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HFE H63D polymorphism is increased in patients with amyotrophic lateral sclerosis of Italian origin

A role for metal-mediated oxidative stress in the pathogenesis of amyotrophic lateral sclerosis (ALS) was proposed in 1994 in the first studies of familial ALS mutant superoxide dismutase 1, and interference with iron homoeostasis is now postulated.[1] The HFE gene on chromosome 6 is a mean corpuscular haemoglobin class I-like molecule related to iron regulation. Mutations in the coding region cause hereditary haemochromatosis, a common autosomal recessive disorder of iron metabolism that leads to iron overload in adulthood. Recent reports on HFE mutations in ALS showed contradictory results. Two studies described a higher prevalence of the HFE mutations in ALS than in the control group, and one study did not find any difference between the patients with ALS and the control group.[2–4] We analysed a series of Italian patients with ALS to investigate whether mutations in the HFE gene could represent a risk factor for ALS.

A total of 149 sporadic Italian patients with ALS (mean (standard deviation (SD)) age 59.4 (9.7) years) according to El Escorial criteria for clinically definite or probable ALS were consecutively recruited to this study. Control samples were obtained from 168 healthy people, matched by age (difference of 5 years), sex and ethnic origin (Italian region of birth) to patients with ALS. Patients and controls were informed about the objectives of the study, and informed consent was obtained. The study was approved by the institutional ethics committee. Blood samples were collected, and DNA was purified with a 6100 Nucleic acid Prep Station (ABI PRISM™). Rapid detection of H63D, C282Y and S65C, the three most common mutations in the HFE gene, was performed using the pyrosequencing technique (Pyrosequencing AB, Uppsala, Sweden). This assay is based on a duplex polymerase chain reaction (PCR) in which exons 2 and 4 are amplified together. The exon 2 PCR product is used for S65C and H63D polymorphisms, and the exon 4 PCR product for the C282Y mutation. The mutation analysis was subsequently carried out in a triplex assay by means of three pyrosequencing primers in one well. Forward PCR primers for each reaction were modified with biotin at the 5′ terminus to capture single-stranded templates from PCR products for pyrosequencing. The following PCR primers were used: for exon 2, ex2-F 5′-ggc tac gtg gat gac cag c-3′ and ex2-R 5′-gag ttc ggg gct cca cac-3′; for exon 4 ex4-F 5′-cct ggg gaa gag cag aga t-3′ and ex4-R 5′-cag atc aca atg agg ggc tg-3′. The primers used for sequencing were: 5′-gct gtt cgt gtt cta tg-3′ for exon 2 and 5′-ggg gaa gag cag aga t-3′ for exon 4. The PCRs were performed for 45 cycles, with initial denaturation at 95℃ for 10 min, followed by 95℃ for 30 s, annealing for 30 s and extension at 72℃ for 30 s. The final extension was at 72℃ for 5 min. Bound biotinylated single-stranded DNA was prepared following the protocols provided by the manufacturer (PSQ96 sample preparation kit; Pyrosequencing AB). SNP/AQ analysis was performed automatically on a PSQ™96 MA system using enzymes and reagents from an SNP Reagent kit (Pyrosequencing AB).

The group with ALS included 65 women and 84 men (mean (SD) age 61.1 (11.1) years), and the control group included 66 women and 102 men (mean (SD) age 60.7 (9.2) years). Table 1 shows the findings for the three SNPs H63D, C282Y and S65C. Analysis of HFE mutations showed a higher frequency of the mutated allele H63D in patients with ALS than in controls (28.8% *v* 14.8%; $p = 0.004$). The odds ratio conferred by the presence of the H63D allele was 2.25 (95% confidence interval 1.30 to 3.93). An increased frequency was also found in patients with ALS when all three mutations were combined (33.3% *v* 17.3%; $p = 0.002$). No significant differences were found between patients with the H63D allele and patients with wild-type HFE gene considering age of onset (63.4 (SD 9.3) *v* 60.2 (SD 11.9)), sex (22 men and 21 women *v* 62 men and 44 women), type of onset (33 spinal and 10 bulbar *v* 80 spinal and 26 bulbar) and disease duration (median survival time, 783 *v* 993 days).

Our data support the hypothesis that the change in iron metabolism may confer susceptibility to neurodegenerative diseases such as ALS. Our results are consistent with those found in the USA,[2] and in Ireland and Britain.[4] Interestingly, the second study reported an odds ratio of 1.85 (95% confidence interval 1.35 to 2.54) for the presence of the heterozygous H63D polymorphism, a value similar to that found in our population. In Europe, the C282Y allele has a north to west frequency-decreasing gradient, with higher frequencies reported in Ireland (28.4%) and Britain (17.4%) and lower frequencies in Italy (3.2%) and Greece (2.6%). Conversely, the H63D allele has a higher frequency in southern Europe (Spain, 32.3%) and a lower frequency in the Celtic populations (5%).[5] These marked ethnic differences may explain the negative findings of one study on patients with ALS in Texas, USA,[3] in which no matching for ethnic origin was performed.

The possible role of the H63D polymorphism in ALS is unclear. In a human neuronal cell line transfected with genes carrying the HFE mutations, the H63D polymorphism induced a decreased expression of SOD1, α-tubulin and β-actin;[2] these events can cause a disruption of axonal transport, a factor implicated in ALS pathogenesis. Alternatively, the H63D polymorphism may determine a subclinical increase in intracellular iron, possibly related to neurone oxidative damage. Further studies on the analysis of iron metabolism in patients with ALS are needed to elucidate the role of the H63D polymorphism as a genetic risk factor for sporadic ALS. An alternative possibility could be a linkage disequilibrium of ALS with an unknown gene located near the HFE locus.

Table 1 Frequency of HFE polymorphisms in patients with amyotrophic lateral sclerosis and controls

	Patients with ALS		Controls	
Polymorphisms	**No**	**%**	**No**	**%**
H63D/wild type	41	27.5	25	14.8
H63D/H63D	2	1.3	–	–
C282Y/wild type	5	3.3	3	1.8
S65C/wild type	2	1.3	1	0.6
Wild/wild type	99	66.4	139	82.7
Total	149	100	168	100

AlS, amyotrophic lateral sclerosis.

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G Restagno, F Lombardo
Molecular Genetics Unit, Department of Clinical Pathology, Children's Hospital, Torino, Italy

P Ghiglione, A Calvo
Department of Neuroscience, University of Torino, Torino, Italy

E Cocco, L Sbaiz
Molecular Genetics Unit, Department of Clinical Pathology, Children's Hospital, Torino, Italy

R Mutani, A Chiò
Department of Neuroscience, University of Torino, Torino, Italy

Correspondence to: Professor A Chiò, Department of Neuroscience, University of Turin, via Cherasco 15, Torino 10126, Italy; adriano.chio@unito.it

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Causes of death in multiple system atrophy

Multiple system atrophy (MSA) is a heterogeneous neurodegenerative disorder, with a clinical presentation combining extrapyramidal,

cerebellar, autonomic or pyramidal symptoms. There are two major subtypes: MSA-P, with a clinical predominance of parkinsonism, and MSA-C, with a clinical predominance of cerebellar symptoms. Although various factors have been proposed to predict survival in MSA, including age at onset and several phenotypic features,[1] the terminal/end of life events have never been systematically studied. We present our results from a study on the causes of death in a series of pathologically confirmed, definite MSA cases.

All patients registered with the University of Miami/NPF Brain Endowment Bank (UM/BEB) donation programme with a diagnosis of neuropathologically confirmed, definite multiple system atrophy (MSA; n = 21) were included in this study. Pertinent information was gathered by two prospectively filled questionnaires used as part of the UM/BEB's recruitment process: (a) the UM/BEB Parkinson's disease registry form, a 128-item, self-administered questionnaire on demographics, environmental exposures, personal and family history, comorbid conditions, activities of daily living, clinical and treatment details; and (b) the ''agonal state'' form, a 25-item questionnaire on events covering the 48 h before death completed by the treating doctor/nurse. For comparisons, each MSA case was closely matched for age at disease onset ($\pm$2 years) and sex with a Parkinson's disease brain donor by an investigator blinded to the disease status and clinical information. Medical, hospital and hospice records of brain donors were also collected on an annual basis and all disease-related information was extracted by two independent clinical investigators (blinded to the aims of the study), and entered into separate databases that were checked for consistency. Brain removal, autopsy, fixation and sectioning were performed according to standard protocols. Postmortem diagnosis of MSA and Parkinson's disease were based on well-accepted criteria.[2 3] For statistical analysis, Mann–Whitney U test for two samples was used in non-parametric comparisons, and χ^2 tests with Yates' corrected p value and two-tailed Fisher exact p values in the comparison of proportions, as appropriate. The study was approved by the local institutional review board.

Table 1 shows the demographics and primary causes of death of all patients. Patients with MSA had significantly shorter disease duration (p = 0.02) than matched patients with Parkinson's disease, and most presented with parkinsonian-predominant symptoms. None of the patients had a predominantly autonomic presentation. In all, 15 of 21 (71.4%) patients with end-stage MSA had permanent in-dwelling balloon (Foley) catheters because of symptoms of urinary incontinence for at least 6 months before death; 13 (61.3%) had recurrent lower urinary tract infections (LUTIs). The recurrence of infections did not correlate with the presence of permanent Foley catheters; 4 of 13 (30.8%) patients with LUTIs did not have permanent Foley catheters. Two patients with MSA used clean intermittent self-urinary bladder catheterisation. Of the 13 patients with recurrent LUTIs, 5 (38.5%) died as a result of their infections. In addition, 7 (33.3%) patients had recurrent ($\geq$2) episodes of aspiration and 8 (38.1%) had percutaneous gastrostomy (PEG) feeding tubes inserted because of swallowing/feeding difficulties and aspiration. The recurrence of aspirations was independent of PEG tubes, as 4 of 7 (57.1%) patients with PEG continued having episodes of aspiration after PEG. Of patients with recurrent aspirations, one died as a result of acute aspiration and two as a result of aspiration pneumonia.

Sudden death related to MSA was reported in 8 (38.1%) patients. In seven patients, sudden death was characterised as cardiopulmonary arrest of otherwise unknown aetiology, and in one as acute aspiration during sleep. In all, 5 of 6 (23.8%) patients with reported symptoms of laryngeal stridor as part of their disease died suddenly. Two of the patients with stridor had a permanent tracheostomy. Sudden death was reported in one of them. Skin infections in the form of complicated pressure ulcers were present in 6 (28.6%) patients. However, skin infections were not associated with death in any case. Marked weight loss ($\geq$10% of premorbid weight) was reported in 16 (76.2%) patients. Weight loss was considerably less common in patients with PEG (5–31.2%) compared with those without PEG (11–68.8%). Three patients died as a result of weight loss and wasting. In contrast with Parkinson's disease, all patients with MSA died as a result of events related to their disease. One patient with MSA died from intestinal perforation after PEG tube misplacement.

More than one third of patients with MSA in this study died suddenly. Several mechanisms for sudden death have been proposed in MSA. The combination of passive glottic narrowing by selective paralysis of the vocal cord abductors and active narrowing by adductor activation during inspiration[4] have been associated with stridor and acute airway obstruction. Furthermore, patients with MSA show minimal to no chemosensitivity to hypoxia (especially during sleep) possibly owing to the degeneration of brain stem respiratory centres. These may explain sudden death in patients with MSA even after tracheostomy.[5]

Dysphagia caused by delays in the oral and pharyngeal phases of swallowing, in combination with laryngeal (airway and sensory) and oesophageal sphincter disturbances may lead to both aspiration pneumonia and acute aspiration.[6] PEG tube feeding prevented considerable weight loss and wasting, but not the recurrence of aspirations and aspiration pneumonia. Additional measures, such as improvement of oral/dental hygiene and proper patient postprandial and sleep positioning,[7] may be considered to decrease mortality from aspiration. An additional finding of this study is the high prevalence of weight loss among patients with end-stage MSA. Weight loss is a risk factor for mortality in chronically ill patients.[8] Dietary adjustments, early swallowing studies and PEG tube feeding may reduce mortality in patients with MSA.

Neurogenic lower urinary tract dysfunction is considered a valuable diagnostic tool for MSA.[9] Urinary urgency or incontinence (storage disorder) and incomplete emptying or urinary retention (voiding disorder) may occur simultaneously and lead to intractable LUTIs, which are major causes of morbidity in this disorder.[10] More than half of our patients reported recurrent LUTIs and a large number died from complications related to LUTIs. Frequent urological monitoring and treatment of complications, in addition to the use of clean intermittent self-urinary bladder catheterization, may reduce the risk of LUTI-associated mortality.

In summary, all patients with MSA died from disease-related events, with sudden death and infections being the most common. We propose that careful screening for laryngeal stridor, neurogenic bladder dysfunction and dysphagia with aggressive treatment may increase total survival time in patients with MSA. More studies on the patient are warranted. Research efforts should be directed towards the development of more efficient identification and prevention strategies for the major complications of MSA.

Table 1 Characteristics and primary causes of death

	MSA (n = 21)	PD (n = 21)
Sex	10M/11F	10M/11F
Mean (SD) age at disease onset (years)	59.4 (10.1)	59.9 (8.9)
Mean (SD) disease duration (years)*	8.5 (4.7)	13.4 (8.2)
MSA type at presentation (%)		
MSA-P	16 (76.2)	
MSA-C	5 (23.8)	
Permanent balloon (Foley) catheters (%)†	15 (71.4)	7 (33.3)
Percutaneous gastrostomy (%)	8 (38.1)	3 (14.3)
Tracheostomy (%)	2 (9.5)	None
Sudden death (%)‡	8 (38.1)	3 (14.3)
Cause of death (%)		
Cardiopulmonary arrest	7 (33.3)	1 (4.8)
Urinary tract infection	5 (23.8)	None
Aspiration pneumonia	2 (9.5)	None
Infectious pneumonia	2 (9.5)	7 (33.3)
Acute aspiration	1 (4.8)	1 (4.8)
Wasting syndrome	3 (14.3)	1 (4.8)
Cerebrovascular accident	None	3 (14.3)
Myocardial infarction	None	2 (9.5)
Cancer	None	2 (9.5)
Other	1 (4.8)	1 (4.8)

F, female; M, male; MSA, multiple system atrophy; MSA-C, MSA with a clinical predominance of cerebellar symptoms; MSA-P; MSA with a clinical predominance of parkinsonism; PD, Parkinson's disease.
Results in the comparisons between MSA and PD groups were significant for *p = 0.02, †p = 0.03 and ‡p = 0.02.

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Spiridon Papapetropoulos, Alexander Tuchman, Daniel Laufer
Department of Neurology, University of Miami, Miller School of Medicine, Miami, USA

Athanassios G Papatsoris
2nd Department of Urology, School of Medicine, University of Athens, Athens, Greece

Nektarios Papapetropoulos
Department of ENT, Tameside General Hospital, Ashton under Lyne, Lancashire, UK

Deborah C Mash
Department of Neurology, University of Miami, Miller School of Medicine, Miami, USA

Correspondence to: Dr Papapetropoulos Spiridon, Department of Neurology, University of Miami, School of Medicine, Room 4004, 1501 NW 9th Avenue, Miami, FL 33136, USA; spapapetropoulos@med.miami.edu

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Pisa syndrome after unilateral pallidotomy in Parkinson's disease: an unrecognised, delayed adverse event?

Dystonic lateroflexion of the trunk, also referred to as Pisa syndrome, pleurothotonus or a lean to the side, was originally described in association with prior exposure to neuroleptics. However, axial deformities (Pisa syndrome, camptocormia and antecollis) are also well recognised but poorly understood features of multiple system atrophy or late-stage Parkinson's disease. Here, we report on three patients with longstanding Parkinson's disease who, 4–9 years after a left pallidotomy, developed a Pisa syndrome to the right.

Case histories

The first patient, now 72 years old, was diagnosed with Parkinson's disease at age 44 years, after initially presenting with pain in his right arm and leg. The right side always remained the more affected and the dyskinesias that developed after 4 years of levodopa treatment were also more pronounced on the right side. Because of progressive motor fluctuations, a left-sided pallidotomy was performed after 17 years of disease, which resulted in abolition of the right-sided dyskinesias and an improvement in the tremor and rigidity on the right. Eight years after the pallidotomy, 25 years after disease onset, he gradually developed a lean to the right, which showed some diurnal fluctuation and responded modestly to dopaminergic treatment. When ''on'', he still remains independent for most daily activities. Parkinson's disease dementia has recently been diagnosed.

In the second patient, now 63 years old, Parkinson's disease was diagnosed at the age of 47 years when he first noticed decreased dexterity and a tremor of his right hand. He developed limb dyskinesias (more on the right side than on the left) after only 1 year of levodopa treatment. After unsuccessful alternative drug regimens, a left-sided pallidotomy was performed after 6 years of disease. The dyskinesias on the right completely disappeared and a beneficial effect on tremor and walking were documented. Fifteen years after his first symptoms, and about 9 years after surgery, a lean to the right evolved that was unresponsive to dopaminergic drugs. Over the past year, he has developed features of early Parkinson's disease dementia. He uses a wheelchair for outdoor activities only.

The third patient, now 69 years old, noticed a tremor of his right hand when he was 43 years old. Dyskinesias, mainly on the right, became apparent 3 years after levodopa treatment. Seventeen years after onset, he underwent a left-sided pallidotomy. The dyskinesias on the right side subsided, and he also experienced ''off''-period improvement and better balance. In his 21st year of disease, 4 years after the pallidotomy, he developed a mild torticollis to the right. Around the same time, he started to gradually develop a lean to the right (fig 1), sometimes causing him to fall out of a chair. Mild Parkinson's disease dementia was established recently. He is still able to walk unsupported.

Comment

The outcome and follow-up after a median 14 months of 26 patients with Parkinson's disease who underwent a unilateral medial pallidotomy in our hospital in 1995–96 have been reported previously.[1] Here, we describe the further follow-up of three of these patients, because they developed a marked lean (Pisa syndrome) to the right side 4–9 years after a left pallidotomy, at disease durations of 15–25 years. The truncal lateroflexion came on gradually, and showed some diurnal fluctuation and dopamine responsiveness in only one patient. In all patients, signs and symptoms started and remained more pronounced on the right side, which was also the more dyskinetic side, hence the choice of a left-sided pallidotomy. Despite the long disease duration, mobility was still relatively well preserved, particularly in patients 1 and 3, and the dyskinesias continued to be less severe on the side contralateral to the pallidotomy.

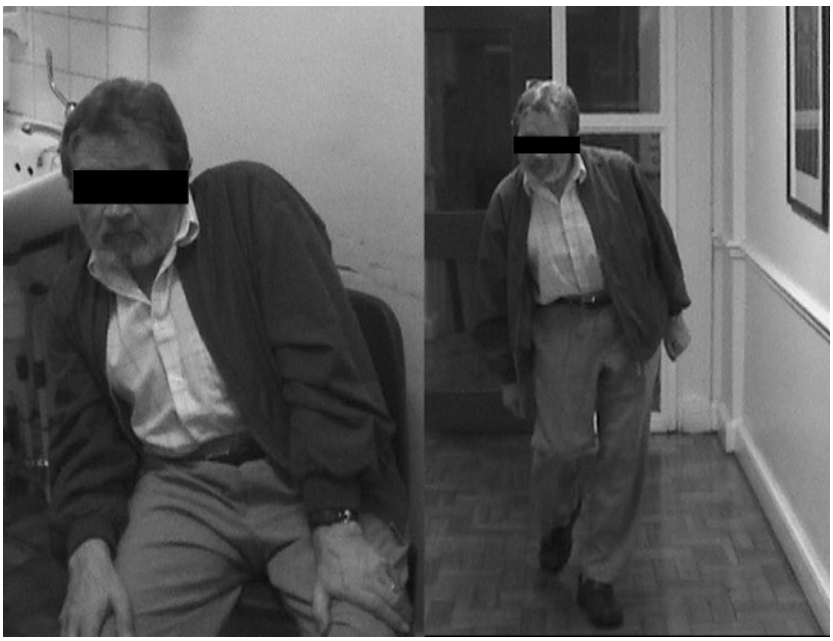

Figure 1 A 69-year-old man with a 26-year history of Parkinson's disease underwent a left pallidotomy 9 years ago. Four years after this procedure, he gradually developed a lean to the right. These photographs show the marked lean to the right, which is present during both sitting and walking, as well as a mild head tilt to the left. Informed consent was obtained for publication of this figure.