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Minimum birth prevalence of mitochondrial respiratory chain disorders in children

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Summary

Mitochondrial respiratory chain disorders comprise a group of perhaps several hundred different genetic diseases. Each individual disorder is rare, but collectively they account for substantial use of health care resources. However, few accurate data on prevalence are available due to problems such as variation in clinical presentation, age of onset, referral practices and limitations of diagnostic methodologies. With this retrospective study, we aimed to determine the minimum birth prevalence of respiratory chain disorders that have onset in childhood, that is the proportion of births that will have onset of symptoms caused by a respiratory chain defect by 16 years of age. Of the 1 706 694 children born in the three south-eastern states of Australia (New South Wales, Victoria and South Australia) between January 1st 1987 and December 31st 1996, samples from 430 were referred for investigation of a respiratory chain disorder. Definite diagnosis of a respiratory chain disorder was made in 86 cases based on defined clinical, pathological, enzyme and molecular criteria. Age at presentation ranged from 0 to 129 months (median 4 months). The total data set predicts a minimum birth prevalence for respiratory chain disorders in children of 5.0/100 000 [95% confidence interval (CI) 4.0–6.2]. A significantly higher figure of 58.6/100 000 (95% CI 34.7–92.6) was noted for Australian families of Lebanese origin. Clinical awareness of respiratory chain disorders and investigation methods have improved since 1987, but not all affected children would have been recognized as such from the more recent years. The minimum birth prevalence of 6.2/100 000 (95% CI 4.5–8.4) for the 43 patients born between 1991 and 1994 is thought to be a more accurate estimate for respiratory chain disorders presenting in childhood. Combining our data with a previous study on prevalence of adult-onset respiratory chain disorders predicts a minimum birth prevalence of 13.1/100 000 or 1/7634 for respiratory chain disorders with onset at any age.

Keywords: birth prevalence; respiratory chain disorders; mitochondria; children

Abbreviations: CI = confidence interval; mtDNA =mitochondrial DNA

Introduction

Disorders of the mitochondrial respiratory chain were first identified in 1962 (Luft *et al*., 1962) and since then a vast range of clinical conditions have been added to this group of diseases. They are regarded as probably the most frequent cause of metabolic abnormality in paediatric neurology (Zeviani *et al*., 1996), but may more commonly present with non-neurological symptoms such as failure to thrive or hepatic, cardiac, renal, gastrointestinal, endocrine, haematological or other symptoms (Munnich *et al*., 1996). Respiratory chain disorders are claimed to occur with a frequency of 1/5000 to 1/10 000 births (Robinson, 1993; Bourgeron *et al*., 1995; Smeitink *et al*., 1998). Until recently (Applegarth *et al*., 2000; Chinnery *et al*., 2000; Darin *et al*., 2001), however, none of these figures were based on actual data. Realistic estimates are still complicated by the clinical and genetic variability of these disorders. It is likely that many patients with respiratory chain disorders are not referred for investigation, as initial symptoms are often non-specific and can masquerade as phenocopies of other diseases (Zeviani *et al*., 1996). Complete ascertainment is

unlikely since, as stated by Munnich *et al.* (1996), 'a respiratory-chain deficiency can theoretically give rise to any symptom, in any organ or tissue, at any age and with any mode of inheritance'.

Even when a suspected patient is referred appropriately, it can be difficult to achieve a correct diagnosis (Trijbels *et al*., 1993; Thorburn, 2000; Thorburn and Smeitink, 2001). Screening tests such as blood lactate level and muscle morphology can be normal in patients with confirmed respiratory chain disorders (Robinson, 1993; Kirby *et al*., 1999). Mutation analysis is complicated by the complexity of the mitochondrial respiratory chain, which is composed of 13 subunits encoded by mitochondrial DNA (mtDNA) and over 60 subunits encoded by nuclear genes. A large number of other nuclear genes are required for mitochondrial protein import and assembly, and regulation of mtDNA replication and expression (Shoubridge, 2001). In the last decade, most of the focus has been on disorders caused by mutations in mtDNA (Dahl and Thorburn, 2001; Shoubridge, 2001) and pathogenic mtDNA mutations are found in many or most adults with a suspected respiratory chain disorder (Shoffner, 1996; Chinnery and Turnbull, 1997). However, mtDNA mutations are identified in only a small proportion of children investigated (Shoffner, 1996; Lamont *et al*., 1998; Thorburn, 2000), the majority of whom probably have a defect encoded by the nuclear genome (DiMauro, 1999). Approximately 25 nuclear genes have been shown to cause respiratory chain disorders, but these account for only a minority of patients (Shoubridge, 2001) so paediatric diagnosis continues to rely mostly on enzymatic and functional investigations, and interpretation of these results is often difficult (Trijbels *et al*., 1993; Thorburn, 2000; Thorburn and Smeitink, 2001). It is thus necessary to classify many patients as having only probable or possible respiratory chain disorders even though they may be regarded as having a very high clinical suspicion (Walker *et al*., 1996; Bernier *et al*., 2002).

A further problem in establishing the frequency of respiratory chain disorders is that many of the specialist diagnostic centres have overlapping and incomplete regions from which they are referred patients. For the last 15 years, our centre (The Murdoch Children's Research Institute) has been the only major Australian centre focused on the diagnosis of respiratory chain disorders in children. In conjunction with referring clinicians and pathologists, we believe we have almost complete ascertainment of children who have been investigated for a respiratory chain disorder in south-eastern Australia (New South Wales, Victoria and South Australia) for this period. We emphasise that the clinical variability and diagnostic issues described above mean that it is unlikely any centre could ascertain all patients in a large population with respiratory chain disorders. The aim of this study was thus to estimate the minimum birth prevalence for respiratory chain disorders that have onset in childhood, that is the proportion of births that will have onset of symptoms caused by a respiratory chain defect by 16 years of age.

Methods

In a retrospective analysis, we evaluated all children born in south-eastern Australia between 1987 and 1996 who were referred to our centre for investigation of a possible respiratory chain disorder. This geographic region was selected because each of the three states in the region had an integrated tertiary centre specialising in clinical and biochemical evaluation of patients with suspected inborn errors of metabolism, which was expected to have virtually complete ascertainment of all children in that state suspected of a respiratory chain disorder. Each centre had a consistent pattern of referring samples from all such children to our centre for investigation since before 1987. The time period chosen allowed for ascertainment of all children who had presented and been investigated before 5 years of age and corresponds to approximately a third of our total respiratory chain diagnoses.

Details of the methods used for respiratory chain enzyme, functional (i.e. fibroblast ATP synthesis and growth on galactose media) and molecular studies have been described elsewhere (Rahman *et al*., 1996; Kirby *et al*., 1999). The diagnoses were established according to an objective classification scheme based on assigning major or minor criteria for clinical, pathological, enzymatic, functional, molecular and metabolic parameters (Table 1). Individual criteria were modified from an adult classification system (Walker *et al*., 1996) for use in the classification of paediatric cases (Bernier *et al*., 2002). Only patients classified as having a definite diagnosis by these conservative criteria were included. Age at presentation and biopsy were obtained from the clinical reports. For children with more than one tissue used in the investigation, the tissue sample that conclusively established the diagnosis was chosen for the calculation of the time between presentation and diagnosis.

Birth rates were obtained from the birth registries of the three states (New South Wales Midwives Data Collection, Epidemiology and Surveillance Branch, NSW Health Department; Perinatal Data Collection Unit, Melbourne, Victoria; and Pregnancy Outcome Unit, Adelaide, South Australia). There is mandatory registration of births in the three states from the 20th gestational week onwards and the data include all live and stillbirths. Each data registry records the country of birth of the mother and, when we discuss patients whose mothers were born in Lebanon, the same counting criteria are applied.

Confidence intervals (CIs) of 95% for estimates of birth prevalence were estimated from Poisson distributions. Relative risk estimates were made using EpiInfo Version 6 (Centers for Disease Control and Prevention, Atlanta, GA, USA). Mann–Whitney *U* tests for comparing medians of the different groups and Kaplan–Meier cumulative probability survival analyses (for calculating the proportion of unaffected cases at any time point) were performed using SPSS Version 10 (SSPS Inc., Chicago, IL, USA).

Table 1 *Summary of diagnostic classification scheme*

	Major criteria	Minor criteria
1. Clinical	Clinically complete respiratory chain encephalomyopathy or mitochondrial cytopathy	Symptoms compatible with a respiratory chain defect
2. Histology	>2% ragged red fibres in skeletal muscle	Smaller numbers of ragged red fibres or widespread electron microscopic abnormalities of mitochondria
3. Enzymology	Cytochrome-c oxidase negative fibres (>2% if <50 years of age or >5% if >50) or residual activity of a respiratory chain complex <20% in a tissue, <30% in a cell line or <30% in two or more tissues	Antibody-based demonstration of a respiratory chain defect or residual activity of a respiratory chain complex 20–30% in a tissue, 30–40% in a cell line or 30–40% in two or more tissues
4. Functional	Fibroblast ATP synthesis rates >3 SD below mean	Fibroblast ATP synthesis rates 2–3 SD below mean or fibroblasts unable to grow in galactose media
5. Molecular	Nuclear or mtDNA mutation of undisputed pathogenicity	Nuclear or mtDNA mutation of probable pathogenicity
6. Metabolic	–	One or more metabolic indicators of impaired respiratory chain function

The details of this classification scheme are described by Bernier *et al*. (2002). Identification of two major or one major and two minor criteria allows a definite diagnosis. Identification of one major and one minor or of at least three minor criteria allows a probable diagnosis.

Results

Patient characteristics

From the cohort of 430 referred cases, there were 86 patients diagnosed with a definite respiratory chain defect (34 girls and 52 boys) according to the established criteria (Bernier *et al*., 2002) for the period from January 1987 to December 1996. Table 2 lists the pathological diagnoses and shows that molecular diagnoses were obtained in 20 out of the 86 patients (23%), including 10 patients with mtDNA mutations, six with mutations in the autosomal SURF1 gene and four with mutations in the X chromosomal G4.5 gene. Mutations in the G4.5 gene cause Barth syndrome and related mitochondrial cardiomyopathies, and the gene product has been shown to be involved in the turnover of mitochondrial cardiolipin (Vreken, *et al*., 2000). It is debatable whether such patients should be classified as having a primary or secondary respiratory chain disorder. One of our four cases was originally classified as having a definite respiratory chain disorder because he had a major enzyme criterion (<20% complex I activity in skeletal muscle) and minor clinical and histology criteria. Only subsequently was he found to have a G4.5 mutation. Since the other three G4.5 cases had pathogenic mutations in the same gene, we classified them as definite with a major molecular criterion and two or more minor criteria for clinical, histology and metabolic features.

In 14 patients (17%), the diagnosis was established in tissue obtained post-mortem. The definite diagnosis was established in muscle in 48 patients (56%), in liver in 23 (27%), in fibroblasts in 10 (12%), in blood in three (3%) and in heart in two patients (2%). Sixty-eight patients had multiple tissues studied, of whom 32 appeared to have a systemic defect (i.e. expressed in all tissues studied) and 36 had some degree of tissue specificity.

For the total cohort, age at presentation ranged from 0 to 129 months (median 4.0 months), age at diagnostic biopsy from 0 to 139 months (median 11.4 months) and time between presentation and diagnostic biopsy from 0 to 99 months (median 5.3 months). Figure 1 depicts the age of presentation in the total patient group and in various subgroups. Age at presentation was significantly higher in patients with a mtDNA mutation compared with those without an identified mtDNA mutation ($P < 0.005$, Mann–Whitney U test), but there was no significant difference between patients with and without a nuclear gene mutation identified (Fig. 1B). These data are consistent with previous suggestions that most children with respiratory chain disorders have nuclear gene defects (Shoubridge, 2001) and that mtDNA mutations tend to cause later onset of disease (Rubio-Gozalbo *et al*., 2000). However, it should be noted that the ranges of age at presentation overlapped for all subgroups (Fig. 1).

Changes in practice during the study period

Our diagnostic services for respiratory chain disorders have existed since 1987, but in the first few years of the study period, the biopsies investigated were mainly fibroblasts. For the 29 children born prior to 1991, nine (31%) had diagnoses based on analysis of blood or fibroblasts, compared with only four of the 57 (7%) children born after 1990. Thus, most children born prior to 1991 did not have muscle or liver biopsies investigated, meaning that tissue-specific enzyme

Table 2 *Enzyme and molecular defects in patients*

Enzyme or molecular defect	Total group	Lebanese patients
Respiratory chain complex I	27*[1]	3
Respiratory chain complex II	1	0
Respiratory chain complex III	4*	3*
Respiratory chain complex IV	20**[2]	9**
Combined respiratory chain defect	25**	3
Other mtDNA mutations	5[3]	0
Barth syndrome G4.5 gene	4[4]	0

*Each asterisk represents one pair of siblings. There were six pairs of siblings in the total group and three in the Lebanese group. [1]Includes five patients with pathogenic mtDNA mutations (G3242A, T3250C, C3303T, G13513A, G14459A). [2]Six of the 20 patients have pathogenic SURF1 mutations; there are no SURF1 mutations in the Lebanese group. [3]Two patients with T8993G and one each with T8993C, A3243G and mtDNA re-arrangement. [4]Two cousins plus two unrelated patients.

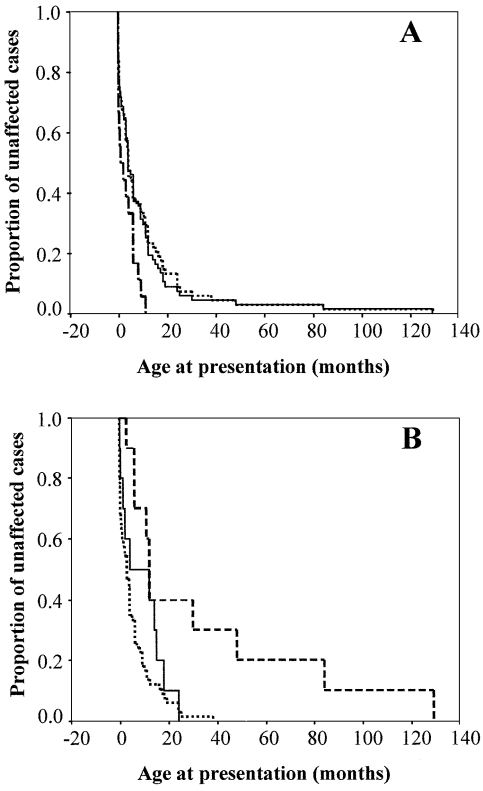

Fig. 1 Age at presentation of patients with respiratory chain disorders. Kaplan–Meier graphs showing age at presentation as the proportion of patients remaining unaffected with increasing age. (**A**) Age at presentation in the total patient group (solid line, median 4.0 months, $n = 86$), Lebanese patients (dashed line, median 1.5 months, $n = 18$) and non-Lebanese patients (dotted line, median 4.0 months, $n = 68$). (**B**) Age at presentation in patients with an identified mtDNA mutation (dashed line, median 12.0 months, $n = 10$), an identified nuclear gene mutation (solid line, median 8.0 months, $n = 10$) and with no mutation identified (dotted line, median 3.0 months, $n = 66$).

defects would not have been detected. In addition, relatively crude combined assays for complexes I + III and II + III were used prior to 1992 and these linked enzyme assays can be less sensitive than assays for the individual complexes (Taylor *et al*., 1993). Of the 86 patients diagnosed, six had initial studies on skin fibroblasts prior to 1992 that were regarded as normal. Subsequently, a tissue-specific respiratory chain enzyme defect was identified in skeletal muscle or liver from three of these patients. The other three patients had normal results for complex I + III and II + III assays, but were subsequently shown to have complex I (two cases) or complex III defects when re-analysed using assays for the individual complexes.

Since 1992, assays for the individual respiratory chain enzymes have been used and biopsies of tissues other than fibroblasts (mainly skeletal muscle and liver) have become our preferred source for investigation. We would therefore expect a higher number and proportion of patients to have been diagnosed in more recent years. However, two factors may counter this trend. The increased awareness of respiratory chain disorders is reflected in the increased referral rate since 1993, meaning that more children with a lower degree of clinical suspicion may be being investigated. Secondly, children with later clinical presentations may not have been investigated yet if born in the more recent years.

The net effect of these changes was that there was no significant fluctuation in the diagnosis rate over the study period, although the referral rate has increased slightly (Fig. 2).

Ethnicity and prevalence

Of the 86 patients, 18 were of Lebanese origin (12 boys and 6 girls). Age at presentation in this group was significantly lower than in non-Lebanese patients (Fig. 1A, $P < 0.05$, Mann–Whitney U test), ranging from 0 to 11 months (median 1.5 months), age at diagnostic biopsy from 0 to 45.5 months (median 6.5 months), time between presentation and diagnostic biopsy from 0 to 42.5 months (median 3.6 months). A variety of enzyme defects were identified among the Lebanese patients (Table 2) and consanguinity was present in 11 out of 15 Lebanese pedigrees and four out of 65 other pedigrees.

Table 3 *Minimum birth prevalence of respiratory chain defects in children*

	Diagnoses	Births	Minimum birth prevalence (per 100 000)	95% CI
1987–1996				
Total	86	1 706 694	5.0	4.0–6.2
NSW	48	865 324	5.5	4.1–7.3
VIC	29	644 232	4.5	3.0–6.5
SA	9	197 138	4.6	2.1–8.7
1991–1994				
Total	43	692 668	6.2	4.5–8.4
NSW	25	351 775	7.1	4.6–10.5
VIC	15	261 222	5.7	3.2–9.4
SA	3	79 671	3.8	0.8–11.1

NSW = New South Wales; SA = South Australia; VIC = Victoria.

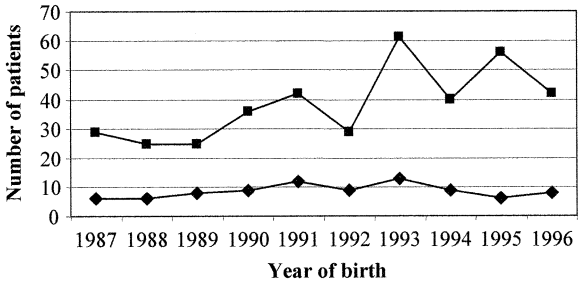

Fig. 2 Numbers of referrals (squares) and diagnoses (diamonds) for each birth year.

Table 3 lists the numbers of diagnoses and births for each state and the calculated minimum birth prevalences for respiratory chain disorders for two time intervals (1987–1996 and 1991–1994), giving a minimum birth prevalence of 5.0/100 000 (95% CI 4.0–6.2) and 6.2/100 000 (95% CI 4.3–8.1), respectively. There was no significant difference in minimum birth prevalence between the three different states. A striking difference in minimum birth prevalence was noted, however, for children of mothers born in Lebanon. In both time periods, the crude minimum birth prevalence of respiratory chain disorders is 12-fold higher in this group than in the total population ($P < 0.0001$ in both time periods, Table 4).

A significant demographic change that has occurred in the referral region is the increase in maternal country of birth for mothers from 'Asia (including the Middle East)' from ~8% of births in 1987 to ~12% in 1996, with small decreases in the proportion of mothers born in Australia, Oceania, UK and Europe. This is mostly accounted for by an increase in mothers born in South-East Asia; the number of mothers who were born in Lebanon remained constant between 1987 and 1996, representing 1.8 ± 0.2% of all mothers in each year. It seems unlikely that these demographic changes have had a substantial impact on respiratory chain birth prevalence in the region.

Discussion

Any attempt to estimate the occurrence of respiratory chain disorders in a population is clearly dependent on the reliability of the diagnoses, completeness of referrals and a geographically defined population to use as the denominator. This study fulfils these criteria. Establishing a reliable diagnosis is more of a concern for respiratory chain disorders with onset in childhood than those with onset in adulthood, since most adult patients are diagnosed by identification of a recognized pathogenic mtDNA mutation (Chinnery and Turnbull, 1997). Most children with respiratory chain disorders are diagnosed by finding a respiratory chain enzyme defect, and the wide variety of methods and normal ranges used by different centres creates concern that individual centres may over- or under-diagnose respiratory chain disorders. We have used a conservative diagnostic classification (Bernier *et al*., 2002) based on the rationale that, for a definite diagnosis, one must have support for the diagnosis from at least two types of evidence (Walker *et al*., 1996).

Definite diagnoses were obtained in 20% of the children investigated and the most common enzyme defects were complex I, complex IV and combined respiratory chain defects. The diagnostic yield and proportion of each enzyme defect are similar to a previous large study (Munnich *et al*., 1996) in which 23.5% of children were diagnosed, but substantially less than the value of ~70% reported elsewhere (Shoffner, 1996). Pathogenic mtDNA mutations were identified in 12% of diagnosed cases and pathogenic nuclear gene mutations in a further 12%.

The group of patients of Lebanese origin is remarkable in their frequency of respiratory chain disorders, showing a minimum birth prevalence of 58.6/100 000 (95% CI 34.7–92.6). A very high prevalence of respiratory chain disorders has also been reported in the Saguenay-Lac St Jean region of Quebec, where ~50/100 000 have a distinct form of complex IV deficiency. In that case, the cause is a founder effect, since virtually all cases of this clinically and biochemically homogeneous disorder are homozygous for a common mutation in the *LRPPRC* gene (Mootha *et al*., 2003).

Table 4 *Minimum birth prevalence of respiratory chain defects in children of Lebanese origin*

	Diagnoses	Births	Minimum birth prevalence (per 100 000)	95% CI
1987–1996				
Total	86	1 706 694	5.0	4.0–6.2
Lebanese	18	30 279[1]	58.6[2]	34.7–92.6
Non-Lebanese	68	1 676 415	4.1	3.2–5.2
1991–1994				
Total	43	692 668	6.2	4.5–8.4
Lebanese	9	12 426	71.4[3]	32.2–135.7
Non-Lebanese	34	680 242	5.0	3.5–7.0

[1]In South Australia, the mother's country of birth has only been recorded since 1991, so the numbers of births to women born in Lebanon for the period 1987–1990 is unknown. We assumed that, in each year, it was equal to the mean value for 1991–1996 of 45.2 per annum. [2]For the 1987–1996 time period, the relative risk of having a child with a respiratory chain disorder for Lebanese couples was 11.8 (95% CI 7.1–19.6) compared with the total population risk ($P < 0.0001$). [3]For the 1991–1994 time period, the relative risk of having a child with a respiratory chain disorder for Lebanese couples was 11.7 (95% CI 5.7–23.9) compared with the total population risk ($P < 0.0001$).

In contrast, our Lebanese patients are not homogeneous with respect to their region of origin within Lebanon or in religious background, clinical presentation or enzymatic diagnosis. Therefore, it appears that the Australian Lebanese community has a higher incidence of several different respiratory chain diseases rather than there being a single founder effect. This finding is of interest because many physicians may confuse mitochondrial disease with mitochondrial inheritance. It now appears that most children with respiratory chain disorders do not have mutations in the mitochondrial genome, but rather in the nuclear genome (Rubio-Gozalbo *et al*., 2000; Thorburn, 2000). Most respiratory chain disorders in children will show autosomal recessive inheritance and thus may be common, but overlooked, in other ethnic groups with a high incidence of consanguinity.

We believe we have almost complete ascertainment of children diagnosed with a definite respiratory chain disorder residing in south-eastern Australia. For children born in the 10-year period from 1987 to 1996, our crude data predict a minimum birth prevalence for respiratory chain disorders in the paediatric population of 5.0/100 000 (95% CI 4.0–6.2) (86 patients out of 1 706 694 births). As described in Results, improvements in diagnostic approaches mean that we are likely to have missed some diagnoses of children born in the first few years of the study period. It is also likely that some children born in the last few years of the study period will not yet have been diagnosed. Because of these opposing trends, we regard the years from 1991 to 1994 to be more representative of the real minimum birth prevalence, being 6.2/100 000 (95% CI 4.5–8.4) (43 patients out of 692 668 births). While this figure is clearly still likely to be an underestimate, it is comparable with the only two previous estimates. As part of a larger study on many inborn errors of metabolism, Applegarth *et al*. (2000) estimated the prevalence of respiratory chain disorders to be 3.2/100 000 (95% CI 1.7–5.5) for 408 667 births in British Columbia between 1988 and 1996. Darin *et al*. (2001) estimated the prevalence of respiratory chain disorders (diagnosed prior to 6 years of age) to be 8.9/100 000 births (95% CI 5.3–14.0) for 202 446 births in western Sweden between 1984 and 1992. In addition to the 18 cases identified in the Swedish study, Darin *et al*. (2001) also reported a range of patient demographics in a larger group of 32 children, many of which were similar to our study. For example, median age of onset was 3 months (cf. 4 months), proportion of children with a pathogenic mtDNA mutation was 19% (cf. 12%), proportion of children with a molecular diagnosis was 25% (cf. 23%), patients with a mtDNA mutation tended to have a later onset, and the most common enzyme defects were complex I and combined respiratory chain defects.

The criteria we use for definite diagnosis are conservative and have stricter cut-off values than many other centres use. The clinical diversity of respiratory chain disorders also makes it likely that some symptomatic children will not be referred for investigation or that they will not be referred until an older age when other symptoms may develop. Thus, although we have essentially complete ascertainment of diagnosed cases, it is unlikely that we or any other centre would have complete ascertainment of children with symptoms caused by a respiratory chain disorder. Given the problems of ascertainment and accurate diagnosis, we regard a birth prevalence of 10/100 000 or 1/10 000 as the most plausible estimate for respiratory chain disorders presenting in childhood.

A minimum point prevalence of 6.9/100 000 was recently reported for mitochondrial disease caused by pathogenic mtDNA mutations in the north-east of England (Chinnery *et al*., 2000). Essentially all of the patients had disease onset after 4 years of age, in contrast to our patient group who, with only two exceptions, had onset before 4 years of age. These

two patients presented at ages seven and 10.8 years with Kearns–Sayre syndrome and MELAS (mitochondrial myopathy, encephalopathy, lactacidosis and stroke) syndrome, respectively, and both had common mtDNA mutations. A point prevalence for adult onset disease is roughly equivalent to the total birth prevalence of the same disorder when there are no marked differences in lifespan, migration or contributing etiological factors. Patients with mtDNA mutations are, if anything, likely to have a shorter lifespan than those without, so the figure of 6.9/100 000 can be regarded as a minimum birth prevalence for adult onset respiratory chain disease. This value can therefore be added to our estimate of 6.2/100 000 to give a documented total minimum birth prevalence of 13.1/100 000 or 1/7 634 for respiratory chain disorders with onset at any age. Allowing for incomplete ascertainment, these two studies together suggest that a figure of 20/100 000 or 1/5 000 births is a conservative realistic estimate for the minimum birth prevalence of respiratory chain disease.

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