Relations between genotype and phenotype in German patients with the Machado-Joseph disease mutation

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Abstract

Objective—Machado-Joseph disease (MJD) is an autosomal dominant cerebellar ataxia with extensive phenotypic variability originally described in families of Portuguese ancestry. Recently, the mutation causing the disease has been identified as an expanded CAG trinucleotide repeat. In this study relations between genotype and phenotype were investigated.

Methods—A series of 180 German patients with degenerative forms of ataxia were clinically and genetically examined. Patients bearing the MJD mutation were assigned to three phenotypes: phenotype 1 characterised by early onset and dystonia or pronounced rigidity associated with ataxia and spasticity. Main symptoms in phenotype 2 were ataxia and spasticity. In phenotype 3 onset was relatively late and peripheral neuropathy accompanied ataxia. Clinical and molecular data were correlated.

Results—An expanded CAG array was found in 42 patients from 22 families. Repeat length of CAG varied between 67 and 80 CAG motifs and showed an inverse correlation with the age of onset. For the development of phenotype 1 early onset (< 20 years) seemed more decisive than extensive repeat length. Phenotype 2 was present in all patients with more than 73 CAG motifs and onset between 20 and 40. Phenotype 3 developed in most patients with less than 73 CAG motifs and onset was regularly beyond the age of 40. Intrafamilial variability of both repeat length and phenotype was large reflecting meiotic instability of the expanded CAG repeat.

Conclusion—The MJD mutation is the most frequent cause of dominantly inherited ataxia in Germany. Variations in repeat lengths substantially influence age of onset as well as phenotype but cannot explain why MJD characteristics of Portuguese families such as "bulging eyes", dystonia, and rigidity are essentially missing in German families. Despite the genotypic and phenotypic relations found in this study a reliable individual prognosis of the course of the disease is not possible at a presymptomatic stage.

Keywords: Machado-Joseph disease; spinocerebellar ataxia type 3; clinical features; molecular genetics

The spinocerebellar ataxias (SCAs) are a heterogeneous group of neurodegenerative disorders with a wide phenotypic variability. Recently, several genes causing genetically distinct forms of SCA have been mapped chromosomally.¹⁻⁸ For spinocerebellar ataxia type 1 (SCA1) and Machado-Joseph disease (MJD) the mutations causing disease have been identified as expanded CAG triplet repeats.^{9,10} CAG repeat expansions also cause spinobulbar muscular atrophy,¹¹ Huntington's disease,¹² and dentatorubral pallidolysian atrophy (DRPLA).¹³ Inverse correlation of repeat length and age of onset has been shown for all of these disorders.¹⁴⁻¹⁸ Furthermore, the CAG repeat is unstable during transmission from parent to offspring with a tendency for expansion.¹⁹⁻²⁴ The elongation of the CAG repeat in later generations results in earlier onset and a more severe course of the disease, a phenomenon known as anticipation.²⁵⁻²⁸ The differentiation of various subtypes of SCA at their genetic background gives an opportunity to redefine their clinical phenotypes and to study the relation between genotype and phenotype.

Machado-Joseph disease is an autosomal dominantly inherited spinocerebellar degeneration originally described in families of Portuguese ancestry.²⁹⁻³¹ Pathological changes include neuronal loss and gliosis in the cerebellum (especially in the dentate nucleus but sparing the cerebellar cortex), the spinal cord (spinocerebellar tracts, Clark's columns, anterior horn cells, and the posterior columns, but not the corticospinal tract, although clinically pyramidal signs are frequent), the substantia nigra, red nucleus, subthalamic nucleus, cranial nerve nuclei, and peripheral nerves.³²

The clinical presentation of MJD is very pleomorphic. Three subtypes have been described with the following characteristics³³: type 1 is characterised by pronounced pyramidal and extrapyramidal signs besides the common features of cerebellar ataxia and ophthalmoplegia. Type 1 shows earlier onset (20-30 years) and a more rapid course of the disease. The clinical presentation of type 2 is limited to cerebellar and pyramidal deficits without extrapyramidal signs. Age of onset in type 2 is intermediate (20-45 years). Type 3 is characterised by pronounced peripheral signs in addition to ataxia and shows later onset (40-60 years) with rather slow progression.

"Bulging eyes" due to lid retraction, dystonia,

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Figure 1 Correlation between age of onset, repeat length, and clinical phenotype in 42 patients of German ancestry with MJD/SCA3. A linear correlation between age of onset and number of CAG motifs was obtained ($r = -0.82$, $P < 0.0001$).

and faciolingual fasciculations are minor but rather characteristic features of MJD.³⁴

German families with ataxia and bearing the MJD mutation present with a phenotype distinct from patients with MJD of Portuguese or Japanese ancestry³⁵ but similar to French families described as spinocerebellar ataxia type 3 (SCA3).³⁶ Recently, we proved that despite phenotypic differences both SCA3 and MJD are caused by mutations in the *MJD1* gene.³⁷

Here we present detailed clinical data for 42 patients with German ataxia bearing the MJD/SCA3 mutation and a study of the relation between the size of the expanded CAG repeat and the clinical presentation.

Materials and methods

A continuous series of 180 patients with degenerative forms of ataxia were clinically examined by one of us (LS) and blood samples were obtained for genetic analysis. The series included 77 patients from 45 families with a history of dominant inherited disease. All patients with autosomal dominant cerebellar ataxias (ADCAs) presented with symptoms such as cerebellar ataxia, external ophthalmoplegia, pyramidal and extrapyramidal features, polyneuropathy, and mild dementia consistent with ADCA type I according to the classification of Harding.³⁸

Genomic DNA was extracted from peripheral white blood cells.³⁹ Amplification of the CAG repeat by the polymerase chain reaction (PCR) was essentially carried out as described by Schöls *et al.*³⁷ Briefly, 100 ng genomic DNA was amplified by PCR using the primer sequences MJD52 and MJD25¹⁰ in a total volume of 10 μ l. Amplification was carried out for 30 cycles with denaturation at 95°C for one minute, annealing at 58°C for one minute, and extension at 72°C for one minute, in a Robocycler (Stratagene). Formamide was added to a final concentration of 1% to avoid non-specific amplification products. Products from PCR were electrophoresed in 6% denaturing polyacrylamide gels and sizes were determined by comparison with an M13 sequencing ladder. We included the AAG and two CAA variant triplets in determining the number of CAG repeats.

Statistical analysis of the relations between age of onset and CAG repeat number on the affected alleles was evaluated by linear regression analysis.

Results

An expanded CAG repeat in the MJD gene was found only in patients with a family history of dominant inheritance and not in patients with autosomal recessive or sporadic disease. From the 45 ADCA kindreds included in this study five families with 11 patients had already been shown to have SCA1 based on the identification of the SCA1 mutation.¹⁴

The MJD mutation was found in 42 patients from 22 families with autosomal dominant cerebellar ataxia. All families were of German origin and no relation to Portuguese or Japanese ancestors could be found.

All 42 patients with MJD/SCA3 were heterozygous with one allele in the normal range (14–30 CAG motifs) and the other with an expanded trinucleotide repeat. Repeat lengths of the expanded alleles varied between 67 and 80 CAG motifs with 73 CAG repeats being the most often expanded allele size (eight patients).

Age of onset was inversely correlated with CAG repeat length ($r = -0.82$, $P < 0.0001$, fig 1). Patients with the same repeat length differed by up to 21 years in their age of onset (fig 1, table 1). For example, in patients with 73 CAG motifs onset varied between 29 and 50 years of age.

Characteristic signs of MJD as described in patients of Portuguese ancestry³⁴ were not prominent in German patients bearing the MJD mutation. "Bulging eyes", rigidity, and dystonia were seen only in one of 42 patients. Faciolingual myokymia was present in a mild form in nine of 42 patients in our series, but similar facial movements were found in patients with ADCA not carrying the MJD mutation. Thus the German patients with the MJD genotype presented with the SCA3 phenotype.³⁶

Initial complaints were problems with equilibrium and unsteadiness of gait in 38 of 42 patients with SCA3/MJD. Four patients reported diplopia as the first symptom, which preceded gait ataxia by up to 10 years. At the time of examination ataxia of gait, stance, and limbs were major clinical signs in all patients independent of repeat length (table 1). Cerebellar dysarthria (36 of 42) and cerebellar oculomotor signs such as gaze evoked nystagmus (41 of 42) and impaired smooth pursuit (38 of 42) were common.

As well as cerebellar dysfunction, peripheral neuropathy was a major clinical sign in most patients with relatively milder repeat expansions up to 72 CAG motifs (table 1). Muscle cramps and teasing dysesthesiae were frequent complaints in these patients. Peripheral neuropathy signs included distally pronounced wasting of muscles in the feet and hands with mild paresis. Sensory loss was most pronounced in temperature discrimination followed by loss of vibration sense. Apart from two patients in whom pyramidal signs were more prominent than peripheral disorder all patients with less than 73 CAG

Table 1 Correlation of CAG repeat length and clinical features in 42 German patients bearing the MJD mutation

(CAG)n repeats	67	68	69	↓	69	70	70	70	71	71	↓	72	72	73	73	73	73	73	↓	73	73	↓	74	74
Age of onset (y)	55	50	56	51	50	44	42	41	46	40	50	47	41	50	49	45	42	38	37	35	29	40	39	22
Duration (y)	13	12	1	2	8	6	21	3	14	25	8	9	8	19	8	25	1	18	20	5	15	5	6	11
Sex	M	F	M	M	F	F	F	M	M	M	F	M	F	F	F	F	F	F	F	M	M	M	F	M
Ataxia	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦
Peripheral neuropathy	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦
Spasticity																								
Ophthalmoplegia																								
Swallowing problems																								
Faciolingual myokymia																								
Rigidity																								
Bradykinesia																								
Choreoathetosis																								
Dystonia																								
Incontinence																								
Phenotype	3	3	3	3	3	2	3	3	3	3	3	2	3	3	3	3	2	2	2	2	2	2	2	2
Disability: ambulant	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ambulant with support																								
Wheelchair bound							+							+		+		+	+					

♦ = major sign; ◆ = minor sign; ↓ = members of the SCA3/MJD kindred presented in fig 2.

repeats could be grouped as type 3 patients according to Coutinho and Andrade.³³

Patients with 73 CAG motifs formed an intermediate group from their clinical appearance. The four patients with comparatively late onset (50, 49, 45, 42 years) showed more peripheral signs, as type 3 patients and the four patients with earlier onset (38, 37, 35, 29 years) had more pronounced spasticity, as type 2 patients (see below).

In 21 patients exhibiting more than 73 CAG motifs peripheral neuropathy was rarely a prominent problem (table 1). Instead spasticity evolved, especially in the legs (20 of 21). The gait was often characterised by stiffness (19 of 21). Hyperreflexia (16 of 21), extensor plantar responses (13 of 21), and spinal automatisms (11 of 21) were frequent signs. Due to the predominance of spasticity with ataxia we grouped these patients as type 2 after Coutinho and Andrade.³³

Phenotype 1 (pronounced extrapyramidal features, especially rigidity and dystonia in combination with spasticity and ataxia) was seen in only two patients of our series. One patient had considerable rigidity combined with gross spasticity resulting in extreme stiffness which caused disablement of walking and most voluntary movements after 15 years of the disease. She reported that the clinical picture had changed during the disease. Unsteadiness of gait and clumsy hands had been the main problems during the first eight years, thereafter progressive stiffness became most disabling. The other type 1 patient was the only patient with significant dystonia in our series. After nine years of disease he developed abnormal posturing of the right arm during walking whereas his movements were inconspicuous (apart from ataxia) when he was sitting or standing. Dystonic movements were not found by the same neurologist after a disease duration of seven years.

The expanded alleles of these type 1 patients harboured 79 and 78 CAG repeats respectively. Remarkably, four patients with 80 CAG repeats did not show type 1 features despite their longer repeat expansions and durations of disease up to 10 years. They presented with classic type 2

phenotypes (table 1).

Extrapyramidal involvement was clinically apparent only in a few other patients. One had a mild form of rigidity not warranting the classification as type 1. Two patients had bradykinesia but not as a prominent sign and without rigidity. Another patient presented with involuntary choreoathetotic movements of the fingers after a duration of disease of 18 years. Two patients, carrying 70 and 73 CAG repeats, developed myoclonus, especially of the proximal muscles of all limbs after 20 and 19 years of disease. Myoclonus was present in the relaxed patient as well as during voluntary movements. Both patients were already bedridden when myoclonus developed.

Bulbar and pseudobulbar signs, especially swallowing problems, were not serious in type 3 patients with repeat lengths of less than 72 CAG units. Only patients with a disease duration of more than 20 years developed mild problems in swallowing. By contrast, all patients with 72 or more CAG motifs (mostly type 2 and type 1 patients) developed dysphagia when the duration of the disease exceeded five years. Similarly, external ophthalmoplegia was less frequent in type 3 patients and was often associated with swallowing problems and longer duration (table 1).

Urge incontinence was a problem in eight of 42 patients without being correlated with repeat lengths, spasticity, or disease duration.

Figure 2 Family tree reduced to affected members of a large SCA3/MJD kindred with wide variation of CAG repeat expansions and clinical appearance (see table 1).

important to recognise the influence of age of onset on clinical appearance. In our series this is seen best in the group of patients with 73 CAG repeats. Those with later onset (> 40 years) present as type 3 and those with earlier onset (< 40 years) as type 2. Age of onset may be even more important for the development of subphenotype 1 as our type 1 patients had the earliest onset (< 20 years) but not the longest repeat expansions of our series. A similar tendency exists in the data of Maruyama *et al.*²⁴ However, the few type 1 patients in both studies preclude reliable evaluations.

Coexistence of two or more subphenotypes in one family has been reported for large MJD kindreds.^{41,42} Here we present a family with SCA3/MJD with clinical variations warranting the classification in different subtypes even among sibs (type 2 *v* type 3; fig 2, table 1). Phenotypic variability also occurred in patients with similar repeat lengths as is seen for patients with 73 CAG repeats included in this study (table 1).

Therefore, a reliable individual prognosis of the course of the disease at a presymptomatic stage or in the beginning of the disease is not possible either on the basis of the repeat lengths or based on family history despite demonstrable genotype and phenotype correlations in general.

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