Association of the H63D polymorphism in the hemochromatosis gene with sporadic ALS

Abstract—Iron misregulation promotes oxidative stress and abnormally high iron levels have been found in the spinal cords of patients with ALS. The authors investigated whether HFE gene polymorphisms, linked to hemochromatosis, are associated with ALS using two independent populations of patients with sporadic ALS and controls (totaling 379 patients and 400 controls). They found that the H63D polymorphism is overrepresented in individuals with sporadic ALS (odds ratio 1.85, CI: 1.35 to 2.54).

NEUROLOGY 2005;65:934–937

E.F. Goodall, BSc; M.J. Greenway, MB; I. van Marion, BSc; C.B. Carroll, PhD, MRCP; O. Hardiman, MD, FRCPI; and K.E. Morrison, DPhil, FRCP

Many theories of ALS pathogenesis have been proposed, among them that of increased oxidative stress. Elevated levels of iron have been found in ALS spinal cord tissue,[1] and this may contribute to oxidative damage via the ability of iron to generate reactive oxygen species through the Fenton reaction.

Iron homeostasis in humans is tightly controlled as both iron overload and deficiency have severe physiologic consequences. Hereditary hemochromatosis (HH) is a genetic disorder resulting in accumulation of unchelated iron in parenchymal tissues and organ damage. Two principal polymorphisms in the *HFE* gene, C282Y and H63D, have been identified as the cause of classic HH.[2] Although the exact function of HFE is unknown, reports suggest a role in sensing body iron levels via its interaction with the transferrin receptor (TfR).[3] The most frequently identified polymorphism in HH is the C282Y variant, which disrupts cell surface expression of HFE and therefore the interaction with TfR.[3] Another common HFE polymorphism is the lowly penetrant H63D, often occurring in HH patients with a heterozygous C282Y variant.[2] Both of these polymorphisms in the heterozygous state have been reported in association with higher iron concentrations.

Given the evidence of both oxidative stress and disrupted iron homeostasis in ALS, we investigated whether this disease is associated with the HFE polymorphisms. We report an association between the H63D polymorphism and ALS, suggesting that disrupted iron metabolism may be one factor that initiates or propagates motor neuron damage in ALS.

Methods. *Subjects.* A total of 166 individuals with sporadic ALS were recruited through the Birmingham Motor Neurone Disease (MND) Care and Research Centre, Queen Elizabeth Hospital, U.K. An additional 213 patients with sporadic ALS were enlisted from an independent Irish population. All conformed to a diagnosis of definite or probable ALS, according to the El Escorial criteria for ALS. Control samples were obtained from healthy individuals (192 in Birmingham and 208 in Ireland). Fifty-one percent of the Birmingham controls and 10% of the Irish controls were spouses of patients with ALS. The other controls in each population were healthy, unrelated individuals. Informed consent was obtained before blood sampling, and the study was approved by the South Birmingham Local Research Ethics Committee and Beaumont Hospital Ethics Committee. All patients and controls were white.

Genotyping. DNA was extracted from venous blood samples according to standard protocols using NucleonII kits (Amersham). DNA (50 ng) from the Birmingham samples was amplified by PCR primers flanking the H63D and C282Y polymorphisms (Alta Bioscience, University of Birmingham, UK), as described by others.[4] The *HFE* polymorphisms were detected by restriction enzyme digestion with Rsa1 and Mbo1 for C282Y and H63D (New England Biolabs). DNA samples from six patients with HH known to be homozygous or heterozygous for the H63D and C282Y polymorphisms were amplified and digested as controls. DNAs from the Irish samples were amplified and genotyped using an alternative method.[5] Consistency of genotyping was assessed by reanalysis of 10% of the Birmingham samples by this alternative method.

Statistical analysis. The observed genotypes were compared using the Fisher's exact test. Analyses were interpreted as significant if the distributions differed by $p < 0.05$. Presentation with bulbar or limb symptoms and the men vs women breakdown of patients with ALS with and without the HFE variants were compared using Fisher's exact test. Age at disease onset was investigated using the Student t test. All statistical analyses were performed using the SPSS software package.

Results. Genotype and allele frequency results for the HFE polymorphisms in the study patients and controls are shown in tables 1 and 2. Genotype frequencies did not deviate from those predicted by Hardy-Weinberg equilibrium in either patients or controls in both data sets, and no sex differences were apparent (table 1). Genotypes at the H63D locus of patients with ALS and controls were compared using Fisher's exact test, pooling individuals either heterozygous (CG) or homozygous (GG) for the disease allele because of the low numbers of homozygous individuals (five patients with ALS vs two controls from Birmingham and 10 patients vs three controls from Ireland). This showed a significant difference in genotypes, with 130 (34%) of the patients with ALS in the combined data set

From the Department of Clinical Neuroscience (E.F. Goodall, I. van Marion, Drs. Carroll, and Morrison), The Medical School, University of Birmingham, Birmingham, U.K.; Department of Clinical Neurological Sciences (Drs. Greenway and Hardiman), Royal College of Surgeons in Ireland, Dublin, Ireland; and Department of Neurology (Dr. Hardiman), Beaumont Hospital, Dublin, Ireland.

Funding from the Midlands Neuroscience Teaching and Research Fund, the University Hospital Birmingham Charities (I.V.M.) and the Medical Research Council (E.F.G.) is acknowledged.

Disclosure: The authors report no conflicts of interest.

Received March 4, 2005. Accepted in final form June 8, 2005.

Address correspondence and reprint requests to Prof. K.E. Morrison, Institute of Biomedical Research, The Medical School, University of Birmingham, Edgbaston, Birmingham, B15 2TT, U.K.; e-mail: k.morrison@bham.ac.uk

 Copyright © 2005 by AAN Enterprises, Inc.

Copyright © by AAN Enterprises, Inc. Unauthorized reproduction of this article is prohibited.

Table 1 *Genotype and allele frequency data for the H63D polymorphism*

	Birmingham		Ireland		Combined	
	ALS, n = 166	Controls, n = 192	ALS, n = 213	Controls, n = 208	ALS, n = 379	Controls, n = 400
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Genotypes						
Wild type (CC)	113 (68)	151 (78.6)	136 (63.8)	161 (77.4)	249 (65.7)	312 (78.0)
Heterozygous (GC)	48 (28.9)	39 (20.3)	67 (31.3)	44 (21.2)	115 (30.3)	83 (20.8)
Homozygous (GG)	5 (3.0)	2 (1.0)	10 (4.7)	3 (1.4)	15 (4.0)	5 (1.3)
Pooled (GC, GG)	53 (31.9)	33 (21.4)	77 (36.0)	47 (22.6)	130 (34.3)	88 (22.0)
	$p = 0.030$		$p = 0.003$		$p < 0.001$	
Allele frequency						
C	274 (82.5)	341 (88.8)	339 (79.6)	366 (88.0)	613 (80.9)	707 (88.3)
G	58 (17.5)	43 (11.2)	87 (20.3)	50 (12.0)	145 (19.1)	93 (11.6)
	$p = 0.018$		$p = 0.001$		$p < 0.001$	
Analysis by gender of patients	Male, n = 104	Female, n = 62	Male, n = 119	Female, n = 94	Male, n = 223	Female, n = 156
Wild type (CC)	69 (66.3)	44 (71.0)	73 (61.3)	63 (67.0)	142 (63.7)	107 (68.6)
Pooled (GC, GG)	35 (33.7)	18 (29.0)	46 (38.7)	31 (33.0)	81 (36.3)	49 (31.4)
	$p = 0.607$		$p = 0.473$		$p = 0.379$	
Gender of controls	Male, n = 59	Female, n = 133	Male, n = 133	Female, n = 75	Male, n = 192	Female, n = 208
Wild type (CC)	45 (76.3)	106 (79.7)	101 (75.9)	60 (80.0)	146 (76.0)	166 (79.8)
Pooled (GC, GG)	14 (23.7)	27 (20.3)	32 (24.1)	15 (20.0)	46 (24.0)	42 (20.2)
	$p = 0.574$		$p = 0.605$		$p = 0.399$	

having at least one G allele compared to only 88 (22%) of controls. Evaluation of the allele frequencies at the H63D locus likewise showed an association between the G allele and ALS ($p < 0.001$). The control group H63D genotype frequencies did not significantly differ between the Birmingham and Irish populations and were consistent with those of other studies of control individuals in these populations.[6,7] The odds ratio (OR) conferred by the presence of a mutant G allele for the combined data set was evaluated as 1.85 (CI: 1.35 to 2.54).

In contrast, there was no difference between patients and controls for the C282Y polymorphism ($p = 0.466$ for the Birmingham group and $p = 0.905$ for the Irish group) (see table 2). No significant differences were found between HFE genotype and mode of onset (limb vs bulbar) nor in age at onset of disease in either population or in the combined data set (table 3).

Discussion. There is increasing evidence to suggest a role for disrupted iron metabolism in a range

Table 2 *Genotype and allele frequency data for the C282Y polymorphism*

	Birmingham		Ireland		Combined	
	ALS, n = 166	Controls, n = 192	ALS, n = 213	Controls, n = 208	ALS, n = 379	Controls, n = 400
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Genotypes						
Wild type (GG)	143 (86.1)	159 (82.8)	167 (78.4)	165 (79.3)	310 (81.8)	324 (81.0)
Heterozygous (GA)	22 (13.3)	31 (16.1)	46 (21.6)	40 (19.2)	68 (17.9)	71 (17.8)
Homozygous (AA)	1 (0.6)	2 (1.0)	0 (0)	3 (1.4)	1 (0.3)	5 (1.3)
Pooled (GA, AA)	23 (13.9)	33 (17.2)	46 (21.6)	43 (20.7)	69 (18.2)	76 (19.0)
	$p = 0.466$		$p = 0.905$		$p = 0.783$	
Allele frequency						
G	308 (92.8)	349 (90.9)	380 (89.2)	370 (88.9)	688 (90.8)	719 (89.9)
A	24 (7.2)	35 (9.1)	46 (10.8)	46 (11.1)	70 (9.2)	81 (10.1)
	$p = 0.748$		$p = 0.913$		$p = 0.607$	

Copyright © by AAN Enterprises, Inc. Unauthorized reproduction of this article is prohibited.

Table 3 *Age at disease onset and mode of disease onset in patients with ALS with and without the H63D polymorphism*

	Birmingham			Ireland			Combined	
	Mean	SD	Range	Mean	SD	Range	Mean	SD
Age at onset (y)								
H63D genotypes								
Wild type (CC)	59.91	10.96	28–81	56.68	12.69	22–84	57.81	12.18
Non-wild type (GC, GG)	58.53	10.53	38–77	54.64	14.26	25–90	56.14	13.04
		$p = 0.493$			$p = 0.304$			$p = 0.249$

	Birmingham		Ireland		Combined	
	Limb, n = 110	Bulbar, n = 56	Limb, n = 164	Bulbar, n = 49	Limb, n = 292	Bulbar, n = 111
Mode of onset	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
H63D genotypes						
Wild type (CC)	78 (70.9)	35 (62.5)	106 (64.6)	30 (61.2)	198 (67.8)	70 (63.1)
Non-wild type (GC, GG)	32 (29.1)	21 (37.5)	58 (35.4)	19 (38.8)	94 (32.2)	41 (36.9)
	$p = 0.294$		$p = 0.735$		$p = 0.409$	

of neurodegenerative disorders.[8] Considering ALS, a previous report investigating the prevalence of C282Y and H63D polymorphisms in American patients with ALS and controls did not find any associations, but this study was limited by low numbers (51 patients, 47 normal controls) of ethnically diverse cases and controls.[9] Another recent report, comprising 121 patients with ALS and 133 controls did report an association of the H63D polymorphism with ALS.[4] However, there were difficulties with the control group used in this later study as it consisted of individuals with neuromuscular diseases other than ALS, and the control group allele frequencies were different from those previously reported.

Key issues to be addressed in any association study include the number of cases and controls investigated; the validity of the control population; the replication of the findings in an independent population and a consideration of whether the association are biologically plausible. In this study, we first investigated polymorphisms in the *HFE* gene in 166 patients with sporadic ALS and 192 control individuals from Birmingham and found differences in the H63D variant at the $p = 0.03$ level of significance. We then replicated our results in an independent Irish population, and the combined data set gives an OR for the H63D polymorphism of 1.85. Given the key role of iron in oxidative reactions, there is biologic plausibility for iron homeostasis proteins to have an influence in ALS.

It is not clear why the H63D polymorphism and not C282Y is linked to ALS in our study. The molecular mechanisms whereby either polymorphism leads to HH are not fully understood. The C282Y polymorphism seems to alter the interaction of HFE with β2 microglobulin, with secondary loss in intracellular transport of HFE leading to decreased expression on the cell surface.[3] H63D is thought to effect the formation of a salt bridge within HFE, altering binding to the transferrin receptor.[3] Of possible relevance to this mutation and ALS, the mechanism for iron transport across the blood-brain barrier is thought to be via receptor-mediated endocytosis of the iron-transferrin complex by capillary endothelial cells.[10]

Our study, the first to show a significant association of *HFE* gene polymorphisms in ALS compared with appropriate controls in two populations, suggests that further investigation of iron homeostasis pathways in ALS pathogenesis is warranted. This may also have implications for the use of antioxidants as therapeutic agents in ALS, as patient responses may differ based on HFE genotype.

Acknowledgment

The authors gratefully acknowledge the role of Dr. H.S. Pall in referring patients with ALS and their carers for this study and Dr. R. Raha-Chowdhury for providing DNA samples from patients with hemochromatosis as positive controls for polymorphism detection. The authors also thank Dr. S. Ennis, Professor A. Green, and the staff of the National Centre for Medical Genetics, Ireland for their assistance.

References

1. Ince PG, Shaw PJ, Candy JM, et al. Iron, selenium and glutathione peroxidase activity are elevated in sporadic motor neurone disease. Neurosci Lett 1994;182:87–90.
2. Feder JN, Gnirke A, Thomas W, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet 1996;13:399–408.
3. Fleming RE, Sly WS. Mechanisms of iron accumulation in hereditary hemochromatosis. Annu Rev Physiol 2002;64:663–680.
4. Wang X, Lee S, Simmons Z, et al. Increased incidence of the Hfe mutation in amyotrophic lateral sclerosis and related consequences. J Neurol Sci 2004;227:27–33.
5. Slott MK, Fellowes AP, Upton JD, Burt MJ, George PM. Simple multiplex PCR for the simultaneous detection of the C282Y and H63D hemochromatosis (*HFE*) gene mutations. Clin Chem 1999;45:426–428.
6. Chambers V, Sutherland L, Palmer K, et al. Haemochromatosis-associated HFE genotypes in English blood donors: age-related frequency and biochemical expression. J Hepatol 2003;39:925–931.

Copyright © by AAN Enterprises, Inc. Unauthorized reproduction of this article is prohibited.

7. Ryan F, Vaughan J. Haemochromatosis mutation analysis in a normal Irish population. Br J Biomed Sci 2000;57:315–316
8. Thompson KJ, Shoham S, Connor JR. Iron and neurodegenerative disorders. Brain Res Bull 2001;55:155–164.
9. Yen AA, Simpson EP, Henkel JS, Beers DR, Appel SH. HFE mutations are not strongly associated with sporadic ALS. Neurology 2004;62: 1611–1612.
10. Moos T. Brain iron homeostasis. Dan Med Bull 2002;49:279–301.

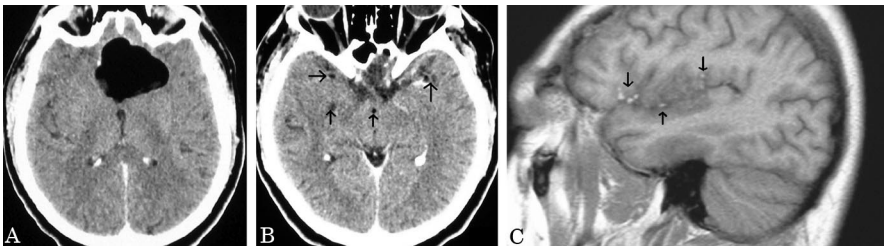

Figure. (A) CT scan shows voluminous frontal cyst. (B) Multiple hypodense lesions corresponding to fatty droplets (dashed arrows). (C) T1-weighted MRI shows hyperintense images in the sylvian valley corresponding to fatty droplets due to dermoid cyst rupture in subarachnoid space (dashed arrows).

Chemical meningitis in reaction to subarachnoid fatty droplets

S. Castro, MD; G. Castelnovo, MD; A. Lebayon, MD; S. Fuentes, MD; S. Bouly, MD; and P. Labauge, PhD; Nimes, France

A 50-year-old man with no medical history presented to our department with a month-long history of worsening headaches with fever. Clinical examination revealed hyperthermic meningeal syndrome. CSF examination found 900 white cells/mm^3 including 95% neutrophils, hyperproteinorachia (125 mg/dL), and hypoglycorachia (1.6 mmol/L) with normoglycemia.

Encephalic CT scan and MRI revealed an aspect of ruptured frontosellar dermoid cyst, with evidence of fatty material within the subarachnoid space (figure). This condition can mimic septic meningitis and is a rare cause of recurrent puriform aseptic meningitis[1,2]

The tumor was surgically removed and the patient was discharged without further problems.

Copyright © 2005 by AAN Enterprises, Inc.

Disclosure: The authors report no conflicts of interest.

Address correspondence and reprint requests to Dr. G. Castelnovo, Neurological Department, Hopital Caremeau, CHU Nimes, 2 Av du Prof Debre, Nimes, France; e-mail: giovanni.castelnovo@chu-nimes.fr

1. Miller D. Case report: dermoid cyst of the frontal lobe with intraventricular rupture. J Neurol Neurosurg Psychiatry 1950;13:63–65.
2. Stendel R, Pietila TA, Lehmann K, Kurth R, Suess O, Brock M. Ruptured intracranial dermoid cysts. Surg Neurol 2002;57:391–398.

Copyright © by AAN Enterprises, Inc. Unauthorized reproduction of this article is prohibited.

Neurology®

Chemical meningitis in reaction to subarachnoid fatty droplets

S. Castro, G. Castelnovo, A. Lebayon, et al.

Neurology 2005;65;937
DOI 10.1212/01.wnl.0000181350.70430.4f

This information is current as of September 26, 2005

Updated Information & Services	including high resolution figures, can be found at: http://n.neurology.org/content/65/6/937.full
References	This article cites 2 articles, 1 of which you can access for free at: http://n.neurology.org/content/65/6/937.full#ref-list-1
Permissions & Licensing	Information about reproducing this article in parts (figures,tables) or in its entirety can be found online at: http://www.neurology.org/about/about_the_journal#permissions
Reprints	Information about ordering reprints can be found online: http://n.neurology.org/subscribers/advertise

Neurology ® is the official journal of the American Academy of Neurology. Published continuously since 1951, it is now a weekly with 48 issues per year. Copyright . All rights reserved. Print ISSN: 0028-3878. Online ISSN: 1526-632X.

AMERICAN ACADEMY OF NEUROLOGY®