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Localization of the Candidate Gene D-Amino Acid Oxidase Outside the Refined 1-cM Region of Spinocerebellar Ataxia 2

To the Editor:

Spinocerebellar ataxia 2 (SCA2) is one form of the neurodegenerative autosomal dominant cerebellar ataxias (for review, see Harding 1983) and has been linked to chromosome 12q (Gispert et al. 1993*b*) in 25 previously described and 13 new families from a founder collective of >500 patients in Holguín, Cuba (Auburger et al. 1990; Gispert et al. 1993*a*). Although SCA2 in most patients cannot be distinguished from other spinocerebellar ataxias by clinical criteria, in some patients it exhibits a particular phenotype with early neuropathy/late slow saccades and late myoclonus (Orozco et al. 1990). Autopsy in 11 patients demonstrated olivo-ponto-cerebellar atrophy with a selective sparing of the dentate nucleus (R. Estrada, J. Galarraga, G. Orozco, A. Nodarse, and G. Auburger, unpublished data). Complete allelic association within the Holguín population was established with the microsatellite D12S105 (Hernández et al. 1995), and the candidate region was determined to be within a 6- cM region distal to the marker D12S84, contrasting previous reports by Pulst et al. (1993) and Lopes-Cendes et al. (1994) and according to preliminary data between D12S84 and D12S1329 (Allotey et al. 1994).

The D12S105 sequence (hs262xb9.seq) including 342 bp representing the region of maximal allelic association in the Cuban SCA2 founder effect was subjected to sequence homology analysis at the European Molecular Biology Laboratories database and yielded an almost perfect match with 99.70% similarity with intron 1 of the human D-Amino acid Oxidase (DAmOx) gene, which has previously been shown to be linked to all SCA2 pedigrees worldwide with no recombination (Hernández et al. 1995). The small sequence differences were the result of length variations in the four primitive repeat motifs contained in this intron. DAmOx has previously been mapped by chromosome specific hybrids to chromosome 12 and pseudogenes have not been detected (Fukui and Miyake 1992). Primers designed from exons 1 and 10 of the DAmOx cDNA-sequence (Momoi et al. 1988) gave specific PCR products in the CEPH-YAC838f5 corresponding to the locus D12S105, confirming the physical localization and providing the investigation with an excellent candidate gene for SCA2, since DAmOx is expressed specifically in spinocerebellar tissue (Horiike et al. 1985) without its function being known. A mutation in this DAmOx gene would fit well with previous hypotheses on the pathomechanism of spinocerebellar degeneration, since oral loading tests with glutamate in such patients have demonstrated a decreased metabolism of amino acids glutamate and aspartate (Plaitakis 1982) and since accumulation of the excitotoxic neurotransmitter glutamate is known to lead to cerebellar Purkinje neuron death (Meldrum and Garthwaite 1990), matching well the morphological changes observed in SCA2.

Within intron 1 of the DAmOx gene a second $(AC)_n$ microsatellite is found that we termed D12S105a (AFM-262xb9a). With flanking primers 5′-AGCAGTTGA-GAGGATTGAGAGG-3′ and 5′-GCAAGCTTGGAG-

Table 1

Haplotypes in the Ancestors of Each of 13 Large Cuban SCA2 Families Together with Intrafamilial Recombinant Haplotypes (Written Always to the Left of the Ancestral Haplotype)

MABe	PL	Fi	Ry	Es	Ve	LB	In	Su	Al	Mu	Pu	So	Microsatellite
ND	4	4	5←5	ND←5	5	4	4	5	NI	5	4	4	IGF1
ND	2	ND	5←2	ND←10	3	2	2	8	2	2	2	1	D12S78
ND	4	4	4←4	ND←8	4	4	9	4	4	4	4	4	PAH-VNTR-BglII
1	1	1	ND←1	ND←1	1	1	1	1	1	1	1	1	D12S338
4	4	ND	7←4	ND←7	4	4	4	4	4	4	4	4	D12S353
3	ND	3	ND←3	ND←4	4	3	3	3	4	4	4	4	D12S1331
8	8	8	ND←8	ND←8	8	8	8	8	8	8	8	8	D12S330
6	6	6	ND←5	ND←6	6	6	6	6	6	6	6	6	D12S317
1	1	1	1←2	9←1	1	1	1	1	1	1	1	1	D12S84
5	5	5	5←5	NI←5	5	5	5	5	5	5	5	5	D12S105
5	5	ND	6←5	4←5	ND	5	5	5	5	5	5	5	D12S105a
3	3	3	4←3	3	3	3	3	3	3	3	3	3	D12S1328
													SCA2
7←9	9	9	9	9	9	9	9	9	9	9	9	9	D12S1329
6←11	11	ND	11	11	11	ND	11	11	11	11	11	11	D12S1333
3←3	3	3	3	3	3	3	3	3	3	3	3	3	D12S1332
4←5	6←5	5	5	5	3	5	5	5	5	5	5	5←6	D12S1330
4←4	9←4	4	4	4	4	4	4	4	4	4	4	5←3	D12S354
5←5	3←5	3	3	5	6	5	5	5	5	5	5	5←5	D12S79
5←2	4←2	5	2	ND	3	2	2	2	2	2	2	2←2	D12S369
ND	ND	6	5	3	3	2	6	5	ND	ND	ND	ND	D12S366
ND	4←4	4	2	4	4	ND	ND	ND	4	4	NI	ND	D12S349
ND	3←3	3	4	3	3	5	3	7	7	4	3	8←9	D12S86

NOTE.—ND = no data; and NI = not investigated.

TATGTATCC-3′ we performed genotyping in 25 Cuban SCA2 families and 2 families from the French Caribbean (Martinique) with probable SCA2 (Belal et al. 1994). Seven alleles were observed yielding a heterogeneity index of .79. In addition, six recently developed microsatellites in the SCA2 candidate interval of 6 cM between D12S84 (AFM116xb8) and D12S79 (AFM067yc5) andeight further new microsatellites were genotyped in these families: D12S129 (Montgomery et al. 1993), D12S338 (AFM291wd9), D12S353 (AFM304wg5), D12S1331 (AFM340xg1), D12S330 (AFM086xd7), D12S317 (AFM065ye9), D12S1328 (AFM240we1), D12S1329 (AFM291xe9), D12S1333 (AFM154tc5), D12S1332 (AFMa128yf1), D12S1330 (AFM312yb1), D12S354 (AFM304wh5), D12S369 (AFM142zc5), D12S366 (AFM351tb1), D12S349 (AFM299zd5) (see Gyapay et al. 1994; CEPH database). This analysis identified recombinant events in families Rs and Es and placed the SCA2 gene to the telomeric side of D12S1328 (see table 1 and fig. 1*a*), excluding conclusively the DAmOx gene from the SCA2 region with a distance of ~1 cM between the D12S105a microsatellite and SCA2. Fine mapping of the SCA2 locus was helped further by the clinical characterization of a previously undescribed Cuban SCA2 family named Be, which consists of 4 branches ABe, MABe, HBe, and CoBe and contains 155 affected members. Within the branch MABe a crossing-over event was identified that places the SCA2 mutation centromeric to D12S1329, thus reducing the candidate region to ~1 cM (see fig. 1*b*) between flanking markers D12S1328 and D12S1329 (map according to Généthon, unpublished data). It is important to note that further analysis of these Rs and MABe recombinants by using newly generated microsatellites from the 1-cM candidate region will be able to reduce the candidate region even further.

In contrast, the investigation of potential distantly related SCA2 families seemed to be of limited value for genetic mapping: Whereas the ancestral haplotype D12S105, D12S105a, D12S1328, D12S1329, D12S1333, and D12S1332 (see table 1) is well preserved among the founders of the 12 published families and 1 new family presented in table 1 and of additional 15 smaller families from Holguín (supporting the notion of a Cuban founder effect), the Cuban D12S1329 allele was not preserved in SCA2 families from France, Tunisia, Canada, or the United States, and the Cuban D12S1328 allele was preserved in only two of six non-Cuban SCA2 families, thus giving little support for a common origin of SCA2 families.

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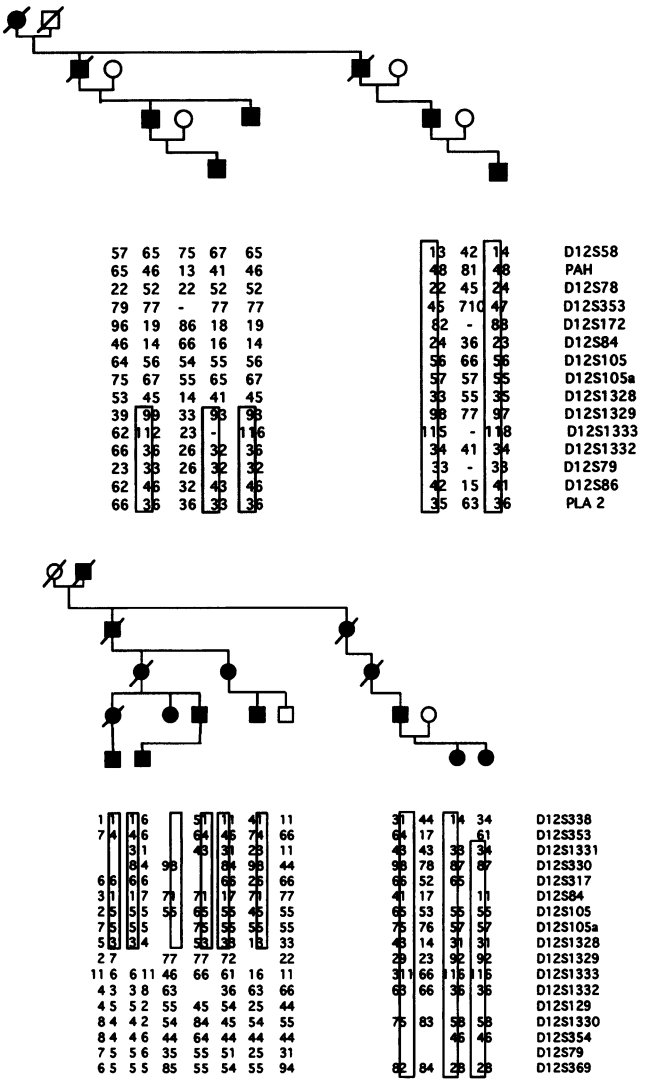

Figure 1 *a*, Haplotype analysis in two meioses representative of two branches of the Rs family from the Holguín SCA2 population. Both the partial and the complete disease haplotype represent typings in five affected individuals of the two family branches. *b*, Haplotype analysis in a recombinant branch of MABe (*left*) and a nuclear pedigree representative of the rest of family Be containing 155 patients (*right*).

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Association Analysis of the Monoamine Oxidase A Gene in Bipolar Affective Disorder by Using Family-Based Internal Controls

To the Editor:

It is well accepted that association studies are a major tool in investigating the contribution of single genes to the development of diseases that do not follow simple Mendelian inheritance pattern (so-called complex traits) (e.g., Lander and Schork 1994). Such major psychiatric diseases as bipolar affective disorder and schizophrenia clearly fall into this category of diseases.

Lim et al. (1994) have recently observed a significant overall association between bipolar affective disorder and alleles of a microsatellite polymorphism at the monoamine oxidase A (MAOA) locus in bipolar patients ($n = 57$) and controls ($n = 59$) of western European extraction. The finding of an overall association was replicated by Kawada et al. (1995) in Japanese patients ($n = 58$) and controls ($n = 68$), although individ-