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Report

A Major Locus for Myoclonus-Dystonia Maps to Chromosome 7q in Eight Families

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Myoclonus-dystonia (M-D) is an autosomal dominant disorder characterized by myoclonic and dystonic muscle contractions that are often responsive to alcohol. The dopamine D2 receptor gene (*DRD2*) on chromosome 11q has been implicated in one family with this syndrome, and linkage to a 28-cM region on 7q has been reported in another. We performed genetic studies, using eight additional families with M-D, to assess these two loci. No evidence for linkage was found for 11q markers. However, all eight of these families showed linkage to chromosome 7 markers, with a combined multipoint LOD score of 11.71. Recombination events in the families define the disease gene within a 14-cM interval flanked by D7S2212 and D7S821. These data provide evidence for a major locus for M-D on chromosome 7q21.

Myoclonus-dystonia (M-D), previously known as "idiopathic hereditary myoclonus," "hereditary essential myoclonus," or "myoclonic dystonia" (Lang 1997 [MIM 159900]), is a rare movement disorder that is transmitted as an autosomal dominant trait with reduced penetrance. M-D usually starts in childhood or adolescence and is characterized clinically by involuntary lightning jerks (myoclonus) that respond dramatically to alcohol and by dystonia, frequently involving the neck and arms (Mahloudji and Pikielny 1967; Quinn 1996; Gasser 1998). Because of the prominence of both myoclonus and dystonia, M-D has been grouped under the "dystonia plus" category (Fahn et al. 1998), thus differentiating it from early-onset generalized dystonia (DYT1) and other primary dystonias.

Previously, it was reported that a missense change (Val154Ile) in a conserved region of the D2 dopamine receptor (*DRD2*) on chromosome 11q23 cosegregated with M-D in one large family (Klein et al. 1999). However, sequence analysis of the *DRD2* gene in 17 M-D patients from other families and linkage analysis using chromosome 11 markers surrounding this gene in another four clinically similar families were negative, suggesting locus heterogeneity for this disorder (Durr et al. 2000; Klein et al. 2000). This idea was confirmed when

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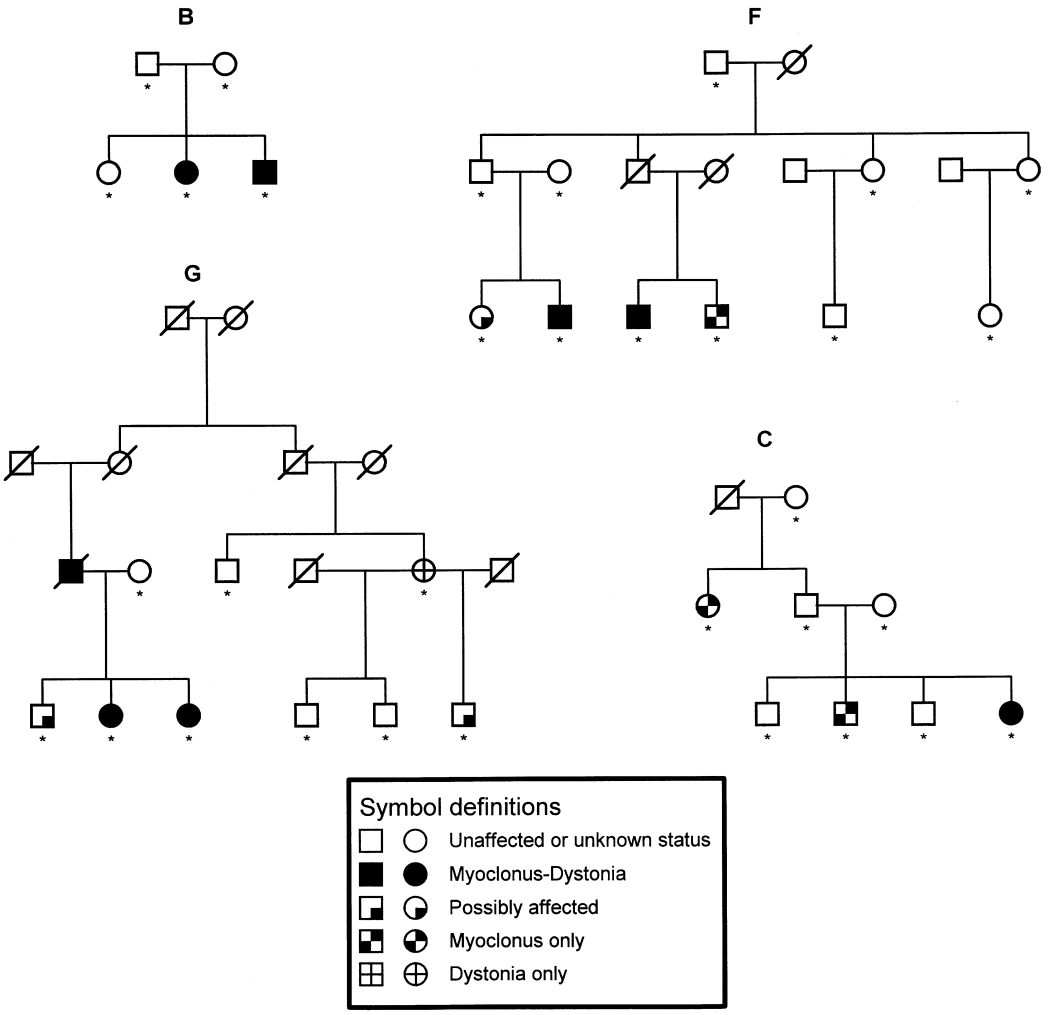

Figure 1 Pedigrees of families not described elsewhere in which M-D is linked to chromosome 7. An asterisk (*) denotes DNA samples, and slashes denote deceased individuals.

a second M-D locus was mapped to a 28-cM region on chromosome 7q21-q31 in a single family (Nygaard et al. 1999).

In the present study, we evaluated eight families (A–H) with M-D for linkage to the known M-D loci. In addition, two candidate genes (*GNG11* and *GNGT1*) in the chromosome 7q region were screened for mutations.

The various local IRBs approved these studies. After giving informed consent, 107 members of eight families with M-D underwent neurological examination by movement disorders specialists. Secondary myoclonus and dystonia were excluded by both history and lack of additional neurological signs in all cases and by brain imaging in selected cases. Index patients were videotaped and the tapes were reviewed by a member of the clinical team (S.B.B.) masked to pedigree and genotype status. The diagnoses of dystonia and myoclonus were established according to published criteria (Mahloudji and Pikielny 1967; Bressman et al. 1989; Quinn 1996). Forty-two individuals were diagnosed with M-D (number of affected individuals with DNA sample, family A: 4; family B: 2; family C: 3; family D: 2; family E: 7; family F: 3; family G: 3; and family H: 18). The phenotype in these eight families is consistent with the phenotype of the families described elsewhere (Klein et al. 1999; Nygaard et al. 1999). The mean age at onset was 8.1 ± 5.9 years (range 1–37 years). Both the myoclonus and the dystonia, whether present individually or together, were most prominent in the neck and arm; other sites involved included the trunk and face. Seven of the families had at least one affected family member with known response to alcohol. Detailed clinical findings, including pedigrees, for the following families have been described elsewhere: families A and D (Klein et al. 2000), family E (Kurlan et al. 1988), and family H (Kyllerman et al. 1990). Pedigrees of the families not described else-

Table 1

Two-Point LOD Scores for Chromosome 7 Markers in M-D Families

Marker (Location) and Family[a]	Z at θ = .00	.01	.05	.10	.20	.30
D7S2204 (90.95):						
A	.00	.00	.00	.00	.00	.00
B	ND					
C	ND					
D	.25	.25	.22	.18	.11	.05
E	−2.21	−2.03	−1.14	−.66	−.25	−.09
F	ND					
G	−2.56	−1.11	−.48	−.25	−.08	−.03
H	ND					
Total	−4.52	−2.89	−1.40	−.73	−.22	−.07
D7S2212 (95.43):						
A	.00	.00	.00	.00	.00	.00
B	.00	.00	.00	.00	.00	.00
C	.55	.53	.47	.39	.24	.12
D	.19	.18	.14	.10	.04	.01
E	−1.88	−.20	.38	.54	.53	.37
F	.47	.45	.38	.30	.17	.07
G	.73	.71	.62	.52	.34	.18
H	3.86	3.79	3.49	3.09	2.28	1.44
Total	3.92	5.46	5.48	4.94	3.60	2.19
D7S2410 (100.81):						
A	.60	.59	.54	.47	.32	.17
B	.00	.00	.00	.00	.00	.00
C	.57	.55	.48	.40	.25	.12
D	.26	.25	.22	.18	.11	.05
E	.30	.30	.28	.26	.21	.15
F	.90	.88	.79	.68	.45	.23
G	.70	.68	.60	.50	.32	.17
H	4.84	4.75	4.39	3.93	2.94	1.88
Total	8.17	8.00	7.30	6.42	4.60	2.77
D7S646 (104.86):						
A	.60	.59	.54	.47	.32	.17
B	.00	.00	.00	.00	.00	.00
C	.31	.30	.26	.22	.14	.07
D	.22	.21	.17	.12	.05	.01
E	1.82	1.79	1.66	1.49	1.13	.73
F	.78	.76	.69	.59	.39	.20
G	.70	.68	.60	.50	.32	.17
H	4.53	4.44	4.09	3.64	2.67	1.63
Total	8.96	8.77	8.01	7.03	5.02	2.98
D7S657 (104.86):						
A	.00	.00	.00	.00	.00	.00
B	.00	.00	.00	.00	.00	.00
C	.15	.14	.12	.10	.06	.03
D	.22	.21	.17	.12	.05	.01
E	1.82	1.79	1.66	1.49	1.13	.73
F	.49	.47	.40	.32	.18	.07
G	.80	.78	.69	.58	.38	.20
H	5.17	5.08	4.71	4.24	3.22	2.11
Total	8.65	8.47	7.75	6.85	5.02	3.15
D7S1820 (105.92):						
A	.60	.59	.54	.47	.32	.17
B	.00	.00	.00	.00	.00	.00
C	.56	.54	.48	.39	.24	.11
D	.00	.00	.00	.00	.00	.00
E	.31	.30	.28	.26	.21	.15
F	.17	.17	.14	.11	.06	.03
G	.63	.61	.53	.44	.28	.14
H	3.93	3.87	3.59	3.22	2.41	1.56
Total	6.20	6.08	5.56	4.89	3.52	2.16
D7S2482 (108.59):						
A	.60	.59	.54	.47	.32	.17
B	ND					
C	.57	.55	.48	.40	.25	.12
D	ND					
E	1.20	1.18	1.09	.98	.72	.43
F	.22	.21	.18	.14	.07	.03
G	.83	.81	.72	.61	.40	.22
H	.86	.84	.78	.69	.52	.35
Total	4.28	4.18	3.79	3.29	2.28	1.32
D7S821 (109.12):						
A	.00	.00	.00	.00	.00	.00
B	ND					
C	.57	.55	.48	.40	.25	.12
D	.21	.21	.17	.12	.05	.01
E	1.82	1.79	1.66	1.49	1.13	.73
F	.61	.60	.54	.47	.32	.17
G	−2.73	−1.03	−.41	−.19	−.05	−.02
H	3.06	3.00	2.75	2.42	1.74	1.05
Total	3.54	5.12	5.19	4.71	3.44	2.06
D7S1799 (113.92):						
A	−3.00	−1.39	−.72	−.44	−.19	−.08
B	.30	.29	.26	.22	.13	.06
C	ND					
D	.23	.22	.18	.13	.05	.01
E	ND					
F	ND					
G	.68	.66	.58	.48	.30	.16
H	−2.36	.65	1.74	1.96	1.75	1.24
Total	−4.15	.43	2.04	2.35	2.04	1.47

(*continued*)

Note. ND = not done.

[a] Map locations are based on a combination of the Marshfield Medical Research Foundation map and the Genetic Location Database.

where (families B, C, F, and G) are shown in figure 1. The mode of transmission was consistent with autosomal dominant inheritance with reduced penetrance in all eight families.

DNA was extracted from venous blood samples and genotyping was performed according to a standard PCR protocol (Research Genetics), using a LICOR automated sequencer. The following markers were used on chromosome 7: D7S2204 – 4.48 cM – D7S2212 – 5.38 cM – D7S2410 – 4.05 cM – D7S646 – 0.0 cM – D7S657 – 1.06 cM – D7S1820 – 2.67 cM – D7S2482 – 0.53 cM – D7S821 – 4.80 cM – D7S1799. The following markers were used on chromosome 11: D11S2000 – 3.98 cM – D11S1391 – 0.57 cM – D11S897 – 0.00 cM – D11S1986 (*DRD2* is located between D11S1391 and D11S897/1986). Map order was obtained from the Genetic Location Database, and distances are based on the Marshfield Medical Research Foundation database.

We conducted two-point and multipoint linkage anal-

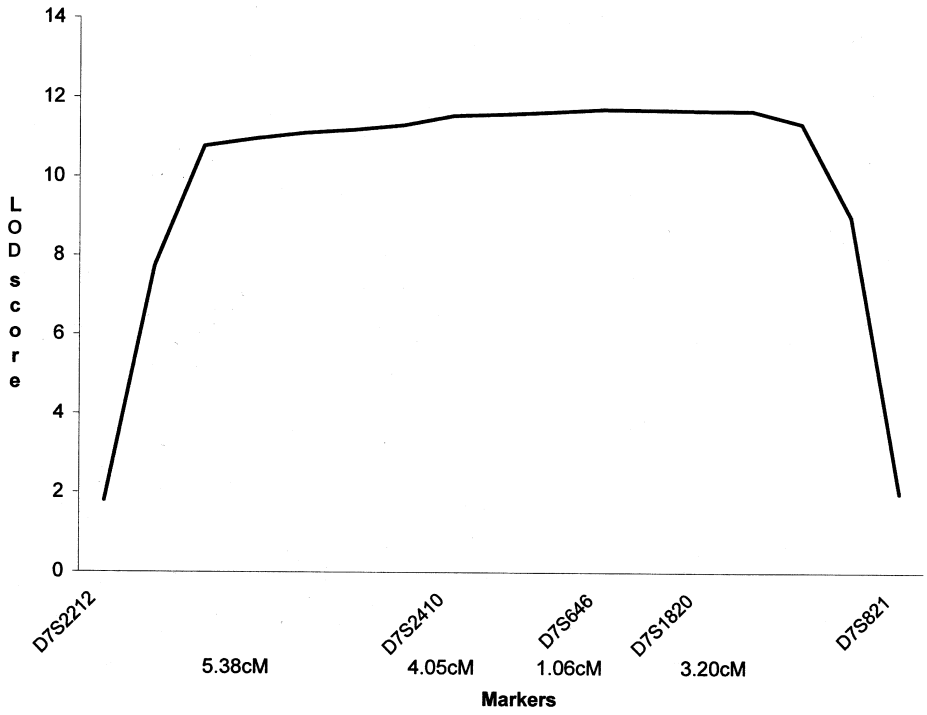

Figure 2 Multipoint LOD scores for all eight families. Markers used and their map positions are listed on the X-axis. Z_{max} is 11.71 at marker D7S646, although most of the region between D7S2212 and D7S821 reveals a LOD score >10.

yses, using the VITESSE program (O'Connell and Weeks 1995) and assuming autosomal dominant inheritance of a rare allele (frequency .0001) with reduced penetrance. Since reliable estimates of penetrance are not available for this disorder, we used an "affecteds-only" model in which only those with features of definite myoclonus, definite dystonia, or both were considered affected. All others were coded as "unknown" for disease status, and penetrance was assumed to be very low (1%). Marker allele frequencies were assumed to be equal. Two-point LOD scores were calculated for both chromosome 7 and chromosome 11 markers within the two M-D regions.

No positive linkage to the region of *DRD2* (chromosome 11q) could be demonstrated in any of these eight families (data not shown; Klein et al. 2000). However, chromosome 7 markers were consistent with linkage in all eight families (table 1), with the maximum combined two-point LOD score (Z_{max}) at D7S646 ($Z = 8.96$ at recombination fraction [θ] 0.0). Five markers that span the region, including D7S2212, D7S2410, D7S646, D7S1820, and D7S821, were used in a multipoint linkage analysis. Results are presented in figure 2 and show a maximum combined multipoint LOD score of 11.71 at D7S646. Critical crossover events that define the gene location occurred in an affected individual (family G, individual 416) and an obligate gene carrier (family E, individual 416) and are shown along with other key individuals in figure 3. Specifically, there are crossovers between D7S2212 and D7S64 in family E (D7S2410 is uninformative) and between D7S2482 and D7S821 in family G. This narrows the candidate region to a 14-cM interval between D7S2212 and D7S821 and substantially reduces the initially reported region of 28 cM (Nygaard et al. 1999). There was no shared (partial)

Table 2

Primer Pairs for Exon Amplification of *GNGT1* and *GNG11*

Gene and Exon	Primer (5′→3′)	
	Forward	Reverse
GNGT1:		
Exon 1	CTCCAGCAATTCATGCATAG	ACTCCCACAGTATCTCTAGC
Exon 2	CTGGCATCTGGAGTATTCTG	CACGTACATGCTTAGTAGTA
GNG11:		
Exon 1	GCCTTCAGTTGTTTCGGGA	CAGGAAAAATCGGACCCTGC
Exon 2	CATTAAGGCGTAGGGACAAAG	AGTGAGCAAAGATGAGTAGC

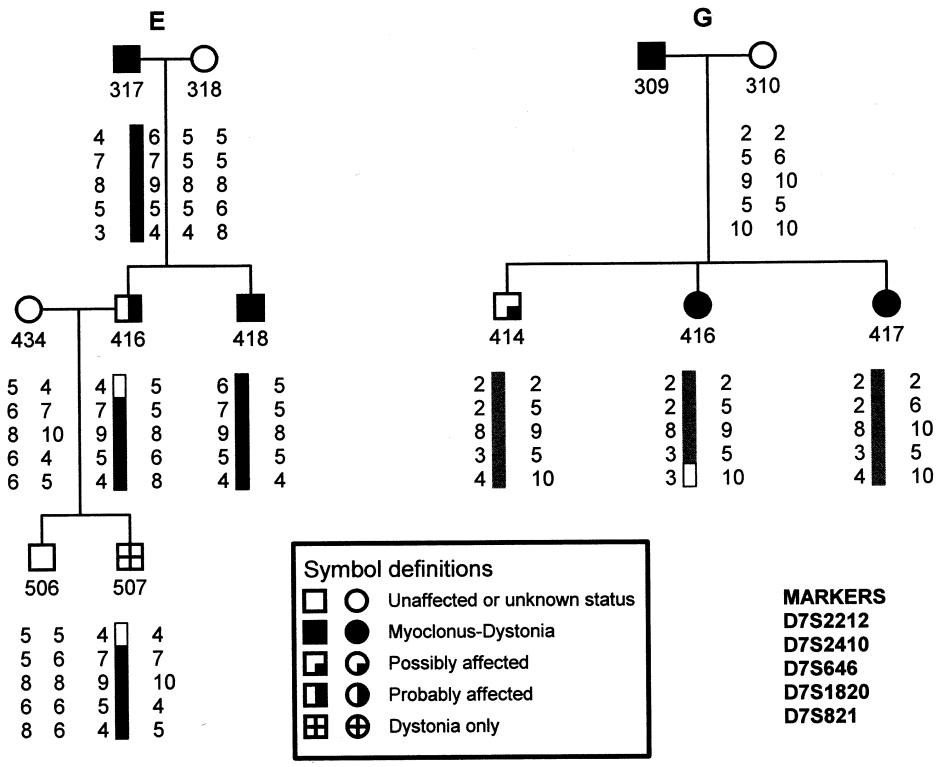

Figure 3 Partial pedigrees for the families possessing critical recombination events. The shaded chromosomes are the disease-bearing chromosomes. In family E, individual 416 has a crossover between D7S2212 and D7S646, with D7S2410 being uninformative. In family G, individual 416 has a recombination between D7S821 and D7S1820. Taken together, these crossovers place the M-D locus between D7S2212 and D7S821, an interval of ~14 cM. The distances between markers are given in the text.

haplotype within the linked region across families, rendering a recent common founder mutation unlikely.

Large parts of the chromosome 7q region have already been sequenced and well characterized (Bouffard et al. 1997), thereby facilitating the search for candidate genes. On the basis of the apparent involvement of *DRD2* in one family with M-D, the two gamma subunits of guanine nucleotide-binding proteins (G-proteins *GNG11* and *GNGT1*) that are located in this region of chromosome 7 (National Center for Biotechnology Information accession number AC002076) represented appealing candidates for mutation screening. G proteins are involved as modulators or transducers in various transmembrane signaling systems and thus could interact with the dopamine D2 receptor in second messenger pathways.

We designed primer pairs to allow bidirectional sequencing of the two exons of each gene (table 2). Genomic DNA from one affected member of each family or from control individuals was amplified using standard PCR conditions. Dideoxy cycle sequencing of PCR products was performed with the Thermo Sequenase Fluor Primer Cycle Sequencing Kit (Amersham). No mutations were found in the coding regions of these two genes in any of the families. However, in *GNGT1,* a G→A transition at base pair 148 (E50K) was detected in families E, H, and C. When other affected members of these families were typed, this change was not inherited with the disease. In addition, this same variation was seen in control individuals. Both of these findings suggest that the E50K change is a polymorphism. Previously, Nygaard et al. (1999) excluded sequence alterations in the gene for the human metabotropic glutamate receptor type 3 (*GRM3*) in another family with M-D linked to 7q. Since the candidate region is still quite large and contains many genes, linkage studies, currently in progress, of additional families with M-D and identification of more markers in the region will be needed for further fine mapping of this locus.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Center for Medical Genetics, Marshfield Medical Research Foundation, http://research.marshfieldclinic.org/genetics/

Genetic Location Database, http://cedar.genetics.soton.ac.uk/public_html/ldb.html

National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for M-D [MIM 159900])

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