The significance of the differences in clinical and biochemical data between infants according to their genotype was analysed using *t* tests and χ^2 analyses.

Results

Table 1 gives the number of infants with cystic fibrosis identified by neonatal screening each year, and the incidence of the disease in East Anglia between 1981 and 1990. Regression analysis showed a significant decrease ($p<0·001$) in the incidence of cystic fibrosis at an average rate of 7·5% each year over this period. During the decade 238 990 infants were screened of whom 107 have cystic fibrosis. Children with cystic fibrosis who were born elsewhere and moved into the region were excluded from the analysis. The number of infants with cystic fibrosis identified each year ranged between eight and 14 (mean 11), resulting in an incidence of the disease in East Anglia of one in 2234 over the 10 years of the study.

Guthrie cards were available and suitable for genotypic analysis in 82 infants (table 2). Of 164 chromosomes studied 135 (82%) had the δF508 mutation. Genotype frequencies did not differ significantly from those expected for Hardy-Weinberg equilibrium. There were 54 (66%) homozygotes (δF508/δF508) and 27 (33%) heterozygotes (δF508/other), and one child who did not have the δF508 mutation (other/other). Thus most (99%) of the affected children had at least one chromosome bearing the δF508 gene mutation. There was no significant difference in the mean birth weights and gestational ages of the three groups.

Analyses of the differences in mode of presentation were restricted to the two major groups (δF508/δF508 and δF508/other). There was no significant difference in first or second immunoreactive trypsin concentrations between the two groups. At 4 months 78% of the two groups still had immunoreactive trypsin concentrations in the diagnostic range. Thereafter their rate of decrease was similar in the two groups. The mean age of diagnostic sweat test (5·7 weeks) was not significantly different between the two groups, nor was the mean sweat osmolality or sodium concentration. Seventy five per cent of homozygous infants and 56% of heterozygous infants were identified by immunoreactive trypsin screening alone ($p>0·05$). A greater proportion of the heterozygotes than the homozygotes had neonatal meconium ileus (22 *v* 17%), but this difference was not significant ($p>0·05$). A significantly greater proportion of the former presented with failure to thrive or respiratory symptoms before the second immunoreactive trypsin assay, however ($p<0·01$). By the time of the confirmatory sweat test 71% of the homozygotes and 67% of the heterozygotes had symptoms. Respiratory symptoms accounted for the majority (35%) in the homozygotes, whereas the heterozygotes presented in equal proportions with respiratory and gastrointestinal symptoms.

Of the 107 children with cystic fibrosis, eight were siblings of affected children. Of the 40 families studied since April 1985 (whose 43 children were the subjects of a prospective intervention study[1]), their affected child was, in the case of 12 couples, the first born; in one family both the first and second born were affected. In one family both the first and third born were affected; in 21 couples the second born alone was affected. In four couples it was the third born alone (including one of twins); and in one family both the second and fourth born were affected (table 3).

Nine sets of parents of affected children (23%) went on to have nine further liveborn children (table 4). Four couples each had one further

Table 3 Birth order of 43 children with cystic fibrosis born of 40 couples who had at least one affected child

Birth order	No of children with cystic fibrosis	No of normal children	Total
First born	14	26	40
Second born	23	9	32
Third born	5	4	9
Fourth born	1	0	1
Total	43	39	82

Table 4 Outcome of pregnancies of nine of 40 couples who already had at least one child with cystic fibrosis and who chose to have further pregnancies

	Antenatal test	No antenatal test	Total
No of pregnancies	8	4	12
Infant with cystic fibrosis	1	2	3
Normal infant	4	2	6
Cystic fibrosis abortus	1	0	1
Normal abortus	2	0	2

unaffected child after antenatal testing, and one couple had their second affected child. Four couples had pregnancies without antenatal tests, resulting in the birth of two children with cystic fibrosis and two normal children. One couple had a therapeutic abortion (affected conceptus), and there were two spontaneous abortions (normal conceptus) after a chorionic villus biopsy sample.

Of the 40 families, 36 were married couples. Office of Population Census and Statistics (OPCS) birth statistics for women giving birth within marriage provide data on the number of previous liveborn children from which distribution of sibship size can be predicted. For East Anglian mothers during the study period the average sibship size is estimated to be 2·45 children. In the 40 families studied here the average number of children was 2·05. There were more than the expected number of sibships of one or two children and fewer sibships of three or more children ($p<0·05$). In most families there was only one child affected by cystic fibrosis, either the only child or the last born. There was a significant birth order effect ($p<0·001$).

Discussion

We report here the decreasing incidence of cystic fibrosis in East Anglia over the last 10 years, and on the genotypic and phenotypic characteristics of the 82 infants for whom full data were available. During a decade when the birth rate in the region has increased by 20%, the incidence of cystic fibrosis has apparently halved (table 1). In East Anglia the screening programme for cystic fibrosis has worked effectively and only one in 10 000 children has been subjected to an unnecessary sweat test.[7] As far as we know only one child with a normal neonatal blood immunoreactive trypsin concentration, according to present diagnostic criteria, was subsequently found to have cystic fibrosis. The observed incidence represents complete ascertainment insofar as that is possible. A designated paediatrician in each district of East Anglia is responsible for the documentation of each child identified.

The efficiency of the screening procedure as experienced in East Anglia has not been universally shared; factors, preanalytical and analytical, which might contribute significantly to variations in the specificity and sensitivity of the test have been reviewed.[8] In centres where such problems exist a combination of immunoreactive trypsin measurement and genotyping performed on the dried blood spot could, depending on the number of cystic fibrosis mutations included in the analysis, result in an improvement in screening test efficiency.[9]

Early detection of cystic fibrosis by neonatal screening offers parents of an affected child the opportunity of counselling before further pregnancies, and of subsequent antenatal testing. Genetic counselling has been available throughout the decade, a period during which methods for prenatal diagnosis and carrier detection have been introduced, and latterly, increased in accuracy and availability.[5 10-12]

Using linear regression analysis, and adjusting the number of cases of cystic fibrosis identified each year to correspond to the same number screened each year, the decline in incidence was found to be highly significant. It is unclear why the number of children born with cystic fibrosis each year throughout the decade has decreased.

In the 40 families ascertained by newborn screening since 1985 the smaller sibship size and significant birth order effect suggest that early diagnosis and counselling have discouraged some couples from having more children. It can be shown, however, that this alone can only reduce the birth incidence by at most about 20%. From OPCS birth statistics for East Anglia it can be estimated that the proportion of couples having another child when they already have one, two, three, or four children is respectively 92, 41, 31, and 40%. If 100 cystic fibrosis couples conformed to this pattern they would have a total of 61 children with cystic fibrosis (table 5) as compared with 49 if they had no further children after their first affected child. Although genetic counselling and prenatal diagnosis have undoubtedly reduced the number of children born with cystic fibrosis, it is not clear that this entirely accounts for the decrease in incidence over the last 10 years. Other possible contributing factors are incomplete ascertainment, reduction in the rate of consanguinity, and decrease in gene frequency due to population migration. None of these would be expected to have a large effect and it is possible that sampling artefact has exaggerated the trend.

We found no significant difference in the biochemical and early clinical features of infants with cystic fibrosis detected by neonatal immunoreactive trypsin screening according to their genotype. Genotyping of children with cystic fibrosis and examination of its relation with clinical status have been undertaken in several other regions of the world.[13-18] Kerem *et al*,[14] in a cross sectional study of 293 affected subjects of all ages, reported an association between possession of the δF508 allele and pancreatic insufficiency and postulated that certain alleles (including δF508) which conferred more severe disease were recessive to those which had a milder clinical expression. They found no direct correlation between genotype and pulmonary function, except in those with pancreatic sufficiency who had better lung function regardless of genotype. The association between specific mutations at the cystic fibrosis locus and pancreatic phenotype cannot be absolute, however, because several subjects have been described who are homozygous for

Table 5 Pregnancy outcome of 100 cystic fibrosis carrier couples, (a) following East Anglian pattern of reproductive behaviour (see text), and (b) having no further children after their first affected child (results in parentheses). All results rounded to whole numbers

Birth order (n)	*No of couples having nth child*	*No children of birth order n with cystic fibrosis*
1	100	25
2	92 (69)	23 (17)
3	38 (21)	9 (5)
4	12 (5)	3 (1)
5	5 (1)	1 (0)
Total No of children with cystic fibrosis		61 (49)

the δF508 allele but remain pancreatic sufficient.[14 16 18 19] In addition, the range of clinical expression of the disease within each genotype group is wide. Three of the 82 children reported here were judged clinically, during childhood, not to require pancreatic enzyme supplements. One of these children did not carry the δF508 allele, and had neonatal blood immunoreactive trypsin and sweat sodium concentrations which were lower than the means of the other two groups; however, he was symptomatic by the time the second immunoreactive trypsin assay was performed. The other two children were homozygous for the δF508 allele.

The birth weights of the infants reported here did not differ significantly from those of 48 normal infants born in East Anglia.[20] This finding conflicts with earlier reports[21] of low birth weight in cystic fibrosis, and suggests that, although pathophysiological abnormalities may be found in the affected fetus as early as 28 weeks,[22] they do not cause intrauterine growth retardation. We have shown that by the sixth postnatal week most infants (69%) had symptoms of cystic fibrosis irrespective of genotype (table 2). This result is comparable with the 85% of infants screened by Wilcken *et al*,[23] 80% screened by Roberts *et al*,[24] and 58% screened by Wesley *et al*.[25] The incidence of meconium ileus of 19% is also similar to those described by these groups. We can offer no evidence on either clinical or biochemical grounds that exocrine pancreatic function decreases more rapidly in infants homozygous for δF508. Non-genetic factors, including the quality of care and treatment received, especially in the early years, must have a substantial influence on the severity and natural history of the disease.

Although neonatal screening may identify infants with the disease soon after birth, it can only have a modest impact on the incidence of cystic fibrosis, through genetic counselling of couples with an already affected child. Until methods for the identification of heterozygotes are widely available, it is unlikely that a further substantial decrease in the incidence of cystic fibrosis will be seen.

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