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

Ludger Schöls, Georgios Amoridis, Jörg T Epplen, Mathias Langkafel, Horst Przuntek, Olaf Riess

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. For spinocerebellar ataxia type 1 (SCA1) and Machado-Joseph disease (MJD) the mutations causing disease have been identified as expanded CAG triplet repeats. CAG repeat expansions also cause spinobulbar muscular atrophy, Huntington’s disease, and dentatorubral pallidolysian atrophy (DRPLA). Inverse correlation of repeat length and age of onset has been shown for all of these disorders. Furthermore, the CAG repeat is unstable during transmission from parent to offspring with a tendency for expansion. 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. 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. 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.

The clinical presentation of MJD is very pleomorphic. Three subtypes have been described with the following characteristics: 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,
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 (ADCA)s presented with symptoms such as cerebellar ataxia, external ophthalmoplegia, pyramidal and extrapyramidal features, polyneuropathy, and mild dementia consistent with ADCAtype 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 Schols et al.37 Briefly, 100 ng genomic DNA was amplified by PCR using the primer sequences MJDS2 and MJDS210 in a total volume of 10 μl. 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 (Stratagen). 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.34

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 myokymia was not 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 paresthesia. 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...
patients had more signs, repeats, intermediate problem prominent The according to onset (50, responses especially rigidity features, patients these as especially 21). Hyperreflexia movements two only of the most stiffness in inconspicuous found. 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 choreathetotic 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).

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

![Figure 2](http://jnnp.bmj.com/)

**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).

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

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<th>68</th>
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</table>

* = major sign; • = minor sign; † = members of the SCA3/MJD kindred presented in fig 2.
Molecular genetic data from the original publication \(^{10}\) and further families of Portuguese and Japanese ancestry with classic MJD phenotypes \(^{22,23}\) did not differ from the repeat expansions in German families. Hence, the phenotypic differences of MJD and SCA3 remain unexplained at the molecular level but may be caused by the influence of other genes differing between patients with the MJD phenotype and those with the SCA3 phenotype. Haplotype analyses at flanking markers are conserved among Japanese and Azorean patients with MJD. Similar studies comparing Portuguese patients with the MJD phenotype and German patients with the SCA3 phenotype are under way to approach the question of common founders of the disease in these populations.

Despite clinical differences the criteria defining subphenotypes of MJD as elaborated by Coutinho and Andrade \(^{23}\) are well applicable to patients with the SCA3 phenotype, because clinical differences between SCA3 and MJD do not concern the criteria for subtypes apart from dystonia. However, we suggest a minor modification for the subclassification of patients with "pure" ataxia. Some of these patients develop more peripheral signs (type 3), others more pyramidal signs (type 2) later in the disease. In our experience noticeable spasticity develops, if at all, rather early (<5 years) in the disease. Long standing "pure" ataxia is combined with rather slow progression and ends as type 3. Therefore, we classified patients with SCA3/MJD with "pure" ataxia as type 3 patients and not as type 2 as recommended in the original report. \(^{3,31}\) Ophthalmoplegia does not seem to be helpful for the assignment of different subtypes in German patients with SCA3/MJD and was not strictly correlated with the expanded repeat length.

Some patients seem to start as type 2 and others as type 3 phenotypes. Some type 2 patients develop features of type 1 later in the disease and some type 3 patients change to intermediate type 2 or 3 appearance. This transitional form of disease was first described by Barbeau and coworkers. \(^{40}\) We never saw any patient with initial prominent spasticity (type 2) to develop a type 3 phenotype later on.

Using the subclassification recommended here (table 2) we found strong correlations between repeat lengths and subphenotypes in our series of patients with SCA3/MJD. Maruyama and coworkers \(^{34}\) did a similar study in Japanese patients with MJD using the original scheme. \(^{32,33}\) In their study a substantial overlap existed concerning repeat lengths between type 2 and type 3 patients. The stronger correlation of repeat length and subtype in our study most likely results from a different grouping of ataxic patients without major peripheral or pyramidal signs as type 3 instead of type 2 as mentioned above.

This study confirms an inverse correlation between CAG repeat length and age of onset. According to our data the repeat length accounts for about 70% \((r^2 = 0.67)\) of total variation in the age of onset. Besides the correlation of age of onset and repeat length it is

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### Table 2

<table>
<thead>
<tr>
<th>Type 1</th>
<th>Type 2</th>
<th>Type 3</th>
</tr>
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<tbody>
<tr>
<td>Age of onset (y)</td>
<td>10–30</td>
<td>20–45</td>
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<tr>
<td>Disease progression</td>
<td>Rapid</td>
<td>Intermediate</td>
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<td>Major clinical features:</td>
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<td>Corebellar ataxia</td>
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<td>+ to + +</td>
</tr>
<tr>
<td>Peripheral neuropathy</td>
<td>0 to +</td>
<td>0 to +</td>
</tr>
<tr>
<td>Pyramidal signs</td>
<td>+ to + +</td>
<td>+ to + +</td>
</tr>
<tr>
<td>EPS (rigidity, dystonia)</td>
<td>+ to + +</td>
<td>+ to + +</td>
</tr>
</tbody>
</table>

0 = absent; + = mild; + + = moderate; + + + = severe; * = at onset; EPS = extrapyramidal symptoms.
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. 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. 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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