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Machado-Joseph Disease in Pedigrees of Azorean Descent Is Linked to Chromosome 14

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Summary

A locus for Machado-Joseph disease (MJD) has recently been mapped to a 30-cM region of chromosome 14q in five pedigrees of Japanese descent. MJD is a clinically pleomorphic neurodegenerative disease that was originally described in subjects of Azorean descent. In light of the nonallelic heterogeneity in other inherited spinocerebellar ataxias, we were interested to determine if the MJD phenotype in Japanese and Azorean pedigrees arose from mutations at the same locus. We provide evidence that MJD in five pedigrees of Azorean descent is also linked to chromosome 14q in an 18-cM region between the markers D14S67 and AACT (multipoint lod score +7.00 near D14S81). We also report molecular evidence for homozygosity at the MJD locus in an MJD-affected subject with severe, early-onset symptoms. These observations confirm the initial report of linkage of MJD to chromosome 14; suggest that MJD in Japanese and Azorean subjects may represent allelic or identical mutations at the same locus; and provide one possible explanation (MJD gene dosage) for the observed phenotypic heterogeneity in this disease.

Introduction

Machado-Joseph disease (MJD) is an inherited degenerative disorder of the human central and peripheral nervous systems that is characterized clinically by ataxia, spasticity, parkinsonism, dystonia, disorders of eye movement, sensory loss, muscle weakness, and fasciculations. This constellation of clinical features can be present to varying degrees in members of the same family and/or at different times in the illness. Neuropathologically, MJD is epitomized by the selective degeneration of neurons in the substantia nigra, dentate nucleus of the cerebellum, peripheral nerves, and neurons giving rise to the spinocerebellar and pyramidal tracts of the spinal cord (Nakano et al. 1972; Cunha et al. 1988; Rosenberg 1992; Spinella and Sheridan 1992; Sequeiros and Coutinho 1993). MJD is thought to have originated from founders in the Iberian Peninsula who migrated to the Azores and then to several other countries including North and South America (Sequeiros and Coutinho 1993). In recent years a similar clinical and pathological disorder has been described in populations of non-Azorean descent (Cooper et al. 1983; Sakai et al. 1983; Jain and Maheshwari 1990). It is unknown whether these represent (*a*) new or different genetic defects or (*b*) further examples of the original genetic defect through migration of Portuguese navigators (Spinella and Sheridan 1992).

The genetic defect underlying MJD, which is inherited as an autosomal dominant trait, remains unknown but is clearly different from that involved in other spinocerebellar degenerations (Carson et al. 1992; Silviera et al. 1993). However, recent genetic linkage studies have mapped a locus for MJD, in five pedigrees of Japanese origin, to a 29.5-cM region of chromosome 14q, between D14S53 and D14S51 (Takiyama et al. 1993). In view of the genetic heterogeneity in other dominantly inherited spinocerebellar ataxias (Gispert et al. 1993; Orr et al. 1993), we were interested to determine whether MJD pedigrees of Azorean descent also segregated a genetic defect on chromosome 14. To address this question we investigated the inheritance of nine polymorphic markers from 14q24-qter in five pedigrees of Azorean descent. Our results provide three new observations. First, they confirm the previous report of linkage of MJD to this region and thus argue against locus heterogeneity between the Japanese and Azorean MJD pedigrees examined to date. Second, our results allow the MJD locus to be more narrowly defined. Third, our results shed some light on putative mechanisms for the highly variable disease phenotype.

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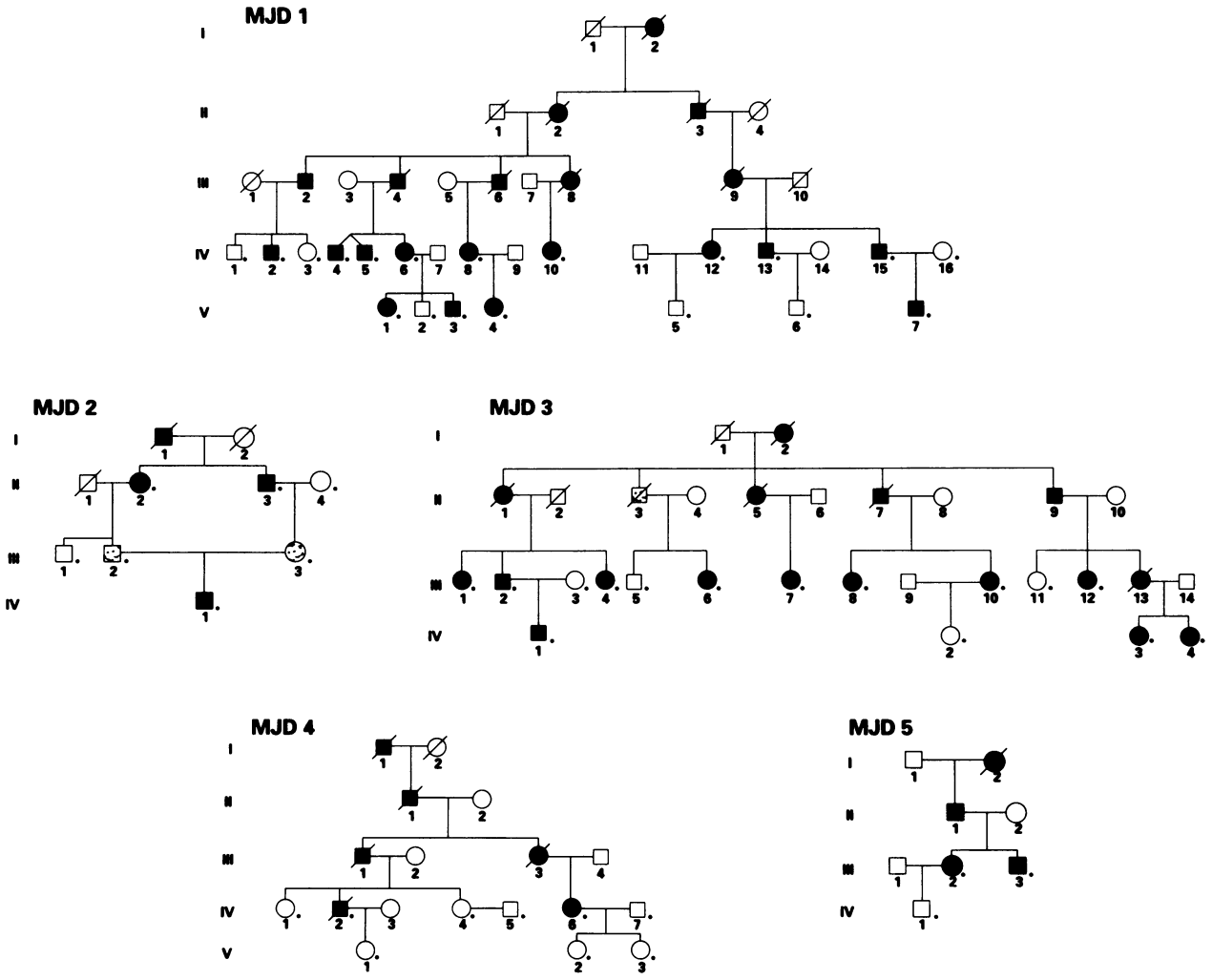

Figure 1 Pedigree diagrams for MJD pedigrees. ■ = Affected male; O = asymptomatic transmitting female parent; and □ = asymptomatic male. Marbled symbols represent putative transmitting parents. A black dot beneath a subject indicates that genotype data are available.

Subjects and Methods

Subjects

Members of five pedigrees with MJD were ascertained through referral to clinics at the University of Toronto and at the Hospital de Ponta Delgada (fig. 1). The clinical status of symptomatic and asymptomatic pedigree members was ascertained through direct clinical examination by a qualified neurologist and, in most instances, by the use of ancillary tests (computed tomography, magnetic-resonance-imaging scanning, etc.). Pedigree members, including spouses, were classified as either MJD affected or asymptomatic. The risk for MJD in currently asymptomatic family members was deduced from the subject's age by using an age-at-onset correction (see below). A detailed description of the clinical phenotypes observed in members of the MJD2 pedigree is to be published elsewhere (Lang et al., in press).

Genotyping Studies

Genomic DNA was isolated from buffy-coat leukocytes or from transformed lymphoblast lines as described elsewhere (St George-Hyslop et al. 1992). The genotype of each subject was ascertained for the polymorphic simple-sequence-repeat (SSR) loci D14S53 (Wang and Weber 1992), D14S55 (Wang and Weber 1992), D14S48 (Wang and Weber 1992), D14S67 (Weissenbach et al. 1992), AFM224xb4 (J. Weissenbach, unpublished data), AFM120xc1 (J. Weissenbach, unpublished data), D14S81 (Weissenbach et al. 1992), AACT (Byth and Cox 1993), D14S65 (Weissenbach et al. 1992), and D14S51 (Wang and Weber 1992) by using the published PCR primer sequences for each locus and the methods for PCR amplification and PAGE as described elsewhere (St George-Hyslop et al. 1992). Samples from affected members from different pedigrees and samples from normative Azorean

controls were run on the same gel to ensure that allele scoring was consistent not only between different pedigrees but also between MJD affected and normal subjects who were included in order to assess allele frequencies in the Azorean population.

Statistical Analyses

Pairwise lod score (Z) values were calculated from the observed genotype and phenotype data by using the LINKAGE (version 4.9) package of programs. The initial maximum-likelihood parameters assumed an autosomal dominant pattern of inheritance with age-dependent penetrance in accordance with previously published epidemiologic studies (Barbeau et al. 1984; Sequeiros and Coutinho 1993). An MJD gene frequency of 1/4,000 was used, on the basis of published estimates of the disease frequency in the Azorean island of San Miguel, from which the MJD pedigrees in our data set originated (Sequeiros and Coutinho 1993). Marker-allele frequencies were deduced from unrelated spouses of family members who had no family history of MJD and who were of Azorean origin (n=38). These allele frequencies did not differ significantly from those published for random Caucasian populations. To allow the inclusion of genetic data from currently asymptomatic family members, including spouses, we calculated a curve depicting cumulative age-dependent risk for MJD, using data from our own pedigrees and from the published data of Barbeau et al. (1984) (n=179 subjects). This curve depicts ~2% penetrance at age <15 years, 50% penetrance by age 38 years, and 98% penetrance at age >65 years. All Z values were recalculated using disease phenotype information from only affected individuals and spouses.

Multipoint analyses were conducted using the three most informative loci—D14S67, D14S81, and AACT—and relative genetic map distances based on published linkage maps (Weissenbach et al. 1992), the CHLC report (GDB G00-043-978), and unpublished genetic linkage analyses on CEPH pedigrees (D. W. Cox, unpublished data; J. Weissenbach, unpublished data). To simplify the maximum-likelihood calculations the genotype data were recoded to four alleles by using the method of Braverman (1985).

Results

The initial report of linkage of MJD in five Japanese pedigrees used markers D14S53, D14S55, D14S48, and D14S51. In our pedigrees these markers were not sufficiently informative to permit definitive proof of linkage, although all gave positive Z values at high recombination fraction (θ) values ($Z<+1.5$; $\theta\geq.05$) (table 1). However, testing of five newer SSR markers from this region (D14S67, AFM120xc1, D14S81, AACT, and D14S65) did provide significant cumulative evidence for genetic linkage at D14S81 and AFM224xb4 (table 1). Furthermore, D14S81 generated significant evidence for linkage in a single pedigree (MJD3) (table 1). Positive but nonsignificant scores at higher θ values were also generated at D14S67 and AACT, which flank these markers centromerically and telomerically (table 1). AFM120xcl, however, was uninformative (table 1).

To determine the position of the MJD locus in our pedigrees, relative to the currently available chromosome 14 markers, which have not yet all been placed upon the same linkage map, we first determined the relative physical order of these markers, using a battery of somatic cell hybrids and flow-sorted translocation chromosomes bearing breakpoints in the 14q2-qter region (Beroud et al. 1993; D. W. Cox, G. D. Billingsley, and V. Nguyen, unpublished data). These analyses predict the order to be cen–D14S53–D14S55 – (D14S67 – D14S48 – AFM224xb4) – D14S81 – AACT–D14S65–D14S51–tel but do not unambiguously resolve the order of D14S67, D14S48, and AFM224xb4. We did not position AFM120xc1 on this physical map, because it was uninformative in our MJD pedigrees (two alleles; heterozygosity <50%). However, AFM120xc1 has been placed 3 cM centromeric to D14S81 (J. Weissenbach, unpublished data). In order to carry out multipoint analyses, we next determined that the genetic distances between markers in this region were as follows: D14S53–10 cM–D14S55–2 cM–D14S48/D14S67–15 cM–D14S81–3 cM–AACT/PI–6 cM–D14S65–D14S51 (Weissenbach et al. 1992; CHCL report (GDB G00-043-978); D. W. Cox, G. D. Billingsley, and V. Nguyen, unpublished data; J. Weissenbach, unpublished data). The multipoint analyses were then performed using data from the three most informative loci (D14S67, D14S81, and AACT). To simplify the calculations we did not include data from AFM120xc1 and D14S48, because these markers were uninformative in our pedigrees. Similarly, data from AFM224xb4 were not included, both because its position relative to D14S67 and D14S48 is not proved and because it did not detect any recombinants with MJD and thus would not provide useful relational mapping information. The D14S67-D14S81-AACT-MJD multipoint analysis both confirmed the two-point evidence for linkage of the Azorean form of MJD to chromosome 14q and suggested placement of MJD in the interval between D14S67 and AACT (Z=+7.00 near D14S81), with odds ratios of 1,737:1 against placement centromeric to D14S67 and 41.6:1 against placement telomeric to AACT (fig. 2). Direct inspection of the recombination events in our pedigrees confirms the statistical analyses because (1) D14S67 detects a set of five recombinants that, together with additional recombinants, are also detected by D14S55 and D14S53 (not shown) and (2) AACT detected two different recombinants that were also detected by D14S65 and D14S51 (along with additional recombinants). Repeating these analyses by using penetrance data only for known affected subjects and married-

Table 1

Z Values between MJD and Chromosome 14q Markers

Locus	Pedigree(s)	Z Values at θ = .00	.01	.05	.10	.20	.30	.40
D14S53	All	−∞	−1.39	−.03	.56	.81	.77	.37
D14S55	All	−∞	.20	.93	1.16	1.17	.86	.36
D14S48	All	−1.12	1.03	1.00	.87	.69	.35	.10
D14S67	MJD 1	−∞	−.75	−.12	.13	.22	.20	.07
	MJD 2	.11	.11	.11	.11	.12	.10	.07
	MJD 3	−.67	1.97	1.96	1.80	1.58	1.02	.40
	MJD 4	.60	.53	.45	.37	.29	.15	.04
	MJD 5	.24	.20	.17	.13	.10	.05	.01
	Total	−∞	2.06	2.57	2.54	2.31	1.52	.59
AFM224xb4 ..	MJD 1	1.73	1.48	1.23	.99	.77	.38	.11
	MJD 2	.01	.09	.12	.14	.13	.11	.07
	MJD 3	2.36	2.64	2.50	2.25	1.94	1.21	.46
	MJD 4	.76	.66	.56	.46	.37	.19	.05
	MJD 5	−.08	−.07	−.06	−.05	−.04	−.02	.00
	Total	4.78	4.80	4.35	3.79	3.17	1.87	.69
AFM120xc1 ...	All	.01	.05	.06	.05	.00	.00	.00
D14S81	MJD 1	2.60	2.31	2.02	1.72	1.43	.85	.36
	MJD 2	−.04	.06	.10	.12	.12	.10	.06
	MJD 3	4.13	3.73	3.30	2.87	2.41	1.45	.52
	MJD 4	−.28	−.20	−.14	−.10	−.07	−.03	.00
	MJD 5	−.14	−.11	−.08	−.06	−.04	−.02	.00
	Total	6.27	5.79	5.20	4.55	3.85	2.35	.94
AACT	MJD 1	−∞	.63	.93	.97	.91	.61	.24
	MJD 2	−.37	−.19	−.08	−.01	.04	.07	,06
	MJD 3	2.26	2.03	1.78	1.52	1.24	.70	.25
	MJD 4	−.35	−.23	−.16	−.11	−.08	−.03	−.01
	MJD 5	.17	.14	.11	.09	.07	.03	.01
	Total	−∞	2.38	2.58	2.46	2.18	1.38	.55
D14S65	All	−∞	−2.83	−1.51	−.80	−.48	−.30	−.16
D14S51	All	−∞	−.08	.65	.86	.88	.61	.23

Note.—The probable physical map order of these markers is cen-D14S53-D14S55-(D14S67-D14S48-AFM224xb4)-AFM120xc1-D14S81-AACT-D14S65-D14S51-tel. Individual family-specific Z values are displayed only for those markers rendering significant or suggestive cumulative Z values.

in members caused a modest reduction (<10%) in Z values and did not alter the conclusions of linkage or placement of MJD (Z=+7.00 at D14S81).

The initial report of linkage of MJD to chromosome 14q in Japanese MJD pedigrees had suggested the presence of linkage disequilibrium at D14S42, D14S43, D14S53, D14S48, and D14S51 (Takiyama et al. 1993). In contrast, in the present study we were unable to detect any locus at which all MJD-affected subjects segregated the same allele. Indeed, even at the two loci (D14S81 and AFM224xb4) without apparent recombination events, we observed three different alleles cosegregating with MJD at both loci in the five MJD pedigrees analyzed here.

Finally, in addition to confirming the initial linkage report and improving the precision of the localization of the MJD gene, we were also interested in the mechanisms underlying widely varying clinical phenotypes in this disease. In this regard, we noticed that subject IV-1 in pedigree MJD2 developed MJD at the unusually early age of 16 years and is now severely affected by MJD at age 22 years (fig. 3). Significantly, subject IV-1 is the offspring of a consanguineous union in which both parents (III-2 and III-3) are asymptomatic cousins (current ages 49 years and 46 years, respectively) but whose own parents (II-2 and II-3) are both mildly affected by MJD (both with onset age ~60 years) (fig. 3). In comparison, the usual age at onset in MJD is in the 3d, 4th, and 5th decades (mean±SD 38.4±11.5 years), with only 7% of subjects being penetrant by age 20 years (comparable with the situation in subject IV-1) and with 79% being so by the age of 50 years (comparable with the situation in subjects III-2 and III-3) (Barbeau et al. 1984). The detailed descriptions of the clinical phenotypes

of these subjects will be published elsewhere (Lang et al., in press). Analysis of the extended haplotype between D14S67 and AACT for members of this pedigree revealed that subject IV-1 is homozygous for all markers between and including AFM224xb4 and AACT and has inherited these haplotypes by direct descent from both affected grandparents.

Discussion

Our results confirm the initial report of linkage of MJD to chromosome 14 and provide direct evidence that MJD in Azorean subjects is likely to be the same illness as that observed in Japanese subjects. We cannot tell, from the current results alone, whether the Japanese form of MJD is an allelic but independent MJD mutation or whether the Azorean and Japanese forms arise from the same mutation. The latter possibility exists because MJD, like familial amyloidotic polyneuropathy Andrade type, may have been brought to Japan by Portuguese navigators (Sakaki et al. 1989; Rosenberg 1992). We also cannot formally exclude other, less likely possibilities—e.g., the possibility that two linked MJD genes might exist on chromosome 14 (within an interstitial karyotypic anomaly or perhaps as members of a gene family).

Second, by mapping the MJD locus to an 18-cM region (D14S67-D14S81-AACT) our results improve the precision of the localization of MJD provided in the initial report (Takiyama et al. 1993). However, because the resolution of available genetic maps in this region is still crude, further refinement in positioning of MJD should be possible once additional markers have been isolated, particularly from the 10–15-cM interval between the D14S67/D14S48/AFM224xb4 cluster and AFM120xc1/D14S81.

Both the age at onset and the severity of this disease are quite variable (Barbeau et al. 1984; Rosenberg 1992). A few instances of progressively more severe disease with earlier onset in subsequent generations have been observed (Rosenberg 1992). This phenomenon is most easily explained by "anticipation," a phenomenon that is clearly present in one other dominantly inherited ataxia—namely, spinocerebellar ataxia type 1 (SCA1) (Orr et al. 1993). In SCA1 the molecular defect associated with anticipation is the amplification of an unstable CAG trinucleotide repeat (Orr et al. 1993). It is conceivable that the anticipation observed in MJD may arise from a similar mechanism. However, the observation that the putative MJD homozygote in pedigree MJD2 has a more severe phenotype than do the affected grandparents (and the currently asymptomatic parents) raises the possibility that other subjects with severe disease may also be homozygotes. The existence of disease homozygotes in small population isolates, such as those in the Azores, would not be unexpected. We cannot, of course, exclude the obvious possibility that subject IV-1 in MJD2 both is homozygous for MJD and has an extreme pathologic expansion of a putative trinucleotide repeat, compared with this subject's other affected relatives, who have later ages at onset. However, this dichotomy should be easily resolved by further molecular and phenotypic studies on additional inbred kindreds.

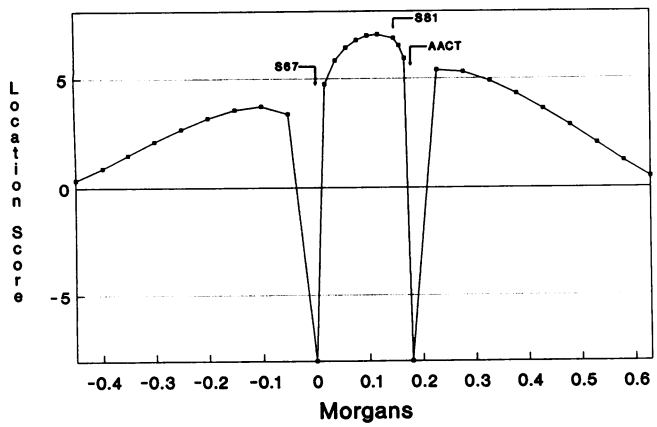

Figure 2 Multipoint analyses using D14S67, D14S81, and AACT

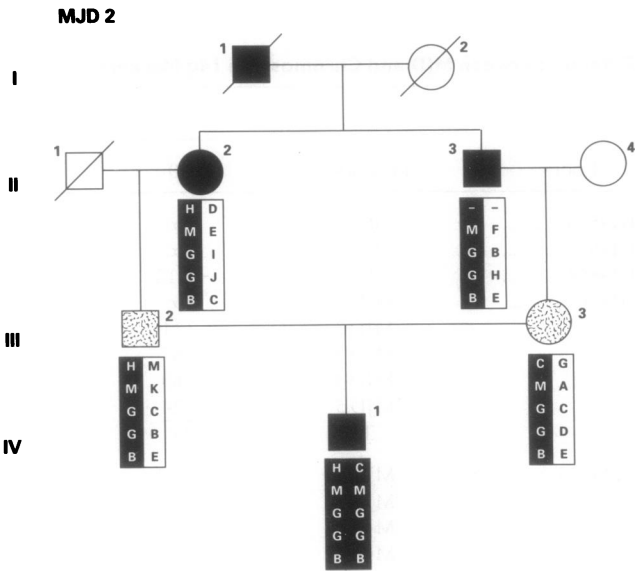

Figure 3 Genotype data at the markers D14S67, AFM224xb4, D14S81, AACT, and D14S65, for members of the MJD2 pedigree. The dark vertical haplotype represents the putative MJD chromosome in this pedigree. Subject IV-1, who is severely affected in comparison with both affected grandparents and with the currently asymptomatic transmitting parents, is homozygous for all markers below D14S67. A probable recombination event has occurred between MJD and D14S67 in this pedigree.

Acknowledgments

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