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Prevalence of Spinocerebellar Ataxia Type 2 Mutation among Italian Parkinsonian Patients

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Abstract: We evaluated the prevalence of the SCA2 mutation among 224 Italian patients affected by typical Parkinsonism, including 145 sporadic and 79 familial forms. Pink1, Parkin, and LRRK2 gene mutations had been excluded previously. Molecular testing for the CAG expansion at the SCA 2 locus was performed on leukocyte DNA. Cloning and sequencing of the expanded allele was performed in patients positive for the SCA2 expansion. A 38 CAG expansion was detected in 1 of 79 families studied. The proband, a male age 67, and his sister, age 69, were both affected by a benign form of L-dopa–responsive Parkinsonism not associated with cerebellar signs. The inheritance was autosomal dominant. The CAG expansion was stable through meiotic transmission: sequence analysis showed that the CAG stretch was interrupted by 3 CAA. Our study shows that CAG expansion at the SCA 2 locus may represent a genetic cause of familial L-dopa–responsive Parkinsonism among Italian patients. The stability of the pathological CAG expansion detected in this family was related to the presence of CAA interruptions. These findings, together with literature data, suggest that the molecular intrinsic structure of the expanded allele may modulate the phenotypic expression of the SCA2 mutation. © 2006 Movement Disorder Society

Key words: SCA2; Parkinson's disease; CAG repeats

Spinocerebellar ataxia type 2 (SCA2) is an autosomal dominant cerebellar ataxia caused by an abnormal CAG expansion within the coding region of the SCA2 gene, on chromosome 12 (12q23-24.1).[1] The SCA2 gene encodes protein *ataxin 2*, whose function is still unknown; normal alleles contain from 14 to 31 repeats and are frequently interrupted by one or more CAA triplets, whereas the expanded alleles contain a pure uninterrupted stretch of 34 to 59 CAG repeats.[2]

Limb and gait ataxia, slow saccades, and hyporeflexia are considered the typical clinical features of SCA2; however, extrapyramidal signs, cognitive disturbances, and signs of motor neuron involvement have also been described.[1]

Recently, it has been reported that Parkinsonism may represent a predominant clinical manifestation in SCA2 patients, with a phenotypic spectrum ranging from typical levodopa-responsive PD to Parkinson-plus phenotype.[3,4] The SCA2 "parkinsonian" phenotype is usually associated with a smaller pathological CAG expansion compared to the SCA2 "spinocerebellar" phenotype.[5]

The prevalence of the SCA2 mutation among familial Parkinsonisms ranges in different ethnicities between 1.5% and 8% of cases, being the highest among Chinese populations.[3] The prevalence of the SCA2 mutation among Italian parkinsonian patients has not been investigated to date. We have screened a large series of clinically defined Italian parkinsonian patients who are on file in the movement disorders database of our center.

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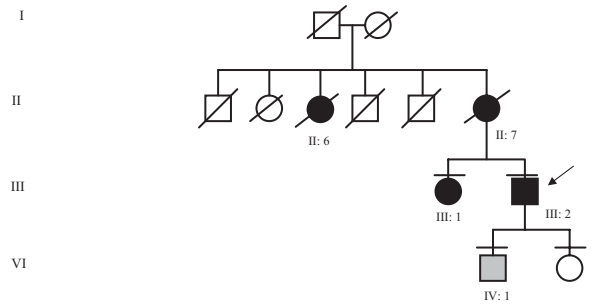


FIG. 1. Pedigree of the family affected by spinocerebellar ataxia type 2 (SCA2)-related Parkinsonism. Black symbols indicate the affected members, the gray symbol the presymptomatic carrier. The arrow marks the proband; the horizontal slashes indicate subjects who have been examined and tested for SCA2 mutation.

PATIENTS AND METHODS

We performed molecular genetic screening for the SCA2 mutation on 224 consecutive patients affected by Parkinson's disease diagnosed according to commonly used criteria,[6] including 145 sporadic and 79 index cases of familial forms; among the familial cases, 29 were compatible with autosomal dominant and 31 with autosomal recessive transmission, whereas in 19 patients, it was not possible to establish a clear transmission pattern. Mutations in Pink1 and Parkin genes as well as the common mutation of LRRK2 gene had been previously excluded.

Molecular testing for the SCA2 mutation was performed on DNA extracted from leukocytes as previously reported.[1] Informed consent was obtained in agreement with the guidelines of the Ethical Committee of Our Institution.

For sequence analysis of the SCA2 alleles, polymerase chain reaction products were electrophoresed through a 2% agarose gel, and the bands corresponding to the normal size and expanded alleles were excised from the gel under ultraviolet light, purified with QIAquick kit (Qiagen), subcloned in pGem-T-easy vector (Promega) using "TA cloning" strategy, and sequenced by automatic sequencer ABI 3730 sequencer (MWG BIOTECH, DNA Sequencing Facility).

RESULTS

None of the patients affected by sporadic Parkinsonism had a pathological CAG expansion at the SCA2 locus. Among 79 familial cases, 1 patient carried a 38 CAG expansion at the SCA2 locus.

Family history was compatible with an autosomal dominant transmission (Fig. 1): the proband's sister (Fig. 1, Patient III-1) had also received a diagnosis of PD. Their mother (Fig. 1: Patient II-7), dead at age 75 of a cardiac attack, presented upper limb resting tremor since she was in her sixties, and her sister (Fig. 1, Patient II-6) was reported to have tremor and dementia during her elderly years.

The proband (Fig. 1, Patient III-2), a 67-year-old man, since age 48 had noticed resting tremor in his lower limbs, predominant in the left leg. Soon after he also complained of fatigue, mild drooling, and upper limb resting tremor, more severe in the left hand. L-Dopa was introduced 2 years later, with marked benefit. At last evaluation, he had a mild parkinsonian syndrome. Neurological examination performed without medications (in the *off* condition) revealed tremor at rest, more severe in the lower limbs, slight hypomimia, mild axial and limb rigidity, mild bradykinesia (prevalent in left hemibody), and slight bending posture. The Motor score of Unified Parkinson's Disease Rating Scale (UPDRS III) was 21.

His sister (Fig. 1, Patient III-1), since age 57 complained of right-hand resting tremor. Three years later, Parkinson's disease was diagnosed, and L-dopa was introduced with clinical improvement. At last evaluation, at the age of 69, in the *off* condition, she presented with severe hypomimia, asymmetric resting tremor of upper limbs, and flexed posture; gait was slow, and she got up from a chair with some difficulties. Her UPDRS III score was 16. Neither of the 2 patients complained of any motor fluctuations or dyskinesias.

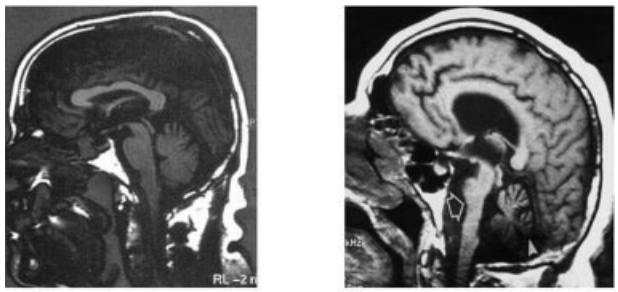
Acute challenge with 250 mg of L-dopa produced a significant improvement of UPDRS III scores both in the proband (57%) and in his sister (63%). No gait or limb ataxia was present in either patient, and eye movements and cranial nerves were normal.

Neuropsychological tests, somatosensorial, visual, and motor evoked potentials, and peripheral nerve conduction were all normal. Brain magnetic resonance imaging (MRI) did not show signs of cerebellar or brainstem atrophy (Fig. 2). DAT scan showed a presynaptic defect in the nigrostriatal pathways, as commonly seen in idiopathic Parkinson's disease (Fig. 3).

SCA2 molecular testing documented a heterozygous pathological expansion of 38 CAG repeats in the proband and in his sister; the pathological allele was also stably transmitted to the 32-year-old proband's son (Fig. 1: Patient IV-1), who is currently asymptomatic. Cloning and sequence analysis of the expanded alleles in the three carriers confirmed these data and documented that three CAA repeats interrupted the expanded CAG stretch.

DISCUSSION

This study performed on a large cohort of parkinsonian patients including 145 sporadic and 79 familial cases, confirms that SCA2 mutation may exclusively



A B

FIG. 2. Mid-sagittal T1 brain magnetic resonance imaging (MRI) scan of the proband (A) documents the absence of pontocerebellar atrophy, which in contrast is evident in the corresponding MRI images of a typical spinocerebellar ataxia type 2 (SCA2) patient (B, marked by arrows).

manifest as L-dopa–responsive Parkinsonism. In agreement with the literature, we detected the SCA2 mutation only among familial cases, with an estimated prevalence of approximately 1% among Italian patients.

In the family testing positive at the SCA2 molecular screen (Fig. 1), the clinical phenotype overlapped that of idiopathic PD: in particular, both affected patients manifested a benign disease form, characterized by slow progression of the symptoms and by the absence of L-dopa–induced motor fluctuations or dyskinesias, despite the long disease duration (19 years in Patient II-1 and 12 years in Patient II-2, respectively).

As previously reported in SCA2 patients presenting with a pure parkinsonian clinical phenotype,[7] brain MRIs did not show cerebellar atrophy (Fig. 2), whereas DAT scans showed a presynaptic defect in the nigrostriatal pathway (Fig. 3).

The presence of parkinsonian signs in SCA2 patients is not surprising, because the neuronal loss in the substantia nigra represents, together with cerebellar atrophy, a neuropathological hallmark of SCA2.[7] Moreover, functional neuroimaging studies have recently demonstrated the occurrence of nigrostriatal degeneration in SCA2 patients even in absence of parkinsonian signs.[8] However, it is difficult to explain the reason why the SCA2 mutation may cause in some families either a predominant or an exclusive involvement of the substantia nigra.

According to the higher predominance of the parkinsonian SCA2 phenotype documented in specific ethnic groups (i.e., Chinese), it was initially suggested that the genetic background could modulate the pathogenic effect of the SCA2 mutation.[4] On the other hand, it is important to point out that, independently from the patients' ethnic background, in general, patients with SCA2-related Parkinsonism carry a smaller pathological expansion (from 35 to 42 CAG) compared to SCA2–ataxic patients.[3]

In agreement with these data, also in our patients, we found a pathologically expanded allele carrying 38 CAG repeats: moreover, the allele size remained stable through meiotic transmission. Cloning and sequence analysis of the expanded allele showed that three CAA repeats interrupted the CAG tract (data not shown). An interrupted, genetically stable 39 CAG expansion was detected in another SCA2 family in which the affected members clinically manifested a pure parkinsonian phenotype without cerebellar signs.[9]

Conversely, molecular genetic studies performed in SCA2 ataxic patients document that the CAG tract in the expanded alleles is uninterrupted, whereas the presence of CAA interruptions characterizes the normal alleles, which are usually stable during meiotic transmission.[10] Taken together, these data suggest that the phenotypic expression of the SCA2 mutation may be influenced by the intrinsic molecular structure of the pathologically expanded repeat.

Supporting this hypothesis, recent data coming from molecular and structural analysis of the SCA2 gene transcripts showed that the (CAA) interruptions modulate the CAG repeat folding in the normal SCA2 transcripts, whereas the lack of CAA interruptions in the expanded mutant alleles may allow the formation of single-hairpins, which could exert a toxic effect by sequestering RNA-binding proteins.[11]

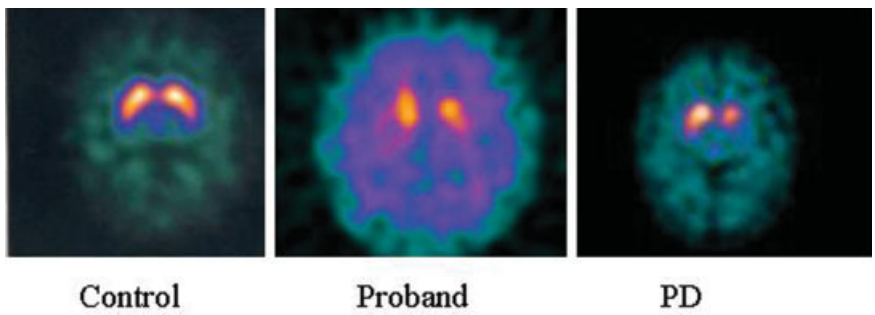


FIG. 3. Transaxial iodine-123 fluoropropyl beta-carbomethoxy-3 beta-(4-iodophenyltropane) ([^{123}I]FP-CIT) single-photon emission computed tomography scans at the level of basal ganglia in a control (left), the proband (middle) and a Parkinson's disease patient (PD, right). [^{123}I]FP-CIT binding is markedly reduced in the proband with a pattern similar to that observed in Parkinson's disease. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Further data coming from clinical and experimental models will be helpful to clarify this issue. In conclusion, this study confirms that SCA2 mutation may manifest as a benign form on familial Parkinsonism also among Italian patients, and we suggest including this molecular test in the genetic screening of autosomal dominant Parkinsonisms.

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