Electronic letter

Eight years' experience of direct molecular testing for myotonic dystrophy in Wales

Siv Fokstuen, Jenny Myring, Linda Meredith, David Ravine, Peter S Harper

EDITOR—Myotonic dystrophy is the most common muscular dystrophy of adult life, with a frequency of around 3–15 per 100 000 in most European populations.¹ It is characterised by multisystemic involvement and extreme intra- and interfamilial variability in age at onset and degree of severity, which may range from subjects with cataract alone to severely affected infants with neonatal hypotonia, respiratory distress, and mental retardation; most patients have symptomatic onset in adult life. The disorder is now recognised as resulting from an expanded and unstable CTG trinucleotide repeat sequence in the 3' untranslated region of the myotonic dystrophy protein kinase gene on chromosome 19,^{2–4} which has provided a highly accurate direct molecular test for diagnosis, replacing earlier testing by linkage analysis. Testing is now available as a service in medical genetic centres throughout the UK and in most other developed countries. However, in contrast to Huntington's disease,⁵ for which numerous studies have been reported and where established protocols for presymptomatic testing exist,⁶ there are only limited data for the experience of presymptomatic testing in myotonic dystrophy as a service, based mainly on the use of linked genetic markers^{7–9} and no specific guidance regarding counselling approaches. Most reports of molecular analysis as an aid to primary diagnosis have either been selected case reports or research studies.

In the present paper, we report our eight year results of direct mutation testing for myotonic dystrophy performed in Wales. The data provide a basis to discuss the outcomes and referral patterns for diagnostic, presymptomatic, and prenatal testing in our centre. Counselling issues related to presymptomatic testing are discussed in detail in an accompanying paper.¹⁰

Methods

From March 1992 to June 2000, the laboratory records of the Institute of Medical Genetics, Cardiff, showed a total of 526 requests for direct molecular testing for myotonic dystrophy, 292 of whom were referrals from Wales, 226 from other centres in the UK and Ireland, and eight from abroad. For the purpose of this study we only included the requests from within Wales, which has a population of 2.9

million and is served by a single medical genetics service. Samples studied for research purposes (analysed by a separate laboratory within the Institute) were excluded. Out of the 292 samples, 213 were referred by clinical geneticists from the Institute of Medical Genetics, Cardiff and 79 by other clinicians, mainly by paediatricians (n=43) or neurologists (n=29), but also by other medical specialists (n=7). Pedigree and clinical data, the reason for referral (diagnostic, prenatal, or presymptomatic), and the molecular results from the 213 referrals from the Institute were obtained by review of the medical records. The clinical data on the subjects tested were divided into the following categories: symptomatic, equivocal, or normal. Molecular testing was considered as diagnostic in the presence of neuromuscular and/or other patient complaints possibly related to myotonic dystrophy. Presymptomatic testing was defined according to the patient's perception. Thus, we not only included completely healthy subjects at risk (n=69) in this group, but also people who at the time of testing considered themselves to be healthy although there proved to be features related to myotonic dystrophy at clinical examination (n=9). This situation occurred in five parents (four mothers and one father) and two grandparents of newly diagnosed children and in two persons at risk.

To analyse the 79 referrals from clinicians outside the Institute of Medical Genetics, the request form was checked and when there was insufficient information a short questionnaire was sent out to the referring clinician. A total of five cases remained without adequate information, reducing the total of analysed investigations to 287.

DNA ANALYSIS

Genomic DNA was prepared by standard methods. Southern blot analysis and PCR were used to analyse the (CTG)_n repeat within the *DMPK* gene. Repeat sizes up to approximately 80 repeats were determined by PCR and analysed by 6% PAGE followed by blotting and hybridisation with a ³²P labelled (CTG)₆ oligo. The larger expansions were determined by Southern blot analysis, using *EcoRI* digested DNA and a ³²P labelled DNA probe, pM10M6, from the region D19S95.¹¹

Institute of Medical Genetics, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN, UK
S Fokstuen*
J Myring
L Meredith
D Ravine
P S Harper

Correspondence to:
Professor Harper,
harperps@cardiff.ac.uk

*Present address: Division of Medical Genetics, Centre Medical Universitaire, 1 Rue Michel-Servet, 1211 Geneva 4, Switzerland,
siv.fokstuen@hcuge.ch

Figure 1 Repeat sizes.

Results

Fig 1 shows the distribution of CTG repeat number for the samples where accurate sizing was possible, analysed since direct molecular analysis was initiated as a service in 1992. It can be seen that a clear separation exists between the normal and abnormal ranges, with no result falling into an ambiguous category. Two samples with 60 repeats were the smallest abnormal expansions seen. No examples of mosaicism (other than “smeared” bands on

Southern analysis for some larger expansions in adults) were observed.

Table 1 gives the referral sources and general categories of the 287 requests for molecular testing from Wales over the eight year period. There were 205 diagnostic investigations, 78 presymptomatic tests, and only four prenatal diagnoses (two amniocenteses and two chorionic villus samplings). The outcomes of the diagnostic tests with positive and negative family history, respectively, are summarised in tables 2 and 3. Most diagnostic requests with a positive family history were performed to confirm the clinical diagnosis in members of already well known families. The positive detection rate of diagnostic requests with negative family history was low (34/122), most likely because of use of the test as part of a differential diagnosis, as discussed below.

Of the six diagnostic requests with inconclusive family history, three were confirmed as affected. The other three, all referred by neurologists and with an equivocal clinical status, proved to have a normal result.

Surprisingly, nine out of 77 (11.7%) clinically abnormal patients with a positive family history had a normal molecular result (table 4). Two subjects (Nos 7 and 8), both from the same family, were subsequently diagnosed as cases of PROMM (proximal myotonic myopathy) and have been described elsewhere.¹² So far, no alternative diagnosis has been established in the remaining seven cases. Case 6, a 40 year old woman, had a sister with confirmed myotonic dystrophy. Her mother was normal and her father has so far refused to be tested. On clinical examination, she was considered to have percussion and relaxation myotonia and her grip was definitely weak; there were no cataracts or facial weakness. Electromyography gave a normal result. The remaining six patients in table 4 showed equivocal symptoms, insufficient to make a definite clinical diagnosis.

In all four prenatal investigations, the mother was the affected parent. Two tests showed abnormal results. In one case, the parents elected to continue the pregnancy and a congenitally affected infant was born and in the other case the pregnancy was terminated.

Table 1 Type of molecular testing for myotonic dystrophy in 287 cases originating from Wales

Referral source	Diagnostic (symptomatic subjects)			Presymptomatic	Prenatal	Total
	Positive FH	Negative FH	Inconclusive FH			
Clinical Geneticists	73	63	1	72	4	213
Paediatricians	2	34	—	3	—	39
Neurologists	2	20	5	2	—	29
Others	—	5	—	1	—	6
Total	77	122	6	78	4	287

Table 2 Outcome of diagnostic tests with positive family history

Referral source	Normal	Abnormal	No result	Total
Clinical Geneticists	9	63	1	73
Paediatricians	—	2	—	2
Neurologists	—	2	—	2
Others	—	—	—	—
Total	9	67	1	77

Table 3 Outcome of diagnostic tests with negative family history

Referral source	Normal	Abnormal	No result	Total
Clinical Geneticists	33	29	1	63
Paediatricians	34	—	—	34
Neurologists	15	4	1	20
Others	4	1	—	5
Total	86	34	2	122

Table 4 Diagnostic tests with positive family history but normal molecular results

Case No	Age (y)—sex	Clinical status	EMG	Diagnosis
1	Newborn—F	Equivocal	—	—
2	17—M	Equivocal	—	—
3	18—F	Equivocal	—	—
4	20—F	Equivocal	—	—
5	26—F	Equivocal	—	—
6	40—F	Symptomatic	Normal	—
7	41—M	Symptomatic	Myotonia	PROMM
8	63—M	Symptomatic	Myotonia	PROMM
9	57—F	Equivocal	—	—

F = female; M = male; EMG, electromyography; PROMM, proximal myotonic myopathy.

Table 5 Referral source, age, and outcome of presymptomatic tests (n=78)

Referral source and age (y)	Normal	Abnormal	Total
Clinical Geneticists			
0-15	—	—	—
16-20	8	—	8
21-30	18	2	20
31-40	12	6	18
41-50	9	2	11
51-60	6	1	7
61-70	1	3	4
71-80	2	1	3
>80	—	1	1
Paediatricians			
31-40	3	—	3
Neurologists			
17-20	1	—	1
31-40	1	—	1
Others			
31-40	1	—	1
Total	62	16	78

Table 6 Presymptomatic tests with abnormal molecular results

Case No	Age (y)—sex	Test situation	Clinical examination	Repeat size
1	19—M		Normal	350
2	23—M		Percussion and grip myotonia	300
3	29—M		Equivocal clinical features	150
4	33—F	Mother of affected child	Grip and percussion myotonia, mild facial and hand weakness	150
5	33—F	Mother of affected child	Grip myotonia, slight facial weakness	150
6	37—F	Mother of affected child	Delayed relaxation of grip	Smear: 300–400
7	37—M		Normal	200
8	39—M		Normal	60
9	47—F	Mother of affected child	Normal	80
10	48—F	Mother of affected child	Weakness at wrists	150
11	52—M		Normal	80
12	61—M	Grandparent	Diabetes, cataract, some stiffness in hands	65
13	65—M	Father of affected adult son	Bilateral cataract	100
14	69—F	Grandparent	Normal	67
15	75—F	Grandparent	Normal	80
16	84—F	Grandparent	Cataract	75

M = male, F = female.

The results of the 78 presymptomatic molecular investigations are given in table 5. The youngest person tested was 16 years old. Only six requests were from clinicians other than clinical geneticists from the Wales Medical Genetics Service. The three requests from paediatricians were for testing of completely healthy mothers of symptomatic children. Neither the diagnostic tests in the children nor the presymptomatic tests in the mothers showed an abnormality. Details about the 16 subjects with an abnormal presymptomatic test result are summarised in table 6. All 16 subjects felt symptom free at the time of testing, although nine of them showed clinical features on examination that were potentially related to myotonic dystrophy. Those with a repeat size of 100 or less were either clinically normal or suffered only from cataracts.

Discussion

The data presented here reflect the experience of diagnostic, presymptomatic, and prenatal testing for myotonic dystrophy in Wales since the availability of direct mutation analysis as a service in 1992. The majority of investigations (205) were done for diagnostic reasons. Although affected mothers run a high risk of giving birth to congenitally affected infants,¹³ prenatal diagnosis was only requested in four instances. In earlier reports from this centre,⁷ where a total of 16 prenatal diagnostic tests with linkage analysis were undertaken over a three year period (1986–1989), the referral sources covered a large part of the UK, rather than just the population of Wales.

An important finding of the overall test outcomes in this series is the lack of equivocal repeat size lengths (37–50 CTG repeats). Direct molecular analysis for myotonic dystrophy can therefore be considered as a very precise test to differentiate normal or disease associated alleles. This is in contrast to Huntington's disease and other CAG repeat disorders, where normal and disease size ranges are extremely close and where overlap may occur.¹⁴ In myotonic dystrophy, any equivocal range alleles would be likely to be found in clinically normal subjects in ancestral generations, who are unlikely to request presymptomatic testing unless systematic study

or “cascade screening” of extended families is undertaken.

The finding of a normal molecular result in symptomatic patients with a positive family history (cases 6, 7, and 8 in table 4) emphasises the relevance of molecular testing even in patients with definite clinical symptoms. The patients with equivocal neuromuscular abnormalities in table 4, however, might not have been genotyped in the absence of any family history as their clinical findings appear less suggestive of myotonic dystrophy without the pedigree information. The potentially misleading nature of minimal or equivocal clinical features of myotonic dystrophy has been noted previously. A “partial syndrome” of myotonic dystrophy, composed mainly of minor ophthalmological and electromyographic abnormalities, has been claimed to exist, based mainly on a study of a large Labrador kindred.¹⁵ However, subsequent molecular analysis showed that most such subjects in this and other kindreds did not carry the myotonic dystrophy mutation.^{8, 16} By contrast, Ashizawa *et al*,¹⁷ analysing lens opacities alone in a series of 98 subjects at risk, found 100% specificity for opacities considered characteristic, when compared with a prediction from closely linked DNA markers. Outside the family context, lens opacities are less specific, Giordano *et al*¹⁸ finding apparently characteristic lens opacities in 10 of 1400 healthy subjects, none of whom showed the myotonic dystrophy mutation.

It should also be noted that the great majority of diagnostic tests in this series done in the context of a definite family history (73 out of 77) were requested by clinical geneticists, who may have been less familiar with the assessment of minor clinical features than neurologists.

This lack of specificity of symptoms may also be responsible for the high percentage (67.5%) of normal molecular results within the group of diagnostic tests with negative family history. Of interest also was the comparison of the outcome between the different referral sources. All 34 diagnostic tests with a negative family history referred by paediatricians proved to give a normal result. This may be explained by the high proportion of tests performed as part of the differential diagnosis of other severe

childhood neuromuscular disorders. The proportion of abnormal results without family history was also low when requested by neurologists (four out of 20), again suggesting use of testing in a wide range of muscle disease. By contrast, the proportion of abnormal results in this group when requested by clinical geneticists (29 out of 63) was considerably higher. Most patients with a normal result referred by a clinical geneticist (16 out of 33) had an age range of 0-15 years, reflecting again the frequent use of the test as part of a differential diagnosis in children with motor and/or developmental delay of uncertain cause.

The fact that only six out of 78 presymptomatic blood samples were referred by clinicians other than clinical geneticists reflects that for late onset disorders in general the need for genetic counselling before presymptomatic testing is well established. It may also in part be because of the special interest and research involvement in myotonic dystrophy in the Institute of Medical Genetics. Molecular laboratories should always inform the referring clinician about the need and the significance of full genetic counselling before presymptomatic testing, as discussed in the accompanying paper.¹⁰

Table 5 shows that no presymptomatic test has been carried out on minors, though this does not mean that there was no demand for testing healthy children at risk. The counselling and general issues relating to childhood testing for late onset disorders have received considerable debate²⁰ and are especially complex in such a variable disorder as myotonic dystrophy, as outlined in the accompanying paper.

The outcome of presymptomatic testing in this series (16 out of 78, 20.5% abnormal) indicates that penetrance of the mutation is already high by adult life, as indicated previously by a number of studies, and in contrast to such disorders as Huntington's disease where the proportion with abnormal results approaches the 50% expected for first degree relatives of an affected patient. If those asymptomatic subjects (nine) with suspicious or definite clinical abnormalities (table 6) are removed, then the proportion of abnormal results in clinically normal subjects is reduced to seven out of 69 (10.1%). This value is similar to that of two earlier studies; a report of linkage based presymptomatic testing from our centre showed 8.6% (seven out of 81) to be abnormal,⁹ while a research study of family members⁸ showed a corrected value of 8.3% (nine out of 139). However, the present series is not precisely comparable with these two studies, since the prior risk was not always 50%; in the five cases where mothers were tested because of an affected child, there was a higher likelihood of an abnormal result, while in another case a grandparent was the closest affected relative. These results emphasise both the importance of careful clinical assessment before a sample is sent for molecular analysis and also that adult first degree relatives who are clinically normal should appreciate that their chance of an abnormal result is only around 10%.

- Since the identification of the specific gene defect in 1992, direct molecular testing for the myotonic dystrophy mutation has developed as a widespread service in clinical practice, both in the primary diagnosis of symptomatic patients and for presymptomatic and prenatal prediction.
- Between 1992 and 2000, the Molecular Diagnostic Laboratory of the Institute of Medical Genetics, Cardiff has performed a total of 526 tests; 292 of these have been tests for people living in Wales (population 2.9 million). A total of 213 of the samples from Wales were referred by clinical geneticists and 79 by other medical specialists, mainly paediatricians and neurologists. Data on the reason for referral, the pedigree and clinical status, and the outcome of testing were available for 287.
- As no test result showed an equivocal repeat size, direct mutation analysis for myotonic dystrophy can be considered as a very accurate and specific test to differentiate normal and disease associated alleles. There were 205 diagnostic investigations on symptomatic patients (104 abnormal), 78 presymptomatic tests (16 abnormal, 62 normal), and four prenatal diagnoses (two abnormal, two normal).
- In 122 symptomatic patients with a negative family history only 34 proved to have the mutation, reflecting the lack of specificity of clinical symptoms in myotonic dystrophy. A normal result was also found in nine out of 77 symptomatic patients with a positive family history. Only seven of the 16 subjects with an abnormal presymptomatic test result showed no clinical abnormality on examination, supporting the high penetrance of the myotonic dystrophy mutation by adult life and indicating the importance of careful clinical assessment in relation to presymptomatic testing.

It is interesting to note that all seven subjects with an abnormal presymptomatic test result of less than 100 repeats were either clinically normal or suffered only from cataracts (table 6). This genotype/phenotype correlation previously reported²¹ is of value in interpreting an abnormal result, since the tested subject can be reassured that a molecular result of less than 100 repeats indicates a low risk for significant clinical symptoms. Since the main implication for such patients will be the intergenerational amplification of the CTG repeat from anticipation,^{22 23} it would seem appropriate to consider them as gene carriers rather than affected persons. For the 69 clinically normal subjects in this series, only two (2.9%) showed a clinically significant expansion of over 100 repeats, considerably less than the 10% chance of an abnormal genotype, a result also noted by Brunner *et al.*¹⁹

In conclusion, our experience has confirmed that direct molecular analysis for the myotonic dystrophy mutation has proved to be of

considerable value in a service setting for this relatively frequent genetic disorder. Its use and interpretation in both diagnosis and prediction, however, need to be taken in conjunction with thorough clinical assessment and in the light of the extreme variability in clinical features shown by the disorder.

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paper¹⁰ can be found on
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