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NATURAL SELECTION AT THE MJD LOCUS: PHENOTYPIC DIVERSITY, SURVIVAL AND FERTILITY AMONG MACHADO-JOSEPH DISEASE PATIENTS FROM THE AZORES

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Summary. Machado-Joseph Disease (MJD) is an autosomal dominant neurodegenerative disorder of adult onset, associated with the expansion of a (CAG)n tract in the coding region of the causative gene, localized on 14q32.1. Machado-Joseph Disease shows non-Mendelian features typical of other triplet repeat disorders, including clinical heterogeneity, variable age at onset and anticipation. Three phenotypes have been proposed (clinical types 1, 2 and 3). Type 1 is associated with early age at onset and a high repeat number of the CAG sequence, and Types 2 and 3 have later onset and lower numbers of CAG repeats. This paper investigates whether there is selection against the MJD gene, acting through differential survival, nuptiality and fertility associated with clinical type and age at onset. The study sample comprised 40 MJD patients from the Azores (Portugal) having fully documented reproductive histories and known dates of death. The proportion of married patients of each clinical type increased from 0·22 among Type 1 patients, to 0·40 in Type 2 and 0·95 in Type 3. Age at onset and length of survival were also associated with marital status, with the married cases having later mean age at onset and longer mean survival time. In the whole sample, clinical type was associated with fertility, with significantly fewer children born to Type 1 patients. Among married patients clinical type was not associated with age at marriage, reproductive span or number of children. No reduction of fertility was detected among married patients in whom the onset of MJD was below the age of 50. The authors' interpretation of these results is that the high-repeat CAG haplotypes associated with early age at onset and clinical Type 1 are selected against through reduced survival and fertility. The fertility component of selection is mediated by nuptiality rather than marital fertility.

Introduction

Machado-Joseph Disease is an autosomal dominant neurodegenerative disorder, usually characterized by gait ataxia and limitation of eye movements, among a wide range of clinical features, which all usually arise during adult life (OMIM 109150). Coutinho (1992) observed a mean age at onset of 40·2 years in a large Portuguese sample, but ages of onset ranging from 6 to 70 years have been reported (Coutinho, 1992; Sequeiros & Coutinho, 1993). There are a number of such disorders showing late-onset neurodegeneration and dominant inheritance, the best known being Huntington's Disease. This combination of features is especially problematic for families in which the disease occurs, since dominant inheritance implies that someone with a parent carrying the gene has a 50% chance of inheriting it, and yet the late onset means that an individual will frequently not know whether they themselves carry the gene until after they have married and reproduced. Molecular diagnosis before onset can remove this uncertainty, but brings its own problems (Meissen *et al*., 1991; Decruyenaere *et al*., 1996).

From the earliest reports, MJD has been recognized as pleomorphic in its clinical presentation (Nakano, Dawson & Spence, 1972; Woods & Schaumburg, 1972; Rosenberg *et al.*, 1976). On the basis of clinical presentation and age at onset, three MJD phenotypes were proposed by Coutinho & Andrade (1978). Type 2 is the basic presentation, with progressive ataxia, pyramidal signs and ophthalmoplegia, having a mean age at onset of 40·5 years in the large Portuguese series studied by Coutinho (1992). Type 1 is characterized by early onset (mean age 24·3 years; Coutinho, 1992) with marked pyramidal and extrapyramidal signs. Type 3 has later onset (mean age 46·8 years; Coutinho, 1992), and is associated with additional distal muscular atrophies and sensory loss.

The MJD locus has been assigned to the long arm of chromosome 14 (Takiyama *et al.*, 1993), and is associated with the expansion of a (CAG)n tract in the coding region of the causative gene, localized on 14q32.1 (Kawaguchi *et al.*, 1994). The expansion of CAG repeats in coding regions is known to be a common feature of a number of neurodegenerative disorders (Tsuji, 1996). Such 'triplet repeat disorders' share a number of characteristic features whose inheritance cannot be explained by Mendelian principles, including clinical heterogeneity, variable age at onset, and anticipation (earlier age at onset in successive generations).

The number of triplet repeats seems to influence aspects of clinical presentation, particularly age at onset (Andrew *et al.*, 1993; Snell *et al.*, 1993; Koide *et al.*, 1994; Ranum *et al.*, 1994). In MJD, a strong inverse correlation between the number of triplet repeats and age at onset has been demonstrated (DeStefano *et al.*, 1995; Dürr *et al.*, 1995; Maciel *et al.*, 1995; Maruyama *et al.*, 1995). Anticipation has been reported by several authors studying different series of patients (Maciel *et al.*, 1995; Sousa *et al.*, 1997), and is associated with an expansion of triplet repeats between parents and children, the difference in CAG repeat length being greater in paternal than in maternal transmission. Type 1 MJD shows a larger degree of expansion in CAG repeats than the other clinical types. It has also been reported that a patient homozygous for the expanded triplet repeat had juvenile onset and more severe symptoms of the disease (Lang *et al.*, 1994; St-George-Hyslop *et al.*, 1994; Takiyama *et al.*, 1995).

A survey of MJD in the nine islands of the Azores (total population 237,795) identified 103 cases, which represents a prevalence of 1 in 2309 individuals, and in the Azorean island of Flores (population 4329) the disease has its highest worldwide prevalence (Lima *et al.*, 1997b), with 1 in 103 people affected. Even though MJD is now considered to be one of the most common autosomal dominant spinocerebellar degenerations (Takiyama *et al.*, 1993; Kawaguchi *et al.*, 1994; Tsuji, 1996), it is clearly over-represented in the Azores. As a comparative figure the nationwide survey of Japanese patients by Hirayama *et al.* (1994) can be cited, which estimated a frequency of about one MJD patient per million of population. The evolutionary processes responsible for the high frequency of MJD in the Azores are most likely to be founder effect and genetic drift, as has previously been shown to be the case when high frequencies of hereditary disorders occur in isolated populations (Morton, 1980; O'Brien *et al.*, 1988). However, a previous study has demonstrated that there is not a unique set of founders for all Azorean MJD patients (Lima *et al.*, 1998).

The main focus of this paper, however, is another evolutionary process, natural selection. Analysis of other expansion disorders, namely Huntington's Disease and Spinocerebellar Ataxia Type 1 (Frontali *et al.*, 1996), has suggested that natural selection may act against the MJD gene through the differential fitness of CAG repeat length in the causative genes. Under the model proposed by Frontali *et al.* (1996), most of the alleles with the largest expansions are lost at each generation and are replaced by those in the low/medium expansion range. Such a mechanism of selective loss of the largest numbers of repeats would be expected to balance the inter-generational expansion of triplet repeats reported above. A case-control survey of MJD families undertaken to discover whether natural selection acted in the same way in MJD found no reduction in their reproductive output compared with unaffected families (Lima *et al.*, 1997a). That survey was limited, by the study design, to the fertility of married MJD patient controls, and could not consider whether MJD patients survived and married at the same rate as unaffected individuals.

The purpose of the present study is to take a series of clinically diagnosed MJD patients for whom complete reproductive histories have been recorded, and to test for associations between the clinical features of the disease phenotype and survival, nuptiality and marital fertility. The variables subsumed in the clinical features include clinical type and two of its components: age at onset and length of survival. The hypothesis is that in a disease of this nature, of variable but generally late age at onset, and imposing no apparent physiological or biological limitation on reproduction, reduction in fitness will usually be mediated by social behaviour. In cases of exceptionally early onset, however, it might occur that the sufferer died or became incapacitated before the normal age at marriage, thus obviating the behavioural component of selection. Behavioural selection against the MJD gene may occur if someone who already has the disease, or someone who is an undiagnosed member of an MJD family, is unwilling or unable to marry. Additionally, selection may occur if the disease develops in a patient who is married but still of reproductive age, and the couple decide to curtail their reproduction at that point.

Such behavioural choices will be to an extent the prerogative of individuals and couples, but will also be subject to societal norms of behaviour, through the reported stigma attaching to undiagnosed members of MJD families, as well as to those

already showing symptoms (Boutté, 1987). The authors suggest that through these mechanisms the gene may influence the fitness of its carriers, and hence predict an association between the clinical features of MJD patients and their reproductive success. They predict that clinical type, and age at onset in particular, will affect survival, nuptiality and marital fertility.

Methods

The series studied comprised 40 clinically confirmed MJD patients born in the Azores between 1901 and 1979, for whom dates of birth, marriage (if applicable) and death were available. The sample of patients analysed corresponded to all patients for whom there was a complete set of data at the time of the study. Of the 40 patients, 28 were married, and their complete reproductive history was fully documented. Cross-checking among the various genealogical sources, both oral and written, showed that, as far as could be ascertained, the unmarried patients had no children. This sample represented, at the time of the study, the largest group of individuals for whom all pertinent data were available. For the married patients the following demographic variables were calculated: age at marriage, number of offspring and duration of reproductive span (number of years between marriage and birth of the last child). The information was obtained from an extensive demographic and genealogical database of the Azorean MJD families built at the University of the Azores. The database and the software used for the analysis (ANALYPOP) were developed by E. Labelle under the supervision of F. M. Mayer (University of Quebec in Montreal) and have been described in detail in Lima *et al.* (1997b).

The diagnosis of MJD was determined by clinical examination by an experienced neurologist, using established diagnostic criteria (Lima & Coutinho, 1980). Age at onset, clinical type (assigned according to the classification proposed by Coutinho & Andrade (1978)) and survival time (number of years between age at onset and time of death) were the clinical variables describing the phenotypic heterogeneity of MJD. After testing for associations between demographic and clinical variables the consistency of the present sample with the larger Portuguese series studied by Coutinho (1992) was established to give confidence in the wider applicability of the results.

Results

Table 1 shows the characteristics of the sample divided by clinical type. Type 1 patients had a mean age of onset of 23·2 years, significantly younger than Type 2 ($t = -2{\cdot}96$, $p = 0{\cdot}01$) and Type 3 ($t = -5{\cdot}89$, $p = 0{\cdot}000$). Age at onset of Type 2 was 42·1 years, and that of Type 3 was 50·9 years, but the difference between them was not significant ($t = -1{\cdot}76$, $p = 0{\cdot}104$). Among the whole sample, survival time was 12·1 years in Type 1 patients, significantly shorter than that of Type 3, who survived for an average of 16·7 years. The survival of Type 2 patients, at 13·0 years, was not significantly different from either Type 1 ($t = -0{\cdot}34$, $p = 0{\cdot}740$) or Type 3 ($t = -1{\cdot}65$,

Table 1. Clinical type and demographic features of MJD patients

	Whole sample						Married cases only					
Clinical type	*n*	Age at onset	Age at onset (PC)[a]	Proportion married	Survival	Children	*n*	Age at marriage	Age at onset	Survival	Reproductive span	Children
Type 1	9	23·2	24·3	0·22	12·1	0·56	2	23·5	34·5	17·0	12·0	2·50
Type 2	10	42·1	40·5	0·40	13·0	2·40	6	23·5	37·3	12·2	7·2	2·40
Type 3	21	50·9	46·8	0·95	16·7	3·48	20	24·8	50·5	17·0	11·4	3·48
Significance tests		*t* test		Kendall's tau-c	*t* test	Mann–Whitney *U*		*t* test	*t* test	*t* test	*t* test	Mann–Whitney *U*
Type 1 vs Type 2		**		***	—	—		—	*	—	—	—
Type 1 vs Type 3		***			*	**		—	**	—	—	—
Type 2 vs Type 3		—			—	—		—	—	—	—	—

[a]Values from Coutinho (1992).
*p<0·05; **p<0·01; ***p<0·001; —, not significant.

Fig. 1. Machado-Joseph Disease profile by marital status.

$p=0{\cdot}110$). Among the married cases there were no significant differences in survival time between the clinical types.

The proportion of married patients of each clinical type increased from 0·22 among Type 1 patients, to 0·40 in Type 2 and 0·95 in Type 3. This association of marital status with clinical type was statistically significant (Kendall's $\tau=-0{\cdot}615$, $p=0{\cdot}000$). The association of marital status with clinical type means that some of the components of clinical type, namely age at onset and length of survival, were also associated with marital status, with the married cases having a later mean age at onset (46·5 years versus 33·1, $t=2{\cdot}13$, $p=0{\cdot}05$) and longer mean survival time (16·0 years versus 11·8, $t=2{\cdot}61$, $p=0{\cdot}01$).

Table 1 shows that when the whole sample, both married and unmarried, was considered the mean number of children per patient was also related to clinical type, with significantly fewer children born to Type 1 than to Type 3 (Mann–Whitney $U=43{\cdot}5$, $p=0{\cdot}010$). The differences in the number of children born to Type 2 and Type 3 patients, and to Type 1 and Type 2 patients, were not statistically significant, with $U=97{\cdot}0$ ($p=0{\cdot}499$) and $U=25{\cdot}5$ ($p=0{\cdot}113$) respectively.

To explore the relationship between age at onset, age at death and marital status, age at death for all cases is plotted against their age at onset of MJD in Fig. 1. The mean age at marriage was 24·4 years. The sample included three cases of childhood onset, at ages 5, 6 and 8 years, who died at ages 13, 18 and 15 years respectively. There were a further nine cases with onset below the age of 35 (these will be referred to as 'early-onset' cases), of whom five (56%) remained unmarried. There were 28 cases with onset at 35 years of age or more ('late-onset' cases), and of these four (14%) were unmarried. Leaving aside the three juvenile-onset cases, there was a significant association between early and late onset and marital status ($\chi^2=6{\cdot}3$,

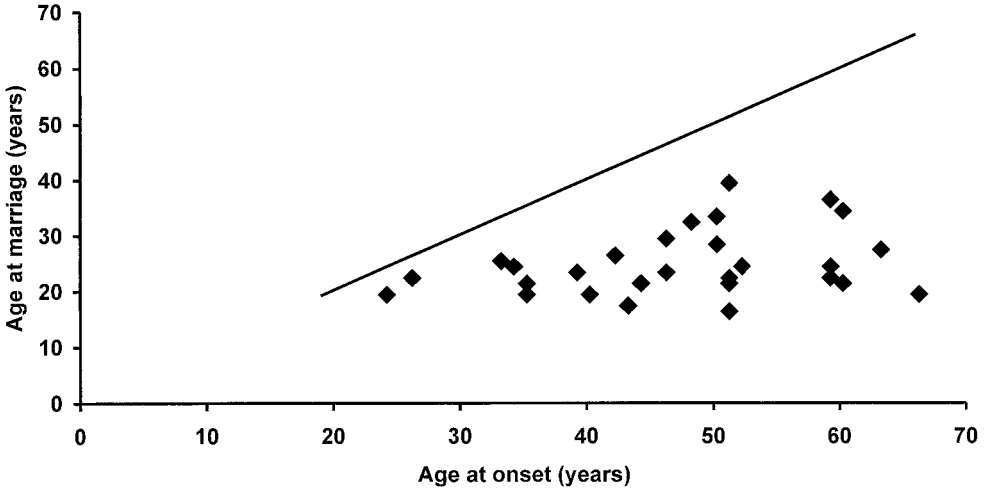

Fig. 2. Age at marriage in relation to age at onset of MJD.

$p=0{\cdot}01$). In order to illustrate further the importance of early onset in inhibiting marriage, age at marriage is plotted against age at onset in Fig. 2. The trend line shows where the data points would fall if age at marriage were equal to age at onset. In fact the mean age at onset ($42{\cdot}5 \pm 15{\cdot}7$) was much higher than mean age at marriage ($24{\cdot}4 \pm 4{\cdot}9$) but there was some overlap between their distributions. Nevertheless, all the data points lie below the trend line, indicating that there were no cases in which marriage occurred after onset.

There were 28 married patients, so testing for associations with clinical type within this group was hampered by small sample sizes. The aim of the study was to discover whether termination of reproduction at onset of MJD symptoms caused any difference in marital fertility between the clinical types. Table 1 shows that there was no difference in age at marriage between the three clinical types. Type 3 patients, however, had a significantly later age at onset than Type 1 ($t=-2{\cdot}58$, $p=0{\cdot}018$) and Type 2 ($t=-2{\cdot}87$, $p=0{\cdot}008$), while Type 1 and 2 did not differ in age at onset ($t=-0{\cdot}78$, $p=0{\cdot}789$). There was thus on average a longer period between marriage and disease onset among Type 3 patients than among Types 1 and 2. However, no association between clinical type and length of survival or reproductive span of the married patients was detected. Nor was there any difference in the number of children born to patients of each clinical type. For Type 1 versus Type 2 $U=3{\cdot}5$ ($p=0{\cdot}429$), for Type 1 versus Type 3 $U=17{\cdot}0$ ($p=0{\cdot}779$), and for Type 2 versus Type 3 $U=53{\cdot}0$ ($p=0{\cdot}700$).

In order to explore further the question of whether marital fertility might have been curtailed by couples deciding not to have more children after the onset of MJD in one partner, the following additional test was devised. On the assumption that reproduction is naturally completed by the age of 50, the sample of married patients was divided into two groups: those with onset below 50 years of age, and those with onset at 50 years or more. If onset of the disease after marriage led people to stop having children whilst still of reproductive age, a smaller mean family size in the

'onset under 50' age group would be predicted. There was no such effect either on reproductive span ($t = -0{\cdot}679$, $p = 0{\cdot}5052$) or on family size, with an average of 3·53 children born to the 'onset under 50' group and 3·77 to the 'onset at 50 and over' group (Mann–Whitney $U = 75{\cdot}0$, $p = 0{\cdot}8242$).

Comparison of the results presented here with data from Coutinho (1992) shows consistency in the proportion of clinical types, with just over half the patients presenting with Type 3 MJD. Average age at onset in the present study was 42·5 years (range 5 to 66 years), with a mean duration of 14·7 years, slightly shorter than observed by Coutinho (1992). This discrepancy can perhaps be explained by the inclusion in the present study of the childhood-onset cases with their short life span. In the present sample, there is a marginally later age at onset of Types 2 and 3 than in Coutinho (1992): 42·1 years versus 40·5 years for Type 2 and 50·9 years versus 46·8 years for Type 3. This may be due to chance or might reflect slight differences in diagnostic practice. Although the age at marriage of MJD patients in this study was in accordance with previously published data, the mean number of offspring (3·6) was lower than previously reported (Lima *et al.*, 1997a). The earlier study had included 96 unions involving an MJD patient, marriages that took place in the Azores between 1850 and 1950, producing an average of 6·3 children per couple. The authors believe that the discrepancy in family size can be attributed to the later period of marriage (1921–1970) in the present study. Livi Bacci (1971) has recorded a decline in marital fertility in Portugal from the mid-nineteenth century, and Rocha (1991) has documented the twentieth century reduction in marital fertility in the Azores. Although the number of marriages per decade is very small, even within these data, this decline is detectable, falling from 7·5 children per marriage in the 1920s to 3·7, 3·2 and 3·6 in subsequent decades, and reaching an average of 2·6 in the 1960s. The general agreement between the results reported here and by Coutinho (1992) and Lima *et al.* (1997b) suggests that the patients analysed in the present study are representative of Portuguese cases in general.

Discussion

There are two mechanisms by which this selection occurred: firstly, and only occasionally, there were juvenile-onset cases who died before reproductive age, and secondly, there were those who lived long enough to marry but failed to do so. Figure 1 shows that for the juvenile-onset cases, the disease itself was an effective barrier to survival and reproduction apparently without any need to invoke the moderating effect of marriage. The juvenile-onset cases died at ages 13, 15 and 18, however, whilst the youngest age at marriage in the sample was 17 years. This suggests that although the mechanisms may be categorically distinct, there can be an overlap between their operation in practice, with juvenile-onset cases who survive long enough to reach marriageable age remaining unmarried notwithstanding their maturity.

For those patients who did not marry, even though they survived until adulthood, there are a number of possible explanations for their celibacy. They might simply happen not to have married, just as a proportion of the unaffected population would have remained single. The proportion of unmarried patients was too high at 43% (or 32% if those with childhood onset who died by 18 years of age are discounted) for

this to be an adequate explanation for all cases. Celibacy rates for the general and regional Portuguese population (Livi Bacci, 1971) show that in the period 1900–1960 the proportion of single females aged 50–54 years fell from 21·4% to 16%, with the proportion of men declining from 13% to 10·4%. Figures for the proportion of single females aged 50–54 years within the Azores were slightly higher than these general figures in the administrative district of Angra and slightly lower in the district of Ponta Delgada. In view of these rates, it seems likely that 'natural' celibacy can provide only a partial explanation for the large proportion of unmarried MJD patients.

The unmarried patients might have intended to marry, but failed to do so because the onset of the disease came before they had chosen a partner, and once symptoms appeared they or their potential mates decided against marriage. Alternatively, they might have remained celibate regardless of age of onset, either by their own choice, restrained by the knowledge that they were at risk of developing MJD, or avoided as marriage partners by others for the same reason. Celibacy in these situations can be referred to as either 'symptom-based' or 'restraint-based'. These possibilities can be distinguished by referring to Fig. 1. If fear of later occurrence were as potent a deterrent to marriage as the development of symptoms itself, no difference in the distribution of age at onset between the married and unmarried cases would be expected. It can be recalled, however, that there was a skewing of unmarried cases towards early age at onset, with 56% unmarried in the group with onset below 35 years of age compared with only 14% unmarried among those with onset at 35 years of age or more. This discrepancy in proportions suggests that at least some of those unmarried cases with MJD onset at ages 27, 29, 31, 33 and 34 were single people within the normal range of age at marriage whose intention had been to marry, but who were prevented from doing so by onset of the disease. Unfortunately, these data do not allow complete confidence in this judgement, since in general the age at marriage in this sample was lower than the age at onset of the disease.

There is circumstantial evidence of such behaviour in the distributions of age at marriage and age at onset of MJD. Figure 2 shows these data plotted for each of the married cases. Although the mean age at onset ($42{\cdot}5 \pm 15{\cdot}7$) was much higher than mean age at marriage ($24{\cdot}4 \pm 4{\cdot}9$), as stated previously, there was considerable overlap between their distributions. Yet there were no cases in which marriage occurred after onset. This can be interpreted to indicate that people did not get married once the symptoms of MJD had become apparent. The skewing of the unmarried cases towards the earlier age of onset, together with the fact that there was no case in which marriage took place after onset, implies that for the early-onset cases onset of the disease played a part in the decision not to marry. In practice, perhaps some of the early-onset unmarried cases had delayed marriage in case symptoms developed, only to be confirmed in their celibacy at the onset of MJD. In such a case 'restraint-based celibacy' would be overtaken by 'symptom-based celibacy' later in life. With regard to the later-onset unmarried cases it is assumed that their condition is a combination of 'natural' celibacy perhaps together with some element of restraint, though it might be questioned whether 'natural' celibacy is a state consistent with the consciousness of being at risk. In a dataset of this sort it is impossible to identify the reason why any particular individual remained unmarried, and it is difficult to attribute celibacy

exclusively to one or other cause, since once symptoms of MJD have developed the concepts of 'restraint-based' and 'natural' celibacy can no longer apply. Thus, as has been seen in the case of juvenile-onset cases living to marriageable age, one cause may give way to another during the life course.

For the second prediction, that clinical type and age of onset would reduce marital fertility through the premature cessation of reproduction after onset of symptoms, no support was found. Comparison of the number of children born to married patients of the three clinical types showed no difference in reproductive output between them, even though the Type 3 patients survived significantly longer on average than Types 1 and 2. Furthermore, the test devised to examine the effects of disease status in cutting short the reproduction of married patients after onset showed no difference in family size between those with onset during the reproductive period and those with onset after the age of 50. At a descriptive level the subject of age at onset and continued procreation can be pursued still further. Among the seven married patients with age at onset of 35 years or younger, the mean family size was 4·00, and among the four in whom the disease started before the age of 35, mean family size was 4·50. Thus, no evidence was found that early onset of the disease after marriage led to a reduction in marital fertility, even though reproduction continued after onset of the disease in only three cases.

The authors' interpretation of these data is that selection against MJD is mediated by marital status alone, rather than through reduction of marital fertility. It is not clear at what stage the decision not to marry takes place. In some cases, it may be that the development of symptoms prevents marriage; in others, that the family legacy of MJD reduces the likelihood of marriage, either through self-imposed restraint, or through an aversion exercised by potential marriage partners. The case-control approach used by Lima *et al.* (1997b) might be used to confirm this finding if reproductive output could be measured in terms of the number of grandchildren rather than children, though in view of the difficulty in calculating pedigrees to this level in a population with such a high emigration rate as the Azores, a surrogate measure such as 'children who marry' could be taken as an alternative. Another approach would be to compare the nuptiality and reproductive success of affected individuals with that of their unaffected sibs.

To summarize, the results show that clinical type is associated with reproductive success, as predicted. However, this effect appears to be entirely due to the lower rate of marriage among Type 1 MJD patients. Among married patients there was no dependence of fertility on clinical type or age at onset. In other words, natural selection against the MJD gene seems to have operated solely through the lack of reproductive success of the unmarried cases.

These results have significance for the debate about the regulation of the number of CAG repeats, and the assessment of the number of CAG repeats in Azorean MJD patients will provide the opportunity to integrate molecular features with their reproductive and clinical characteristics. Frontali *et al.* (1996) proposed a model of selective loss in each generation of the majority of alleles with the largest number of repeats and their replenishment by the expansion of alleles in the low-to-medium repeat range. Such a mechanism of selective elimination of the largest CAG repeat numbers is entirely consistent with the results of the present study, which has

demonstrated natural selection against early-onset forms of MJD associated with the most highly repeated CAG sequences.

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References

Andrew, S., Theilmann, J., Almqvist, E., Norremolle, A., Lucotte, G., Anvret, M. *et al.* (1993) DNA analysis of distinct populations suggests multiple origins for the mutation causing Huntington Disease. *Clin. Genet.* **43**(6), 286–294.

Boutté, M. (1987) The stumbling disease: A case study of stigma among Azorean-Portuguese. *Social Sci. Med.* **24**(3), 209–217.

Coutinho, P. (1992) *Doença de Machado-Joseph-Tentativa de definição*. Dissertação de Doutoramento, Universidade do Porto, Porto.

Coutinho, P. & Andrade, C. (1978) Autosomal dominant system degeneration in Portuguese families of the Azores Islands: A new disorder involving cerebellar, pyramidal, extrapyramidal and spinal cord motor functions. *Neurology* **28**, 703–709.

Decruyenaere, M., Evers-Kiebooms, G., Boogaerts, A., Cassiman, J. J., Cloostermans, T., Demyttenaere, K. *et al.* (1996) Prediction of psychological functioning one year after the predictive test for Huntington's disease and impact of the test result on reproductive decision making. *J. med. Genet.* **33**(9), 737–743.

DeStefano, A., Farrer, L. A., Maciel, P., Gaspar, C., Rouleau, G., Coutinho, P. & Sequeiros, J. (1995) Gender equality in Machado-Joseph disease. *Nat. Genet.* **11**, 118–119.

Dürr, A., Stevanin, G., Cancel, G., Abbas, N., Chneiweiss, H., Agid, Y. *et al.* (1995) Gender equality in Machado-Joseph disease. *Nat. Genet.* **11**, 118.

Frontali, M., Sabbadini, G., Novelletto, A., Jodice, C., Naso, F., Spadaro, M. *et al.* (1996) Genetic fitness in Huntington's Disease and Spinocerebellar Ataxia 1: A population genetics model for CAG repeat expansions. *Ann. hum. Genet.* **60**, 423–435.

Hirayama, K., Takayanagi, T., Nakamura, R., Yanagisawa, N., Hattori, T., Kita, K. *et al.* (1994) Spinocerebellar degenerations in Japan: A nationwide epidemiological and clinical study. *Acta neurol. scand.* **89** (suppl. 153), 1–22.

Kawaguchi, Y., Okamoto, T., Taniwaki, M., Aizawa, M., Inoue, M., Katayama, S. *et al.* (1994) CAG expansions in a novel gene for Machado-Joseph disease at chromosome 14q32.1. *Nat. Genet.* **8**, 221–228.

Koide, R., Ikeuchi, T., Onodera, O., Tanaka, H., Igarashi, S., Endo, K. *et al.* (1994) Unstable expansion of CAG repeat in hereditary dentatorubral-pallidoluysian atrophy (DRPLA). *Nat. Genet.* **6**, 9–13.

Lang, A. E., Rogaeva, E. A., Tsuda, T., Hutterer, J. & St-George-Hyslop, P. (1994) Homozygous inheritance of the Machado-Joseph disease gene. *Ann. Neurol.* **36**(3), 443–447.

Lima, M. (1996) *Doença de Machado-Joseph nos Açores: Estudo epidemiológico, biodemográfico e genético.* Dissertação de Doutoramento, Universidade dos Açores.

Lima, M., Abade, A., Mayer, F. M. & Coutinho, P. (1997a) Diffusion of a dominant gene: Biodemographic study of the families affected by Machado-Joseph disease in the islands of the Azores (Portugal). *Re. Esp. Antrop. Biol.* **18**, 203–210.

Lima, L. & Coutinho, P. (1980) Clinical criteria for diagnosis of Machado-Joseph disease: Report of a non-Azorean portuguese family. *Neurology* **30**, 319–322.

Lima, M., Mayer, F. M., Coutinho, P. &. Abade, A. (1997b) Prevalence, geographical distribution and genealogical investigation of Machado-Joseph disease in the islands of the Azores (Portugal). *Hum. Biol.* **69**(3), 383–391.

Lima, M., Mayer, F. M., Coutinho, P. & Abade, A. (1998) Origins of a mutation: Population genetics of Machado-Joseph disease in the Azores (Portugal). *Hum. Biol.* **70**, 1011–1023.

Livi Bacci, M. (1971) *A Century of Portuguese Fertility*. Princeton University Press, Princeton.

Maciel, P., Gaspar, C., Silveira, I., Coutinho, P., Loureiro, J. L., Guimarães, J. *et al.* (1995) Machado-Joseph disease: CAG repeat length and clinical features. *J. Neurol.* **242** (suppl. 6), S32.

Maruyama, H., Nakamura, S., Matsuyama, Z., Sakai, T., Doyu, M., Sobue, G. *et al.* (1995) Molecular features of the CAG repeats and clinical manifestations of Machado-Joseph disease. *Hum. mol. Genet*. **4**(5), 807–812.

Meissen, G. J., Mastromauro, C. A., Kiely, D. K., McNamara, D. S. & Myers, R. H. (1991) Understanding the decision to take the predictive test for Huntington disease. *Am. J. med. Genet*. **39**, 404–410.

Morton, N. E. (1980) Genetic epidemiology of isolates. In: *Population Structure and Genetic Disorders*, pp. 43–56. Edited by A. W. Eriksson, H. R. Forsius, H. R. Nevalinna, P. L. Workman & R. K. Norio. Academic Press, London.

Nakano, K., Dawson, D. M. & Spence, A. (1972) Machado disease – A hereditary ataxia in Portuguese emigrants to Massachusetts. *Neurology* **22**, 49–55.

O'Brien, E., Jorde, L. B., Ronnlof, B., Fellman, J. O. & Eriksson, A. W. (1988) Founder effect and genetic disease in Sottunga, Finland. *Am. J. phys. Anthrop*. **77**, 335–346.

OMIM (Online Mendelian Inheritance in Man): http://www.ncbi.nlm.nih.gov/Omim/.

Ranum, L. P. W., Schut, L. J., Lundgren, J. K., Orr, H. T. & Livingstone, D. M. (1994) Spinocerebellar ataxia type 5 in a family descended from the grandparents of President Lincoln maps to chromosome 11. *Nat. Genet.* **8**(3), 280–284.

Rocha, G. (1991) *Dinâmica populacional dos Açores no século XX: Unidade, Permanência, Diversidade*. Dissertação de Doutoramento, Universidade dos Açores, Ponta Delgada.

Rosenberg, R., Nyhan, W. L., Bay, C. & Shore, P. (1976) Autosomal dominant striatonigral degeneration. A clinical, pathologic and biochemical study of a new genetic disorder. *Neurology* **26**, 703–714.

St-George-Hyslop, P., Rogaeva, E., Huterer, J., Tsuda, T., Santos, J., Haines, J. L. *et al.* (1994) Machado-Joseph disease in pedigrees of Azorean descent is linked to chromosome 14. *Am. J. hum. Genet.* **55**, 120–125.

Sequeiros, J. & Coutinho, P. (1993) Epidemiology and clinical aspects of Machado-Joseph Disease. *Adv. Neurol.* **61**, 139–153.

Snell, R. G., MacMillan, J. C., Cheadle, J. P., Fenton, I., Lazarou, L. P., Davies, P. *et al.* (1993) Relationships between trinucleotide repeat expansion and phenotypic variation in Huntington's disease. *Nat. Genet.* **4**, 393–397.

Sousa, A., Coutinho, P., Oliveira, J., Teixeira, C. M., Silva, R. M., Sudarsky, L. A. *et al.* (1997) Anticipation of age-at-onset in Machado-Joseph disease (MJD) – a quantitative genetic study. In: *1st International Symposium on Inherited Ataxias*, Montreal (Abstract).

Takiyama, Y., Igarashi, S., Rogaeva, E. A., Endo, K., Rogaev, E. I., Tanaka, H. *et al.* (1995) Evidence for inter-generational instability in the CAG repeat in the MJD1 gene and

for conserved haplotypes at flanking markers amongst Japanese and Caucasian subjects with Machado-Joseph disease. *Hum. mol. Genet.* **4**, 1137–1146.

Takiyama, Y., Nishizawa, M., Tanaka, H., Kawashima, S., Sakamoto, H., Karube, Y. *et al.* (1993) The gene for Machado-Joseph Disease maps to human chromosome 14q. *Nat. Genet.* **4**(3), 300–304.

Tsuji, S. (1996) Unstable expansion of triplet repeats as a new disease mechanism for neurodegenerative diseases. *Jap. J. hum. Genet*. **41**(3), 279–290.

Woods, B. T. & Schaumburg, H. H. (1972) Nigro-spino-dentatal degeneration with nuclear ophthalmoplegia: A unique and partially treatable clinico-pathological entity. *J. neurol. Sci.* **17**, 149–166.