Mutational Origin of Machado-Joseph Disease in the Australian Aboriginal Communities of Groote Eylandt and Yirrkala

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Objective: To determine whether the presence of Machado-Joseph disease (MJD, also spinocerebellar ataxia type 3 [SCA3]) among Australian aborigines was caused by a new mutational event or by the introduction of expanded alleles from other populations.

Design: We sequenced a region of 4 kilobases (kb), encompassing the CAG repeat within the *ATXN3* gene, in 2 affected Australian aboriginal families and compared them with the Joseph and Machado lineages described before. Full-extended haplotypes (including also more distant single-nucleotide polymorphisms and flanking short tandem repeats) were assessed by segregation and allele-specific amplification. A phylogenetic tree was inferred from genetic distances, and age of the Australasian Joseph-derived lineage was estimated.

Setting: The aboriginal communities of Groote Eylandt and Yirrkala, in the Northern Territories, Australia (local ethics institutional permission was granted, and both community and individual informed consent was obtained).

Subjects: A convenience sample of 19 patients and unaffected relatives, from 2 Australian aboriginal families affected with MJD; 40 families with MJD of multiethnic origins and 50 unrelated Asian control subjects.

Results: The 2 aboriginal families shared the same full haplotype, including 20 single-nucleotide polymorphisms: TTGATC**G**AGC-$(CAG)_{Exp}$-CACCCAGCGC, that is, the Joseph lineage with a G variant in rs56268847. Among 33 families with the Joseph lineage, this derived haplotype was found only in 5 of 16 Taiwanese, all 3 Indian, and 1 of 3 Japanese families analyzed.

Conclusion: A related-extended MJD haplotype shared by Australian aborigines and some Asian families (a Joseph-derived lineage) suggests a common ancestor for all, dating back more than 7000 years.

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MACHADO-JOSEPH DISEASE (MJD) is a neurodegenerative disorder of late onset, first described in families of Portuguese-Azorean extraction in the United States.[1-3] Machado-Joseph disease (also known as "spinocerebellar ataxia type 3" [SCA3]) has been reported in many ethnic backgrounds and is the most common SCA worldwide.[4-6] Though clinically pleomorphic, its first symptom is usually gait ataxia, followed by dysarthria, dystonia, pseudobulbar or bulbar dysphonia, and loss of lower limb coordination.[7] A typical feature of MJD is progressive external ophthalmoplegia, characterized by limitation of upward gaze, followed by limitation of horizontal gaze and convergence.

Machado-Joseph disease is caused by the expansion of a $(CAG)_n$ in the *ATXN3* gene.[8] Normal alleles range from 11 to 44, whereas pathogenic expansions range from 61 to 87 CAGs[8-10] (SCAbase; http://www.scabase.eu).

Two major ancestral origins were described[11]: (1) a worldwide-spread haplotype, TTACAC or the Joseph lineage, and (2) a more recent one, GTGGCA or the Machado lineage, seen mostly in Portuguese.[12] They are associated with families with MJD from the Portuguese Azorean islands of Flores and São Miguel (respectively, the birthplace of the Joseph and the Machado kindreds). The Joseph lineage originated in Asia more than 6000 years ago.[12] It can be found in India, Thailand, Cambodia, and Korea, but it achieves a particularly high frequency in some areas of China and in Taiwan and Singapore.[13-15] Independent mutational events cannot be completely discarded, but the hypothesis

Table 1. SNP-Based Haplotypes of Aboriginal Families With MJD Compared With the Joseph (Flores) and Machado (São Miguel) Lineages

SNP[a]	refSNP ID	Distance From the $(CAG)_n$, bp	Machado Lineage	Joseph Lineage	Yirrkala Family	Groote Family
G/T	rs12590497	12 352	G	T	G/T	T
GT$\underline{T}^{527}$/GT$\underline{C}^{527}$	rs16999141	12 306	T	T	T	T
T/G	rs10146519	11 616	T	G	G/T	G
$\underline{A}^{669}$TG/$\underline{G}^{669}$TG	rs1048755	11 505	G	A	G/A	A
C/T	rs12586535	1249	C	T	T	T
T/C	rs12586471	1125	T	C	C	C
A/G	rs56268847	888	A	A	**G**	**G**
G/A	rs10467858	505	G	A	A	A
C/G	rs10467857	463	G	G	G	G
T/C	rs10467856	452	T	C	C	C
$(CAG)_{exp}$						
$\underline{C}^{987}$GG/$\underline{G}^{987}$GG	rs12895357	1	G	C	C	C
TA$\underline{A}^{1118}$/TA$\underline{C}^{1118}$	rs7158733	132	C	A	A	A
C/A	rs3092822	192	A	C	C	C
C/T	rs77086230	1342	C	C	C	C
C/T	rs79316375	1964	C	C	C	C
G/A	rs8004149	2440	G	A	A	A
G/A	rs111735934	2472	A	G	G	G
C/T	NM_001127696.1:c.946 + 2500C>T	2576	C	C	C	C
A/G	rs7142326	2614	G	G	G	G
C/T	rs74071847	2742	C	C	C	C

Abbreviations: bp, base pair; ID, identification; MJD, Machado-Joseph disease; SNP, single-nucleotide polymorphism.
[a] Alleles in the minus strand.

of an expansion-prone haplotype is less likely.[12] The $(CAG)_n$ length correlates with age at onset in MJD (explaining 45%-76% of its variability).[16,17] In the paternal lineage (with higher instability), 72% of expansions were associated with the TTACAC haplotype, whereas GTGGCA corresponded to 75% of all the contractions.[18] It is important, therefore, to determine the haplotype background of MJD mutations in different populations with MJD and assess their ancestral mutational origins.

In the 1960s, a clinical presentation of SCA was reported in Australian aboriginal families at Angurugu, on Groote Eylandt, and at Yirrkala, on northeastern Arnhem Land, Northern Territories.[19] The molecular diagnosis of MJD was confirmed in 1996.[20] The possibility of a Portuguese ancestry (via the Macassan people of Indonesia) was raised to explain this finding.

The initial human settlement of Oceania dates from 44 000 years ago,[21] but the estimation of a genetic coalescent for Australian aborigines ranges from 60 000 to 119 000 years ago, based on mitochondrial genome variation.[22] The presence of aboriginal people in Arnhem Land is ancient, preceding recorded history, since archaeological evidence supports continuous occupation by these communities for many thousand years,[20] far away from the estimated origin of MJD in Asia.[12] A later introduction of MJD in these aboriginal communities may have occurred (1) through recent trading relationships, for example, during the 16th and 17th centuries, with the Macassan people of Indonesia, who had a partial Portuguese ancestry,[20] or (2) directly from China or other Asian communities, in which the presence of MJD may be older.[12] Alternatively, (3) this might be an independent mutational event, which arose among the aborigines of the Northern Territories, in Australia.

METHODS

MJD FAMILIES

We studied a total of 42 families with MJD from the following countries or areas: Taiwan (n=16), United States (n=5), Portugal (n=3), Japan (n=3), India (n=3), West Indies (n=2), French Guyana (n=1), Germany (n=1), Belgium (n=1), Spain (n=1), Morocco (n=1), Algeria (n=1), Ghana (n=1), and unknown origin (n=1), included in former studies,[11,12] and 2 Australian aborigine families (Groote Eylandt [Angurugu; n=1]) and northeast Arnhem Land [Yirrkala; n=1]).

This study project was approved by the Human Research Ethics Committee–Concord Repatriation General Hospital Zone of the Sydney South–West Area Health Service and by Human Research Ethics Committee of the Northern Territories Department of Health and Community Services and the Menzies School of Health Research. Collective community consent was obtained and individual participants were informed about the research purpose and gave their written consent. All DNA samples were labeled with a numeric code only, to ensure confidentiality of the results.

MJD LINEAGES–GENOTYPING OF A 4-KB REGION ENCOMPASSING THE $(CAG)_n$

Machado-Joseph disease lineages of these families were assessed as in a previous study,[12] genotyping 6 intragenic markers: rs12590497, GT$\underline{T}^{527}$/GT$\underline{C}^{527}$, $\underline{A}^{669}$TG/$\underline{G}^{669}$TG, $\underline{C}^{987}$GG/$\underline{G}^{987}$GG, TA$\underline{A}^{1118}$/TA$\underline{C}^{1118}$, and rs3092822 (**Table 1**). In addition, we identified and genotyped allelic variants along a 4-kb region flanking the MJD expansion. The amplification of 8 small fragments, not including the $(CAG)_n$ tract, allowed equal amplification of both the normal and expanded chromosomes (unbiased given the amplicon length). Reactions were performed using the following primers and annealing conditions: MJDcloF-

MJDclo303R (58°C for 45 seconds); MJDclo269F-MJDclo653R (61°C for 30 seconds); MJDclo716F-MJDclo1200R (61°C for 30 seconds); MJDclo1342F-MJD7 (60°C for 45 seconds); MJDclo1565F-MJDclo2129R (57°C for 30 seconds); MJDclo2552F-MJDclo2706R (61°C for 30 seconds); MJDclo2942F-MJDclo3438R (58°C for 45 seconds); and MJDclo3417F-MJDcloR (61°C for 30 seconds). Amplification reactions were done in a total volume of 12.5 µL, with 0.4 µM of each primer, 200 µM deoxyribonucleotide triphosphates (dNTPs), 1 mM magnesium chloride, 20 mM ammonium sulfate, and 1 U of Taq polymerase (Fermentas; Thermo Fisher Scientific). Polymerase chain reaction products were sequenced using the Big Dye Terminator Cycle kit (Applied Biosystems) and ran in an ABI3100 sequencer (Applied Biosystems), after purification with high-resolution, cross-linked dextran gel filtration columns (Sephadex; GE Healthcare Life Sciences).

DETERMINING PHASE FOR CORE HAPLOTYPES

Phase of single-nucleotide polymorphisms (SNPs) on expanded chromosomes was assessed by segregation, whenever DNA was available from enough relatives. Alternatively, 2 polymerase chain reaction amplifications were done, both encompassing the CAG repeat: one covering the upstream (1256–base pairs [bp]) and the other the downstream (2791-bp) sequences, in *ATXN3*. Amplification was performed using primers MJDcloF and MJD7, and MJD52R and MJDcloR. The reaction was carried out in a total volume of 50 µL, with 0.4 µM of each primer, 200 µM dNTPs, 1 mM $MgCl_2$, 20 mM ammonium sulfate, 1.84% dimethyl sulfoxide, and 1 U of Taq polymerase (BIORON GmbH). Hot-start amplification included an initial denaturation step at 93°C for 3 minutes, followed by 35 cycles, consisting of 92°C for 10 seconds, 60°C for 30 seconds, and 68°C for 1.5 minutes, with increments of 20 seconds per cycle in the last 22 cycles; a final extension of 20 minutes, at 68°C, ended the reaction. Owing to the size of expanded alleles, both reactions amplified exclusively the normal alleles in each patient's sample. Products were submitted to electrophoresis, at 4°C, in a $T_{12}C_5$ polyacrylamide gel, and visualized by silver staining, to confirm specific amplifications. Through direct sequencing, we assessed the phase of normal alleles and could infer phase in expanded chromosomes.

FLANKING (SHORT TANDEM REPEATS [STR]–BASED) HAPLOTYPES AND MUTATIONAL AGE

Next, we genotyped more distant, fast-evolving, polymorphic markers: 4 microsatellites—$(TAT)_n$, $(CA)_n$, $(AC)_n$, and $(GT)_n$—found to be 223-kb and 191-kb upstream, and 21-kb and 190-kb downstream the $(CAG)_n$.[23] Haplotypes were assessed by segregation in the families. We estimated the age of the Joseph-derived lineage based on variation accumulated in these flanking regions, as previously.[12] The probability of change per generation (ε) was given by $\varepsilon = \{1-[(1-c)(1-\mu)]\}$, and the average of mutation/recombination events (λ) equalled εt, where t was the number of generations.

RESULTS

MJD LINEAGES OF THE ABORIGINAL FAMILIES

Both families (Groote Eylandt and Yirrkala) shared the TTACAC (Joseph) lineage that was found in 193 families with MJD from 20 different populations previously studied.[12] These SNPs spread through an approximately 13-kb region encompassing the $(CAG)_n$; this is within a single recombination block in both European and Asian populations (HapMap database; http://hapmap.ncbi.nlm.nih.gov).

Because recombination in this interval is unlikely, we verified whether (1) the 6 previously selected SNPs were powerful enough to discriminate all MJD lineages, or (2) genotyping of additional SNPs within the block would allow identification of new Joseph sublineages. In a region of 4 kb around the CAG expansion, aboriginal and other families with the Joseph core haplotype shared 19 alleles among 20 SNPs genotyped (Table 1).

The full extended haplotype—TTGATC***G***AGC-Exp-CACCCAGCGC—found in the aborigine families (ie, the Joseph lineage with a ***G*** variant in the rs56268847) was also observed only in 9 Asian families with MJD, from Taiwan (n = 5), India (n = 3), and Japan (n = 1).

To evaluate the probability of a de novo expansion in the haplotypic background with the G variant of rs56268847, we analyzed this SNP in patients' normal chromosomes and in noncarrier relatives from MJD families. The G allele was found in only 3 of 60 normal chromosomes analyzed, all from the TTACAC background (total GTGGCA:TTACAC was 0.63:0.37). To estimate the frequency of the G allele, we genotyped 50 additional healthy individuals of Chinese origin. The rs56268847 SNP was in Hardy-Weinberg equilibrium ($P < .99$), presenting low gene diversity (0.12), with the G allele found only in 6 heterozygotes.

The MJD aboriginal haplotype varies from the previously reported Joseph lineage only by this SNP; therefore, a mutation in SNP rs56268847 in the TTGATCAAGC-CACCCAGCGC ancestral background seems more likely than an independent MJD mutational event to explain this Australasian Joseph-derived haplotype.

EXTENDED STR HAPLOTYPES

In the search for more variation around the $(CAG)_n$ repeat that could give us some clue on the introduction of MJD in the aboriginal communities, we analyzed 4, more distant, flanking STRs. The haplotype in *cis* with the expansion was H6:10-24-Exp-12-19 in the Yirrkala family and H9:17-22-Exp-13-19 in the Groote Eylandt family.

Downstream haplotypes were a single step away from each other, whereas upstream STR backgrounds of both families can hardly be explained by single mutation steps; however, a recombination between STR1 and STR2 is likely to have occurred since (1) STR1 and STR2 are located, both in European and Asian populations, in different haplotype blocks (HapMap; http://hapmap.ncbi.nlm.nih.gov); and (2) allele 16 from STR1 was the second most frequent (0.26) in Asian controls, after allele 10 (0.38).[23]

Although we have not found the haplotype resulting from that putative recombination event (16-23-Exp-12-19) in our samples, this seems to be an intermediate haplotype from which H9 of the Groote Eylandt family originated, together with the other 3 haplotypes (H8, H10, and H11) (**Table 2**). When allelic phase was unavailable, we considered the observed intermediate haplotypes and allele/haplotype frequency in Asian controls.[23] Allele 12 of STR3 and allele 19 of STR4 were the

Table 2. Haplotypes of 4 STR Markers Flanking the $(CAG)_n$ at the *ATXN3 Locus*, From Families Sharing the TTGATCGAGC-Exp-CACCCAGCGC Haplotype (the Joseph Lineage With a G variant in the rs56268847 SNP)

Family's Origin	Haplotype	STR1[a]	STR2[a]	STR3[a]	STR4[a]	No. of Families	No. of Steps	No. of Families × No. of Steps
Taiwan	H1[b]	10	22	12/13	19	1	0	0
Japan	H2	10	22	12	16	1	1	1
India	H3	11/16	21/22	12	19	1	1	1
India	H4	11/15	23/26	12/13	19	1	2	2
Taiwan	H5	11	21/23	12	16	1	3	3
Yirrkala	H6	10	24	12	19	1	2	2
India	H7	10	23	18	19	1	2	2
Taiwan	H8	16	23/25	12/14	15	1	4	4
Groote	H9	17	22	13	19	1	5	5
Taiwan	H10	16	24	14	15/19	1	5	5
Taiwan	H11	14	22	13	19	1	8	8

Abbreviations: SNP, single-nucleotide polymorphism; STR, short tandem repeat.
[a] Alleles underlined are those more likely to segregate with the expansion, by assuming the least number of mutation and recombination steps to explain the observed diversity.
[b] H1 was assumed to be the ancestral haplotype, since a minimum number of mutations and recombination events were required to construct a phylogenetic network.

Table 3. Genetic Distances Among the 11 Families With MJD Carrying the Australasian Joseph-Derived Lineage and the Other MJD Populations Where Founder Haplotypes of the Joseph Lineage Have Been Introduced

Location	Portugal	France	Germany	USA	Brazil	Japan	Joseph-Derived Lineage
Portugal	0						
France	0.003	0					
Germany	0	0	0				
USA	0.082	0	0	0			
Brazil	0.005	0	0	0.048	0		
Japan	0.551[a]	0.404[a]	0.363[a]	0.399[a]	0.532[a]	0	
Joseph-derived lineage	0.631[a]	0.406[a]	0.363[a]	0.464[a]	0.592[a]	0.065	0

Abbreviations: MJD, Machado-Joseph disease.
[a] Statistically significant ($P<.05$); genetic distances matrix was estimated with the Arlequin software, version 3.5.[20] The sum of squared size differences (R_{ST}) was applied as the distance method among STR haplotypes, to consider the number of presumed single-step mutations between alleles.

most frequent among families carrying the Joseph-derived lineage. As for STR3, all expanded haplotypes can be explained by only 1 mutational step, except H10: 16-24-Exp-14-15/19 (a Taiwanese family) and H7: 10-23-Exp-18-19 (an Indian family). As for STR4, only 3 families (1 Japanese and 2 Taiwanese) harbored alleles differing from the ancestral 19 (3 or 4 steps away); however, all 3 seemed to share the ancestral allele 12 of STR3, suggesting a common origin for all. More diversity was found in the upstream haplotypes; nevertheless, allele 22 of STR2 (the most frequent) suggested it may be the allele in the ancestral haplotype from which the Joseph-derived lineage emerged. For the most distant STR1, it is difficult to speculate, although allele 10 was the most frequent. An interpopulational pairwise analysis based on these flanking STRs showed that the Joseph-derived families are phylogenetically closer to Japanese families than to any other occidental MJD population (**Table 3**).[24]

AGE ESTIMATION

We aimed at dating the recurrent mutation on rs56268847 in the Joseph lineage; nevertheless, accurate age is difficult to estimate with such a small number of families. Therefore, we intended mainly to compare the expansion time of this Joseph-derived sublineage to that of the Joseph lineage and its introduction in several populations (previously estimated). We analyzed the diversity accumulated on H1, the most probable STR background in which the new lineage arose. Assuming a generation time of 25 years, and based on calculations with the most accurate recombination rate (family data from a previous study[12]), we estimated that the Joseph-derived lineage is 7191 ± 1252 years old. A total of 5 mutation/recombination steps are needed to explain the rise of H6 and H9 haplotypes; this would mean that the presence of MJD in the aboriginal communities is as old as the Joseph-derived lineage in Asia. We cannot exclude the alternative scenario that more than 1 MJD STR-based haplotype background was introduced in those communities.

COMMENT

Kiloh et al[25] reported 13 (possibly 16) cases of a neurological disorder among 1100 tribal aborigines in Groote Eylandt and the adjacent mainland. They mention 2 dis-

tinct clinical pictures (one, in childhood, involving the motor system and remarkably lax ligaments; the other, of later onset, comprising cerebellar, upper motor-neurone, and supranuclear ophthalmoplegia), explained as variants of a single genetic condition.

This was known before and had been attributed to manganese poisoning, mined since the 1960s. From 1990, Tim Burt, MD, from the Menzies School of Health Research, Darwin, studied this so-called Groote Eylandt syndrome.[19] Although there were elevated blood levels of manganese in the local population, no differences were noted between those with and without neurological signs. Also the familial clustering and, often, absence of occupational exposure were noted. Burt et al[26] published a *dominant hereditary ataxia resembling Machado-Joseph disease* (multiple system involvement, sparing of the inferior olives and cerebellar cortex) in Arnhem Land. The prevailing theory blamed the introduction of MJD into the Aboriginal community 300 years ago, by Portuguese-Macassan sailors visiting the island for the trepang harvest. In 1996, the diagnosis of MJD was molecularly confirmed.[20]

The observation that the Groote and Yirrkala families shared the TTACAC haplotype, as do most families with MJD worldwide, does not support a de novo event. We then fully assessed a region of 4 kb around the $(CAG)_n$ in these families and compared them with Portuguese and Asian families from the same (Joseph) lineage. As European, North American, and Brazilian families with TTACAC share a recent common ancestor,[12] the comparison to Portuguese families would allow extrapolation to other Western families.

The 2 aboriginal families with MJD shared an extended SNP background with only 9 Asian families differing in 1 of 20 SNPs from the Western Joseph lineage, which does not support a Portuguese link to explain MJD among aborigines. This SNP background (Joseph lineage with a variant in rs56268847) shared by aborigines and other families with MJD from Taiwan, India, and Japan suggests either an Asian origin of the mutation or an independent event. Under the first hypothesis, either a de novo expansion occurred in the TTGATC**G**AGCCACCCAGCGC haplotype in an Asian population; or there is a single origin for all TTACAC (Joseph lineage) families, including those who carry the ***G*** variant who would share a more recent ancestor. Although we cannot completely rule out the first hypothesis, a SNP recurrent mutation in the Joseph lineage seems more plausible to explain this Joseph-derived haplotype.

In fact, Asia is the suggested place of birth of the Joseph lineage[12]; also, the Joseph-derived haplotype is not restricted to aborigines but also observed in Asia. Diversity accumulates with time, in the more ancient backgrounds, which is in agreement with the SNP mutation (***G*** variant) having occurred in Asia.

We tried then to estimate the age of the origin of this Australasian Joseph-derived haplotype. The fact that we have only 11 families carrying this MJD lineage does not allow a more accurate estimation; however, the high diversity observed with the fast-evolving STRs suggests that the Joseph and Joseph-derived lineages must have diverged early after the occurrence of the de novo expansion at *ATXN3*.

The worldwide distribution and higher diversity of the Joseph lineage, and the lower frequency of this Joseph-derived haplotype within Asia, suggests Joseph as the ancestral lineage; to confirm this hypothesis, we need to enlarge the study of full MJD haplotypes in a larger number of families diagnosed in Asian and other populations.

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