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Linkage Disequilibrium between the Spinocerebellar Ataxia 3/Machado-Joseph Disease Mutation and Two Intragenic Polymorphisms, One of Which, X359Y, Affects the Stop Codon

To the Editor:

Machado-Joseph disease (Takiyama et al. 1993) or spinocerebellar ataxia 3 (Stevanin et al. 1994*a*) (SCA3/MJD), is the most frequent form of autosomal dominant cerebellar ataxia type I, a heterogeneous group of neurodegenerative disorders of unknown etiology (Harding 1993; Sequeiros and Coutinho 1993; Dürr and Brice 1996; Dürr et al. 1996). The responsible mutation has been characterized as an unstable CAG-repeat expansion in the coding region of the MJD1 gene (Kawaguchi et al. 1994; Cancel et al. 1995). The molecular mechanism leading to repeat expansion remains unknown. However, Igarashi et al. (1996) recently suggested, on the basis of an intragenic polymorphism (987G→C) in the MJD1 gene, that an allelic interaction between the normal and expanded chromosomes may be implicated in the instability of the expanded CAG repeat. Until recently, the SCA3/MJD mutation was thought to originate in the Azores Islands. We now know that the disease is not restricted to patients of Portuguese descent and that several different mutations probably are involved, even in Portuguese pedigrees (Stevanin et al. 1995*a;* Takiyama et al. 1995; Gaspar et al. 1996; Iughetti et al. 1996). We report here a new intragenic polymorphism at the stop codon (1118A→C) of the MJD1 cDNA, resulting in the addition of 16 amino acid residues to the C-terminal domain of the gene product, that does not affect the phenotype. Analysis of the haplotypes defined by the 987G→C and 1118A→C polymorphisms revealed the existence of four different haplotypes associated with the SCA3/MJD mutation that have resulted from at least four ancestral mutations.

We have cloned cDNAs corresponding to the normal and expanded MJD1 alleles from a SCA3/MJD patient, by retro-transcription of brain mRNA followed by PCR. Direct sequencing of both cDNAs revealed at codon 359 a polymorphism that differs from the published MJD1 sequence (Kawaguchi et al. 1994) and that corresponds to a 1118A→C substitution (Ter→Y) predicting an SCA3/MJD protein containing 16 additional amino acid residues (YELHVIFALHYSSFPL). This result is in agreement with preliminary results obtained on cDNA from lymphoblastoid mRNA (Trottier et al. 1995) and from a brain library (Goto et al. 1996). The polymorphisms can be detected rapidly by differential PCR with the MJD52 primer (Kawaguchi et al. 1994) and either MJD-TAA (GCAAAAATCACATGGAGCTCT) or MJD-TAC (GCAAAAATCACATGGAGCTCG) for the 1118A→C polymorphism and either MJD-GGG (CTCTGTCCTGATAGGTCCCC) or MJD-CGG (CTCTGTCCTGATAGGTCCCG) for the 987G→C polymorphism. PCR amplifications were performed as described elsewhere (Cancel et al. 1995), except for annealing at 64°C and 63°C for the 1118A→C and 987G→C polymorphisms, respectively. PCR products were separated on 1% agarose gels and were visualized by UV exposure in the presence of 0.5 μg ethidium bromide/ml (fig. 1). PCR products from control subjects who were homozygous for a polymorphism on both normal chromosomes were run on 6% acrylamide gels for better resolution of the alleles and were visualized by autoradiography, after being blotted onto nylon membranes and hybridized with a γ^{32}P-labeled $(CAG)_7$ oligonucleotide.

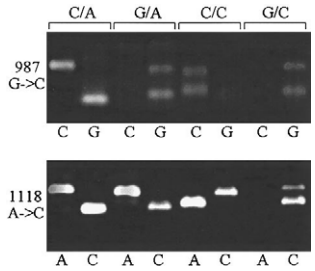

Figure 1 Detection of the four 987G→C/1118A→C haplotypes on expanded SCA3/MJD alleles. The four PCR amplifications (see text) for each subject were run on a 1% agarose gel containing 0.5 μg of ethidium bromide/ml, to separate normal and expanded alleles.

We screened one patient from each of 61 SCA3/MJD families (Stevanin et al. 1995*b;* Dürr et al. 1996): 39 from France, 10 from Portugal, 2 from Morocco, and 1 each from Brazil, Belgium, Algeria, Yemen, Cambodia, French Guyana, French West Indies, Ivory Coast, and Spain, as well as 1 with Spanish and Portuguese origins. The families from French Guyana and Ivory Coast were of Black African ancestry, as was one of the Moroccan kindreds. The exact origin of the mutation in the family with Spanish and Portuguese ancestors, in the Brazilian family, and in the White Caucasian family living in the French West Indies could not be determined. The genealogies of five kindreds of Portuguese origin that were from the rural area of Ribeirao Preto, São Paulo, and carried the same frequent Portuguese surname were traced back to 1820. These families appear to be unrelated, but a founder effect cannot be excluded. The first known patient in the largest family, born in 1820, is of Azorean extraction (Stevanin et al. 1994*b*). Chromosomes from 121 healthy unrelated individuals of French ($n = 56$), northern African ($n = 28$), Brazilian ($n = 26$), and Portuguese ($n = 11$) ancestries, as well as the normal chromosome from each of the index patients, were used to determine allelic distributions. The number of CAG repeats was determined as described elsewhere (Cancel et al. 1995).

In the four control populations (French, northern African, and Portuguese/Brazilian), the C allele at position 1118 was more frequent (~75%) than the A allele (~25%), as shown in figure 2. The C allele was also associated with smaller CAG repeats (from 20 ± 5 to 22 ± 6 CAG repeats in the four populations) than was the A allele (from 24 ± 4 to 25 ± 5 CAG repeats). This difference was significant in French ($P < .001$) and northern African controls ($P < .05$), in whom alleles with 14 and 15 CAG units were exclusively associated with the C polymorphism. In the French and northern African controls, alleles with >33 repeats were of the A type, whereas in the Portuguese/Brazilian control population both polymorphisms were observed in the largest normal alleles. French, northern African, and Black African SCA3/MJD chromosomes carried exclusively the A allele at nucleotide 1118, but alleles A and C were observed in other populations, including the Portuguese (see the appendix).

In the four control populations, 96% of the haplotypes defined by the 987G→C and 1118A→C polymorphisms were GC or CA, showing strong linkage disequilibrium. Haplotype GC represented 97% and 94% of the two major normal alleles, with 14 and 23 CAGs, respectively. Although the CA haplotype was found on only 23% of the normal alleles, it was associated with 73% of those with >33 CAG repeats. Only two (CA and GC) of the four possible haplotypes were detected on 15 alleles with 34–40 CAG repeats. In French ($n = 8$; fig. 3) and northern African ($n = 3$) controls, normal alleles with >33 CAG repeats were associated exclusively with haplotype CA, whereas both CA ($n = 3$) and GC ($n = 3$) were observed in Portuguese and Brazilian subjects. The ancestral haplotypes are probably CA and GC. The very different distribution, with only minimal overlap, of the GC and CA haplotypes in controls suggests that the divergence of these haplotypes is very ancient, and it is reminiscent of that of myotonic dystrophy (Imbert et al. 1993).

The results are strikingly different for the expanded alleles. The four haplotypes are observed on SCA3/MJD chromosomes—but with different distributions, depending on the geographical origin. Three different haplotypes are found on Portuguese SCA3/MJD chromosomes, supporting the hypothesis that the SCA3/MJD mutation in Portuguese/Azoreans has a multiple origin, the most frequent of which (CA) is common to the majority of families with Portuguese ancestry and to all French kindreds. Another haplotype (GA) is shared by the majority of African SCA3/MJD patients, including all of those of Black African origin ($n = 3$). This haplotype is also found in the Yemenite Jewish family originating from a small isolated community near Ta'izz in Yemen (Goldberg-Stern et al. 1994). There is no documentation or historical evidence of Portuguese ancestry in this particular community.

In conclusion, we have described a new intragenic polymorphism, in the stop codon of the MJD1 gene, that is in linkage disequilibrium with SCA3/MJD expansions. The results support the hypothesis of a founder effect, or recurrent mutations, on both the CA haplotype in the French population, as elsewhere had been suggested by analysis with extragenic flanking microsatellites (Stevanin et al. 1995*a*), and on the GA haplotype in SCA3/MJD patients of Black African origin. A founder effect is also suspected in Japanese kindreds (Endo et al. 1996). In Portuguese SCA3/MJD families, there were three different haplotypes. Since recombination events among these polymorphisms, which are only 31 nucleotides apart and close to the SCA3/MJD mutation, are unlikely, the four different haplotypes associated with CAG expansions most probably reflect at least four different ancestral mutations. However, since only one Brazilian kindred of Portuguese ancestry carried the rare CC haplotype, it cannot be excluded that a mutation occurred recently in this family, on a GC haplotype or a CA haplotype. The precise origin of most of the families of Portuguese origin is not known. However, it is of note that the CA haplotype is carried both by Brazilian kindreds with Azorean ancestry and by a Portuguese family of continental origin.

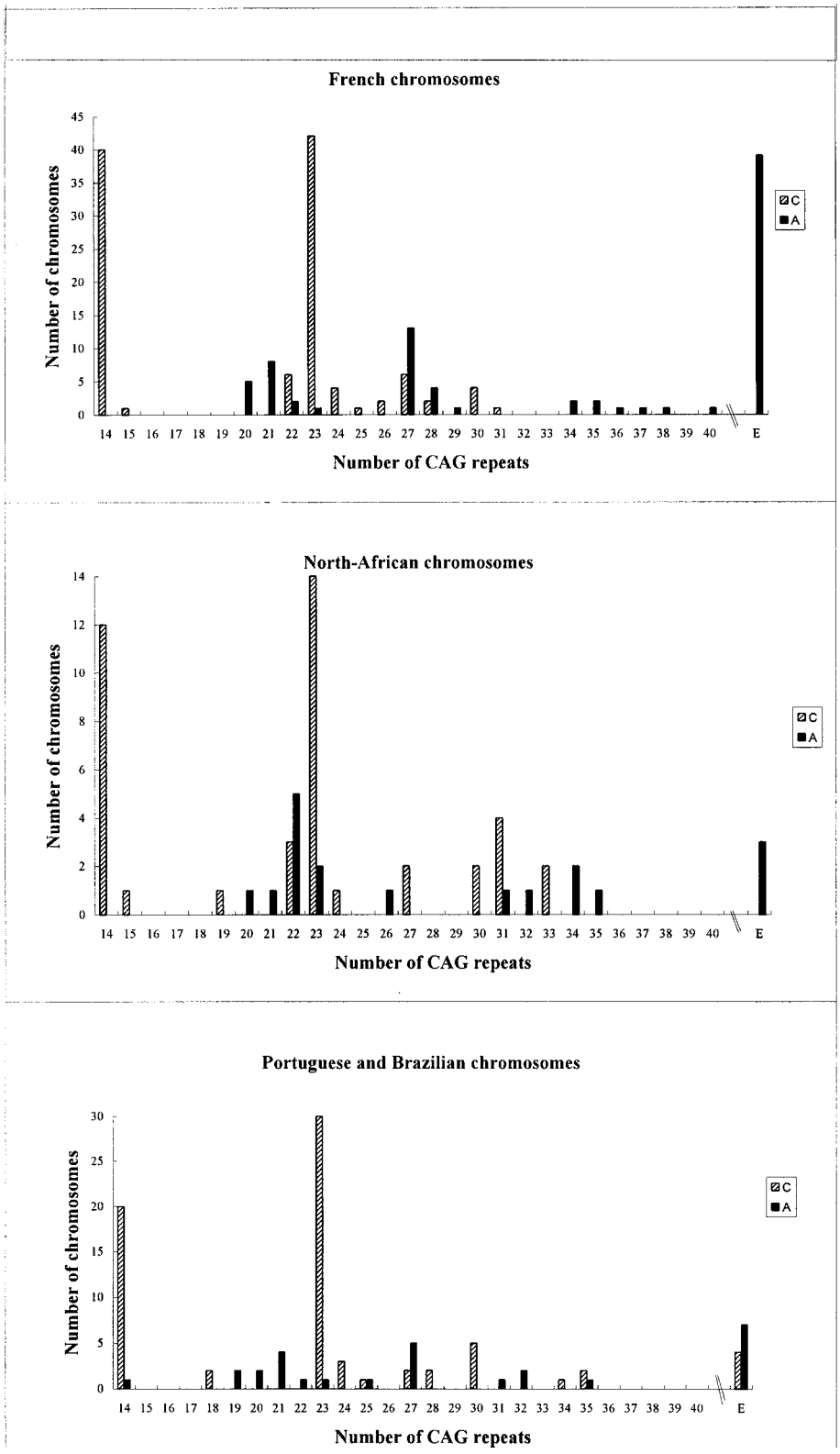

Figure 2 Distribution of the 1118A→C polymorphism according to CAG repeat number, in French, northern African, and Portuguese/Brazilian controls. Portuguese and Brazilian distributions were not statistically different and are represented together. E = SCA3/MJD patients.

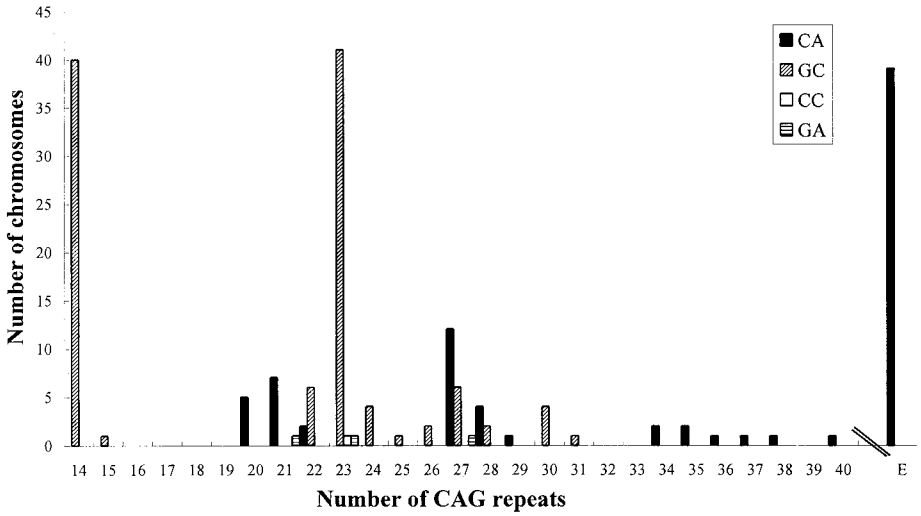

Figure 3 Distribution of the 987G→C/1118A→C haplotypes, as a function of CAG repeat number, on 39 French SCA3/MJD carrier chromosomes (E) and on 148 normal chromosomes from unrelated French subjects.

De novo cases, which, in Huntington disease (HD), permitted identification of the origin of the expansion (Myers et al. 1993; Squitieri et al. 1994), have not been reported yet in SCA3/MJD. However, the distribution of the haplotypes on the largest alleles (>33 repeats) suggests a possible pathogenic mechanism. In French controls, only the CA haplotype is observed on the largest normal alleles and on all SCA3/MJD chromosomes. It therefore is tempting to postulate that, as in HD, de novo expansions occur on these large normal alleles. This hypothesis would explain the complete linkage disequilibrium between the CA haplotype and the SCA3/MJD chromosomes in the French population. However, instability of normal alleles has not been observed yet in SCA3/MJD, even in 16 transmissions of alleles >31 repeats. De novo cases are needed to confirm this hypothesis. Only a few large normal alleles (>34 CAG repeats) have been characterized in Brazilian, Portuguese, or northern African controls: only the CA haplotype was found in northern Africans, but both CA and GC haplotypes were found in the Brazilian and Portuguese controls.

The functional significance of the 1118A→C polymorphism remains unclear, despite the fact that it results in the addition of 16 amino acid residues to the C-terminal domain of the MJD1 gene product. The additional sequence does not carry potential sites for posttranslational modifications such as glycosylation or phosphorylation. Preliminary results show that this polymorphism has no effect on age at onset, clinical presentation, or instability of the expanded CAG repeat (data not shown). Functional analysis in cellular or animal models should, however, be informative.

Appendix

Distribution of Two Intragenic Polymorphisms in 61 SCA3/MJD Families

Polymorphism:				
987G→C	C		G	
1118A→C	A	C	A	C
Ancestry:				
French	39	...	...	...
Portuguese	6	1	...	3
Other Europeans	2	...	...	1
Black Africans	...	...	3	...
Others	3	...	2[a]	1

[a] Includes a Moroccan and a White Jewish Yemenite family.

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A Common mtDNA Polymorphism Associated with Variation in Plasma Triglyceride Concentration

To the Editor:

Many laboratories are investigating the association between nuclear genomic variation and complex human traits (Lander and Schork 1994). However, the extranu-