Leukaemia mortality among relatives of cystic fibrosis patients

L N Al-Jader, R R West, J A Holmes, L Meredith, M C Goodchild, P S Harper

Abstract
A total of 219 families of patients with cystic fibrosis living in Wales were studied for the occurrence of other diseases and for cause of death, and the findings in relation to leukaemia are reported. There were eight deaths due to leukaemia, five of the myeloid type, in first and second degree relatives; this is significantly more than the expected on the basis of national age specific mortality rates. In comparison, mortality among siblings, parents, aunts and uncles, and grandparents from all causes was within the expected. Screening the five patients with myeloid leukaemia for the delta F508 mutation showed that four were carriers of this mutation. It is concluded that carriers of the delta F508 mutation may have an increased risk of developing acute myeloid leukaemia. This could happen through the direct effect of the cystic fibrosis gene itself, or through its influence on another gene, such as the met oncogene, or gene(s) involved in granulocyte function on the long arm of chromosome 7.

Cystic fibrosis is inherited as an autosomal recessive disease for which the most common gene mutation, delta F508, has been defined on 7q31.1 In view of the relative frequency of cystic fibrosis, its variable clinical features, and the multisystem involvement, an association with other diseases might be expected.

As part of a systematic clinical and molecular genetic study of this disorder in Wales a specific inquiry was made about the incidence of various diseases in patients and their relatives. In 1989, we published a preliminary report on this survey showing an increased mortality from leukaemia among first and second degree relatives of 130 families of patients with cystic fibrosis living in Wales. Since then 89 other families have been studied. We report here the principal findings of the whole survey.

Patients and methods
A genetic survey of families of patients with cystic fibrosis in Wales was carried out over a period of 28 months (June 1987–September 1989). Altogether 219 families of 230 patients, representing 75% of all patients with cystic fibrosis living in different parts of Wales, were studied as part of a formal genetic study, set up mainly in order to provide genetic counselling. No bias was exercised in the selection of patients for this study; all were seen during the usual cystic fibrosis clinic sessions when they attended for routine clinical follow up. The diagnosis of cystic fibrosis had been made by the paediatrician, based on typical clinical presentation and features, family history in some cases, and at least one positive sweat test. Multi-generation pedigree and detailed family history were obtained, including age if alive on January 1988, age at death and cause of death (if known), or age at ‘lost to follow up’.

Data on first and second degree relatives were included and grouped: siblings, half siblings, parents, aunts and uncles, and grandparents. The expected and observed numbers of deaths were compared for leukaemia of all types, myeloid leukaemia, and all causes combined. The expected numbers of deaths were obtained by multiplying for each group person years at risk by age (0–4, 5–14, 15–24, etc) with the age specific mortality for England and Wales (1979–88) for leukaemia, myeloid leukaemia, and all causes combined.4 Parents and grandparents of patients with cystic fibrosis had to have survived to reproductive age and the generation intervals for these families average 24–4 years: accordingly person years at risk and expected mortality for parents and grandparents have been estimated from age 24. Siblings, aunts and uncles, however, were at risk of infant and early childhood death, and older aunts and uncles were at risk of higher death rates earlier in the century. Accordingly person years at risk of death and the expected mortality for those aged <5 years were estimated on a cohort of birth basis (in decades). The observed numbers of deaths were compared with the expected, using the Poisson probability distribution.

Carrier status of the eight relatives who died of leukaemia was sought; DNA was extracted from bone marrow slides of three relatives. DNA was also extracted from all relevant members of the affected families and they were screened for the most common cystic fibrosis mutation in Britain, delta F508, by amplification of genomic DNA by the polymerase chain reaction.5 Separation of polymerase chain reaction products was by polyacrylamide gel electrophoresis allowing identification of carriers.

All cases of leukaemia were verified from the original haematological studies.

Results
The families of 230 patients with cystic fibrosis living in Wales were interviewed by LNAJ, identifying 438 parents, 227 siblings, 96 half siblings, 1252 aunts and uncles, and 869 grandparents.
Eight first and second degree relatives of patients with cystic fibrosis had died of leukemia: five with acute myeloid leukemia, two with acute lymphoblastic leukemia, and one with chronic lymphatic leukemia. A grandfather suffering from chronic lymphatic leukemia, who was alive, was not included in the study. Information on age (alive, lost to follow up, or dead) was incomplete for 7% of aunts and uncles and 11% of grandparents; these were assigned according to the age distribution of the appropriate group. The expected number of leukemia deaths for all relatives combined was (3.1) compared with the observed number (8) using the Poisson probability test as shown in table 1 (p<0.02).

Table 1 The expected and observed number of leukemia deaths among first and second degree relatives of patients with cystic fibrosis

<table>
<thead>
<tr>
<th>Relationship</th>
<th>No at risk</th>
<th>Expected deaths</th>
<th>Observed deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sibling</td>
<td>227</td>
<td>0.06</td>
<td>0</td>
</tr>
<tr>
<td>Half sibling</td>
<td>96</td>
<td>0.02</td>
<td>1</td>
</tr>
<tr>
<td>Parent†</td>
<td>438</td>
<td>0.15</td>
<td>2</td>
</tr>
<tr>
<td>Aunt or uncle</td>
<td>1252</td>
<td>0.98</td>
<td>2</td>
</tr>
<tr>
<td>Grandparent‡</td>
<td>869</td>
<td>1.87</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>2882</td>
<td>3.1*</td>
<td>8*</td>
</tr>
</tbody>
</table>

*p<0.02.
†On the basis of age specific death rates for England and Wales 1979-88.
‡For parents and grandparents, person years at risk and expected mortality has been determined from age 24.

The expected numbers of deaths from myeloid leukemia among relatives of patients with cystic fibrosis were also calculated as shown in table 2. The expected number of deaths (1.7) was compared with the observed number (5) using the Poisson probability distribution (p<0.05). These figures for both leukemia and myeloid leukemia become slightly more significant if account is taken of the standardised mortality ratio for leukemia in Wales (0.93) compared with England and Wales (1.00) during the reference period.

Table 3 Individual details of the leukemia cases

<table>
<thead>
<tr>
<th>Case No</th>
<th>Relationship</th>
<th>Age at death</th>
<th>Year of death</th>
<th>Presence (+) or absence (-) of del(5) mutation</th>
<th>Karyotype*</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Father</td>
<td>42</td>
<td>1981</td>
<td>+</td>
<td>XY 46</td>
<td>Acute myeloid leukemia</td>
</tr>
<tr>
<td>2</td>
<td>Mother</td>
<td>37</td>
<td>1986</td>
<td>+</td>
<td>XY 46</td>
<td>Acute myeloid leukemia</td>
</tr>
<tr>
<td>3</td>
<td>Aunt</td>
<td>55</td>
<td>1975</td>
<td>(+)†</td>
<td>ND</td>
<td>Acute myeloid leukemia</td>
</tr>
<tr>
<td>4</td>
<td>Grandfather</td>
<td>73</td>
<td>1954</td>
<td>?</td>
<td>ND</td>
<td>Acute myeloid leukemia</td>
</tr>
<tr>
<td>5</td>
<td>Grandfather</td>
<td>78</td>
<td>1988</td>
<td>+</td>
<td>X 45</td>
<td>Acute myeloid leukemia</td>
</tr>
<tr>
<td>6</td>
<td>Uncle</td>
<td>3</td>
<td>1967</td>
<td>?</td>
<td>ND</td>
<td>Acute lymphoblastic leukemia</td>
</tr>
<tr>
<td>7</td>
<td>Half sister</td>
<td>2</td>
<td>1987</td>
<td>–</td>
<td>‡</td>
<td>Acute lymphoblastic leukemia</td>
</tr>
<tr>
<td>8</td>
<td>Grandfather</td>
<td>75</td>
<td>1975</td>
<td>+</td>
<td>ND</td>
<td>Chronic lymphatic leukemia</td>
</tr>
</tbody>
</table>

*Karyotype done once at diagnosis.
†(+ ) Carrier status by inference.
‡46XX,55XX, +4, +6, +8, +10, +14, +17, +18, +21, +21.
§50% risk of carrying the delta F508 mutation.
ND, not done.
Discussion

Our previous preliminary report showed an excess mortality from leukaemia among first and second degree relatives of patients with cystic fibrosis and the excess was most striking for parents, who are obligatory carriers of the cystic fibrosis gene. The present report on 75% of the Welsh families with a patient with cystic fibrosis still shows an excess mortality from leukaemia and most particularly that the excess was of the myeloid type.

Although the survey has revealed a further living leukaemia case, he is not included in this study as the analysis is of mortality. We also have confirmed that four out of the five patients with acute myeloid leukaemia were carrying the delta F508 mutation.

As this survey was based on a genetic counselling service for families and the analysis is primarily of relatives of living patients with cystic fibrosis, mortality among the patients themselves has not been included in the analysis composition. Although no leukaemia deaths were found among deceased patients with cystic fibrosis in our survey, leukaemia has been reported previously in patients with cystic fibrosis: four children and two adults, three of whom had acute myeloid leukaemia, though acute lymphoblastic leukaemia would be expected to predominate in their age group. As infection remains a major cause of death in patients with cystic fibrosis and serious infection is not unexpected, Biggs and colleagues questioned whether death may occur in early phase of leukaemia, and compared the previous lack of recognition of acute leukaemia in patients with cystic fibrosis to the under-reported incidence of leukaemia in general before the antibiotic era. They also discussed whether the prolonged survival of patients with cystic fibrosis would result in recognition of a predisposition to leukaemia.

Monosomy for chromosome 7 and partial deletion of the chromosome 7 long arm (7q−) are among the common recurring clonal chromosome abnormalities observed in bone marrow cells in patients with acute non-lymphocytic leukaemia or myelodysplastic syndrome. The loss of all or part of chromosome 7 long arm has been associated with a granulocyte chemotaxis defect, a susceptibility to infections, a rapid progression of the disease, and a poor response to treatment. Patients with a 7q− chromosome had a deletion breakpoint in a narrow region between the erythropoietin and plasminogen activator type 1 genes on either side of the cystic fibrosis gene locus. Bone marrow monosomy 7 in children has been associated with a distinct myeloproliferative syndrome that usually evolves into acute myeloid leukaemia. It also appears that a significant incidence (up to 50%) of monosomy 7 in children may be familial.

Metacentric bands mapped to band 7q21-31, is closely linked to the cystic fibrosis gene. Its activation through a DNA rearrangement resulting in the fusion of a translocated promoter region locus on chromosome 1 to the 5 region of sequences derived from the met locus on chromosome 7, is known to occur in vitro when human osteogenic sarcoma cell line is treated with a chemical carcinogen (N-methyl-N-nitro-N-nitrosoguanidine). Met is also amplified and overexpressed in the human gastric tumour cell line, adenocarcinoma of the colon among a few other non-haemopoietic tumour cell lines tested. Similarity has been noted in the structure of the activated met gene with that of the activated abl gene involved in Philadelphia chromosomal translocation occurring in chronic myeloid leukaemia, suggesting that these genes encode aberrant tyrosine kinases that do not respond to normal cellular control mechanisms.

We conclude that carriers of the delta F508 mutation in the cystic fibrosis gene may have an increased risk of developing acute myeloid leukaemia. This could be through the direct effect of the gene or gene product itself or through its influence on another gene, such as the met oncogene, or gene(s) involved in granulocytic function on the long arm of chromosome 7. This influence might occur by the somatic loss of a tumour suppressor gene on the homologous chromosome 7, either through gross chromosomal rearrangements or point mutations.

As a follow up to our study an analysis of all acute myeloid leukaemia patients for frequency of the delta F508 mutation is now being carried out at the University Hospital of Wales. This should give information on whether our findings are of more general relevance outside families affected with cystic fibrosis.

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