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Spinocerebellar Ataxia Type 8: Molecular Genetic Comparisons and Haplotype Analysis of 37 Families with Ataxia

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We reported elsewhere that an untranslated CTG expansion causes the dominantly inherited neurodegenerative disorder spinocerebellar ataxia type 8 (SCA8). SCA8 shows a complex inheritance pattern with extremes of incomplete penetrance, in which often only one or two affected individuals are found in a given family. SCA8 expansions have also been found in control chromosomes, indicating that separate genetic or environmental factors increase disease penetrance among SCA8-expansion–carrying patients with ataxia. We describe the molecular genetic features and disease penetrance of 37 different families with SCA8 ataxia from the United States, Canada, Japan, and Mexico. Haplotype analysis using 17 STR markers spanning an ~1-Mb region was performed on the families with ataxia, on a group of expansion carriers in the general population, and on psychiatric patients, to clarify the genetic basis of the reduced penetrance and to investigate whether CTG expansions among different populations share a common ancestral background. Two major ancestrally related haplotypes (A and A′) were found among white families with ataxia, normal controls, and patients with major psychosis, indicating a common ancestral origin of both pathogenic and nonpathogenic SCA8 expansions among whites. Two additional and distinct haplotypes were found among a group of Japanese families with ataxia (haplotype B) and a Mexican family with ataxia (haplotype C). Our finding that SCA8 expansions on three independently arising haplotypes are found among patients with ataxia and cosegregate with ataxia when multiple family members are affected further supports the direct role of the CTG expansion in disease pathogenesis.

Introduction

Repeat-expansion mutations cause 17 inherited neurological disorders, including fragile-X syndrome (MIM 309550), myotonic dystrophy types 1 and 2 (DM1 [MIM 160900] and DM2 [MIM 602668]), Huntington disease (MIM 143100), and 9 forms of spinocerebellar ataxia (SCAs) (Warren 1996; Zoghbi and Orr 2000; Ranum and Day 2002). Initially, it was thought that expansion mutations caused disease either by decreasing gene expression, as in the case of fragile-X syndrome (Jin and Warren 2000), or by altering the protein coding portion of the gene product, as in the majority of known SCA mutations caused by CAG trinucleotide expansions encoding elongated polyglutamine tracts (Orr 2001). Recently, a growing number of inherited neurological diseases have been shown to be caused by expansions that are transcribed into RNA but not translated into protein, including SCA8 (MIM 603680), SCA10 (MIM 603516), SCA12 (MIM 604326), DM1, DM2, and FXTAS (MIM 309550) (Holmes et al. 1999, 2001; Koob et al. 1999; Matsuura et al. 2000; Hagerman et al. 2001; Liquori et al. 2001; Jacquemont et al. 2003).

SCA type 8 (SCA8) is an inherited neurodegenerative disorder caused by a CTG trinucleotide repeat expansion in a noncoding gene of unknown function. Using our RAPID cloning method, we isolated the SCA8 CTG expansion directly from the DNA of a single patient with ataxia (Koob et al. 1998, 1999). Unlike positional cloning approaches, RAPID cloning does not require

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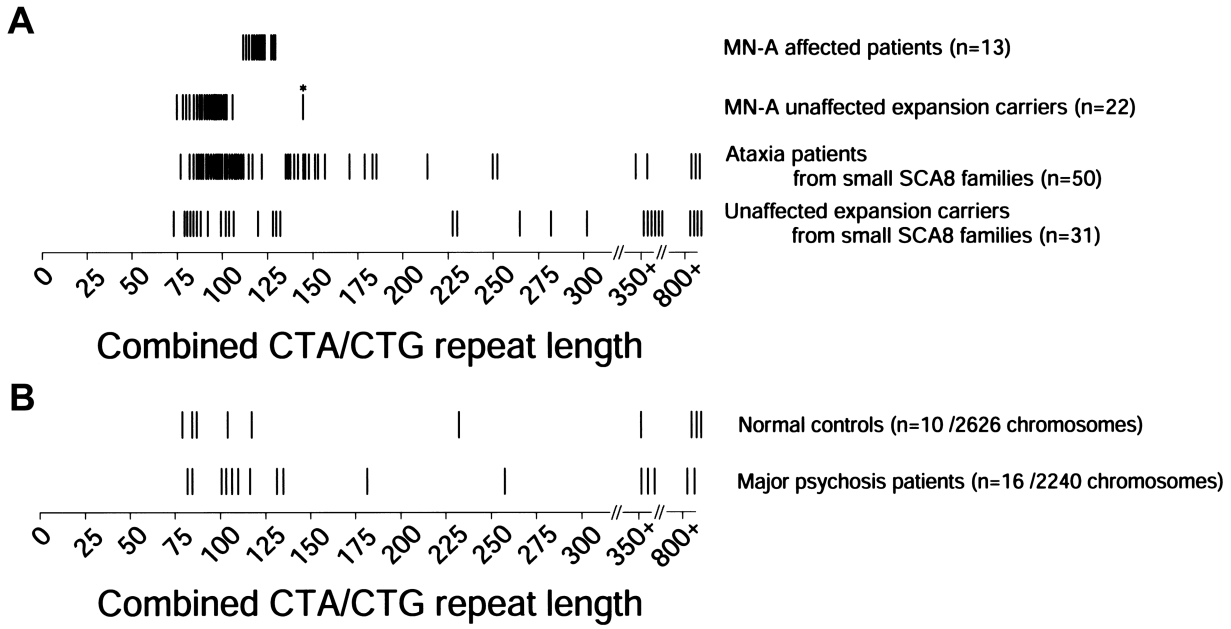


Figure 1 Histograms of the combined CTA/CTG repeat sizes in SCA8 expansion chromosomes of families with ataxia, normal controls, and patients with major psychosis. A vertical bar shows an allele with the CTG expansion. *A*, Histograms of alleles with the expanded repeats (>74) in affected patients and unaffected carriers from the MN-A family and from other small families with SCA8. The number of expanded alleles in each group is shown. The carrier with 143 repeats (marked by an asterisk [*]) is clinically unaffected; however, significant cerebellar atrophy was seen on MRI. *B*, Histograms of expanded repeats in chromosomes of normal controls and patients with major psychosis.

prior linkage data, large pedigrees, or highly penetrant inheritance patterns. The SCA8 expansion was found subsequently in additional families with ataxia, including the seven-generation MN-A family that we described elsewhere (Koob et al. 1999; Day et al. 2000). Affected individuals show slowly progressive, relatively pure cerebellar symptoms such as gait and limb ataxia, ataxic dysarthria, and nystagmus, with variable ages at onset. The CTG expansion is unusual because it was the first untranslated repeat expansion thought to cause ataxia by a gain-of-function RNA mechanism and because it shows dramatic genetic instability and reduced disease penetrance (Koob et al. 1999; Day et al. 2000; Ikeda et al. 2000; Moseley et al. 2000*a*, 2000*b*; Cellini et al. 2002; Topisirovic et al. 2002). Surprisingly, SCA8 expansions have also been found on control chromosomes, leading to the suggestion that the SCA8 expansion in our MN-A family, reported elsewhere (LOD = 6.8, θ = .0) (Koob et al. 1999; Day et al. 2000), may be in linkage disequilibrium with a neighboring disease-causing mutation and that the expansion is a coincidental background finding in the other families with ataxia and SCA8 expansions (Juvonen et al. 2000; Moseley et al. 2000*a*; Stevanin et al. 2000; Vincent et al. 2000*a*, 2000*b*; Worth et al. 2000; Sobrido et al. 2001; Izumi et al. 2003; Schols et al. 2003). To clarify the genetics of SCA8, we performed molecular genetic comparisons of a large number of SCA8 expansion carriers, including the large MN-A family and 36 smaller families with ataxia that are SCA8 positive. Haplotype analysis was performed to determine if the expansions on ataxia chromosomes arose independently of expansions on control chromosomes and to examine whether a mutation in *cis* could cause ataxia or modify SCA8 disease penetrance.

Material and Methods

Study Subjects

We identified 37 families with SCA8 ataxia: 32 families are white and live in the United States or Canada (31 of European and 1 of Central Asian descent), 4 families are from Japan, and 1 family is from Mexico. We obtained informed consent, performed neurological exams, and collected blood samples from 245 individuals, including affected patients (n = 63) and their relatives.

Also analyzed were 13 SCA8-expansion–positive DNA samples sent to Athena Diagnostics for ataxia testing. These samples had no clinical or family identifiers and may include both patients with ataxia as well as unaffected individuals sent for diagnostic testing. In addition, we studied 7 unrelated and apparently unaffected individuals (including members of CEPH families 1334

and 1416) and 14 patients, reported elsewhere, with major psychosis and SCA8 expansions (Day et al. 2000; Vincent et al. 2000*b*). In these two groups without ataxia, ⩾74 combined CTA/CTG repeats (74 is the smallest expansion number found in a patient with ataxia) is the threshold for inclusion in the study. The term "reduced penetrance" that is used throughout this article refers to the lack of clinical symptoms of ataxia among unaffected expansion carriers with affected relatives and among expansion carriers in the general population with no family history of ataxia.

Genetic Analysis

Genomic DNA was isolated from venous blood, with the use of Puregene kit #D-5000 (Gentra Systems). The number of combined CTA/CTG repeats at the SCA8 locus was determined by PCR, as described elsewhere (Koob et al. 1999). Subjects who appeared homozygous by PCR were screened subsequently by Southern blot analysis to detect expansions too large to be amplified. The combined CTA/CTG repeat lengths of very large expansions were estimated by Southern blot analysis. The number of CTA repeats preceding the CTG expansion was determined by sequence analysis in 13 families, as described elsewhere (Moseley et al. 2000*b*).

An *Afl*II polymorphism located 90 bp 3′ of the CTG repeat expansion was discovered in the MN-A family by sequence analysis; it was assayed subsequently by performing the SCA8 PCR (described above) and subjecting the PCR products to *Afl*II digestion (New England Biolabs). Products containing the *Afl*II polymorphism fail to digest.

Development of STR Markers in the SCA8 Region

Di-, tri-, tetra-, and penta-nucleotide repeats exceeding 8 units were identified and developed as candidate polymorphic markers on the basis of the sequence information available from the UCSC genome browser and NCBI databases (Genome Database). PCR primers flanking STR sequences were designed with the use of Primer3. The 5′ end of each forward primer was labeled with [γ-^{33}P] ATP by T4 polynucleotide kinase (New England Biolabs). PCR reactions were as follows: 20–50 ng of genomic DNA was mixed in a 5.5-μl reaction mixture containing 2 pmol of each primer, 10 mM of Tris-HCl (pH 9.0), 50 mM of KCl, 0.1% Triton X-100, 0.01% (weight/volume) gelatin, 200 mM of deoxynucleotide triphosphates, and 0.1 U of Ampli*Taq* DNA polymerase (Applied Biosystems), with variable concentrations of $MgCl_2$ for each marker (see table A1 [online only]). For all markers except D13S318, the PCR reaction was denatured at 94°C for 3 min, followed by 35 cycles (94°C for 45 s, 51°C/54°C/57°C for 45 s, 72°C for 60 s) with a final extension at 72°C for 6 min. For D13S318, the PCR reaction was denatured at 94°C for 3 min, followed by 35 cycles (94°C for 45 s, 55°C for 75 s, 72°C for 75 s) with a final extension at 72°C for 6 min. PCR primers, as well as specific annealing temperatures and $MgCl_2$ concentrations, are described in table A1 (online only). The PCR products were run on 4% denaturing polyacrylamide gels at 65 watts and were visualized by autoradiography. Allele sizes for each marker are given in bp and were determined by comparison with an M13 sequencing ladder.

SCA8 expansion haplotypes were established by determining which allele cosegregated with the SCA8 expansion in each family. The haplotypes of 38 control chromosomes were constructed by determining the alleles that were passed from unrelated spouses to their offspring. In the event that the associated allele for a marker could not be determined unequivocally, both alleles are given.

Haplotypes are presented from centromere to telomere, on the basis of the map position of the markers, as follows: *D13S275-YI18-YI17-YI15-D13S318-YI14-CL2-D13S1296-CL4-CL6-(CTG)$_n$-CL8-CL1-JJ9-JJ10-JJ12-JJ11-D13S135*. Alleles are designated by their size in bp; the SCA8 combined CTA/CTG repeats and isolated CTA repeat tracts are given by the number of repeats. Allele frequencies for each microsatellite marker, calculated from the genotypes of 82 unrelated SCA8-negative control chromosomes, are shown in table 1.

Statistical Analysis

Statistical differences in repeat size among the various clinical groups with SCA8 expansions were calculated by Student's *t*-test (one-tailed). Comparisons of the frequencies of SCA8 expansions in controls with those in patient groups were performed by χ^2 analysis. Analysis of the segregation of the SCA8 expansion among additional affected members (excluding affected probands) of the families with ataxia was performed by χ^2 analysis. Linkage analysis on the small families with ataxia was performed by LINKAGE (v5.1) (Weeks et al. 1995), as described elsewhere (Koob et al. 1999), with the modification that the asymptomatic carriers were assigned a liability class with an expected penetrance of 10%.

Results

Size of Pathogenic and Nonpathogenic SCA8 Expansions

Figure 1 shows the size distributions of SCA8 expansions found in panels of families with ataxia, psychiatric patients, and controls. The repeat sizes found in affected members of a large family with SCA8 ataxia (MN-A; LOD = 6.8, θ = .0) are compared with unaffected MN-A family members that also carry the SCA8 expansion

Table 1

Allele Frequencies of STR Markers

Marker and Allele (bp)	Frequency (%)
D13S275 (n = 78):	
188	2.6
186	2.6
184	30.8
182	62.7
172	1.3
YI18 (n = 78):	
246	1.3
242	2.6
238	1.3
234	3.8
222	3.8
218	12.8
214	27.0
210	17.9
206	20.6
202	3.8
194	1.3
190	3.8
YI17 (n = 76):	
263	11.8
258	3.9
253	15.8
248	40.9
243	25.0
238	2.6
YI15 (n = 72):	
236	12.5
235	1.4
233	9.7
232	4.2
230	44.5
227	8.3
224	19.4
D13S318 (n = 80):	
296	1.3
292	1.3
288	10.0
284	20.0
280	21.3
276	27.3
272	13.8
268	5.0
YI14 (n = 82):	
172	2.4
170	13.4
168	14.6
166	1.2
164	37.9
162	30.5
CL2 (n = 78):	
203	15.4
200	44.8
197	37.2
194	2.6

(*continued*)

Table 1 (continued)

Marker and Allele (bp)	Frequency (%)
D13S1296 (n = 80):	
193	2.5
191	1.3
189	5.0
187	6.3
185	10.0
183	28.6
181	22.3
179	10.0
177	6.3
175	3.8
173	1.3
167	1.3
165	1.3
CL4 (n = 80):	
173	1.3
169	15.0
168	7.5
167	25.0
165	2.5
164	1.3
163	20.0
158	2.5
157	24.9
CL6 (n = 80):	
151	2.5
149	28.8
147	36.1
145	31.3
141	1.3
CL8 (n = 82):	
248	1.2
247	4.9
246	18.3
245	31.7
244	23.2
243	20.7
CL1 (n = 78):	
168	2.6
166	3.8
164	12.8
160	15.4
158	11.5
156	47.5
148	6.4
JJ9 (n = 80):	
202	2.5
200	20.0
199	8.8
198	6.3
196	61.1
194	1.3
JJ10 (n = 80):	
251	18.8
249	17.5
247	62.4
245	1.3

(*continued*)

Table 1 (continued)

Marker and Allele (bp)	Frequency (%)
JJ12 (n = 78):	
176	1.3
174	6.4
172	11.5
170	3.8
168	9.0
166	3.8
164	7.7
162	56.5
JJ11 (n = 80):	
212	2.5
210	7.5
208	5.0
206	16.3
204	51.1
202	11.3
198	6.3
D13S135 (n = 82):	
190	11.0
188	6.1
186	14.6
184	7.3
182	44.0
180	8.5
178	8.5

(fig. 1*A*). Affected MN-A family members have significantly ($P = 5 \times 10^{-9}$) larger expansions (110–127, mean 119) than unaffected MN-A expansion carriers (73–104, mean 90), with the exception of a clinically unaffected 42-year-old individual with 143 repeats whose cerebellum is atrophic on MRI (indicated by an asterisk [*] in fig. 1*A*) (Day et al. 2003). Although all individuals in the MN-A family with >110 repeats show signs of ataxia or cerebellar atrophy, expansion carriers with <110 repeats in the MN-A family (21/35) have shown no signs of ataxia. The tight correlation between repeat size and pathogenesis found in the MN-A family is not found in the broader panel of families with SCA8 ataxia (fig. 1*A*). The reduced penetrance, which appears to be influenced by repeat size for the MN-A family, is much more pronounced in other families with SCA8 ataxia, regardless of repeat length. Among all 37 examined families with ataxia, SCA8 sizes among affected and unaffected expansion carriers can be shorter or longer than the pathogenic threshold found in the MN-A family. These data demonstrate that SCA8 expansions found among patients with ataxia vary dramatically in size and that the presence of an SCA8 expansion cannot be used to predict whether or not an asymptomatic individual will develop ataxia.

SCA8 Expansions in Controls

SCA8 expansions were also found in control samples, including two CEPH families (1334 and 1416). Among 2,626 unrelated control chromosomes analyzed in Minnesota and Canada, we identified 10 SCA8 alleles (0.4%) containing >74 combined CTA/CTG repeats (fig. 1*B*). One of the control expansions was from a grandmother of CEPH family 1416. Medical histories indicate that neither this woman nor her son (54 years old, 800 repeats) are affected by ataxia. All six of the SCA8 expansion carriers in this family (fig. 2*G*) were asymptomatic at the time of clinical evaluation. The expansion-positive individuals in generation III were children when they were evaluated clinically, and, thus, it is not clear if they will be at higher risk of developing ataxia.

Expansions containing >74 combined repeats occurred on 12 (4%) of 292 independent chromosomes in our original collection of probands from genetically undefined families with ataxia. Although the frequency of expansions with >74 combined repeats is significantly higher among unrelated probands with ataxia than in the general population (10/2,626 chromosomes; $P = 4 \times 10^{-25}$), the relative frequency of alleles with >74 combined repeats in the general population appears to occur at a higher frequency than all forms of ataxia (~1/10,000). Taken together, these data suggest that the CTG repeat can cause ataxia but that environmental or genetic modifiers, including repeat length, affect disease penetrance. SCA8 alleles with >74 repeats among patients diagnosed with major psychosis, which were reported elsewhere, are also shown (Vincent et al. 2000*a*) (fig. 1*B*).

Reduced Penetrance in Families with SCA8 Expansions

SCA8 is transmitted in an autosomal dominant pattern with reduced penetrance in the MN-A family, with one copy of the mutation found in affected individuals. In other families, SCA8 shows a complex inheritance pattern in which only a subset of expansion carriers from a given family are affected. Representative pedigrees included in this study are shown in figure 2. The families shown in figure 2*A* and 2*B* appear to transmit ataxia in a dominant pattern with affected individuals in multiple generations. In figure 2*C* and 2*D*, multiple affected individuals were found in a single generation, whereas the families represented in figure 2*E* and 2*F* have only single affected individuals.

In contrast to the relatively large number of affected patients in the MN-A family (n = 13), 25 of the remaining 36 families with ataxia had only a single affected individual, 9 families had two affected individuals, and only 2 families had three affected individuals. Although only a subset of the expansion carriers in the MN-A family develop ataxia (13/35), these data illus-

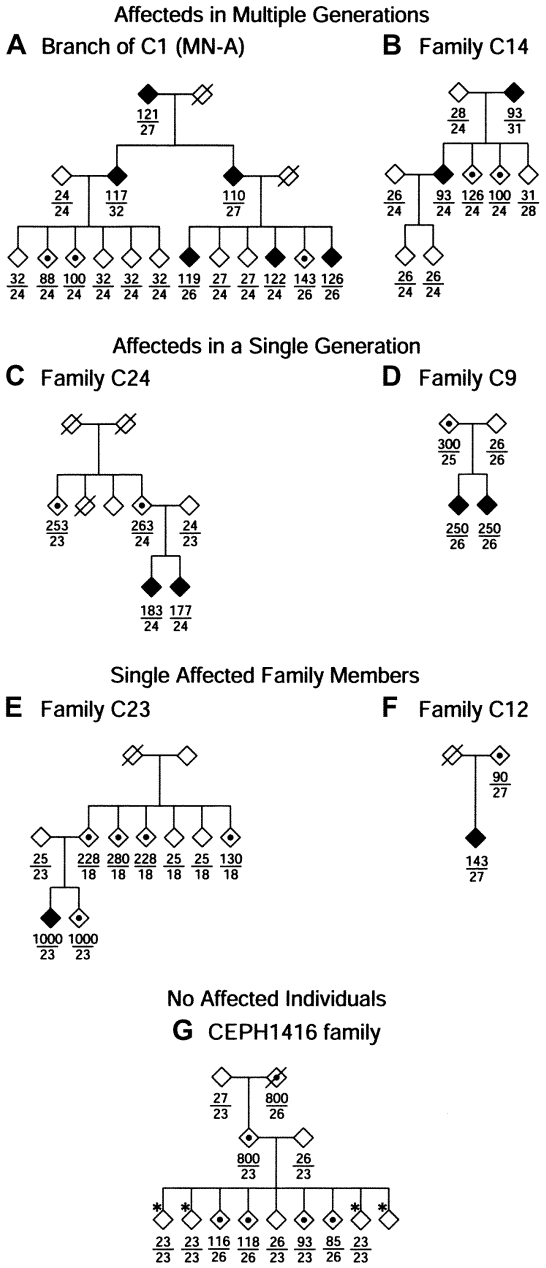


Figure 2 SCA8 pedigrees with varying degrees of disease penetrance. The pedigrees have been altered to preserve confidentiality. Symbols for individuals affected by ataxia are blackened, and unaffected expansion carriers are indicated by symbols with a dot inside them. A diagonal line through a symbol denotes an individual who is deceased. The numbers of the combined CTA/CTG repeat tracts for the expanded and unexpanded alleles are shown below the symbol. Representative pedigrees show affected individuals in multiple generations of a branch of the C1 (MN-A) *(pedigree A)* and C14 *(pedigree B)* families, affected individuals in a single generation of the C24 *(pedigree C)* and C9 *(pedigree D)* families, single affected individuals in the C23 *(pedigree E)* and C12 *(pedigree F)* families, and no affected individuals in the CEPH1416 family *(pedigree G)*. Individuals indicated with an asterisk (*) are negative for CTG expansion by Southern analysis. Reduced penetrance is observed in all of these families.

trate that the penetrance of ataxia appears to be significantly higher in the MN-A pedigree than in the group of 36 smaller families with ataxia that are included in our study.

SCA8 Expansions Cosegregate with Ataxia in Small Families

In the MN-A family, other studies have shown that the cosegregation of the SCA8 expansion and ataxia is highly significant (LOD = 6.8, θ = .00). To distinguish the possibility that the SCA8 expansions are found simply by chance in the 36 additional smaller families with ataxia from the possibility that the expansions do in fact predispose carriers to ataxia, we examined the incidence of the expansion cosegregating with ataxia in family members other than the probands. If, for example, the expansion did not predispose patients to ataxia but was found merely by chance in these 36 families, then we would expect that a 50% frequency of the SCA8 expansion would be found in additional affected first-degree relatives. In contrast, 12 of the 13 affected first-degree relatives available for analysis also inherited the SCA8 expansion, indicating that the expansion cosegregates with ataxia in these small families (P = .0038). The only exception was found in a family (C10, fig. 3*A*) in which two sisters were affected with a form of ataxia clinically distinct from SCA8 by being a markedly more severe disease with rapid disease progression, pronounced choreiform movements, a severe sensory neuronopathy, and neuromyotonic discharges seen by electromyography. Because the striking phenotypic distinctions suggest that a separate disease is segregating in the C10 family, the family was excluded from further analysis. Linkage analysis was performed on the remaining small families with multiple affected individuals. Table 2 shows the results of the linkage analysis of these 10 families with ataxia. Although the highest LOD score for a single family is only 0.34 at a recombination fraction (θ) of .00, the LOD scores were consistently positive and, when combined, exceeded the threshold level of 2.0, considered significant for testing linkage of an X-linked disorder or linkage to a single specific locus (Ott 1991). The cosegregation of the SCA8 expansion among additional affected relatives in the group of small families with ataxia indicates that the SCA8 expansion directly predisposes individuals to developing ataxia.

Haplotype Analysis of SCA8 Expansions

To better understand the origin of the SCA8 expansion and the reduced penetrance of the disease, we performed haplotype analysis on a panel of 37 families with SCA8 ataxia, 13 SCA8-expansion–positive samples that were sent to Athena Diagnostics for ataxia testing, 7 control samples with expansions, and 14 expansion carriers with

psychiatric diseases. A total of 17 polymorphic STR markers were analyzed, including 13 newly developed markers that span a region of ~1 Mb flanking the SCA8 CTG repeat. Figure 4 shows the location of the STR markers relative to the SCA8 CTG-repeat tract and the genomic organization of the *SCA8* gene and the overlapping *Kelch Like 1* (*KLHL1)* gene.

SCA8 Expansions in Whites Arose from a Common Founder

The majority of SCA8 expansion families, in the groups with and without ataxia, are white. Most of the chromosomes carrying SCA8 expansions in white families with ataxia, in Athena samples, in psychiatric patients, and in control subjects have haplotype A or A′ (fig. 3*A*). The core haplotype region defined by 10 consecutive markers flanking the CTG repeat (*YI17-YI15-D13S318-YI14-CL2-D13S1296-CL4-CL6-*$(CTG)_{exp}$*-CL8-CL1*) is nearly identical on haplotypes A and A′, with differences at the D13S1296 and YI15 markers likely explained by microsatellite instability. The differences in the flanking regions of haplotype A and A′ are likely to have resulted from ancestral recombination events at markers YI18 and JJ9, located 284 kb centromeric and 17 kb telomeric from the CTG repeat, respectively. Possible ancestral recombination and instability events accounting for the various related haplotypes are diagrammed in figure 3*B*. The haplotype data demonstrate that the majority of SCA8 expansions in white families with and without ataxia arose from a common ancestral mutation, and that these families share a region of ~290 kb flanking the SCA8 CTG repeat tract. As in other white families, the Central Asian family with SCA8 ataxia (C17; see fig. 3*A*) carried haplotype A with minor variations at two outer markers.

Two Additional Haplotypes Found Among Japanese and Mexican Families with Ataxia

Four unrelated Japanese families with ataxia have an SCA8 expansion haplotype (B) distinct from the two predominant haplotypes in whites (J1–J4; see fig. 3*A*). Two chromosomes from whites with ataxia (family C32 and the C13B chromosome of a patient homozygous for SCA8) have haplotypes similar to haplotype B, with variations likely the result of recombination events at markers flanking the CTG expansion. A third distinct haplotype (C) was found in a Mexican family with ataxia (M1; see fig. 3*A*). These results indicate that independently arising SCA8 expansions are found in families of various ethnic backgrounds who are affected by ataxia.

SCA8 Expansion Haplotypes Relatively Rare in the General Population

To determine if the SCA8 expansion haplotypes were common in the general population, 38 control chromosomes were screened with the use of four consecutive markers flanking the CTG repeat (*CL4-CL6-*$(CTG)_n$*-CL8-CL1*). A total of 26 distinct haplotypes containing these four core markers were found. The most common haplotype, found on five chromosomes (13.2%), was 167-145-$(CTG)_n$-244-156. None of the control chromosomes had haplotypes identical to the core region identified in the major ataxia haplotypes A and A′ in whites (165-147-$(CTG)_n$-246-156). Two control chromosomes (5.3%) had the same core region of haplotype B (168-145-$(CTG)_n$-245-160), and no chromosomes had the same core region of haplotype C.

Clinical Features of Families with SCA8 with Different Haplotypes

The clinical features of the families with SCA8 (grouped by haplotype) are shown in table 3. No obvious phenotypic differences are noted between haplotype groups. The clinical features of the MN-A family (haplotype A, fig. 3*A*) are shown separately because of the large number of affected subjects. Regardless of haplotype group, patients with SCA8 ataxia are characterized by high frequencies of gait ataxia, ataxic dysarthria, limb dysmetria, and gaze-evoked nystagmus, indicating pancerebellar involvement. Pyramidal tract signs and reduced vibratory sense are observed less frequently. Other neurological signs are rare or absent. The clinical features of the families with SCA8 can be summarized as relatively pure cerebellar ataxia. The disease progression is typically very slow, even when onset of ataxia was during the 1st decade of life. When available, MRI scans invariably showed pancerebellar atrophy without brainstem or cerebral atrophy.

Table 2

Linkage Analysis of SCA8 Expansion and Ataxia in Small Families

		LOD Score at θ =						
Family	Haplotype	.00	.01	.05	.10	.20	.30	.40
C2	A	.34	.33	.29	.25	.16	.08	.03
C3	A	.30	.29	.26	.21	.13	.06	.02
C9	A	.15	.15	.13	.11	.07	.03	.01
C11	A	.29	.28	.27	.25	.20	.15	.08
C13	A and B	.26	.25	.22	.19	.11	.05	.01
C14	A	.02	.02	.02	.02	.02	.01	.01
C19	A′	.30	.29	.26	.21	.13	.06	.02
C24	A′	.29	.28	.25	.21	.13	.06	.02
J1	B	.00	.00	.00	.00	.00	.00	.00
J2	B	.07	.07	.05	.04	.02	.01	.00
All		2.02	1.96	1.75	1.49	.95	.51	.20

Group I: Haplotype analysis of 37 SCA8 families

Marker	D13S275	YI18	YI17	YI15	D13S318	YI14	CL2	D13S1296	CL4	CL6		CL8	CL1	JJ9	JJ10	JJ12	JJ11	D13S135	Marker
Repeat Motif	(CA)n	(GATA)n	(AAAAT)n	(AAT)n	(TATC)n	(GT)n	(CAA)n	(CA)n	(GAAA)n	(GT)n	(CTG)n	(CA)n	(GT)n	(GT)n	(CT)n	(GT)n	(GT)n	(CA)n	Repeat Motif
No of Alleles	5	12	6	7	8	6	4	13	9	5		6	7	6	4	8	7	7	No of Alleles
Kb from (CTG)n	974kb	284kb	277kb	156kb	137kb	112kb	72kb	57kb	53kb	10kb	0	1.1 kb	13.6 kb	17kb	20kb	52kb	80kb	97kb	Kb from (CTG)n
C1 (MN-A)	188	214	243	236	280	164	197	175	165	147	73~143	246	156	199	251	174	206	186	C1 (MN-A)
C2	184	210	248	233	284	164	197	175	165	147	80~88	246	156	199	251	174	206	186	C2
C3	184	210	248	233	284	164	197	175	165	147	80~115	246	156	199/196	251/247	174	206	186	C3
C4	184	210	n.d.	n.d.	284	164	197	175	165	147/145	110	246	156	199	251	174	206	186	C4
C5	184/182	210/242	248/243	233/230	284/272	164/162	197/200	175/185	165	147/145	98	246/245	156	199/196	251/247	174/162	206/204	186/180	C5
C6	184	210/242	248	233/230	284/288	164	197	175/183	165/167	147/145	90	246/244	156	199/196	251/247	174/162	206/202	186/182	C6
C7	184	210/214	248/253	233/230	284	164	197	175/183	165/167	147/145	134	246/244	156	199/196	251/247	174/162	206/204	186/180	C7
C8	184/182	210	248	233	284/276	164	197	175	165	147	71~88	246	156	199	251	174	212	186	C8
C9	184	210	248	233	284	164	197	175	165	147	208~750	245	156	199	251	174	206	186	C9
C10	182	214	248	233	284	164	197	175	165	147	130~735	246	156	199	251	172	206	186	C10
C11	184	210/206	248	230	284	164	197	175	165	147	101~118	246	156/158	199/196	251	174	206	186	C11
C12	184	214	248/243	230	284	164	197	175/181	165	147/149	90~143	246/243	156	199/200	251	174	206	186	C12
C13A	182	210	248	230	284/276	164	197	175	165	147	97~120	246	156	199	251	174	206	186	C13A
C14	184	210	248	236	284	164	197	175	165	147/145	93~126	246	156	199	251	174	206	186	C14
C15	184/170	210/242	248/243	236/230	284/272	164	197	175/181	165/167	147/145	91	246/244	156	199/196	251/247	174/162	206/204	186/180	C15
C16	184/188	210/214	248	236/224	284	164/168	197/200	175/179	165/163	147	169	246	156/158	199/196	251/247	176/162	206/204	186/182	C16
C17	184	218	248	233	288	164	197	175	165	147	102	246	156	199	251	174	206	186	C17
C18	184/182	210/218	248/253	233/230	280/272	164/162	197/200	173/181	165/157	147/149	212	245	156	199/196	251/247	174/162	206/204	186/182	C18
C19	184	218	248	233	284	164	197	177	165	147	150	246/245	156	196	247	162	208	180	C19
C20	184	218	248	233	284	164	197	177	165	147	84~588	246	156	196/200	247/251	162/172	206	180	C20
C21	188	218	248/243	236	284	164	197/200	177	165	147	134~445	246	156	196	247	162	206	180	C21
C22	184/182	218	248	236	284	164	197	177	165	147	845~945	246	156	196	247	162	206	180	C22
C23	184	218	248	236	284	164/168	197	177	165	147	130~1110	246	156	196/200	247	162	206	180	C23
C24	182	218	248	236	284	164	197	177	165	147	177~263	246	156	196/200	247	162	206	180	C24
C25	184/182	218/234	248/243	236	284	164	197	177/185	165/169	147/149	1380	246/245	156/158	196	247	162/172	206	180/186	C25
C26A	184	218	248	236	284	164	197	177	165	147	101~950	246	156	196	247	162	206	180	C26A
C27	184	214	248	236	284	164	197	177	165	147	140	246	156	196	247	162	206	180/182	C27
C28	184/188	214/206	248	236/230	284/268	164/162	197/200	177/181	165/157	147	146~1130	245	156/164	196/200	247/251	162/172	206	180/186	C28
C29	182	222/206	248/258	236	284/280	164	197	177/181	165/167	147/145	75	246/244	158/164	196/200	247	162/172	206/202	182/186	C29
C26B	182	206	258	236	284	164	197	177	165	147	101~950	246	156	196	247	162	206	180	C26B
C30	182/170	218/210	248/253	236	288/276	164/170	197/200	177/173	165/169	147/149	150	246	156/158	196/198	247	162	206	182	C30
C31	182	214	248	230	288	168	200	181	165	147	138	246	156	196	247	166	210	188	C31
C32	182	210	238	230	288	162	200	185	168	145	80~84	245	160	198	249	168	212	190	C32
J1	184/182	214	248	230	284	164	200	179/183	168	145	89~105	245	160	198/196	249	168/162	210/204	192/182	J1
J2	184	214	248	230	284	164	200	179	168	145	95~99	245	160	198	249	168	210	192	J2
J3	184/182	214/210	248/238	230/233	284/276	164/162	200/197	179/183	167/157	145	155	245/244	162/168	198/200	249/251	168/172	214/204	190/178	J3
J4	182	218	248	230	284	162	200	185	168	145	95~136	245	160	198	249	166	210/208	190	J4
C13B	184	218	243	227	284/276	162	200	181	168	145	97~120	245	160	196	249	168	204	182	C13B
M1	182	214	248	233	284	168	203	185	163	149	100	244	148	198	249	168/164	216	190	M1

C: Caucasian SCA8 families
C13A/C13B and C26A/C26B represent haplotypes of the two different expansion chromosomes in these homozygous individuals.
J: Japanese SCA8 families
M: Mexican SCA8 family

Group II: Haplotype analysis of 13 diagnostic ataxia samples

Marker	D13S275	YI18	YI17	YI15	D13S318	YI14	CL2	D13S1296	CL4	CL6		CL8	CL1	JJ9	JJ10	JJ12	JJ11	D13S135	Marker
Repeat Motif	(CA)n	(GATA)n	(AAAAT)n	(AAT)n	(TATC)n	(GT)n	(CAA)n	(CA)n	(GAAA)n	(GT)n	(CTG)n	(CA)n	(GT)n	(GT)n	(CT)n	(GT)n	(GT)n	(CA)n	Repeat Motif
No of Alleles	5	12	6	7	8	6	4	13	9	5		6	7	6	4	8	7	7	No of Alleles
Kb from (CTG)n	974kb	284kb	277kb	156kb	137kb	112kb	72kb	57kb	53kb	10kb	0	1.1 kb	13.6 kb	17kb	20kb	52kb	80kb	97kb	Kb from (CTG)n
AD1	184	210/242	248	233/230	284/288	164	197	175/183	165/167	147/145	88	246/244	156	199/196	251/247	174/162	206/202	186/182	AD1
AD2	184/182	210/206	248/263	233/224	284	164/168	197/200	175/179	165/163	147	86	246	156/158	199/196	251/247	174/162	206/204	186/190	AD2
AD3	184	210/206	248/243	233/236	284/288	164	197	175/183	165/167	147/145	56	246/244	158/154	199	251	174/170	206/208	186/196	AD3
AD4	184/182	214/194	248	233/230	284/276	164/162	197/200	175/181	165/157	147	103	246/245	156	199/196	251/247	174/162	206/204	186/180	AD4
AD5	184/182	210/206	248/238	230	284/280	164/168	197/203	175/183	165/163	147/149	89	246/243	156/158	199/196	251/247	174/162	206/204	186/182	AD5
AD6	184	210/202	248/263	233/224	288/280	164/168	197/200	175/179	165/163	147	105	246	156	199/196	251/247	174/162	206/204	186/182	AD6
AD7	184/182	210/214	248/243	233/224	288/272	164/168	197/200	175/181	165/157	147	137	246/245	156/154	199/200	251	174/168	206/204	186/178	AD7
AD8	184/182	214/206	248/258	236/224	284/280	164/168	197	175/179	165/167	147/145	69	246/244	156/158	199/196	251/247	174/164	206/204	186/190	AD8
AD9	182/188	210/190	243/238	230/227	272/268	164/162	197/200	175/181	165/157	147/145	76	246/245	156	199/196	251/247	174/162	206/204	186/180	AD9
AD10	182	210/254	248/223	233/236	284/280	164/162	197/200	181/185	163/157	147/151	62	246/245	152/164	199/200	251	168/166	204	182	AD10
AD11	184/182	218/214	248	236/230	284/280	164	197	177/193	165/157	147/145	849	246/244	156/148	196/200	247/249	162	206/204	180/182	AD11
AD12	184/182	218/210	248	239/227	284/280	164/168	197/203	177/185	165/163	147/149	95	246/243	156/158	196	247	162	206/204	180/182	AD12
AD13	184/182	210/206	238/263	230	288/276	162	200	183/185	168	145	96	245	160	198/200	249	168	216/204	190/182	AD13

Figure 3 Results of haplotype analyses. *A*, Haplotype analysis of several SCA8-expansion–positive families with and without ataxia. The markers in this figure are shown in order of their physical distance from the CTG repeat. *Group I*, Haplotypes of 37 SCA8-positive families in which at least one member has been diagnosed with ataxia. Two predominant and probably related SCA8 expansion haplotypes (A and A′) were found in 18 and 13 families, respectively. Six families, including four from Japan, have a clearly distinct second haplotype (B), and a Mexican family shows evidence for a third independent haplotype (C). The consensus haplotype A is shown in yellow. Minor deviations in repeat size of ±1 repeat unit flanked by markers with conserved allele sizes are indicated by alternative colors (*color key at bottom*). Recombinant

Group III: Haplotype analysis of 5 normal controls and 2 CEPH families

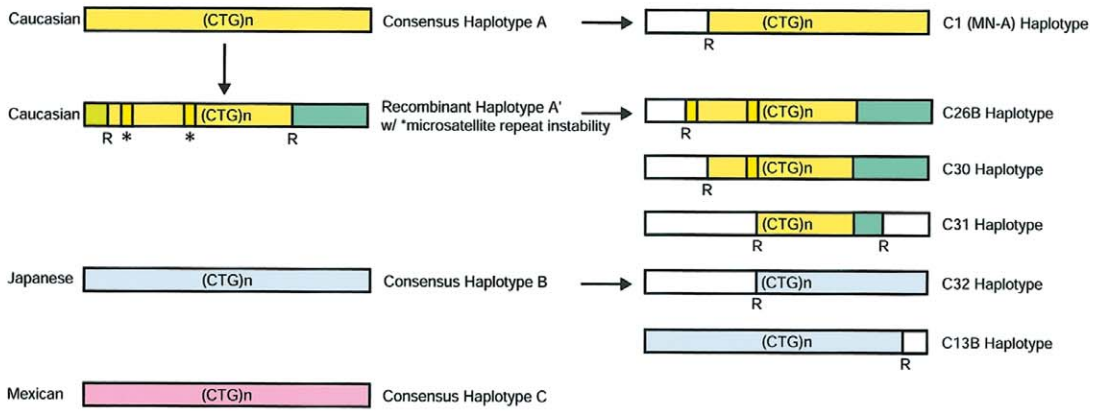
Marker	D13S275	YI18	YI17	YI15	D13S318	YI14	CL2	D13S1296	CL4	CL6		CL8	CL1	JJ9	JJ10	JJ12	JJ11	D13S135	Marker
Repeat Motif	(CA)n	(GATA)n	(AAAAT)n	(AAT)n	(TATC)n	(GT)n	(CAA)n	(CA)n	(GAAA)n	(GT)n	(CTG)n	(CA)n	(GT)n	(GT)n	(CT)n	(GT)n	(GT)n	(CA)n	Repeat Motif
No of Alleles	5	12	6	7	8	6	4	13	9	5		6	7	6	4	8	7	7	No of Alleles
Kb from (CTG)n	974kb	284kb	277kb	156kb	137kb	112kb	72kb	57kb	53kb	10kb	0	1.1 kb	13.6 kb	17kb	20kb	52kb	80kb	97kb	Kb from (CTG)n
N1	184/182	210/206	248	233/224	284/280	164/168	197/200	175/183	165/169	147/149	117	246/247	156	199/196	251/247	174/162	206/204	186/182	N1
CEPH1416	184	210	253	233	284	164	197	175	165	147	85~800	246	156	199	251	174	206	186	CEPH1416
N2	184	214/190	248/243	233/230	284/268	164/162	197/200	171/181	165/157	147	103	246/245	156/164	199/200	251	174	206	186	N2
N3	184/182	210/234	248/243	233/232	280/276	164/168	197	175/191	165/167	147/145	970	246/244	156/164	199/200	251/249	174/168	204/210	186/178	N3
N4	184/182	218/238	248/243	233/232	284/276	164/168	197/200	177/195	165/167	147/149	230	246/244	156/164	196/200	247/251	162/168	206/204	180/178	N4
N5	184	218/210	248/243	236/233	280	164	197	177/191	165/167	147/149	550	n.d.	156	196	247	162	206/204	180/182	N5
CEPH1334	182	222	248	236	284	164	197	177	165	147	160~900	246	156	196	247	162	206	180	CEPH1334

Group IV: Haplotype analysis of 14 major psychosis patients

Marker	D13S275	YI18	YI17	YI15	D13S318	YI14	CL2	D13S1296	CL4	CL6		CL8	CL1	JJ9	JJ10	JJ12	JJ11	D13S135	Marker
Repeat Motif	(CA)n	(GATA)n	(AAAAT)n	(AAT)n	(TATC)n	(GT)n	(CAA)n	(CA)n	(GAAA)n	(GT)n	(CTG)n	(CA)n	(GT)n	(GT)n	(CT)n	(GT)n	(GT)n	(CA)n	Repeat Motif
No of Alleles	5	12	6	7	8	6	4	13	9	5		6	7	6	4	8	7	7	No of Alleles
Kb from (CTG)n	974kb	284kb	277kb	156kb	137kb	112kb	72kb	57kb	53kb	10kb	0	1.1 kb	13.6 kb	17kb	20kb	52kb	80kb	97kb	Kb from (CTG)n
P1	184	210/214	248	233/230	284	164	197	175/181	165/157	147	100	246/245	156/164	199/200	251/249	174	206/204	186/188	P1
P2	184/182	210/214	248	233/236	284/268	164/162	197/200	175/181	165/157	147	103	246/245	156	199/196	251/247	174/162	206/202	186/182	P2
P3	184/182	214/202	248/263	233/224	284	164/168	197/200	175	165/163	147	180	246	156	199	251/247	174	206/204	186/182	P3
P4	184/188	206/250	248/243	233/232	284/276	164/170	197	175/197	165/167	147/145	257	246/244	158/164	199/196	251/247	168/162	204/202	182/178	P4
P5	184/182	210/246	253/228	233/238	284/272	164/170	197/200	175/177	165/155	147	1300	246/244	156/164	199/200	251/249	174/164	206/200	186/174	P5
P6	184/182	210/190	253/243	233/224	288/272	164/170	197	175	165	147	130	246/245	156	199/196	251	174	206/204	186/178	P6
P7	184/182	210/202	248/258	232	272/268	164/168	197/200	175/181	165/157	147/149	600	246/245	156/166	199	251/249	174/168	206/204	186/178	P7
P8	184/182	218/198	248/258	230/239	284/288	164/162	197/203	175/183	165/163	147	1140	246/243	156	199/196	251/247	174/162	206	186/188	P8
P9	184/172	214/238	248/243	233/230	288/276	164/162	197/200	175/183	165/157	147/145	550	246/245	156	196	247/245	162/164	204/210	182/190	P9
P10	184	218	248	233	284	164	197	177	165	147	116	246	156	196	247	162	206	180	P10
P11	184/182	218/202	248/263	233/227	284/276	164/162	197/200	177/183	165/168	147/145	130	246/245	156/160	196/199	247/249	162/168	206/204	180/182	P11
P12	184	218/222	248	236/230	284	164	197	177	165	147	600	246	156	196	247/245	162	206	180	P12
P13	184/188	218	248	236/230	284/280	164/168	197/203	177/185	165/163	147/149	107	246/243	156/158	196	247	162	206/204	180/182	P13
P14	184/182	206/246	248	236/230	284	164	197	177/183	165/167	147/145	106	246/244	156	196	247	162	206/202	180/182	P14

Recombinant regions that are not conserved among families are uncolored.

(CTG)n: Size range of the combined CTA/CTG repeat expansions in the family
n.d.: not determined



regions that are not conserved among families are shown in white. Two families (C13 and C26) presented with homozygous expansion-positive patients, and the separate SCA8 haplotypes for these families are indicated. The microsatellite marker name, its repeat motif, and its distance from the SCA8 CTG repeat expansion are shown (*top*). The size range of the combined CTA/CTG repeat expansions in the family (or, in some cases, in a single expansion carrier) are shown. *Group II,* SCA8 expansion haplotypes of 13 samples sent to Athena Diagnostics for testing are either haplotype A or A′, except for a single subject (AD13) with haplotype B. *Group III,* Haplotypes of seven normal control families, including two CEPH families, with SCA8 expansions. Four of these families had haplotype A, three had haplotype A′. *Group IV,* Haplotypes of 14 patients with major psychosis and CTG expansions are either haplotype A ($n = 8$) or haplotype A′ ($n = 6$). *B,* Proposed ancestral origin of SCA8 haplotypes. Proposed ancestral relationship between the major haplotype variants is shown by a small number of ancestral recombination and microsatellite instability events.

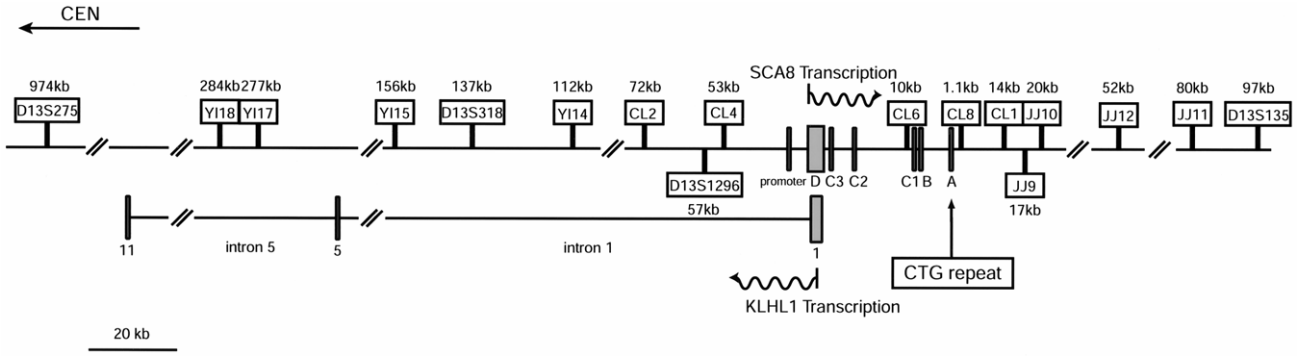


Figure 4 Map of newly developed polymorphic markers in the SCA8 region. Genomic DNA is represented by a horizontal line, and double slashes (//) represent regions in which the map is not drawn to scale. Of the 17 microsatellite markers that were genotyped, 13 were newly developed. The markers and their relative map positions are indicated with their distance from the SCA8 CTG repeat given in kb. Six exons and the promoter region of the *SCA8* gene and exons 1, 5, and 11 of the *KLHL1* gene are indicated by boxes. The CTG repeat exists in exon A of the *SCA8* gene. SCA8 transcript is transcribed in antisense orientation to KLHL1 transcription through exon 1 of KLHL1. Internal scale bar is indicated as 20 kb.

Variations on the MN-A and Other SCA8 Haplotypes

To further investigate possible differences in the MN-A haplotype that may account for the relatively high penetrance found in that family, we analyzed haplotype differences and sequence variations that occurred in the MN-A family but not in the other families with SCA8 ataxia. In the MN-A family (C1; see fig. 3*A*), consecutive allele variations in five markers from D13S318 to D13S275 suggest that the haplotype in the MN-A family diverged from haplotype A through an ancestral recombination event between the D13S318 and YI14 markers. This recombination event may have resulted in a *cis*-modifier that increases disease penetrance being juxtaposed to the SCA8 expansion. Although similar but separate recombination events also occurred on several other chromosomes with haplotypes A′ and B (C26B, C30, C31, C32, and C13B), these events did not appear to increase disease penetrance and would be unlikely to have introduced a similar linked modifier. Although our haplotype data indicate that the expansion chromosomes in whites arose from a single or small number of founder chromosomes, significant size variation of the CTA repeat that flanks the CTG expansion is found on haplotype A, with repeats ranging from 3–17 CTAs (table 4). The CTA portion of the repeat tract has been reported elsewhere to be stable within individual families, and, therefore, the CTA repeat numbers listed in table 4 are likely to be the same within individual families (Moseley et al. 2000*b*). The CTA repeat tract in the MN-A family is much smaller than that in any of the other families with SCA8 ataxia that we analyzed and, hence, is another notable genetic difference.

Further evidence that the region immediately flanking the CTG expansion is highly mutable comes from the identification of an SNP that was found in the MN-A family but not in 17 other families with haplotype A or A′ that were tested. This SNP is located 90 bp immediately 3′ of the CTG expansion and is well within the region of haplotype A conservation. It is possible that—but not yet clear whether—these differences play a role in the increased disease penetrance of the MN-A family. A summary of the sequence variations flanking the SCA8 CTG expansion is shown in table 4.

Possible Role of Unexpanded Allele as Genetic Modifier of Disease Penetrance

To examine the possibility that the second predisposing factor could involve the unexpanded SCA8 alleles, we examined the subset of families with affected sib pairs to determine if, in addition to the SCA8 expansion, they also inherited the same unexpanded allele from the second parent. All five sib pairs available for analysis shared the same unexpanded SCA8 allele, showing a marginally significant result ($P = .025$).

Homozygous Expansion Carriers

Five patients from three different families with ataxia were homozygous for the SCA8 expansion with >74 CTA/CTG repeats. Two of these patients were reported elsewhere as members of a consanguineous branch of the MN-A family. The other homozygous patients were members of families C13 and C26. The C13 family has haplotypes A and B, whereas the C26 homozygote has one consensus haplotype A′ with the second chromosome carrying a version of haplotype A′ with a divergent centromeric end likely representing a recombination event (C26A and C26B, respectively). Clinical infor-

Table 3

Clinical Features of SCA8 Families with Different Haplotypes

	% Affected in Haplotype Group (Abnl/n)[a]				
Clinical Feature	MN-A Family	Haplotype A[b]	Haplotype A′	Haplotype B	All
Gait ataxia	91 (10/11)	92 (11/12)	75 (3/4)	100 (7/7)	91 (31/34)
Limb ataxia	91 (10/11)	86 (18/21)	100 (5/5)	100 (6/6)	91 (39/43)
Dysarthria	100 (11/11)	85 (17/20)	100 (4/4)	100 (7/7)	93 (39/42)
Impaired smooth pursuit	73 (8/11)	76 (19/25)	67 (4/6)	100 (7/7)	78 (38/49)
Nystagmus	73 (8/11)	72 (13/18)	83 (5/6)	71 (5/7)	74 (31/42)
Reduced vibratory sense	45 (5/11)	45 (9/20)	0 (0/6)	33 (2/6)	37 (16/43)
Hyperreflexia	73 (8/11)	40 (8/20)	67 (4/6)	29 (2/7)	50 (22/44)
Hyporeflexia	0 (0/11)	15 (3/20)	0 (0/6)	14 (1/7)	9 (4/44)
Extensor plantar response	18 (2/11)	15 (2/13)	40 (2/5)	0 (0/7)	17 (6/36)
Muscle atrophy	0 (0/11)	8 (1/12)	20 (1/5)	0 (0/7)	6 (2/35)

[a] Abln/n = no. of abnormal individuals/no. of patients with ataxia that were examined.
[b] MN-A family not included.

mation for three of the five homozygous subjects was available and, in each of these cases, the disease symptoms and the clinical course of the disease were similar to affected individuals with a single SCA8 expansion.

Discussion

We reported elsewhere that an untranslated CTG expansion causes SCA8. Although age-dependent reduced penetrance is found in all microsatellite-expansion disorders, the age-independent reduced penetrance of SCA8 is the most difficult genetic feature of the disease to understand. To investigate potential causes of reduced penetrance, we compared the molecular genetic features of 37 different families with SCA8 that were from the United States, Canada, Japan, and Mexico. To determine the role that the expansion and *cis*-acting factors play in predisposing patients to ataxia, high-resolution haplotype analysis was performed on the families with ataxia, as well as on a group of expansion carriers in the general population and on psychiatric patients. High-resolution haplotypes were established by typing 17 microsatellite markers, including 13 new markers we developed that span an ~1-Mb region flanking the SCA8 CTG expansion.

In contrast to other studies with markers 3.2 Mb and 1 Mb centromeric and 0.9 Mb and 1.8 Mb telomeric from the CTG expansion (Koob et al. 1999; Juvonen et al. 2000) that did not show obvious haplotype conservation, the results of our high-resolution haplotype analyses (fig. 3*A*) show two predominant conserved haplotypes (A and A′) among white families with SCA8 ataxia. The core haplotype region defined by the 10 consecutive markers flanking the CTG repeat (*YI17-YI15-D13S318-YI14-CL2-D13S1296-CL4-CL6-*$[CTG]_{exp}$*-CL8-CL1*) is nearly identical on haplotypes A and A′, with differences at the D13S1296 and YI15 markers likely caused by microsatellite instability. In addition, a distinct haplotype (haplotype B) is conserved among four Japanese and two white families with ataxia (C32 and C13B), and a third haplotype (haplotype C) is found in a Mexican family with ataxia. These results demonstrate that at least three independently arising SCA8 expansions are associated with ataxia.

Interestingly, subjects from Athena Diagnostics with CTG expansions (whom we presume were tested because of clinical ataxia, but about whom we have no clinical information), normal controls (including two CEPH families), and patients with major psychosis have the same haplotypes (A and A′) found in white families with ataxia, except for a single subject (AD13) from Athena Diagnostics who shows haplotype B. These results indicate that, among whites, CTG expansions in normal controls and patients with major psychosis have the same ancestral origin as the families with SCA8 ataxia. The reason that these expansion-positive controls and patients with major psychosis do not exhibit ataxia is not yet understood.

Evidence that the expansion itself predisposes families to ataxia, independent of *cis*-acting modifiers includes: (1) the high frequency of ataxia among SCA8 expansion carriers versus controls ($P = 4 \times 10^{-25}$); (2) the common clinical phenotype among affected families; (3) the cosegregation of the expansion and ataxia in small families with multiple affected individuals, excluding the MN-A family ($P = .0038$); and (4) a LOD score of 6.8 at $\theta = .00$ in the MN-A family and a combined LOD score of 2.02 at $\theta = .00$ among 10 small families with multiple affected individuals. Because the SCA8 expansion was isolated from a single patient with ataxia by our RAPID cloning method (Koob et al. 1998, 1999) instead of a positional cloning approach that depends on large families, it is not surprising that the genetic characteristics and disease penetrance do not follow the pattern of SCAs defined elsewhere.

The penetrance of the SCA8 expansion is much higher

Table 4

Highly Mutable Sequences Flanking the SCA8 CTG Expansion

Haplotype, Ethnic Origin, and Family	No. of CTA Repeats[a]	No. of CTG Repeats	G/A SNP[a,b]
A, white:			
C1 (MN-A)	3	70~140	G
C2	8	72~80	A
C3	8	72~107	A
C8	17	54~71	A
C9	ND	208~750[c]	A
C10	12	118~723	A
C11	9	92~109	A
C12	12	78~131	A
C13A	ND	97~120[c]	A
C14	12	81~114	A
C15	ND	91[c]	A
C16	ND	169[c]	A
C22	ND	845~945[c]	A
C23	ND	130~1,110[c]	A
A′, white:			
C24	9	168~254	A
C26A/B	ND	101~950[c]	A
C29	ND	75[c]	A
C30	ND	150[c]	A
B, Japanese:			
J1	9	80~96	ND
J2	8	87~91	ND
J3	8	147	ND
J4	8	87~128	ND
B, white:			
C13B	ND	97~120[c]	A
C, Mexican:			
M1	ND	100[c]	ND

[a] ND = not determined.

[b] G/A SNP identified 90 bp 3′ of the CTG expansion in the MN-A family.

[c] Number of CTA repeats is unknown; therefore, values represent the combined number of CTA/CTG repeats.

in the MN-A family than in the other families with ataxia that we describe here or that have been reported elsewhere in the literature (Mosemiller et al. 2003). In contrast to the relatively large number of affected patients in the MN-A family ($n = 13$), 25 (~70%) of the small families with ataxia in our study had only a single affected individual, 9 families had two, and only 2 families had three. Although the expansion in the MN-A family arose from the same founder as the other white families with an SCA8 expansion of haplotype A, several genetic differences between the MN-A family and the small families with ataxia may contribute to the increased disease penetrance in the MN-A family.

First, the affected chromosome in the MN-A family (C1) has haplotype A, with a superimposed centromeric recombination event that occurred between the D13S318 and YI14 markers. It is possible that this recombination event, which occurred 112–137 kb 5′ of the CTG repeat or ~70 kb upstream of the suggested promoter region of SCA8 (Benzow and Koob 2002), could account for the relatively high disease penetrance in the MN-A family. Although it appears that this recombination event would be too far upstream to act as an enhancer for the currently defined promoter, it is possible that alternative upstream promoter elements have not yet been identified and that the presence of this alternative chromosomal region 5′ of the SCA8 transcript could increase SCA8 transcriptional activity. Altered SCA8 expression may increase the toxic effects of the resultant CUG-containing transcripts, resulting in the relatively high penetrance of the disease in the MN-A family. Substantial evidence supporting a model of RNA pathogenesis has accumulated in the myotonic dystrophy field, in which dominant effects of CUG and CCUG repeat-containing transcripts have been shown to be the primary disease mechanism (Philips et al. 1998; Mankodi et al. 2000; Tapscott 2000; Liquori et al. 2001; Tapscott and Thornton 2001; Kanadia et al. 2003). A similar disease mechanism may be responsible for SCA8. Alternatively, the recombinant chromosomal region could harbor other genetic modifiers, including those of the overlapping *KLHL1* gene (Koob et al. 1999; Nemes et al. 2000).

Additional genetic variations in the MN-A family that are present within the conserved portion of the haplotype A include: the SNP located 90 bp 3′ of the CTG, the relatively short CTA repeat tract containing only 3 repeats, and interruptions within the CTG portion of the repeat (Moseley et al. 2000*b*). It is not yet clear whether the 5′ recombination event, or the other sequence variations we have identified, increase disease penetrance in the MN-A family. However, the sequence variations we have observed, in addition to changes in overall repeat length, indicate that both the CTG repeat tract and the region immediately flanking the CTG expansion are highly mutable.

In addition to possible *cis*-modifiers that our haplotype results suggest play a role in increasing disease penetrance in the MN-A family, we have examined the possible involvement of the unexpanded SCA8 allele as a *trans*-modifier in the other families with SCA8. Among five available affected sib pairs, all five inherited the same unexpanded allele from the other parent ($P = .025$). This result suggests the possibility that a subset of SCA8 alleles not containing the expansion may increase the likelihood that the disease will be expressed. On review of the literature, we found six additional sib pairs with complete genotype results for the unexpanded SCA8 allele (Juvonen et al. 2000; Silveira et al. 2000; Brusco et al. 2002; Schols et al. 2003). Four of these sib pairs share the same unexpanded allele, and two do not (not significant), if combined with our data $P = .034$. Additional data will be needed to determine

whether or not this suggestive trend is found in a larger group of patients.

The reduced penetrance commonly seen in families with SCA8 could be the result of modifying genetic factors similar to those reported in other diseases, including amyotrophic lateral sclerosis (ALS). In familial ALS1 (MIM 105400), identical point mutations within the *SOD1* gene result in remarkable variation in disease onset within and between families. Recently, *VEGF* was shown to act as a genetic modifier in patients with sporadic and familial ALS (Lambrechts et al. 2003). Homozygous variations in the *VEGF* promoter reduce circulating VEGF levels and increase the risk of developing ALS (Lambrechts et al. 2003). Similarly, a clarification of modifiers in degenerative ataxias could help us to understand the reduced penetrance of SCA8.

The data presented here provide additional pieces of a complex puzzle, by describing in detail the repeat ranges found in various SCA8 expansion populations. Furthermore, although not all expansions cause ataxia, in families in which multiple members are affected, the expansion cosegregates with the disease in the additional affected relatives ($P = .0038$). Although the majority of the families were too small for linkage analysis, the combined LOD score for all of the clinically similar families with multiple affected individuals was consistent with the SCA8 expansion causing disease (LOD = 2.02). In addition, our haplotype and sequence analyses have uncovered several genetic variations in the MN-A family that may play a role in increasing disease penetrance in that family. Finally, the discovery that SCA8 expansions among patients with ataxia arose independently on three different haplotypes suggests that independently arising SCA8 expansions can cause ataxia. Although this additional information helps clarify the genetic complexities of ataxia, further analysis in cell culture and animal models will be needed to understand the molecular mechanisms involved in SCA8 and the reasons for the reduced penetrance.

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Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:

Genome Database, http://www.gdb.org
NCBI, http://www.ncbi.nlm.nih.gov/
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/
Primer3, http://www.broad.mit.edu/cgi-bin/primer/primer3_www.cgi
UCSC Genome Browser, http://www.genome.ucsc.edu/

References

Benzow KA, Koob MD (2002) The KLHL1-antisense transcript (KLHL1AS) is evolutionarily conserved. Mamm Genome 13:134–141

Brusco A, Cagnoli C, Franco A, Dragone E, Nardacchione A, Grosso E, Mortara P, Mutani R, Migone N, Orsi L (2002) Analysis of SCA8 and SCA12 loci in 134 Italian ataxic patients negative for SCA1-3, 6 and 7 CAG expansions. J Neurol 249:923–929

Cellini E, Piacentini S, Nacmias B, Forleo P, Tedde A, Bagnoli S, Ciantelli M, Sorbi S (2002) A family with spinocerebellar ataxia type 8 expansion and vitamin E deficiency ataxia. Arch Neurol 59:1952–1953

Day J, Dalton JC, Ikeda Y, Ranum LPW (2003) MRI studies in SCA8: cerebellar atrophy in unaffected expansion carriers partially explains reduced penetrance. Am J Hum Genet Suppl 73:548

Day JW, Schut LJ, Moseley ML, Durand AC, Ranum LPW (2000) Spinocerebellar ataxia type 8: clinical features in a large family. Neurology 55:649–657

Hagerman RJ, Leehey M, Heinrichs W, Tassone F, Wilson R, Hills J, Grigsby J, Gage B, Hagerman PJ (2001) Intention tremor, parkinsonism, and generalized brain atrophy in male carriers of fragile X. Neurology 57:127–130

Holmes SE, Hearn EO, Ross CA, Margolis RL (2001) SCA12: an unusual mutation leads to an unusual spinocerebellar ataxia. Brain Res Bull 56:397–403

Holmes SE, O'Hearn EE, McInnis MG, Gorelick-Feldman DA, Kleiderlein JJ, Callahan C, Kwak NG, Ingersoll-Ashworth RG, Sherr M, Sumner AJ, Sharp AH, Ananth U, Seltzer WK, Boss MA, Vieria-Saecker AM, Epplen JT, Riess O, Ross CA, Margolis RL (1999) Expansion of a novel CAG trinucleotide repeat in the 5′ region of PPP2R2B is associated with SCA12. Nat Genet 23:391–392

Ikeda Y, Shizuka M, Watanabe M, Okamoto K, Shoji M (2000) Molecular and clinical analyses of spinocerebellar ataxia type 8 in Japan. Neurology 54:950–955

Izumi Y, Maruyama H, Oda M, Morino H, Okada T, Ito H, Sasaki I, Tanaka H, Komure O, Udaka F, Nakamura S, Kawakami H (2003) SCA8 repeat expansion: large CTA/CTG repeat alleles are more common in ataxic patients, including those with SCA6. Am J Hum Genet 72:704–709

Jacquemont S, Hagerman RJ, Leehey M, Grigsby J, Zhang L, Brunberg JA, Greco C, Des Portes V, Jardini T, Levine R, Berry-Kravis E, Brown WT, Schaeffer S, Kissel J, Tassone F, Hagerman PJ (2003) Fragile X premutation tremor/ataxia syndrome: molecular, clinical, and neuroimaging correlates. Am J Hum Genet 72:869–878

Jin P, Warren ST (2000) Understanding the molecular basis of fragile X syndrome. Hum Mol Genet 9:901–908

Juvonen V, Hietala M, Paivarinta M, Rantamaki M, Hakamies L, Kaakkola S, Vierimaa O, Penttinen M, Savontaus ML (2000) Clinical and genetic findings in Finnish ataxia patients with the spinocerebellar ataxia 8 repeat expansion. Ann Neurol 48:354–361

Kanadia RN, Johnstone KA, Mankodi A, Lungu C, Thornton CA, Esson D, Timmers AM, Hauswirth WW, Swanson MS (2003) A muscleblind knockout model for myotonic dystrophy. Science 302:1978–1980

Koob MD, Benzow KA, Bird TD, Day JW, Moseley ML, Ranum LPW (1998) Rapid cloning of expanded trinucleotide repeat sequences from genomic DNA. Nat Genet 18: 72–75

Koob MD, Moseley ML, Schut LJ, Benzow KA, Bird TD, Day JW, Ranum LPW (1999) An untranslated CTG expansion causes a novel form of spinocerebellar ataxia (SCA8). Nat Genet 21:379–384

Lambrechts D, Storkebaum E, Morimoto M, Del-Favero J, Desmet F, Marklund SL, Wyns S, et al (2003) VEGF is a modifier of amyotrophic lateral sclerosis in mice and humans and protects motoneurons against ischemic death. Nat Genet 34:383–394

Liquori C, Ricker K, Moseley ML, Jacobsen JF, Kress W, Naylor S, Day JW, Ranum LPW (2001) Myotonic dystrophy type 2 caused by a CCTG expansion in intron 1 of ZNF9. Science 293:864–867

Mankodi A, Logigian E, Callahan L, McClain C, White R, Henderson D, Krym M, Thornton CA (2000) Myotonic dystrophy in transgenic mice expressing an expanded CUG repeat. Science 289:1769–1773

Matsuura T, Yamagata T, Burgess DL, Rasmussen A, Grewal RP, Watase K, Khajavi M, McCall AE, Davis CF, Zu L, Achari M, Pulst SM, Alonso E, Noebels JL, Nelson DL, Zoghbi HY, Ashizawa T (2000) Large expansion of the ATTCT pentanucleotide repeat in spinocerebellar ataxia type 10. Nat Genet 26:191–194

Moseley ML, Schut LJ, Bird TD, Day JW, Ranum LPW (2000*a*) Reply. Nat Genet 24:215

Moseley ML, Schut LJ, Bird TD, Koob MD, Day JW, Ranum LPW (2000*b*) SCA8 CTG repeat: en masse contractions in sperm and intergenerational sequence changes may play a role in reduced penetrance. Hum Mol Genet 9:2125–2130

Mosemiller AK, Dalton JC, Day JW, Ranum LPW (2003) Molecular genetics of spinocerebellar ataxia type 8 (SCA8). Cytogenet Genome Res 100:175–183

Nemes JP, Benzow KA, Moseley ML, Ranum LPW, Koob MD (2000) The SCA8 transcript is an antisense RNA to a brain-specific transcript encoding a novel actin-binding protein (KLHL1). Hum Mol Genet 9:1543–1551 (correction/addition 9:2777)

Orr HT (2001) Beyond the Qs in the polyglutamine diseases. Genes Dev 15:925–932

Ott J (1991) Analysis of human genetic linkage. The Johns Hopkins University Press, Baltimore

Philips AV, Timchenko LT, Cooper TA (1998) Disruption of splicing regulated by a CUG-binding protein in myotonic dystrophy. Science 280:737–741

Ranum LPW, Day JW (2002) Dominantly inherited non-coding microsatellite expansion disorders. Curr Opin Genet Dev 12:266–271

Schols L, Bauer I, Zuhlke C, Schulte T, Kolmel C, Burk K, Topka H, Bauer P, Przuntek H, Riess O (2003) Do CTG expansions at the SCA8 locus cause ataxia? Ann Neurol 54: 110–115

Silveira I, Alonso I, Guimaraes L, Mendonca P, Santos C, Maciel P, Fidalgo De Matos JM, Costa M, Barbot C, Tuna A, Barros J, Jardim L, Coutinho P, Sequeiros J (2000) High germinal instability of the (CTG)n at the SCA8 locus of both expanded and normal alleles. Am J Hum Genet 66:830–840

Sobrido MJ, Cholfin JA, Perlman S, Pulst SM, Geschwind DH (2001) SCA8 repeat expansions in ataxia: a controversial association. Neurology 57:1310–1312

Stevanin G, Herman A, Durr A, Jodice C, Frontali M, Agid Y, Brice A (2000) Are (CTG)n expansions at the SCA8 locus rare polymorphisms? Nat Genet 24:213 (author reply 24: 215)

Tapscott SJ (2000) Deconstructing myotonic dystrophy. Science 289:1701–1702

Tapscott SJ, Thornton CA (2001) Reconstructing myotonic dystrophy. Science 293:816–817

Topisirovic I, Dragasevic N, Savic D, Ristic A, Keckarevic M, Keckarevic D, Culjkovic B, Petrovic I, Romac S, Kostic VS (2002) Genetic and clinical analysis of spinocerebellar ataxia type 8 repeat expansion in Yugoslavia. Clin Genet 62:321–324

Vincent JB, Neves-Pereira ML, Paterson AD, Yamamoto E, Parikh SV, Macciardi F, Gurling HM, Potkin SG, Pato CN, Macedo A, Kovacs M, Davies M, Lieberman JA, Meltzer HY, Petronis A, Kennedy JL (2000*a*) An unstable trinucleotide-repeat region on chromosome 13 implicated in spinocerebellar ataxia: a common expansion locus. Am J Hum Genet 66:819–829

Vincent JB, Yuan QP, Schalling M, Adolfsson R, Azevedo MH, Macedo A, Bauer A, DallaTorre C, Medeiros HM, Pato MT, Pato CN, Bowen T, Guy CA, Owen MJ, O'Donovan MC, Paterson AD, Petronis A, Kennedy JL (2000*b*) Long repeat tracts at SCA8 in major psychosis. Am J Med Genet 96: 873–876

Warren ST (1996) The expanding world of trinucleotide repeats. Science 271:1374–1375

Weeks D, Sobel E, O'Connell J, Lange K (1995) Computer programs for multilocus haplotyping of general pedigrees. Am J Hum Genet 56:1506–1507

Worth PF, Houlden H, Giunti P, Davis MB, Wood NW (2000) Large, expanded repeats in SCA8 are not confined to patients with cerebellar ataxia. Nat Genet 24:214–215

Zoghbi HY, Orr HT (2000) Glutamine repeats and neurodegeneration. Annu Rev Neurosci 23:217–247