Screening for Duchenne muscular dystrophy

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SUMMARY A programme was introduced in Wales to screen all 18 month old boys who were not yet walking for raised creatine kinase activity within the existing community developmental screening programme. During an 18 month period 25 229 such boys were identified of whom 19 930 (79%) had a Denver developmental screening test and 338 (1.7%) of these were not walking. Two hundred and five of those who did not walk (61%) had creatine kinase activity assayed and two cases of Duchenne muscular dystrophy were detected.

We conclude that screening boys of 18 months who do not walk is worthwhile if the opportunity arises, but that a population based screening programme of this type is not justified as detection rates will be unacceptably low.

Duchenne muscular dystrophy is a serious X linked recessive disorder for which there is no treatment. It is the commonest of the muscular dystrophies with an incidence of about 1 in 4000 male births.1,2 Recently there have been great advances in the understanding of the molecular genetics of Duchenne muscular dystrophy and accurate and reliable antenatal diagnostic tests are now available for carriers of the disease.3-5 Now that the defect in the basic protein has been recognised, there must be hope that treatment will follow.6 Discovering whether or not the female relatives of affected boys are carriers, together with prenatal diagnosis, are the principal methods of preventing Duchenne muscular dystrophy. These can be improved by careful family studies and the use of genetic registers. Early diagnosis of affected boys is crucial, as the earlier that carriers among female relatives are discovered the greater the opportunity for preventing secondary cases. Despite the importance of early diagnosis the mean age at diagnosis for new cases often remains unsatisfactorily late.7-9

Detection of new cases at birth is feasible by newborn screening of all boys.10-13 This has not found general favour because, as there is no treatment at present for Duchenne muscular dystrophy, it does not fulfil the traditional requirements.14 It has, however, been justified on financial grounds as the creatine kinase assay used is cheap, and prevention (compared with management) is cost effective.15,16 As a compromise Gardner-Medwin et al proposed that screening might be restricted to boys who are not walking by the age of 18 months.9 This age was selected because 97% of normal boys are walking,17 whereas only 50% of boys with Duchenne muscular dystrophy are doing so.8 This would mean fewer tests and less anxiety for parents, particularly as their concern regarding development might already be aroused by the delay in walking.

We report our experience of setting up and running a screening programme for Duchenne muscular dystrophy covering the whole of Wales in which all boys unable to walk four independent steps by the age of 18 months were tested by having creatine kinase assays carried out on blood taken by finger print sampling.

Patients and methods

The screening programme was coordinated by a specialist nurse in this department who was supported by a clinical geneticist, a consultant paediatrician, a clinical research fellow, and the principal scientist responsible for the regional newborn screening programme. Before starting the programme all health authorities were visited and an initial discussion between the chief administrative medical officer of the district, the nursing administration, and the project staff was held to discuss the aims and protocol of the programme. Subsequently the team was invited back to some districts for further discussion with general practitioners, health visitors, and the community medical services. The project was discussed with consultant paediatricians.
through the Welsh Paediatric Society, and ethical permission was obtained from the relevant bodies.

The screening programme was based on the routine Denver developmental screening test\textsuperscript{20,19} that should be performed on all children in Wales by the health visitor. Incorporated into this was a simple item that elicited whether or not the boys could walk four independent steps. The age at which different health authorities performed this screening varied. Those districts that screened with the Denver test earlier than 18 months arranged to reassess boys who were not walking again at that age. If they failed this initial screening test they were referred to the local community paediatric service for a general assessment.

At this consultation the family was counselled about the possibility of muscular dystrophy and the boy offered a finger prick blood test for creatine kinase. This was taken on the same filter papers used for newborn phenylketonuria screening and was posted to the newborn screening laboratory at the Institute of Medical Genetics where the assay was performed. All results were communicated to the community paediatric service. Positive results were confirmed on a sample of venous blood taken by the local consultant paediatrician or general practitioner. Referral to a paediatric neurologist was then arranged for repeat serum creatine kinase assay, muscle biopsy, and electromyography to confirm the diagnosis. After confirmation of the diagnosis genetic counselling was offered and extended family analysis performed.

The programme was monitored using computer data obtained from the Welsh Health Common Services Authority. This computer has a record of all children born in Wales and provided the numbers of 18 month old boys in each district. As part of the screening programme each health authority was sent each month a list of all the boys in that area that would reach the age of 18 months during that month. These were then issued to the health visitors who returned the computer print out indicating which boys had been screened and which were not walking four independent steps. These were collected by a person nominated locally to be in charge of the screening programme, and then sent to the central coordinating officer. From this monitoring system the following figures were obtained for each area: the number of 18 month old boys; the number who had undergone the Denver test; the number not walking; the number who had had a creatine kinase assay; the number of positive tests; and the number diagnosed as having Duchenne muscular dystrophy.

All the boys diagnosed during this study period were assessed with the Griffiths's mental development scales.\textsuperscript{20}

All other cases of Duchenne muscular dystrophy diagnosed clinically and born during the dates of the cohort studied were identified through genetic counselling services provided throughout Wales.

**Biochemical Method**

Creatine kinase was measured fluorimetrically on a 0-5 cm spot punched from the finger prick filter paper specimen. The assay was in a final volume of 200 \( \mu l \) containing the following: glucose 20 mmol/l; magnesium acetate 10 mmol/l; adenosine diphosphate 3 mmol/l; nicotinamide adenine dinucleotide phosphate 2 mmol/l; diadenosine pentaphosphate 440 \( \mu l \); hexokinase 2500 U/l and glucose-6-phosphate dehydrogenase 3000 U/l in imidazole buffer 100 mmol/l, pH 6-7. The blood spot was eluted for one hour at 30°C in 200 \( \mu l \) of reaction mixture without substrate. Creatine phosphate was added in a 20 \( \mu l \) volume and the incubation continued for 30 minutes; 100 \( \mu l \) were diluted to 2 ml with carbonate buffer (500 mmol/l, pH 11-0) and the fluorescence of the reduced form of nicotinamide adenine dinucleotide phosphate in this solution was measured.

Blood samples from both normal and affected boys were assayed concurrently and creatine kinase Lin-Trol (Sigma) was used as a creatine kinase standard. The method gave a normal range with a mean (SD) of 163 (98) U/l, \( n=205 \), range 0-385 U/l, during the 18 month period for which data are complete. The three cases detected gave values of 6685, 5274, and 9005 U/l, respectively.

**Results**

This study ran for two years from January 1986 to January 1988 and concerned boys born between July 1984 and July 1986 (table 1). To make the programme operational required considerable effort and there was initial resistance in some districts. Because of this the data are not complete for the first six months.

Taking the 18 month period July 1986 to January 1988 (screening boys born between 1 January 1985 and 1 July 1986) where full returns are available, 25229 boys were identified from the computer returns. The returns indicated that 19930 of these (79%) had had the Denver developmental screening test and of these 338 (1.7%) were not walking. Two hundred and five of the 338 (61%) of the boys who were not walking had a creatine kinase test.

There was, however, considerable variation between districts. One district screened 89% of boys with the Denver test and 76% of those who were not walking with the creatine kinase assay. Another district only screened 27% with the Denver test.
Screening for Duchenne muscular dystrophy

Table 1 Data for 18 month period 1 July 1986 to 1 January 1988

<table>
<thead>
<tr>
<th>District</th>
<th>No of 18 month old boys</th>
<th>Percentage who had Denver test</th>
<th>Percentage not walking</th>
<th>Percentage tested for creatine kinase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3726</td>
<td>89</td>
<td>1.9</td>
<td>76</td>
</tr>
<tr>
<td>2</td>
<td>1784</td>
<td>53</td>
<td>2.0</td>
<td>136</td>
</tr>
<tr>
<td>3</td>
<td>3229</td>
<td>72</td>
<td>1.5</td>
<td>86</td>
</tr>
<tr>
<td>4</td>
<td>2017</td>
<td>67</td>
<td>2.0</td>
<td>52</td>
</tr>
<tr>
<td>5</td>
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<tr>
<td>6</td>
<td>973</td>
<td>27</td>
<td>1.9</td>
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<tr>
<td>7</td>
<td>2950</td>
<td>76</td>
<td>1.7</td>
<td>64</td>
</tr>
<tr>
<td>8</td>
<td>4210</td>
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<td>44</td>
</tr>
<tr>
<td>9</td>
<td>3226</td>
<td>84</td>
<td>1.7</td>
<td>83</td>
</tr>
<tr>
<td>Total No</td>
<td>25229</td>
<td></td>
<td>1.7</td>
<td>61</td>
</tr>
</tbody>
</table>

Mean value

Table 2 Subscale quotients on the Griffiths's mental development scale

<table>
<thead>
<tr>
<th>Subscale</th>
<th>Case No 1</th>
<th>Case No 2</th>
<th>Case No 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at assessment (months)</td>
<td>29</td>
<td>26</td>
<td>18</td>
</tr>
<tr>
<td>Locomotor</td>
<td>51</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Personal and social</td>
<td>58</td>
<td>73</td>
<td>86</td>
</tr>
<tr>
<td>Hearing and speech</td>
<td>53</td>
<td>65</td>
<td>52</td>
</tr>
<tr>
<td>Eye and hand coordination</td>
<td>68</td>
<td>75</td>
<td>89</td>
</tr>
<tr>
<td>Performance</td>
<td>68</td>
<td>71</td>
<td>78</td>
</tr>
<tr>
<td>General quotient</td>
<td>60</td>
<td>73</td>
<td>83</td>
</tr>
</tbody>
</table>

CASE REPORTS OF BOYS DIAGNOSED THROUGH SCREENING PROGRAMME

Case 1

This boy was the second boy in the family. He was sitting without support at 8 months, cruising at 18 months, and not walking four independent steps until 28 months. When the diagnosis was made his older brother was also suspected as having Duchenne muscular dystrophy at the age of 5 years 6 months, and had obvious clinical features of the disease. Venous creatine kinase activities for both boys were grossly raised at 15650 U/l (case 1) and 12450 U/l, respectively. Muscle biopsy and electromyography on the older brother confirmed the diagnosis. On analysis of DNA both boys show deletions with c-DNA probe cf56A, permitting specific prenatal diagnosis in a future pregnancy.

Case 2

This boy was an only child who sat up unaided at 6 months, crawled at 10 months on his front, and at 12 months on his knees. He was standing with support at 12 months, walking with support at 14 months, and walking independently at 20 months. On examination he was generally hypotonic with no pseudohypertrophy or other specific abnormal signs. Creatine kinase activity was raised at 15750 U/l, electromyography and muscle biopsy confirmed the diagnosis. The mother's risk of being a carrier is 46% based on creatine kinase and pedigree analysis. Analysis of DNA has so far not shown a deletion.

Case 3

This boy was an only child who sat unsupported at 10 months, crawled at 12 months, was standing with support at 15 months, and walking independently at 19 months. On examination no specific abnormalities were detected, but he died suddenly at home.

according to the computer returns, but did screen all those who were not walking (albeit only 4). One district, despite testing 84% with the Denver test, only tested 16% of those who were not walking with the creatine kinase assay. One district sent in more creatine kinase tests than the recorded number of boys who were not walking.

In the 18 month period for which complete data are available two boys were diagnosed through the screening programme. Three other boys in the cohort were diagnosed clinically as having Duchenne muscular dystrophy before screening at 18 months.

In the full two year study three boys were identified as having Duchenne muscular dystrophy through the screening programme, and four boys with Duchenne muscular dystrophy born between 30 June 1984 and 30 June 1986 were also identified, giving a total of seven cases identified in the two year cohort. The case histories of these seven cases are summarised below. The developmental quotients obtained on the Griffiths's mental development scales are shown in table 2.

Table 2 Subscale quotients on the Griffiths's mental development scale

<table>
<thead>
<tr>
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<td>71</td>
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<td>60</td>
<td>73</td>
<td>83</td>
</tr>
</tbody>
</table>
while asleep. The creatine kinase activity was 9005 U/l and a muscle biopsy taken at necropsy confirmed the diagnosis. The cause of death was aspiration, thought to have occurred during a febrile convulsion. The mother’s risk of being a carrier is 99% based on creatine kinase and pedigree data. No DNA was available for analysis.

CASE REPORTS OF BOYS DIAGNOSED CLINICALLY
Case 4
This boy’s disease was diagnosed at the age of 18 months with his older brother who had been investigated for developmental delay and clumsiness at the age of 3 years 6 months. He was walking at 15 months and his older brother at 18 months. Creatine kinase activity was raised at 8600 U/l. A creatine kinase activity of 9450 U/l, muscle biopsy and electromyography on the older boy confirmed the diagnosis. DNA analysis of the two boys has so far not shown any deletion.

Case 5
This boy’s disease was diagnosed at the age of 10 months when he was being investigated for failure to thrive. He was sitting unsupported at 10 months and walked unaided at 18 months. The creatine kinase activity was 15 200 U/l, electromyography and muscle biopsy confirmed the diagnosis. The mother’s risk of being a carrier is 99% based on creatine kinase and pedigree data. DNA analysis showed a deletion with c–DNA probe cf56A.

Case 6
This boy’s disease was diagnosed at the age of 1 year. He was sitting unsupported at 6 months but not walking unaided until 20 months. The mother, who had a brother with Duchenne muscular dystrophy was a potential carrier and had received genetic counselling but had not had antenatal diagnostic tests. A creatine kinase estimation was done at the mother’s request when he was 1 year old, and was raised at 7620 U/l. The mother is an obligate carrier. DNA analysis has so far failed to show a deletion.

Case 7
The boy was the first boy in the family, and had an older sister. He was sitting without support at 9 months, cruising at 10 months, and walking without help at 17 months. He was investigated as he was considered to be late walking at 17 months. Creatine kinase activity was raised at 14 562 U/l and diagnosis was confirmed by muscle biopsy and electromyography. The mother’s risk of being a carrier is 36% based on creatine kinase and pedigree data. DNA analysis has so far failed to show a deletion.

Discussion
During the 18 months that the screening programme was running fully, one can estimate from the population statistics that theoretically seven boys should have been born with Duchenne muscular dystrophy. Five have already been discovered, two through the screening programme (cases 2 and 3) and three on clinical grounds (cases 5, 6, and 7). Of the three diagnosed clinically, two were walking before 18 months and would have been missed by the programme. At present therefore 40% of the known cases have been detected by the programme, but this figure may well decrease if further cases are found in this cohort.

At the outset it was theoretically expected that at best 50% of cases would be detected, if even this figure cannot be achieved it makes the screening programme less worthwhile. If one of the main advantages of early diagnosis is secondary prevention, then the fewer cases picked up through the screening programme, the smaller the percentage of possibly preventable cases. It has been calculated that 15–20% of secondary cases can be prevented by newborn screening. This figure falls to less than 10% if screening is performed at 18 months assuming a 50% detection rate, and will be considerably less than 10% if the detection rate falls much below 50%. The impact for secondary prevention of a programme of the type described will therefore be small.

In our study out of a total of seven cases two would have been preventable by earlier diagnosis. Deletions of the dystrophin gene have been found in one of these families meaning that accurate antenatal diagnosis is available to them. In this family it was the detection of the younger boy through the screening programme that led to the older brother being diagnosed.

The reasons why less than 50% of cases were detected are that the uptake of developmental screening was low with only 79% of boys receiving a Denver developmental screening test; only 61% of the boys who were not walking had a creatine kinase assay and, lastly, three cases were diagnosed before 18 months. This is unusual and we believe that in one case the existence of the programme had heightened awareness of the condition leading to earlier diagnosis.

There were 335 boys who were not walking; of the boys that did not have a creatine kinase assay some may already have had alternative diagnoses and in some the parents may have declined the offer of a creatine kinase assay. The actual reason for the test not being done in individual cases was not recorded. The 205 boys that did have the assay may therefore
be a selected group. There were no false positives with the assay, and though only 205 assays were carried out two cases were diagnosed. This ratio of one diagnosis in 100 tests is acceptable for a simple, cheap, assay for a serious condition. The creatine kinase assay is reliable and its use in screening for Duchenne muscular dystrophy has been reported in several studies.\textsuperscript{22,23} In Scheuerbrandts et al.'s voluntary screening programme the false positive rate was only 0.065%.\textsuperscript{25}

Three of the boys with Duchenne muscular dystrophy in this study are appreciably delayed developmentally, two are marginally delayed, and the other two are below average. Four of the boys also show delay in language development. It could therefore be argued that they would have been picked up anyway if all boys with developmental delay are investigated for Duchenne muscular dystrophy, assuming that developmental delay is recognised efficiently in the community.

The incidence of boys who were not walking (1.7%) is less than that reported by Neligan and Prudham\textsuperscript{17} even though our criteria were less strict (4 independent steps as opposed to 10). This difference may be explained by the general trend for developmental abilities to improve,\textsuperscript{24,25} or it may reflect an under reporting of boys who were not walking on the computer returns.

The logistic problems of this study were considerable. There were problems of acceptability both with parents of children with delayed walking and with their doctors. Despite considerable efforts many children missed the Denver screening test and of those who were not walking an appreciable number did not have a creatine kinase assay. We believe, therefore, that a formal screening programme such as ours for detecting Duchenne muscular dystrophy is probably not justified for the returns we achieved. Nevertheless we do believe that it is essential that creatine kinase assays are done as part of the assessment of developmental delay in all boys.

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### References


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