Searching for modulating effects of SCA2, SCA6 and DRPLA CAG tracts on the Machado-Joseph disease (SCA3) phenotype

Machado–Joseph disease (MJD), or SCA3, is an autosomal dominant cerebellar ataxia of adult onset. First described in pedigrees of Azorean ancestry, MJD has a heterogeneous clinical picture (1–4). The mutation associated with this disorder is the expansion of a CAG tract in the coding region of the MJD1 gene (5). Normal chromosomes contain 12–41 CAG repeats, whereas disease causing chromosomes contain 61–86 repeats (6, 7).

A well accepted classification of MJD divides patients into three phenotypes (4, 8). Cerebellar ataxia and progressive external ophthalmoplegia are common features to all types. Type 1 has an earlier onset, usually around 15–20 years, and is characterized by marked pyramidal signs and dystonic posturing. Type 2, the most common, starts often around 30–37 years, and is almost limited to the cerebellar and ocular signs. Type 3 is later in onset, between 37 and 47 years, and progresses with lower motor neuron signs and peripheral neuropathy (8, 9).

The variability in age at onset, the complex and heterogeneous neurologic findings, as well as the existence of three clinical types, indicate that MJD is modulated by modifier factors. Several mechanisms responsible for phenotypic variation can be postulated: (a) the variable number of CAG repeats, (b) flanking and intragene polymorphisms in the disease locus, (c) variations in the normal allele, (d) imprinting, (e) somatic mosaicism, (f) modifying genes, (g) environmental factors (10), and (h) observational biases, if some variation would be due to a non-controlled disease duration, for example (11).

The first and better studied modifying factor was the CAG repeat length itself. Age of onset has been associated with CAG repeat length of the long SCA2 allele (Mann–Whitney U-test, $P < 0.03$, after Bonferroni procedure). Other measures (age of onset, anticipation, clinical types and other neurological signs) were not associated with CAG repeat length of SCA2, SCA6 and DRPLA genes.

Conclusions – The present results show that the CAG tract of SCA2 gene interferes with MJD phenotype. Further studies, with patients of other origins and with typing of other (CAG)n loci, are necessary.
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and in vitro studies indicated that polyglutamine
polyglutamine tracts in proteins otherwise unre-
caused by unstable CAG expansions, encoding
MJD, DRPLA, SCA6, SCA7 and S017 are all
mutational mechanism (13–16). SCA1, SCA2,
sharing both clinical heterogeneity and same
this disease, because MJD is one of a large family
of spinocerebellar ataxias (SCAs), most of them
polyglutamine tracts likely cause them to adopt an
heterozygous for all of them, which resulted in
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huntingtin and also explains part of the variability
in the MJD phenotype.

The question addressed in the present paper is
whether CAG tracts of the several SCA associated
genes would interact with each other, probably
through their polyglutamine products, thus contrib-
utating to the high clinical heterogeneity observed
within each of these closely related disorders.
Based on similar hypotheses, Hayes et al. have
shown that CAG-containing gene RAI1 contributes to the variance in SCA2 age at onset (24), whereas Holbert et al. found that a new protein with a polymorphic (Gln-Ala) tract interacts with huntingtin and also explains part of the variability in age of onset of Huntington disease (25).

Our aim was to verify whether polymorphic
CAG repeats in SCA2, SCA6 and DRPLA loci
would influence MJD phenotype, age of onset as
well as several neurologic characteristics, thus
acting as modifier factors of this disease.

Subjects and methods
The present study was performed in 39 unrelated,
Brazilian patients with a previously detected CAG
expansion in the MJD1 gene. Their clinical and
molecular findings are published elsewhere (9, 11).

All patients were interviewed and examined by
the same physician (LBJ). A standardized proce-
dure included establishing age at onset, disease
duration, first sign of onset and a thorough
neurologic examination. Onset was considered the
time when the patient, or a close relative, was able
to date the appearance of the first symptom.
Patients were classified into three clinical types,
according to the literature (8). Neurologic signs
taken into account were as follows: gait and limb
ataxia, pyramidal syndrome, dysarthria, dyspha-
gia, external ophthalmoparesis, nystagmus, eyelid
retraction, sensitive loss, fasciculations, dystonia,
rigidity/bradykinesia and optic atrophy. Data on
anticipation were obtained from family history,
when reliable, subtracting patient’s age of onset
from that of the affected parent. Statistical analy-
yses of data (clinical histories, heredograms,
neurologic examination and results of molecular
studies) were performed using SPSS for Windows.

Blood samples were obtained, after informed
consent, from all individuals, and genomic DNA
was extracted from lymphocytes as described
elsewhere (26). MJD1, SCA2, SCA6 and DRPLA polymorphic CAG regions were analyzed according
to conditions established previously (27).

The size of the (CAG)n was determined for all
loci – the SCA2, SCA6 and DRPLA loci, besides MJD1. As these loci show polymorphic
CAG lengths, the majority of patients were
heterozygous for all of them, which resulted in
two CAG measurements for each locus. To differ-
entiate each measured CAG tract, we refer to them
as the short and the long allele of a particular gene
(SCA2, SCA6 or DRPLA).

The effect of the length of CAG repeats at the
SCA2, SCA6 and DRPLA loci on age of onset was assessed by the Spearman correlation coefficient.
Their effect on neurologic signs was analyzed by
Mann–Whitney U-test, when the evaluated sign
was absent or present, or by Kruskal–Wallis test,
when the evaluated sign showed more than two
ordinal categories. All statistical results were cor-
corrected, using Bonferroni procedure based on Fin-
er’s modification.

Results
Normal MJD chromosomes had alleles with CAG
tracts ranging from 14 to 38 repeats, the most
frequent allele showing 24 repeats; mean ± SD was
24.5 ± 6. The expanded MJD alleles varied from
71 to 85 repeats, the most frequent one showing 73
CAGs; mean ± SD was 75.6 ± 2.9. The effect of
CAG repeat length of MJD alleles on MJD
phenotype of the present sample is presented
elsewhere (11).

The SCA2 chromosomes (30 MJD patients)
were estimated to have between 19 and 23 CAG
repeats, and the allele with 22 repeats was the most
frequent (90% of this sample). Short alleles ranged
from 19 to 22, while long alleles ranged from 22 to
23 repeats. The SCA6 chromosomes (35 MJD
patients) showed CAG repeat lengths varying from
four to 14, the most frequent allele with 13 repeats. Short alleles showed four to 13, and long alleles, seven to 14 repeats. Analysis of DRPLA chromosomes (39 MJD patients) demonstrated alleles ranging from eight to 21 CAG repeats, and the most frequent comprised 15 CAGs. Short alleles had eight to 15, and long ones, 10–21 repeats. None of them showed a normal distribution.

The CAG length of either short or long alleles at SCA2, SCA6 and DRPLA loci were not associated with age of onset, or with anticipation (Spearman correlation coefficient, 0.17 < \( P < 0.88 \)). When MJD subtypes 1, 2 and 3 were compared according to their CAG repeat lengths at SCA2, SCA6 and DRPLA loci, again no statistical difference was found (Kruskal–Wallis test, 0.108 < \( P < 0.996 \)).

When the role of SCA2, SCA6 and DRPLA loci on the variation of neurologic signs was tested, one positive result was found. Severity of fasciculations was associated with the CAG length of the long SCA2 allele (\( P < 0.03 \), Kruskal–Wallis test after Bonferroni procedure) (Table 1). Patients with 22 repeats in their long SCA2 allele \( (n = 27) \) were mostly free of fasciculations or had only mild intention fasciculations in their faces. Patients with 23 repeats in the long allele \( (n = 3) \) always showed some degree of fasciculations, either of intention or generalized and spontaneous fasciculations. The calculated correlation between these two variables was \( r = 0.507 \).

There was no other association between these \((CAG)n\) lengths and the severity of the neurologic findings (gait and limb ataxia, pyramidal findings, dysarthria, dysphagia, external ophthalmoplegia, nystagmus, eyelid retraction, dystonia, and optic atrophy).

### Discussion

The majority of the polyglutamine diseases strike primarily the cerebellum (cerebellar tracts, nuclei or cortex), the exceptions being Huntington and Kennedy diseases. Intracellular inclusions or aggregates have been found in neurons of patients, and of murine and \textit{in vitro} models of MJD and other polyglutamine diseases (14). While their specific compositions are unknown, the aggregates do contain fragments of proteins with expanded polyglutamine tracts. These may act as polar zippers, joining beta-strands together. There are several arguments that interactions among polyglutamine tracts themselves, after some cleavage, might be the main reason for the aggregate formation (14).

The present study raised the hypothesis that the pathogenic process of the expanded MJD polyglutamine could, in some way, be influenced by the lengths of other, normal, polyglutamine tracts. We used as models those coded at the SCA2, SCA6 and DRPLA loci. The aggregate formation could, for instance, be accelerated or diminished, in general or in some anatomical areas, according to the size of the normal polyglutamine in the SCA2, SCA6 or DRPLA proteins. These could be responsible for some of the clinical heterogeneity in MJD.

Among the studied CAG tracts, chosen to be analyzed as possible modifier factors of MJD phenotype, the CAG tracts of SCA2 gene seemed to be that with the least possibility of being correlated: normal alleles show few variation, of only four CAG repeats – more exactly, from 19 to 23. Besides, 90% of SCA2 alleles had 22 repeats. In spite of the pessimistic foresights due to its poor variation, the only positive finding was one of the SCA2 locus. More specifically, the present work found a significant association between CAG repeat length at the SCA2 gene, and fasciculations.

One can argue that the sample of patients with important fasciculations was very small. However, statistical significance was maintained even after Bonferroni procedure. One can also argue that long SCA2 alleles varied only from 22 to 23 repeats, and it would be difficult to believe that only one CAG repeat would make such a difference for the neurologic evolution. However, the finding has some clinical reasoning, because fasciculations are among the frequent neurologic signs of the SCA2 phenotype (28).

Some evidence of the pleiotropic effects of SCA2 gene was found previously by others. Chataway and collaborators (29) described an excessive transmission of the 22 repeat length SCA2 allele in multiple sclerosis patients. They speculated that the SCA2 gene would contribute epistatically to susceptibility to the disease.

We then conclude that data presented here support the hypothesis that other CAG polymorphic tracts, and specifically SCA2 CAG tract, can interfere with MJD phenotype, and that these results must be confirmed by further studies, with
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MJD patients of diverse ethnic origins, and with typing of other (CAG)n loci.

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