Review

The Pathogenesis of Machado Joseph Disease: A High Manganese/Low Magnesium Initiated CAG Expansion Mutation in Susceptible Genotypes?

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Key words: Machado Joseph disease, manganese, magnesium, CAG, mutation, genotype, pathogenesis

The origin of the progressive spinocerebellar ataxic disorder 'Machado Joseph Disease (MJD)' has been attributed solely to an expansion mutation resulting from an autosomal dominant inheritance of an unstable CAG repeat in chromosome 14q32.1 of the MJD gene that encodes for the synthesis of ataxin 3. The faulty gene has purportedly been disseminated since the Middle Ages into Azorean, Dutch and Makassan communities by an international trading community based in NE-central Portugal. However, following improvements in MJD surveillance, the MJD afflicted families that have been identified in increasing numbers of familial clusters of MJD being discovered around the world—e.g. in Aboriginal, Yemenite, Asian and Japanese populations—cannot be connected back to the original Portuguese founder families, but rather implicates an environmental factor, superimposed on a genetic flaw. An analytical study of the isolated ecosystems supporting both the Portuguese and non-Portuguese MJD affected communities demonstrates a common abnormal hallmark of high manganese (Mn)/low magnesium (Mg) status, suggesting that this aberrant mineral ratio inactivates the Mn/Mg catalyzed endonuclease 1 enzyme in the biosystems of those who are dependent upon these ecosystems. Endonuclease activity is crucial for protecting against the expansion/contraction of the trinucleotide repeats in the genes that encode for proteins such as Ataxin 3—the 'mutant' chaperone protein that hallmarks the central nervous system (CNS) of MJD sufferers. It is proposed that MJD, and possibly the other more common expansion mutation diseases such as Friedrich's Ataxia and Huntingdon's Chorea, are multifactorial diseases caused by a hitherto unrecognised autosomal dominant inherited failure to regulate Mn/Mg metabolism in populations living in high Mn/low Mg ecosystems. Mg supplementation of the 'at risk' populations during the 'in utero' developmental stages could be all that is required to maintain healthy endonuclease turnover, thereby protecting MJD susceptible genotypes against this fatal, progressive neurodegenerative disease.

INTRODUCTION

Machado Joseph disease (MJD) is a progressive spinocerebellar ataxic disorder belonging to the group of polyglutamine neurodegenerative diseases [1–3]. Inhabitants of a single Aboriginal village, Angurugu, on Groote Eylandt, an island off the NE Australian coast have been known, since the late 1960s, to suffer a high incidence of cerebellar, upper motor neurone, brain stem and oculomotor disturbances that manifest as an overlapping variety of amyotrophic, wasting, ataxic, spastic and ophthalmoplegic clinical presentations, resembling MJD [4–7]. The original theory of causation implicated exposure of the Aboriginal population to exclusively high concentrations of manganese (Mn) in the immediate environment surrounding the disease affected village of Angurugu [5,8–10]. After the initial observation by Cowat in 1990 [11] that MJD ran in specific Aboriginal families, the emphasis—as to the etiology—has focussed on the likelihood of a genetic origin of MJD. The astute identification by Burt et al [12,13] of an MJD type of CAG expansion mutation in the greater majority of these victims, led to attribution solely to an autosomal dominant inheritance of an expansion mutation in the CAG repeat in

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Journal of the American College of Nutrition, Vol. 23, No. 6, 715S–729S (2004)
Published by the American College of Nutrition

chromosome 14q32.1. This implicates repercussions of carrying this mutant form of 'ataxin 3' chaperone protein in the full clinical and pathological spectrum of the MJD type of polyglutamine disease, and rejects a role of environmental factors in the pathogenesis of MJD strain of Groote syndrome. The autosomal dominant inheritance of the MJD faulty gene is supported by the speculation that Macassan sailors of Portuguese descent purportedly had sexual encounters with Aboriginal women while visiting the Australian coastline for the trepang harvest, around four hundred years ago [7]. However, both Aboriginal elders and anthropological investigations [14,15] have rejected any sexual association between the MJD affected Aboriginal clans (who reside in Angurugu) and the Macassan sailors. The visiting sailors had camped on beaches on the opposite side of the island, a district that is more than 30 kilometers distant from the hunter-gatherer territory of the MJD-affected 'Lalara' clan [15] (Fig. 1). The Aboriginal clans whose territories cover the districts visited by the Macassans have remained MJD-free.

The original notion that a single genetic cause, e.g. an autosomal dominant inherited mutation is responsible for development of MJD was founded upon the first recorded observations of clinical neuroscientists who had identified a very high incidence of MJD amongst residents of the Fall River coastal district of Massachusetts. These cases involved Portuguese individuals who had invariably emigrated from the Azores in the mid Atlantic [16], islands whose Portuguese inhabitants were later recognised as suffering from the highest incidence rate of MJD in the world [17–19].

However, contrary to the dictates of the conventional consensus that had been formulated upon these early genealogical observations, many further clusters of familial MJD (Table 1) have subsequently been identified amongst a diverse array of ethnic populations around the world, populations who have had

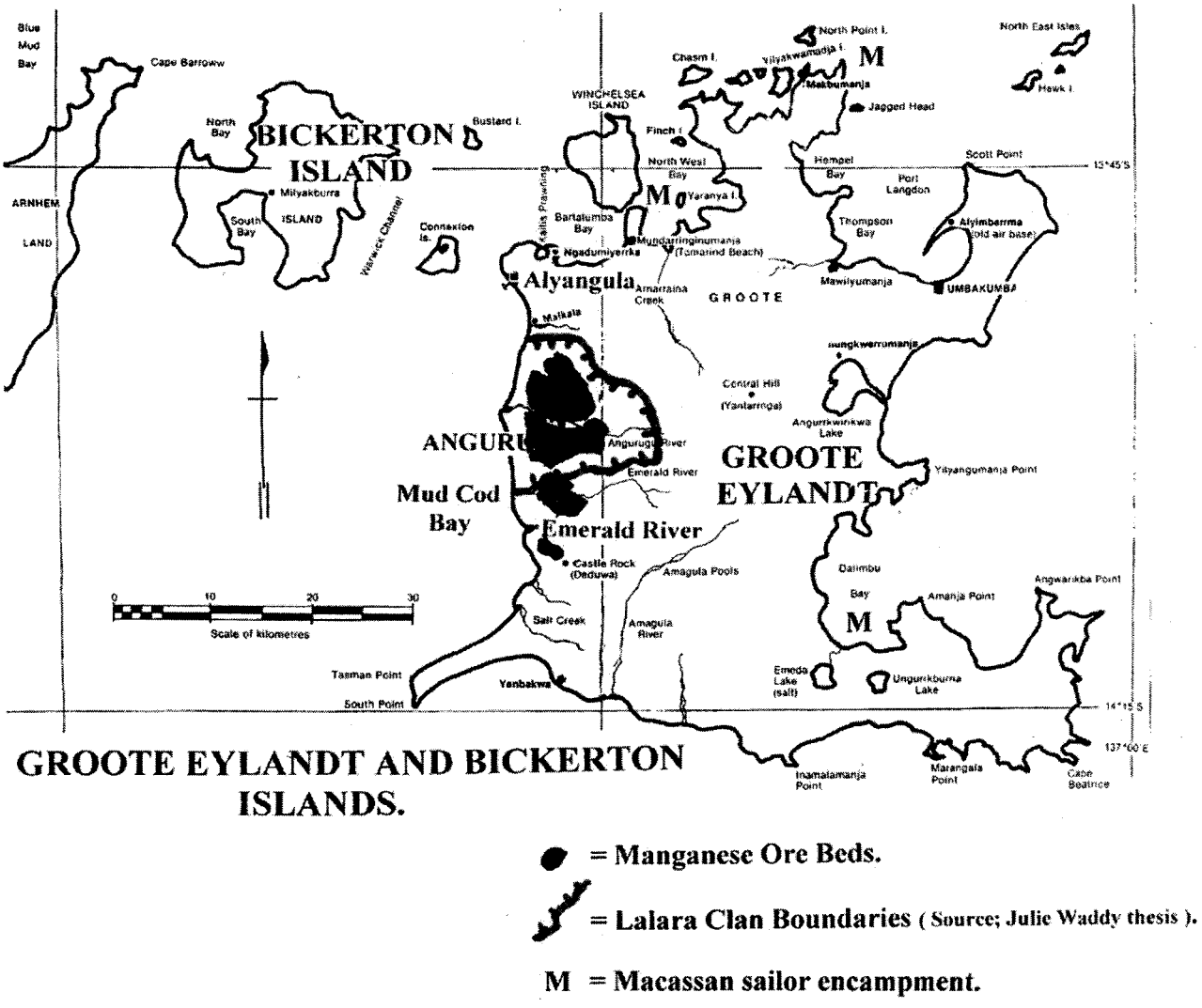

Fig. 1. Map 1.

Table 1. Chronology of the Discoveries of Familial Clusters of Machado-Joseph Disease

1972	Nakado et al	Massachusetts	Azorean
1972	Woods et al	Massachusetts	Azorean
1972	Rosenberg et al	California	Azorean
1978	Coutinho et al	Azores	Azorean
1980	Lima et al	NW Portugal	Portuguese
1980	Healton et al	North Carolina	African
1983	Sakai et al	Japan	Japanese
1984	Sequeiros et al	USA	Italian
1986	Bharuda et al	India	Indian
1986	Yuasa et al	Japan	Japanese
1990	Eto et al	Germany	German
1993	Burt et al	Australia	Aboriginal
1994	Goldburg-Stern	Yemen	Yemenite

no known connection to those Portuguese 'founder' families who had supposedly disseminated the MJD mutation worldwide. For example, MJD clusters appeared in the cited Aboriginal group [7], in Asian Indians [20], Japanese [3,21], in Non Azorean Portuguese [22], in Anglo Saxons [23], in Italian-Americans [24], in Germans [25], in African populations [26], and in a Jewish Yemenite family in Israel, from the recluse mountain community of Taizz [27] (Fig. 2). A wall of steep mountain ridges render this area virtually inaccessible from the Western coastal towns of the Yemen—the settlements where the Portuguese sailors had come to trade. The spatial temporal epidemiology of the MJD familial clusters that have erupted in isolated pockets around the world clearly fails to support the autosomal dominant inheritance hypothesis. Furthermore, some of the non neurological clinical features that have become associated with the early stages of these polyglutamine diseases, such as the aberration in Mg ATP dependent enzymes involved in insulin metabolism [28] (linked to deficient lipoamide or pyruvate dehydrogenase enzyme activity), cannot be attributed to a direct effect of the CAG expansion mutation.

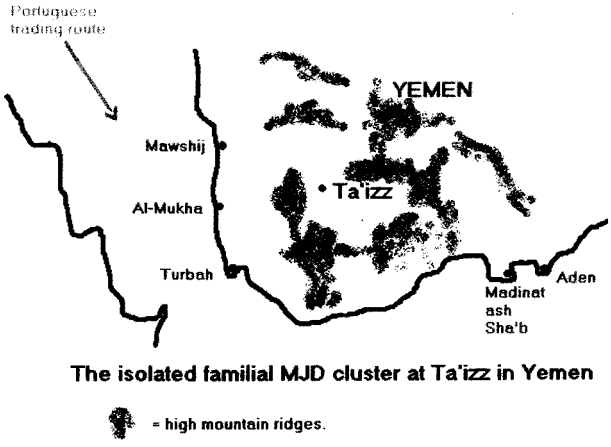

Fig. 2. Map 2.

Despite the fact that neither the genealogy nor the spatio-temporal epidemiology of each new emerging MJD cluster supports the genetic 'single cause' hypothesis, the reductionist paradigm that MJD can only emerge in descendants who are line bred from a founder carrier of an autosomal dominant mutation has continued to gain widespread acceptance.

The debate engendered by the evidence casting doubt on an autosomal dominant inherited mutation, as the single etiologic factor in MJD, suggests that some environmental initiator common to all of these isolated MJD cluster environments could perform a combined eco-genetic role in the etiology of MJD. Presented here is an original theory of causation, that implicates exposure of the Aboriginal population to excessively high concentrations of Mn in the immediate environment surrounding the disease affected village of Angurugu [2–8]. The unique context of the MJD cluster of an unprecedented high incidence rate (approximately 1 in 30) amongst Aboriginal islanders who are unrelated to the MJD susceptible Portuguese family lines and are largely confined to one village, provides an ideal case study for pinpointing the etiological "needle" in the causal "haystack."

This paper examines the origins of the Groote and other clusters of MJD around the world that cannot be solely explained by the conventional genetic theory. It charts the observations amassed from the author's research expeditions to two long standing and prominent MJD cluster locations on Groote Eylandt, N.E. Australia (May 2002) and on the islands of Flores/Sao Miguel/Terceira in the Azores (April 2003), for the purpose of carrying out a comparative environmental analysis of both Portuguese and non-Portuguese MJD affected populations, in the hope of identifying a toxic environmental causal denominator that is common to all MJD cluster locations worldwide. The concept of an environmental initiator as the causal candidate of the MJD expansion mutation has never been considered before.

This study entailed a questionnaire survey of the dietary, occupational and lifestyle status of ten living MJD patients in each cluster location, as well as carrying out analyses of the soils, water and staple foods in the MJD affected ecosystems for the full spectrum of trace elements, and then comparing those results with the analytical results drawn from MJD-free areas occupied by the same ethnic group. The analytical data amassed (Tables 2–4) concerning the Groote Eylandt cluster study confirmed earlier findings of a high Mn/low Mg status [5,8–10] being integral to both the Angurugu ecosystem and the Aboriginal lifestyle. Furthermore, the analytical data amassed from the MJD ecosystems across the Azores [29] (and at Fall River, Massachusetts) (Tables 3–5) revealed the same high level of Mn and low level Mg ecosystem which, in this context, would appear to have derived from the volcanic origins of the local Azorean topsoils—the Azore islands being sited along the mid atlantic tectonic rift line. The levels of Mn are so intense in this region, that seabed mining of Mn nodules has been considered lucrative [29,30]. Other isolated locations

Table 2. Comparative Soil Analyses (22–28 May 2002) between MJD region on Groote Eylandt and MJD-free regions. (Al, Mg, Ca, K, are quoted as weight % oxides, the remainder as ppm)

Sample Location	Al203	Mg0	CaO	K20	Mn	Ba	Sr
Top soil (settlement pre MJD)							
Emerald River	10.35	0.08	0.24	0.13	2373	122	19
Emerald River	8.52	0.06	0.20	0.10	1790	94	16
Top soil (MJD settlement)							
Angurugu Gdns	8.54	0.15	0.07	0.52	84196	1224	258
Angurugu Gdns	8.46	0.22	0.14	0.86	157855	882	361
Angurugu Gdns	8.08	0.24	0.10	1.45	216943	1079	265
Angurugu Gdns	7.35	0.19	0.10	0.93	142596	658	295
Av MJD topsoils	8.36	0.20 (2.50)	0.01 (3.00)	0.94 (2.00)	150397 (400)	961 (250)	952 (150)
Seabed silt							
Mud Cod Bay	0.58	0.69	22.0	0.05	451	48	2187
Bartaluba	0.12	0.42	2.46	1.82	556	106	313
Salt Flats							
Mn Ores							
Mn Pesolites	12.34	0.42	0.14	0.65	227477	9268	1167
Mn dioxide	3.49	0.41	0.13	0.59	323612	12901	1062
Fines							
MJD-free Japan (10 samples)	16.87	2.48	2.97	1.44	874	330	172
MJD-free New Eng (20 samples)	12.13	1.40	1.99	2.20	757	474	98
Average MJD-free	14.50	1.94	2.48	1.57	815	402	135

Table 3. Comparative Soil Analyses (18–27 April 2003) between MJD regions on the Azores, Fall River (USA) and MJD-free regions (Al, Mg, Ca, K are quoted as weight % oxides, the remainder as ppm)

Sample Location	Al203	Mg0	CaO	K20	Mn	Ba	Sr	
Top Soil								
Terra Cha, Terciera	16.19	0.58	1.27	3.00	2051	362	92	garden
Terra Cha, Terciera	15.93	1.41	2.56	2.90	2136	411	167	field
Bretanha, Sao Miguel	17.35	1.51	1.67	3.79	1718	282	158	high field
Bretanha, Sao Miguel	17.39	1.47	1.50	3.38	1803	314	151	garden
Bretanha, Sao Miguel	17.20	1.17	1.46	3.98	1718	236	134	garden
Bretanha, Sao Miguel	17.03	1.79	2.65	3.59	1625	450	267	garden
Ponta Ruiva, Flores	21.01	1.90	3.31	1.23	1393	842	219	garden
Ponta Ruiva, Flores	20.07	0.92	1.19	1.82	1494	2524	242	garden
Ponta Ruiva, Flores	18.90	1.18	2.23	2.68	991	3946	395	yam bed
Santa Cruz, Flores	21.43	1.91	2.50	1.45	1981	676	325	garden
Cedros, Flores	22.12	1.99	1.75	1.77	2345	739	261	garden
Cedros, Flores	20.11	1.84	2.35	0.77	1811	1023	311	yam bed
Ponta Delgada, Flores	21.47	2.37	2.43	1.59	2895	1244	220	yam bed
Ponta Delgada, Flores	19.83	2.32	2.92	1.82	1378	1073	318	garden
Av MJD topsoils	19.00	1.60	2.12	2.41	1810	1009	233	
Fall River, Ma, USA	8.34	0.45	0.73	2.14	1031	590	284	garden
Fall River, Ma, USA	10.52	0.39	0.78	2.13	565	553	358	garden
Subsoil								
Ponta Ruiva, Flores	10.51	12.73	8.62	1.20	1262	279	403	volcanic extrusion
Bretanha, Sao Miguel	15.67	3.37	3.57	3.32	1370	311	274	streambed sedim
Bretanha, Sao Miguel	15.89	0.39	0.66	4.95	1579	69	21	white sandy strata
Bretanha, Sao Miguel	14.43	5.55	9.00	1.75	1339	483	627	volcanic extrusion
Santa Cruz, Flores	14.89	8.13	9.62	0.87	1339	471	598	volcanic ‘pesolite’
Santa Cruz, Flores	15.03	7.59	10.18	0.95	1316	498	597	volcanic extrusion
Santa Cruz, Flores	18.86	6.19	6.79	1.01	1594	794	364	lava bedrock
Santa Cruz, Flores	15.25	8.42	8.00	0.77	1455	439	526	lava
MJD-free Japan	16.87	2.48	2.97	1.44	874	330	172	Av of 10 samples
MJD-free New Eng	12.13	1.40	1.99	2.20	757	474	98	Av of 20 samples
Av MJD-free soils	14.50	1.94	2.48	1.57	815	402	135	

where MJD has ‘sporadically’ emerged in certain families at a high incidence rate, such as across North Central India [20], in the village of Freixo-de-Espada-a-Cinta in North Eastern Portugal [22], in the remote mountain village of Taizz in the Yemen [27] and in the Niigata prefecture of Japan [22] are also regions that lie over geological bedrock formations that are characterized by high levels of Mn and other mineral deposits, as well as low Mg/Ca [31].

Table 4. Comparative Analyses of local Bush Tucker/Vegetation between MJD village on Groote Eylandt and MJD-free regions (May 2002). (All elements quoted as mg/kg except where % w/w is indicated) (samples air dried at 105 degrees C) (mean reference source; NRM Ltd, Coopers Bridge, Bracknell, Berkshire, RG42 6NS, UK)

Samples	Al	Fe	K%	Mg%	Mn	Cu	Ca	Pb	Zn	
yam (MJD)	629	1332	1.2	0.1	1351	16	230	4.9	34	Angurugu
yam (MJD-free)	49	100	0.9	0.1	29	15	783	4.0	19	Bickerton Isle
yam (MJD-free)	6	25	1.1	0.6	22	11	504	3.0	10	Santa Maria
pandanus (MJD)	22	74	1.4	0.1	94	5	1786	5.5	17	Angurugu
pandanus (MJD-free)	29	97	1.8	0.1	70	23	3159	5.0	47	Darwin
Grass (MJD)	52	115	0.6	0.2	430	11	2939	7.1	51	Angurugu
Grass (MJD-free)	67	192	2.0	0.3	88	8	6502	1.8	40	Japan
Grass (MJD-free)	133	323	3.4	0.3	111	25	7400	4.9	68	New Eng
Cycad (MJD)	55	203	3.0	0.3	110	23	2316	7.2	93	Angurugu
Cycad (MJD-free)	30	95	2.7	0.4	60	8	1991	6.3	39	Darwin
Pandanus (MJD)	14	47	1.2	0.8	97	7	1706	2.5	25	Angurugu
Pandanus (MJD-free)	12	31	1.1	0.4	12	12	1893	2.4	22	Darwin
Mean reference	(100)	(200)	(2.0)	(0.37)	(62)	(10)	(4500)	(1.5)	(50)	

Table 5. Comparative Analyses of local Food/Vegetation in MJD clusters on Azores/Fall River, Massachusetts and MJD-free regions (April 2003) (All elements quoted as mg/kg, except where % w/w is indicated) (samples air dried at 105 degrees C)

Samples	Al	Fe	K%	Mg%	Mn	Cu	Ca	Pb	Zn	Ba	Sr	
yam (MJD)	63	136	2.8	0.09	606	3	3715	0.4	16	162	24.6	Ponta Delgada
yam (MJD)	96	70	1.9	0.06	212	4	1422	0.4	7	35	8.5	Bretanha
yam (MJD)	13	39	1.7	0.05	90	3	1749	0.4	6	279	25.3	Ponta Ruiva
yam (MJD)	432	147	1.8	0.08	72	13	1597	0.2	25	239	8.7	Ponta Ruiva
yam (MJD)	27	73	2.0	0.1	549	5	1949	0.3	63	430	22.7	Cedros
Mean yam (MJD)	126	93	2.0	0.07	306	5	2086	0.3	23	229	17.9	
yam (MJD-free)	6	25	1.1	0.06	22	11	504	3.0	10	93	3.8	Santa Maria
yam (MJD-free)	49	100	0.9	0.1	29	15	783	4.0	19	x	x	Bickerton Isle
Araca (MJD)	3	16	1.2	0.16	353	6	6740	0.7	13	29.3	27.6	Ponta Rivia
Grass (MJD)	1009	672	1.7	0.36	346	5	7195	0.9	22	77.3	0	Cedros
Grass (MJD)	51	96	1.9	0.26	353	6	6740	0.7	13	66.1	48.3	Ponta Delgada
Grass (MJD)	74	67	1.1	0.28	426	3	6233	0.5	10	46.1	42.8	Santa Cruz
Grass (MJD)	149	133	3.3	0.28	369	5	5923	0.9	15	9.0	21.7	Bretanha
Grass (MJD)	57	117	3.9	0.21	298	7	7907	2.3	27	9.8	26.2	Terra Cha
Alfalfa (MJD)	72	169	3.3	0.24	345	7	19566	1.1	36	8.1	66.6	Terra Cha
Grass (MJD)	244	474	1.9	0.16	270	16	5660	3.8	126	37.8	25.2	Fall River
Grass (MJD)	55	148	3.6	0.19	31	17	6193	9.5	177	48.1	38.1	Fall River
Grass (MJD)	185	140	5.1	0.09	1765	8	4425	2.2	124	12.4	11.7	Fall River
Grass (MJD-free)	67	192	2.0	0.3	88	8	6502	1.8	40	x	x	Japan
Grass (MJD-free)	133	323	3.4	0.3	111	25	7400	4.9	68	24.2	16.2	New Eng
Mean reference	(100)	(200)	(2.0)	(0.37)	(62)	(10)	(4500)	(1.5)	(50)	(10)	(20)	

THE WORKING HYPOTHESIS

The proposed theory on the origins of MJD amalgamates the key facets of the two main theories that currently predominate the general consensus on the origins of the MJD cluster amongst the Aboriginees on Groote Eylandt. For the first time, it is suggested that the primary cause of the MJD expansion mutation is initiated by external environmental/dietary factors that bring about an excess of Mn and deficiency of Mg in the biosystem. In this respect, the MJD expansion mutation develops whenever MJD susceptible genotypes are dependent upon environments that are characterized by this abnormal mineral ratio. The genetic susceptibility prerequisite could be exacerbated by the interbreeding amongst the MJD communities, since they are largely located in geographically and socially isolated island/mountain environments. Mg ions are thousands of times more abundant than Mn ions inside normal cells. Whenever the balance of this Mg to Mn ratio is upset, the resulting inactivation of Mn/Mg dependent enzyme groups can cause devastating repercussions for the metabolism of the biosystem. It is well recognised that Mn can readily substitute for Mg binding sites involved in the catalysis of certain key enzymes, and vice versa [32–35]. Efficient catalysis of the Mn/Mg dual activated enzyme groups depends upon the successful conjugation of the correct number of Mn and Mg atoms at their Mn- and Mg-specific binding centers. In this respect, the successful catalysis of these enzymes hinges upon the balanced availability of these metals in the surrounding tissues,

which, in turn, depends upon the levels of availability of these metals in the external environment. Such is the case with crucial enzyme groups which preside over DNA structure and function, where Mn can be substituted for Mg in the binding of two ribosomal subunits as well as the binding of messenger RNA to the whole ribosome [36]. In this respect, excesses of Mn can effect genetic recombination in a manner that is not necessarily in the best interests of the organism. The mutagenic capacity of the high Mn/low Mg ratio is being developed as a pharmaceutical means of arresting reverse transcriptase activity—the enzyme whose activity fuels the pathogenic multireplicating chain reaction that is integral to the AIDS disease process [37]. Endonuclease 1 is one such enzyme whose active centers require a combination of Mg and Mn specific [38] binding for catalysis to proceed. Interestingly, endonuclease 1 is essential for genome integrity in that it protects against the expansion and contraction of the same CAG trinucleotide repeats that are implicated in MJD pathogenesis [38]. Since two Mg ions are required for the successful catalysis of endonuclease 1, it is proposed that the Mn-loaded/Mg-depleted individual fails to activate this enzyme, thereby leading to the development of the expansion mutation of the ataxin 3 protein that has been heralded as the central core of MJD pathogenesis [39]. Successful activation of endonuclease 1 would be further compromised in contexts of environmental Mn contamination which involves the trivalent species of Mn, since Mn ligands on endonuclease 1 specifically require the divalent species of Mn for effective activation, whereas trivalent Mn would fail.

It should also be noted that some very high levels of barium and strontium were also consistently recorded in both the ores, soils and foodchains of the Groote Eylandt and the Azorean MJD clusters (Tables 2–5), and both of these reactive alkali earth elements can also act as Mg replacements, and must therefore be considered, along with Mn, as feasible causal candidates in the pathogenesis of MJD.

Interestingly, research into Friedrich's ataxia—a more common class of triplet mutation disorder than MJD—has identified a key role for metals in the pathogenesis of the disease [40]; where the accumulation of iron in the mitochondria of Friedrich's ataxia sufferers (perhaps as a secondary as opposed to primary causal factor) initiates a series of free radical mediated chain reactions—well known for their ability to exert deleterious mutagenic effects on DNA structure [41].

Procedures for Soil, Water and Vegetation Collection/Analyses

Soil Sample Collection/Analysis Method. Each soil sample comprised a 300 g sample drawn from a mix of 20 columns of dry soil bored with a stainless steel auger; each column having been bored at equal spacings along a W shape spanning an area of approximately 5 acres, the area being representative of the region harvested by the MJD or non-MJD affected population under study. Each column was drawn from the top soil to a depth of 6 inches, having taken care to avoid inclusion of root material/surface organic matter and collection of samples near to gateways, roadsides, animal dung, disturbed/excavated or polluted terrain. The 20 columns were collected into a plastic bag, then mixed into an even homogenate, from which a further sample of no more than 300 g was drawn and placed into a small polythene bag, then sealed, labelled and transported to the laboratories at the Department of Geology, Royal Holloway, University of London, Egham Hill, Surrey TW20 0EX, where samples were dried after arriving at the laboratory, in forced air flow cabinets.

The temperature was maintained below 32°C during the 12 hour drying period and the air was constantly dehumidified. The soil samples were then ground to pass a 2 mm mesh using a hammer mill. The mill was flushed between samples using a small portion of the next sample. Each sample was analyzed by standard ICP/MS analytical procedure where Al, Fe, Mg, Ca, Na, K, Ti, P, Mn are quoted as weight per cent oxides and the remainder of elements quoted as ppm.

Vegetation/Bush Tucker Sample Collection/Analysis Method. Each plant tissue sample comprised a 200 g sample representing tissue collected from approximately 10 pickings/diggings taken at equal spacings in a W shape (where possible) across an area of approximately five acres that was representative of the region harvested by the MJD or MJD-free population under study. Samples were picked dry and away from roadsides, gateways, animal manure, polluted or disturbed terrain, whilst care was taken to avoid inclusion of any root, leaf or soil materials that would not normally get ingested following the customary food preparation practises of Angurugu Aborigines or Azorean islanders.

The tissue was packed directly into plastic bags, lightly sealed, labelled accordingly and transported to the laboratories of the Department of Environmental Sciences at Derby University, Kedleston Road, Derby, DE22 1GB, UK.

Each sample was placed in a plastic sieve and thoroughly washed in deionised water. After removal of any roots or soil, the samples were spread evenly on a drying tray and dried in a 90°C oven to constant weight, and then ground by Christy Norris mill, a small portion of the next sample being used to flush the mill, before collection of the ground material. The samples were then prepared for analysis by dry ashing for non volatile elements and wet digestion in aqua/regia for volatile elements (e.g. selenium). Analyses was by standard ICP scan.

Water Sample Collection/Analysis Method. Separate samples of water were collected from the Angurugu public water supply and the natural spas/water supplies of the Azorean populations. Each sample comprised a total of a quarter liter collection of water into a plastic screw-capped, sterile, acid washed polyethylene bottle. Approximately five 50 ml fillings were consecutively drawn from the sampling source at 5 minute intervals; the first being drawn after the supply had been turned on for at least five minutes, so as to flush out any unrepresentative accumulations of metal, sediment, etc, from

the pipeline before the sample was drawn. The samples were filtered through 0.2 or 0.45 um filters and acidified by addition of a drop of acid to the contents of each polyethylene bottle to prevent cations in the water samples bonding to the wall of the bottle, thereby affecting the accuracy of the various cation levels recorded in the final analysis. Sample bottles were sealed and transported to the laboratories of London University (as for soil) and analyzed by the standard ICP analysis.

Results of Field Investigation into the Origins of the MJD Cluster Amongst the Aboriginal Community of Angurugu on Groote Eylandt

Flaws in the Theory That "The Dissemination of an Autosomal Dominant Inherited 'Expansion' Mutation into the Aboriginal Population Underpins the Pathogenesis of MJD." This theory proposes that an autosomal dominant inheritance of an expanded CAG repeat on chromosome 14q32.1 was introduced into Aboriginal women by Portuguese Macassan sailors who visited Groote Eylandt each rainy season from the early 1600s up until the end of the 1800s [13]. However, both the Aboriginal elders on Groote and the genealogical investigation of anthropologist Peter Spillett [14] have rejected the possibility of any interrelationships between visiting Macassan sailors and the Aboriginal women of the specific clans who are currently affected with MJD. Furthermore, the actual proponents of this theory have failed to identify any sources which provide evidence of Portuguese Macassan interbreeding with the affected Aboriginal kindreds [13]. Furthermore, no cases of MJD have been identified in the Portuguese communities of Indonesia (Macassar, Timor, Jakarta, Flores) from where the MJD gene had supposedly originated [13].

The timing of the Macassan visits between 100 and 400 years ago in relation to the putative introduction of the MJD gene into the Aboriginal community during that period fails to explain why the first case of the MJD strain of Groote syndrome did not emerge until 1968. The clinical manifestation of autosomal dominant mutations have not been associated with such a protracted period of delayed expression before—clinical manifestation of this type of mutation will usually erupt in the first or second generation following the initial introduction of the faulty gene.

Further flaws need to be addressed; why does MJD incidence remain confined to individuals from two clans who were all born into the Aboriginal community of Angurugu (900 head of population) when the hypothetical interrelationships between Aboriginal women and Macassan sailors had occurred along a significant stretch of the Northern Australian coastline? [11,14,42].

Furthermore, why has Groote syndrome largely remained confined to the kindred of a single clan (the Lalara clan) whose traditional hunter gatherer territories [15] are confined to the Mn-rich mid Western shores of the island, when all of the three established encampments of Macassan sailors on the Groote shoreline were located over 30 kilometers away on the far Eastern and Northern shores of the island [11] (Fig. 1).

The MJD type expansion mutation has not been identified in all of those Aboriginal individuals whose clinical presentation has indicated a diagnosis of the MJD type of 'Groote Syndrome' [12]. In this respect, the autosomal dominant theory of MJD causation does not fulfill Koch's postulates.

The author's survey also identified the case of a Caucasian woman who had worked in the Mn sampling laboratory of the mine for several years, until she died of an undefined 'inherited' neurodegenerative wasting condition (personal communication; Groote Eylandt Branch of the Federal Miners Union; May 2002).

Evidence in Support of the Theory That a High Mn/Low Mg Induced 'Expansion' Mutation Underpins the Pathogenesis of MJD.

1. High Mn Prerequisite. The first definite cases of the MJD strain of Groote syndrome erupted during the late 1960s, with the death of the first victim in 1968 [4,5]. There is a spatio-temporal correlation between the emergence and geographical distribution of Groote syndrome and an introduction of the affected population to two significant avenues of excess Mn exposure. The correlation is based upon the assumption of a 'delayed lag', period between exposure and onset of symptoms, given that the classic Mn neuropsychiatric intoxication syndrome invariably presents as a delayed response to the initial Mn exposure event—often emerging years later [33,34,43,44]. This 'delayed lag' period exists between the emergence of Groote syndrome in Angurugu during the late 1960s and the relocation of the Aboriginal community by Missionaries in 1942 from the relatively lower Mn district of the Emerald River Mission [45] (Av; 2081 ppm Mn in soils, Table 2) to the excessively high Mn ecosystem of Angurugu (Av; 150,397 ppm) where the Aboriginees have lived until present day. Furthermore, after 1965 the district immediately surrounding Angurugu developed into a fully operational open cast Mn mine [42]. Angurugu is unique in respect of the fact that its wooden dwellings are actually sited directly on top of the Mn ore bedrock which is exposed at the surface in several locations [9].

The author's questionnaire disclosed that all ten victims who were interviewed had originated from Angurugu—indicating that they had all been chronically challenged by the excessive levels of Mn in that environment, particularly during the vulnerable 'in utero' period, when the embryo has no blood brain barrier protection to regulate CNS uptake of the metal at such an early developmental stage [34]. Furthermore, the survey confirmed that all ten victims had consumed vegetables cultivated in the Mission gardens during their early life. Various analyses programs have consistently shown levels of Mn in the vegetables and soil of the former Mission gardens as

excessive [9,10,45]. The author's analytical data confirms these early findings (Tables 2, 4).

In addition, all MJD victims had also been exposed to a staple dietary intake of locally grown 'bush tucker'; involving yams, pandanus, bush plums and cycad, along with bats and wallaby which also consume cycad, crabs, turtles, shellfish, etc, as 75% of their total early life diet (NB; at a developmental stage when blood/brain barrier function is immature and uptake of Mn into the brain is consequently unregulated [34]. Much of the bushtucker was prepared by cooking in make-shift ovens where the food is covered in soil as part of the customary cooking practise [42]; thereby compounding the potential intensity of Mn intake in these Aboriginal people. All bushtucker consumed was invariably grown from the high Mn soils and seabed areas around Angurugu (Table 2). In particular, the locally grown yams have been shown to concentrate excessive levels of Mn approaching 1000 mg/kg [8–10]. The results of my own analyses also demonstrated excessive concentrations of Mn at 1351 mg/kg in the Angurugu yams, whilst the yams grown on the neighbouring MJD-free Bickerton island yielded forty five fold less at 29 mg/kg Mn (Table 4).

The spatio-temporal epidemiology of MJD incidence can also be correlated with the advent of opencast Mn mining operations in the vicinity around Angurugu, which acts as an additional compounding factor in respect of the intensity of Mn exposure in the local Aboriginal population.

From the 1950s, the Angurugu Aboriginees had been engaged in prospecting the Mn deposits around the area on behalf of the Church Missionary Society [42], but it was not until 1962 that the mining corporation arrived on Groote to start up their own exploratory drilling; whereupon full scale mining operations were instigated in 1965. Interestingly, the first MJD victims had all been employed in the excavation and crushing of the Mn ore at the mine [46], but the author's more recent survey revealed that only 4 out of the ten MJD victims interviewed had been employed in the mine. After the advent of mining operations, Angurugu became host to a constant source of airborne Mn contamination, where the cyclonic winds that are characteristic of the Groote climatic conditions had blown the black Mn dioxide dust from the nearby storage and tailings heaps of the mine into the village [8–11]. Mine workers were naturally exposed to high levels of the dust, although the officially commissioned health and Safety reports confirm that the levels were within 'safety' limits in most, but not all, areas of the workspace [46–48]. Exposure to airborne Mn in steel mill workers, welders and others has been shown to present significant, progressive neuropsychiatric consequences [33,34,43,44,49,50] due to the well recognized inhalatory route of Mn absorption via the nasal-olfactory tract directly into the brain [51]. Two out of three Aboriginal brothers had worked in the sampling mill of the Mn mine without masks, and, interestingly, these two brothers who had worked in the mine had both developed Groote syndrome; the one who wasn't employed by the mine remained disease free [46]. Blood tests had revealed very high concentrations of Mn above 600–700 nmol/L in the neurologically affected Aboriginees, while disease-free Aboriginees and whites showed much lower levels of 150–200 nmol/L in their blood [10,46].

The 'Lalara clan' comprises the sector of the Angurugu population that have suffered 95% of the total MJD cases of Groote syndrome to date. Interestingly, when the map depicting the traditional boundaries of the former tribal territories of the various Groote Aboriginal clans [15] is superimposed over the map depicting the distribution of Mn ore beds on Groote [52], it is clearly illustrated that the traditional hunter gatherer territories of the Lalara clan engulfs approximately 85% of the Mn rich bedrock region (Fig. 1), yet in the Umbakumba vicinity on the opposite Eastern side of the island, where soil Mn has consistently recorded low at 0.002 Mn % dry weight [9,10,45], there is a zero incidence of MJD syndrome amongst the Aboriginal clans who have always roamed those territories.

Other potential sources of Mn exposure were addressed by the survey, such as use of black Mn dioxide in the Aboriginal rock art [42], however, only one out of the ten victims questioned had painted with Mn dioxide.

Two other alkali earth metals, barium (Ba) and strontium (Sr), were also found at unusually high levels of 961 and 952 in the Angurugu garden soils respectively, in relation to the levels of 402 and 135 found in MJD-free areas (Table 2). The levels recorded in samples of the Mn ores were excessive—Ba at 9268/12901 ppm and Sr at 1167/1062 ppm (Table 2).

2. Low Magnesium Prerequisite. Levels of Mg are notoriously very low in the Angurugu ecosystem [8,11], and the author's water, soil and bush tucker analytical data (av Mg in water: 0.6 ppm in solution; av. Mg in soil: 0.20 as % MgO; av Mg in bush tucker such as yams: 0.10% w/w) confirmed these earlier observations over the low Mg status in Angurugu (Tables 2–5).

Furthermore, the survey of the ten Aboriginal victims (Table 6) revealed an excessive lifetime use of culinary salt in all

Table 6. Toxic Common Denominators of Machado-Joseph Disease Cluster Ecosystems

Cluster	Angurugu	Flores/Sao Miguel
Race	Aboriginal	Portuguese
High Mn	Soil/Mine Dust	Soil/Volcano
(Mg Substitute)	Yams in Diet	Yams in Diet
Low Mg	Soil/Local Foods	Soil/Local Foods
	Excess Salt	Excess Salt/High Soil K
	Alcohol	Alcohol
	Acoustic Stress	Acoustic Stress
High Ba/Sr (Mg substitutes)	Soil/Local Foods	Soil/Local Foods
Blood-brain	Mine Explosives	Earthquake
Barrier cells	Electric Storms	Tectonic Shocks
Impaired	Airport Radar	Military Radar
	House Pesticides	Crop Pesticides

cases, where salt is liberally applied over the top of every meal plate. High salt intakes are recognized to deplete the levels of Mg in the biosystem [53]. Alcoholic consumption has also been shown to deplete Mg levels [54], although only half of the MJD victims (the males) questioned had regularly drunk alcohol—alcoholism being a well recognised social problem amongst the male sectors of the Aboriginal communities. These dietary factors can only serve to exacerbate an already severe deficiency status of Mg in the local ecosystem.

3. Other Compounding Eco-Prerequisites. The survey (Table 6) also revealed other common factors shared by all ten MJD victims that could perform a relevant role in the multifactorial etiology. All victims were commonly exposed to insecticides used for controlling cockroaches and flies. They were also exposed to the low frequency radar beacon sited about 0.5 kilometers away at the adjoining Angurugu airport, as well as to the frequent acoustic shock bursts from nearby mine explosions. All of these factors have been shown to increase permeability of the blood brain barrier [55–57], thereby disturbing the regulation of the uptake of metals such as Mn into the brain [34,35,58], which could considerably exacerbate any potential problem of CNS Mn accumulation that may already exist.

Chronic exposure to acoustic shock challenge has also been shown to deplete the levels of Mg within the CNS, due to the intensive demand on energy which utilizes Mg ATP [57]. The low calcium (Ca) levels of the Angurugu ecosystem is another compounding factor that can potentiate the neurotoxicological effects of the high Mn levels [8,38] (Tables 2, 4). In the biosystem that is compromised by an abnormally high Mn to low Ca ratio, Mn will replace Ca in the nerve synapses, leading to neurotoxic effects.

In respect of the low Ca/Mg eco-characteristics of MJD cluster environments, the questionnaire survey of both the Groote and Azorean MJD victims had picked up on the invariably poor health status of the teeth and bones in MJD sufferers. Many victims had noticeably few teeth remaining in their dentitions—also visible in the younger victims who had contracted the disease during their teenage years.

Field Investigation into the Origins of the MJD Clusters Amongst the Portuguese Communities on the Islands of Flores, Sao Miguel and Terceira in the Azores

The islands of Flores, Sao Miguel and Terceira were selected for this eco-analytical MJD cluster study since they represent the three islands in the Azorean Archipelago which host self sufficient populations that demonstrate some of the highest incidence rates of MJD in the world (Flores at 1:106; Sao Miguel at 1:3148; Terceira at 1:6190 prevalence rate) [15–19] (Fig. 3). Although Graciosa island has an MJD prevalence rate of 1:865, the populations on the five remaining islands remain MJD-free.

The sampling focused upon the ecosystems surrounding the specific villages where the MJD founder families had originated and been dependent for their food supplies; Bretanha/Remedics on Sao Miguel island, Ponta Delgada/Ponta Ruiva/Cedros/Santa Cruz on Flores island, Terra Cha/Pedro on Terceira island (Fig. 3).

Particular attention was focused upon Yam consumption during the food sampling programme. The yams were collected from the MJD affected islands, as well as from the MJD-free island of Santa Maria.

The results of the Azorean facet of this study continued to provide hard evidence in support of the theory that a high Mn/low Mg induced 'expansion' mutation underpins the pathogenesis of MJD. Although the topsoil levels of Mn (Av 1810 ppm) in the MJD affected Azorean ecosystems (Table 3) were considerably less concentrated than what must be the 'world record' levels of Mn recorded in Groote topsoils (150397 ppm), the levels of Mn were still excessive (Av 1810 ppm) in relation to mean reference topsoil level of 815 ppm. Furthermore, it was extremely interesting that the consumption of yams (also recording high average levels of Mn 306 mg/kg; low Mg 0.07%) (Table 5) and Mg-depleting salted food products represented the staple daily diet of the MJD affected Azorean islanders, these same dietary practises having been observed in the MJD affected Aboriginal community on Groote Eylandt (Table 6).

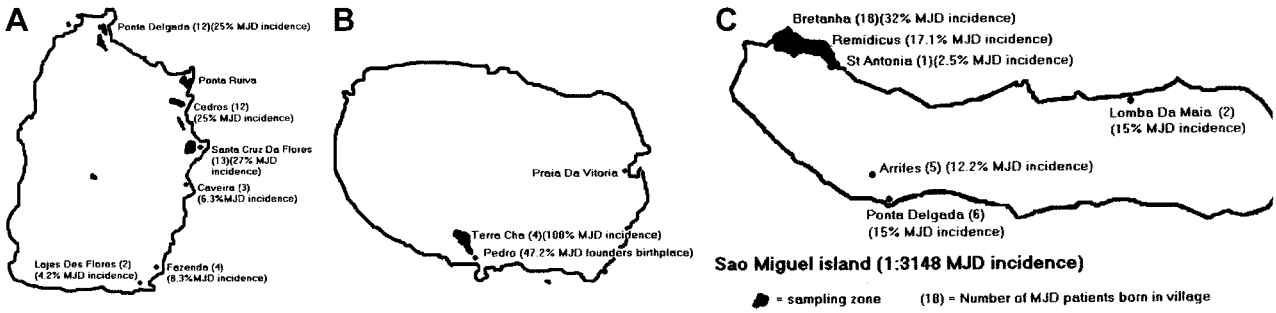

Fig. 3. Maps 3, 4 and 5.

1. High Mn Prerequisite. The volcanic acidic nature of the topsoils on Flores, Terceira probably explained why Mn was consistently high in both the topsoils (1810 ppm) and bedrock samples (1390 ppm) drawn across the Azores (Table 3). It was also interesting that the MJD affected populations tended to reside on specific stretches of the coastline that were closest to the volcanic calderas and lay in the pockets of highest rainfall—explaining the circumstances of enhanced soil acidity and trace mineral leaching that is no doubt responsible for exacerbating the increased availability of Mn as well as the depletion of Mg in the topsoils of these regions. The fact that acid-tolerant hydrangeas tended to predominate in the wild flora of the MJD regions indicates the acidic nature of the soil chemistry caused by the local climatic and other conditions. The local staple foods of the MJD populations were based on yams which produced high mean Mn measurements that averaged out at 306 mg/kg (Table 5) on the MJD affected islands and at 22 mg/kg on the MJD-free Santa Maria isle. It is customary for each Azorean family to maintain their own garden bed of yams which is irrigated by the mountain streams. Other staple foods included meat and milk, sweet potatoes, bananas, pineapples, citrus, corn, pear, grapes, fish and all types of sea food (author's survey 2003).

Much like their MJD counterparts on Groote Eylandt, the questionnaire survey of MJD victims revealed that the local MJD affected Azorean populations had traditionally cooked some foods in 'ovens' burrowed into the earth, drawn their water supplies from the natural 'spas' that were sourced from the volcano caldera lakes and lived in houses that were built out of blocks cut from the volcanic lava beds. In this respect, the self sufficient Azorean islanders had little choice but to naturally furnish every facet of their lifestyles with products that were locally derived from their high Mn ecosystem.

2. Low Mg Prerequisite. Levels of Mg were once again recorded at a below mean level of 1.60% in the topsoils of the Azorean MJD cluster regions, although the levels seemed to be significantly higher in the subsoils (Table 3), probably linked to the presence of a ferromagnesium oxide compound derived from the volcanic ash.

The low Mg in topsoils was well illustrated by the fact that the local farming communities had started to use brands of nitrogen fertilisers that had been automatically fortified with Mg and Ca inclusions; this practise was adopted in order to prevent the formerly common, life threatening metabolic conditions of hypomagnesia and hypocalcemia in their livestock-disorders indicative of Mg and Ca deficiency respectively. Perhaps this practise of fertilizing the local foodchain with Mg may be unwittingly serving as a preventative for MJD in the local human population in the years to come.

The high levels of potassium recorded in soils (Av 2.41%) of the MJD-affected communities (Table 3) could indicate that the high potash factor has induced a 'lock-up' of available Mg in the topsoils of these regions—a well recognized cause of Mg lock up in agricultural soils [59].

Mg was also recorded at very low levels in the staple dietary ingredients such as the yams (0.07 ppm), as well as in the unfertilized pasture grasses grazed by local livestock (Table 5). Similar to the dietary habits of the Groote Aboriginees, use of seafood, culinary salt and food preserved in salt was integral with the dietary customs of the Azorean population (Author's survey 2003). Excessive salt intake impairs the absorption of Mg into the biosystem [53].

3. Other Compounding Eco-Prerequisites. Similar to the Groote MJD ecosystem, there were other environmental prerequisites that must have significantly influenced the Azorean MJD cluster populations (Table 6), such as high input pesticide use on local foodcrops, low frequency sonic shock bursts (from the nearby mid-atlantic rift tectonic earthquake zones and high intensity of thunderstorm activity) and exposure to electromagnetic radiations from the former French military radar station (sited at Ponta Delgada, Flores, that hosts the highest incidence cluster of MJD) since 1966 which was used in conjunction with the French naval vessel "Henri Poincarre" to monitor incoming long range test missile launches that were exploded close to the shores of Flores island.

All of these factors could potentially increase the permeability of the blood brain barrier, thus increasing the rate of uptake/entry of certain metals and their carrier proteins into the brain. The chronic impact of the shock blasts on the biosystem also deplete Mg levels [57].

The MJD Cluster in the Portuguese Populations of the Fall River District of Massachusetts, USA

Samples of vegetation and soil were drawn from the Fall River district, an area where many Azorean emigrants have settled and consequently developed MJD [16]. Levels of Mn were once again high at 688 mg/kg in the vegetation although were normal in the soils (Tables 3, 5), perhaps explained by the contamination of vegetation by the airborne route, via chimney emissions from the high intensity of dye factories, the coal fired power station, refinery, naval docking and other industries operating in this coastal town. Many of the MJD affected Portuguese had actually worked in these dye and clothing fabric factories, and still consumed their customary traditional diet of yams and sea food.

DISCUSSION

The Co-Emergence of Other Types of Neurodegenerative Disease in These High Mn/Low Mg MJD Cluster Environments Suggests That All of These Diseases Could Be Related to a Common Environmental Cause

The close similarities of the MJD and other 'strains' of Groote neurodegenerative syndrome with the established neuropsychiatric profile of classic Mn intoxication (as seen in Mn

miners, steelmill workers, welders, etc) is easily explained according to this hypothesis, although differences exist between the two conditions [5]. However, these variations in the clinical/neuropathological profiles could be explained by the presence of many other idiosyncratic multifactorial prerequisites specific to the context of Groote Eylandt, for instance, prerequisites such as Aboriginal genotype, the valency/magnetic susceptibility/radioactive status of the specific Mn ore [35], routes of Mn absorption, etc [33,51] which would all play a role in dictating the way that the human biosystem reacts to the challenge of high Mn intakes from the external environment; thereby determining the clinical and pathological profile of the specific syndrome that emerges. For instance, some quite extreme differences were observed between the psychiatric perspectives of the clinical profiles of Chilean and Indian Mn intoxicated miners, and these were thought to be linked to the genotype of the exposed individual, mode of Mn exposure and the valency of Mn compounds involved [31].

Furthermore, there are some similarities between the Groote syndromes and the amyotrophic lateral sclerosis, Parkinsonian and Alzheimer-like sufferers in the South Pacific neurodegenerative cluster regions of Guam, Kii Peninsula, West New Quinea [60]—ecosystems which also share the same abnormal high Mn/low Mg mineral template with Groote Eylandt [5,61,62] and the Azores, but the clinical and pathological profiles are not exactly the same [5], nor have any trinucleotide expansion mutations been identified in the South Pacific victims.

Does MJD Syndrome Represent Just One Class of Genetically Dictated Reaction to an Underlying High Mn/Low Mg Imbalance in The Ecosystem?

A wide range of ill defined amyotrophic and ataxic neurodegenerative, psychiatric, teratogenic, mutagenic and 'still birth' complications have erupted in the Angurugu community—often manifesting as a somewhat ill defined, overlapping combination of complications in both the adult and neonatal population of the community [5]. Many of these conditions have been associated with Mn intoxication at some stage in the literature [5,33–35,61–64]. To a lesser extent, there has been overlapping of MJD cases with Alzheimers, Amyotrophic lateral sclerosis and multiple sclerosis incidences in the Azores population. In this respect, it would seem reasonable to propose that the MJD expansion mutation represents just one of several genetically determined modes of reaction to chronic Mn/low Mg exposure—a hitherto unrecognised expression of high Mn/low Mg mediated intoxication.

One study attempted to test the interesting suggestion of an ecogenetic role in the pathogenesis of MJD. The study looked at three groups; MJD affected Aboriginees, their MJD-free relatives and then Aboriginees who were not related to the MJD group in any way. All groups were challenged with the drug dextromethorphan to assess the detoxification enzyme status of the three groups [6]. The results indicated that there were no differences in the levels of enzyme expression across the three groups, but these results told us little in respect of the many other species of detoxification enzyme that are not involved in the specific degradation pathway of dextromethorphan [65], but which are involved in the degradation of many other environmental neurotoxic chemicals.

Although such results have been promoted as a political means of rejecting the role for *any* environmental factor in Groote syndrome, these findings are totally irrelevant in respect of the various different ways in which Mn uptake/transport and excretion may be individually regulated [33–35,58]. A more pertinent study would have drawn comparison between the levels of several detoxification enzyme activities in Caucasian and Aboriginal people.

Another line of argument that is specifically pitched against the Mn causal hypothesis raises an interesting relevant point that requires addressing. Burt states that "although the lesions observed in the MJD Groote syndrome implicates many areas of the brain, it is the substantia nigra that is specifically involved but not the caudate and putamen. However, the opposite is the case in classic Mn toxicity" [6,13]. This point can be addressed by the fact that the neuropathological observations relating to the MJD strain of Groote syndrome and referred to in this comment are only based upon a single autopsy case [13]. Considering the extensive variation of brain lesions observed in Mn induced neuropathology—which are dependent upon duration, intensity and route of Mn exposure, Mn valency, genotype, age of victim and other synergistic variables like iron/Ca/estrogen/Mg status [10,33–35,58] the conclusions for such a statement are very premature. Furthermore, several cases of Mn toxicity have actually revealed extensive neuropathological degeneration in the substantia nigra [66,67] as well as an absence of lesions in the caudate/putamen [68,69].

The Possible Role of High Mn/Low Mg Mineral Imbalance in the Cause of the Excessively Aggressive Behavior That Characterizes the MJD Cluster Communities

There is a unique tendency for extreme excitability and psychotic violence amongst the community of Angurugu; where bouts of unprovoked rage and bizarre murders occur on an almost weekly basis [8–10,64]. Two such murders happened during the author's stay on the island. A report in 1984 by David Biles of the Institute of Criminology in Canberra stated that the rates of imprisonment of young Aboriginal males of Angurugu were many times higher than any other Aboriginal community in Australia [6]. Furthermore the bizarre uninhibited 'psychopathic' nature of the murders is unprecedented amongst the Aboriginal community. Unmotivated aggressive outbursts have also been experienced in some quarters of the Caucasian miners on Groote (personal communications; Groote Eylandt Branch of the Federal Miners Union; May 2002).

Dr. Fabio Medina of the MJD health care centre at Santa Cruz da Flores has confirmed that there is also a tendency towards excessive excitability and violence in the early stage MJD sufferers, as well as amongst the general population on the Azores. Murders are also reported on the islands, perhaps occurring at levels that would exceed the average global rates—given the extremely low population density of the islands. The same levels of extreme aggression can be ascribed to the MJD affected Portuguese communities residing around Fall River in Massachusetts.

The association between elevated brain Mn and aggressive criminal behaviour has been well documented [64]. The term "manganese madness" has been historically ascribed to the insane behaviour of Mn miners in the early stages of Mn neuropsychiatric syndrome [31,33,34,43,44,49,50]. Professor Louis Gottschalk of Psychiatry at California Uni claims "Mn appears to be a marker for violence." Significantly higher levels of Mn have been found in the post mortem brains of death row murderers in relation to brains of normal individuals [71]. The biochemical link with Mn and violence probably stems from the fact that depleted levels of serotonin and loss of serotonin receptors have been identified in the brains of chronic Mn intoxicated animals [72–74]. Furthermore, low levels of Mg in the biosystem has been shown to impair the turn over of serotonin [75]. Low serotonin levels have been associated with violent behaviour [76,77], which could be related to the problem of alcoholism amongst the male population of Angurugu, since alcohol has been shown to invoke aggression in individuals who express low serotonin turn over [78], but alcoholism is no more of a social problem in Angurugu than in any other Aboriginal community [79]. Chronic alcoholism also depletes the levels of Mg in the biosystem [54], which would, in turn, decrease the turnover of serotonin still further [75].

The additional, compounding role of excess Mn as a promoter of the 'insane' nature of the aggression provides a plausible explanation for such a unique cluster of aberrant behaviour in Angurugu.

Can a High Mn/Low Mg Mineral Imbalance Account for All Facets of the Clinical Profile of MJD Polyglutamine Syndrome?

It is suggested that the "all important primary" role of the MJD expansion mutation in the pathogenesis of MJD has been overstated. In respect of Koch's postulates, the fact that a genealogical surveillance program failed to identify the MJD mutation in every single case of provisionally diagnosed MJD [12], suggests that other 'non genetic' eco-influences could be involved in the primary cause of the disease.

In accord with the working hypothesis, the abnormal high Mn/low Mg status of the biosystem could be expected to cause the inactivation of several other specific Mn/Mg catalyzed enzyme systems such as glutamine synthetase [32]. Knock out of glutamine synthetase enables an accumulation of glutamate to neurotoxic levels in the CNS—a mode of excitotoxicity that is well evidenced in the pathogenesis of both MJD and many other classes of amyotrophic/ataxic/polyglutamine neurodegenerative diseases [80,81] that have surfaced on Guam, the Kii peninsula or Groote Eylandt [60]. Mg treatment has been shown to arrest the glutamate mediated neurodegeneration [82].

Interestingly, various conditions stemming from the disordered metabolism of insulin (due to deficiencies in the activities of the pyruvate or lipoamide dehydrogenase enzyme groups [83] have been observed in those suffering from the early stages of the polyglutamine diseases such as MJD.

It is hard to establish any plausible cause-effect association between the CAG expansion mutation and the inactivation of these enzyme systems, but, once again, the proposed causal theory which heralds an underlying Mg deficiency as one of its prerequisites may well account for the disruption of these Mg-ATP dependent enzyme systems [28,78,84] that is a common preclinical complication of these diseases.

High Levels of Alkali Earth Metals/Other Factors in MJD Clusters

It should also be added that the levels of both barium (Ba) and strontium (Sr) were consistently higher in the Groote and Azorean MJD cluster ecosystems in relation to the control region (Tables 2–5). Given the potential of these highly reactive alkali earth metals to affiliate with the types of protein ligand that bind Mg and Ca [85], it is possible that Ba or Sr could replace the Mg vacant domains on endonuclease 1 (eg serving as alternative foreign replacement candidates to Mn), thereby inactivating the capacity of this enzyme to protect against this form of CAG expansion mutation and the pathogenesis of MJD.

The common observation that excess yam consumption had been associated with an unpleasant 'itching' sensation under the skin in all MJD affected cluster communities subjected to the survey, indicates the presence of some hitherto unrecognised form of toxicity (perhaps linked to a natural occurring photosensitiser chemical) resulting from the consumption of yams. Such a chemical could serve as a mutagenic candidate and should also be considered in any further research into potential environmental initiators responsible for the CAG expansion mutation in the pathogenesis of MJD.

CONCLUSION

Laboratory Challenge, Implications for Treatment and Prevention of MJD

This hypothesis could be readily tested 'in vitro' by challenging stem cell cultures that express endonuclease activity with various dose ratios of Mn^{2+}/Mn^{3+} to Mg^{2+} (also running batches of Mg depleted cells that are challenged with Sr and Ba, instead of Mn). Should the hypothesis hold true, then

chelation therapy in the early stages of the disease with a Mn-specific chelating compound, such as EDTA [74], combined with generous Mg supplementation may be sufficient to reactivate endonuclease activity, thereby preventing further CAG expansion mutations from disrupting the pathway of ataxin 3 synthesis. While Mg therapy may help to arrest the MJD disease process, such treatment could obviously never demonstrate the ability to reverse the glutamate mediated neuropathological havoc already reaped by the polyglutamine disease process.

Other positive metabolic benefits could result from the therapeutic correction of the Mn/Mg ratio. For instance, supplementation with Mg compounds that can penetrate the blood brain barrier could reactivate the Mn/Mg glutamine synthetase enzyme, thereby preventing the accumulation of neurotoxic glutamate—another major pathogenic entity that is clearly evident in the MJD disease process. Furthermore, Mg treatment has already been shown to protect brain cells reliably against glutamate NMDA mediated neurodegeneration [82].

Where practically possible, prevention could be achieved by modification of the local environment and diet of the high risk family lines who are known to be susceptible to MJD. In respect to the Aboriginal community at Angurugu, the diet of Mn rich yams could be replaced with yams that have been imported from the low Mn areas nearby—as had been practised in the former pre-MJD times.

Further efforts could be made to curtail the amount of airborne Mn ore fines blowing into Angurugu from the adjoining opencast Mn mine (eg; planting a thicker barrier of 'filtering' trees between the village and the mine workings), however, this problem appears to be gradually abating as the local Mn seams become exhausted and the open cast 'cuts' move further away from the immediate vicinity of the village. One drastic solution is to move the entire village a few miles distant, to an area located off the Mn bedrock. The value of the 'liberated' Mn ore beneath the existing Angurugu village would pay for the construction of a new village several times over!

As a preventative measure for MJD amongst the affected kindred, dietary habits could also be altered; by encouraging Mg rich foods, and, most importantly, adding Mg supplements to the water supplies as well as discouraging excessive salt/alcohol consumption that depletes levels of available Mg in the biosystem [53,54].

One Aboriginal MJD sufferer has started to take Mg citrate supplements at the prescribed daily dose rate since June 2002. His wife and carer have reported dramatic remission of painful muscle spasms/contractions as well as the remission of chronic insomnia and digestive difficulties that directly result from the spasms (personal communication; Jenny Baird, Darwin, Australia).

If the high Mn/low Mg induced MJD causal theory holds true, then it is suggested that straightforward Mg supplementation could be all that is required to maintain the activities of endonuclease 1 and glutamine synthetase; thus preventing the development of both trinucleotide expansion mutations in susceptible genotypes, as well as the accumulation of neurotoxic glutamate in the CNS; thereby preventing the primary initiating event of MJD pathogenesis. Mg supplementation sufficient to maintain optimum Mg levels in the CNS would maintain the balanced activity of these enzymes.

High levels of Mn (at 40 ug/L) have been recorded in the cord blood of an Aboriginal baby whose mother had spent their entire gestation period in the Angurugu environs [8,38]. Although Mn levels were low in the mother's placenta, the high levels in the baby present a very real possibility that the MJD expansion mutation is actually initiated at the vulnerable 'in utero' stages. In this respect, any preventative program ought to ensure that Mg supplementation/Mg fortified drinking water is offered to all woman during their child bearing period in order to provide optimum Mg cover during the 'mutation-sensitive' trimesters of embryonic development.

ACKNOWLEDGMENTS

To Warren Lalara, Jenny Baird, Gayangwa Lalara, Susannah Churchill (nee Cowat), Dennis and Karen Elliot, Kandy Van Cleeve, Brian and Kathy Massey and family of the Anglicare Mission, Murrabuda Warramarrba of the Groote Eylandt MJD cluster, and Maria Oliveirra (Bretanha, Sao Miguel), Dr Fabio Medina, Adriano Nicolau and Maria Lima (Santa Cruz, Flores) of the Azores MJD cluster for their combined assistance with the investigations, questionnaire survey, sample collection, transport, board/lodging and enlightening discussion.

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Received August 5, 2004.