Evidence for anticipation in autosomal dominant limb-girdle muscular dystrophy

M C Speer, J M Gilchrist, J M Stajich, P C Gaskell, C A Westbrook, S K Horrigan, L Bartoloni, L H Yamaoka, W K Scott, M A Pericak-Vance

Abstract

Anticipation, an increase in severity or decrease in age of onset (AO) inherent in the transmission of the disease gene from affected parent to affected child, has been increasingly described in human disease. To assess anticipation in a large kindred in which autosomal dominant limb-girdle muscular dystrophy (LGMD1A) is segregating, age of disease onset was collected from patient interviews of affected family members. A total of 25 parent-offspring pairs, in which the parents are three (3R), four (4R), or five (5R) generations removed from a common founding ancestor, were available for analysis. Life table analyses showed significant decreases in age at first reported symptoms in the offspring of the 3R (χ^2=5.55, p=0.02) and 4R (χ^2=7.81, p=0.005) parents. Pairwise analyses confirmed this decrease with a median decrease of 13 years in transmission to offspring from 3R parents and 18 years in transmission to offspring from 4R parents. The finding of anticipation in this pedigree suggests that the mutation in LGMD1A may be the result of the expansion of an unstable trinucleotide repeat.

(J Med Genet 1998;35:305–308)

Keywords: anticipation; autosomal dominant; limb-girdle muscular dystrophy

Department of Medicine, Duke University Medical Center, Box 3445, Durham, NC 27710, USA
M C Speer
J M Stajich
P C Gaskell
L H Yamaoka
W K Scott
M A Pericak-Vance

Department of Neurology, Rhode Island Hospital, Providence, RI, USA
J M Gilchrist

Department of Medicine, University of Illinois, Chicago, IL, USA
C A Westbrook
S K Horrigan
L Bartoloni

Correspondence to:
Dr Speer.

Received 14 May 1997
Revised version accepted for publication 15 October 1997

The phenomenon of anticipation, where disease severity increases in the transmission of a disease gene from parent to child, has been described by clinicians in a variety of diseases. Since disease severity is difficult to score, anticipation has frequently been studied in terms of an analogue of severity, disease age of onset (AO). The earliest discussions of anticipation focused on schizophrenia[1] and myotonic dystrophy (DM).[2 3] However, Penrose[4] outlined a series of biases in data ascertainment which could lead to the incorrect conclusion of anticipation. Thus, the occurrence of anticipation was challenged and subsequently rejected by geneticists.

The consideration of anticipation as a real genetic phenomenon, although always argued by clinicians to be legitimate, has recently been revived following the discovery of the high correlation of disease age of onset with expansion of a trinucleotide repeat in myotonic dystrophy,[5 6] Huntington's disease,[7] and recently Machado-Joseph disease,[8] among others. These findings led us to investigate the phenomenon of anticipation in a large family in which an autosomal dominant form of limb-girdle muscular dystrophy (LGMD1A; MIM 15900), linked to an approximate 2.5 Mb interval on 5q flanked by D5S479 and D5S594, is segregating.[9]

Materials and methods

FAMILY ASCERTAINMENT AND ASSESSMENT

The family (Duke 39) was originally ascertained in 1984 through a single proband attending the Duke University Medical Center (DUMC) Muscular Dystrophy Association Clinic who was diagnosed with limb-girdle muscular dystrophy. The proband reported a significant family history for LGMD1A, and the family was recruited for participation in genetic linkage studies.[10 11] All known affected family members, regardless of disease severity, were contacted for possible participation in the study, and family ascertainment was continued through all lines of affected subjects. All first degree relatives (sibs and offspring) of affected subjects were approached for participation in the study. To date, 180 family members have been examined. The branches of the family are connected through a common ancestor born in 1836. The family has been followed clinically at DUMC for over 10 years.

Limb-girdle muscular dystrophy in this family segregates as an autosomal dominant disease with age dependent penetrance. Subjects are considered affected when clinical examination shows a characteristic pattern of muscle weakness, particularly weakness of the pelvic and shoulder girdles, absent deep tendon reflexes, and raised creatine kinase blood levels.[10]

Age of onset (AO) was determined by self-report of clinically affected patients following a consistently applied questionnaire documenting common first symptoms, including difficulty climbing stairs, raising hands above the head, and lifting. Three subjects who were obligate carriers reported themselves to be asymptomatic. For these three people, AO was considered to be age at examination, which is an underestimate of AO.

When the pedigree is considered in aggregate, AO data are available on 62 family members. Of these 62, 40 are included in 25 parent-offspring pairs on whom AO data are available on both parent and offspring. Twenty-two of the 62 subjects are not part of a parent-offspring pair. The pairwise data consist of 13 pairs in which the parent is three generations removed (3R pairs) from the common ancestor, 10 pairs in which the parent is four generations removed (4R pairs), and two pairs in

Table 1 Sample characteristics and pairwise age of onset (AO) comparisons. All AO values are reported in years

	3R*	4R	5R
No of affected patients reporting AO†	10	38	14
No of affected patients reporting AO as parents in pairs	8	9	1
Mean AO of parents (SD)	44.2 (14.4)	36.2 (10.9)	—
Median AO of parents (in years)	38.5	38.0	—
No of affected patients reporting AO as offspring in pairs‡	—	13	10
Mean AO of offspring (SD)		29 (7.9)	22 (6.5)
Median AO of offspring	—	28.0	22
Mean decrease in AO of offspring (SD)	16.3 (12.0)	17.1 (8.7)	—
Median decrease in AO of offspring§	13.0	18	—

*The number of generations removed from the common ancestor.
†Sum = 58; two patients reporting AO are in 6R.
‡Sum = 20; two offspring are in generation 6R.
§Includes only parent-offspring pairs.

which the parent is five generations removed (5R pairs) from the common ancestor. The data include observations on five sib pairs. These pairs are consequently non-independent. No person in the pairwise dataset is present as both a parent and an offspring. The 22 affected subjects with known AO who are excluded from the pairwise data either have no offspring and AO data are unavailable on their affected parent (n=5) or have no offspring who are currently symptomatic (n=17); 15 of these excluded subjects are in 4R and four are in 5R. Summary sample characteristics regarding age of onset are shown in table 1.

STATISTICAL ANALYSIS

Pairwise tests to assess AO differences between generations were performed separately for the 13 3R and 10 4R parent-offspring pairs; insufficient data were available to test 5R pairs (two pairs). The data from all 25 available pairs were also pooled across all generations. As AO in these family data are significantly not normal, the non-parametric Wilcoxon signed rank test was used to assess statistical significance.

ASSESSMENT OF BIASES OWING TO "EXCLUDED" FAMILY MEMBERS

Two different strategies were used to test whether the exclusion of the 22 affected subjects with AO data but without parental or offspring AO data would systematically bias any conclusions.

(1) AO data on the subjects from 4R who were a parent in a parent-offspring pair were compared to the AO of the 15 non-paired subjects from 4R, the only generation with a large number of non-paired subjects reporting AO, to test whether the non-paired subjects experienced earlier AO (and presumably more severe disease and thus were therefore less likely to reproduce) than their "included" counterparts. This test was performed using the Mann-Whitney U test.

(2) AO data on all 38 affected subjects in 4R were paired with AO data on all 14 affected subjects in 5R to generate a total of 532 "parent-offspring" pairs, although the majority of the relationships generated in this manner are avuncular. Evidence for earlier onset in offspring than in parents was tested as described above. Although this process does duplicate some data (as pointed out by McInnis *et al*[12]), it does allow an assessment of the robustness of the conclusions.

(3) Comparison of AO in all gene carriers in the 3R, 4R, and 5R generations were performed using life table analysis and evaluated with the Wilcoxon test. For obligate, asymptomatic gene carriers and affected subjects unable to report AO, the age at last examination (ALE) was used as a surrogate for AO. ALE will tend to underestimate the AO for asymptomatic gene carriers. Similarly, ALE will overestimate AO for affected subjects unable to report an AO. This test assessed whether any significant results were because of undetected, systematic exclusion of the subjects from the parental or offspring groups. Furthermore, this test assessed the overall trend of the data across generations regardless of whether affected family members were paired.

All statistical analyses were performed using the SAS statistical package.

DNA ANALYSIS AND RISK ASSESSMENT FOR ASYMPTOMATIC FAMILY MEMBERS

All family members were genotyped using standard methods[11] for D5S594 and D5S479, markers flanking the approximate 2.5 Mb LGMD1A interval. Risk assessment for asymptomatic, at risk family members was performed by visual inspection of the haplotype data and confirmed by computer assessment as calculated with the assistance of the MLINK module of the LINKAGE computer package.[13] Under the assumption of no interference, the probability for a double recombination event leading to an at risk family member carrying the LGMD1A gene when the flanking markers indicated otherwise is approximately $(0.025) \times (0.025)=0.0006$.

Results

All but three parent-offspring pairs showed evidence for earlier onset in the offspring than in the parent. Two parent-offspring pairs reported identical onset ages and in one pair the offspring onset was reported to be one year later than parent onset. The median AO in the 3R parents is 38.5 years and in their 4R offspring is 28; median AO in the 4R parents is 38 and in their 5R offspring is 22 years. Evidence for anticipation is significant in 3R pairs, the 4R pairs, and in the pooled dataset ($p=0.02$, $p=0.008$, $p=0.0006$, respectively). In 3R pairs, the median decrease in AO is 13 years and in 4R pairs the median decrease in AO is 18 years. The decrease in AO between

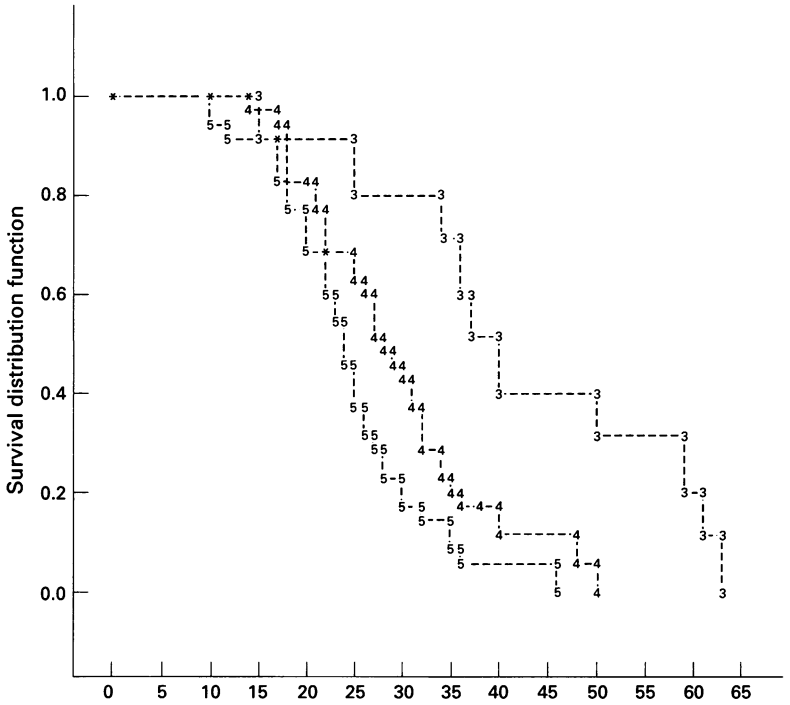

Figure 1 Cumulative survival to patient reported age of onset of symptoms of limb-girdle muscular dystrophy in all gene carriers for the 3R (3), 4R (4), and 5R (5) generations.

offspring of male transmitting parents and female transmitting parents is non-significant (p=0.195) and thus no evidence supporting an imprinting effect was found.

Evaluation of the effect of excluding non-paired patients showed a significant difference in AO between paired 4R parents and non-paired 4R family members (p=0.039), with the non-paired family members reporting a younger AO. However, five family members in this generation are not symptomatic but are at high risk of carrying the LGMD1A gene. When age at examination for these five subjects is included as a surrogate for AO in the analysis, the results are non-significant (p=0.136) Additionally, the consideration of all possible pairs of 4R family members with 5R family members showed significant evidence for anticipation (p<0.0001) suggesting that the trend was significant regardless of the inclusion of the non-paired subjects.

Joint comparison of the AO curves (fig 1) using the Wilcoxon test in life table analysis between all gene carriers in the 3R, 4R, and 5R generations showed a significant decrease in AO in successive generations (Wilcoxon test statistic=11.22, p=0.004). When the analysis was restricted only to those subjects reporting AO, the conclusions are the same (Wilcoxon test statistic=10.17, p=0.006).

Of the family members at risk to have inherited LGMD1A from an affected parent, 15 were identified to have inherited the IL9-D5S178 interval segregating with the disease. All 15 are at least eight years younger than the reported AO of their parents (range 8-19 years younger).

Discussion

The results of this study indicate evidence for anticipation in LGMD1A. The evidence is consistent in the pairwise assessment across the two generations studied (3R and 4R parents and their offspring) and when all available pairs are pooled. The assessment of all possible parental and avuncular pairs of 4R patients with 5R offspring provides further support for anticipation in this pedigree. This approach is a conservative evaluation of the data since, under the hypothesis of anticipation as a result of an expanding trinucleotide repeat, one expects varying onset ages within a sibship or among avuncular relationships associated with the extent of expansion. Furthermore, the trend of anticipation in successive generations is upheld regardless of whether the data are restricted solely to parent-offspring pairs or to all gene carriers.

Consideration of AO data within a single large pedigree such as family 39 effectively eliminates many of the potential underlying biases in data ascertainment which can lead to the incorrect conclusion of anticipation.[3] For instance, the careful, aggressive, long term follow up of such a large family minimises the possibility of selectively ascertaining only pairs of subjects in whom the affected parent has late onset or the affected offspring has early onset. This approach also maximises the probability of detecting "complementary pairs"[4] in which the parent has early onset but the child has later onset, which would provide evidence against anticipation.

One bias inherent in patient reported AO, the tendency for children of affected parents to notice signs of the disease earlier than a parent noticed onset, is difficult to eliminate. This type of bias represents one form of a cohort effect. In principle, such earlier reporting by affected offspring occurs because children are "sensitised" to the existence of the disease in the family. However, it is unlikely that such a bias would occur consistently across this large pedigree. Furthermore, the existence of the disease has been well known in the family for multiple generations, with even the most elderly family members speaking about the expression of the disease in their parents and other relatives, suggesting that such a sensitisation would not be a phenomenon restricted to recent, sampled generations in this pedigree. Another type of cohort effect, whereby disease diagnosis and detection may improve over generations, thereby lowering age at diagnosis in affected patients, is unlikely to be a factor in this study since the measurement is patient reported onset of symptoms rather than age at diagnosis.

Continued long term study of this family will help to determine whether these results are because of presently unobserved and unexplained ascertainment biases. Additionally, the localisation of the disease gene in this LGMD1A family to a region of approximately 2.5 Mb between D5S479 and D5S594[9] has allowed the assessment of gene carrier risk for people within this family. Examination of the data provides no evidence that "complementary pairs" exist but have not been ascertained. When the clinicians are blinded to the genetic risks, it is possible to follow the clinical course of these high risk subjects over the ensuing

years. This long term follow up will allow a more careful assessment of age of onset within this family, as well as the ability to correlate patient reported age of onset with the onset of clinically detectable signs and symptoms.

Are these AO phenomena unique to this single LGMD1A family? Autosomal dominant LGMD is relatively rare and thus limited information exists for comparison. However, supporting evidence for anticipation in LGMD is found in a dominant LGMD family reported by Chutkow *et al.*[14] AO data are reported on two parent-offspring pairs and in both cases, offspring disease onset is earlier than parent onset.

The evidence for anticipation in this pedigree suggests the possibility that the mutation in the gene leading to this muscular dystrophy may be the result of the expansion of a trinucleotide repeat (TNR). LGMD1A, when compared to other diseases with expanding TNRs, is clinically more similar to myotonic dystrophy, in which the unstable DNA sequence is in the 3' untranslated region, than to Huntington disease's, dentatorubropallidoluysian atrophy, spinocerebellar ataxia, or Machado-Joseph disease, each of which exhibits involvement of the brain (for example, ataxia and chorea) and in which the unstable DNA sequence lies within an open reading frame. In view of the tremendous variability in AO within this family, it is likely that other factors, possibly genetic, act to modify disease onset age. Elucidation of the major gene defect will give further insight into the mechanism of anticipation and intergenerational differences in onset age.

The authors would like to acknowledge grants NS 26630 (MAP-V) and grants from the Muscular Dystrophy Association (MCS, MAP-V, and JMG). We also thank Karen Sheff for technical assistance, Peggy Pate, Helen Harbett, and Deborah Gross for assistance with data entry, Eddie Hansen and Dr Jeffery M Vance for assistance with DNA processing, and Roberta Parker for secretarial assistance. The authors also thank two anonymous reviewers for suggestions which considerably improved this manuscript. Most importantly, the authors thank the members of family 39 for their continued interest and participation in these genetic studies in autosomal dominant limb-girdle muscular dystrophy.

1 Mott FW. A lecture on heredity and insanity. *Lancet* 1911;i:1251-9.
2 Raven A, Waring JJ. Studies in dystrophia myotonica. I. Hereditary aspects. *Am J Med Sci* 1929;**197**:595-609.
3 Bell J. Dystrophia myotonica and allied diseases. In: *Treasury of human inheritance*. Cambridge: Cambridge University Press, 1948.
4 Penrose LS. The problem of anticipation in pedigrees of dystrophia myotonica. *Ann Eugen* 1948;**14**:125-32.
5 Harper PS, Harley HG, Reardon W, Shaw DJ. Anticipation in myotonic dystrophy: new light on an old problem. *Am J Hum Genet* 1992;**51**:10-16.
6 Mahadevan M, Tsilfidis C, Sabourin L, *et al.* Myotonic dystrophy mutation: an unstable CTG repeat in the 3' untranslated region of the gene. *Science* 1992;**255**:1253-5.
7 Huntington's Disease Collaborative Research Group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell* 1994;72:971-83.
8 Kawaguchi Y, Okamoto T, Taniwaki M, *et al.* CAG expansions in a novel gene for Machado-Joseph disease at chromosome 14q32.1. *Nat Genet* 1994;**8**:221-8.
9 Bartoloni L, Horrigan SK, Zhang Y, *et al.* Limb-girdle muscular dystrophy 1A: refinement of the 5q31 localization and a physical and genetic map of the interval. *47th Annual Meeting of the American Society of Human Genetics, Baltimore, MD, 28 October-1 November 1997.*
10 Gilchrist JM, Pericak-Vance MA, Silverman L, Roses AD. Clinical and genetic investigation in autosomal dominant limb-girdle muscular dystrophy. *Neurology* 1988;**38**:5-9.
11 Speer MC, Yamaoka LH, Gilchrist JH, *et al.* Confirmation of genetic heterogeneity in limb-girdle muscular dystrophy: linkage of an autosomal dominant form to chromosome 5q. *Am J Hum Genet* 1992;**50**:1211-17.
12 McInnis MG, McMahon FJ, Chase GA, Simpson SG, Ross CA, DePaulo JR. Anticipation in bipolar affective disorder. *Am J Hum Genet* 1993;**53**:385-90.
13 Lathrop GM, Lalouel JM, Julier C, Ott J. Strategies for multilocus linkage analysis in humans. *Proc Natl Acad Sci USA* 1984;**81**:3443-6.
14 Chutkow JG, Heffner RR, Kramer AA, Edwards JA. Adult-onset autosomal dominant limb-girdle muscular dystrophy. *Ann Neurol* 1986;**20**:240-8.