

SHORT REPORT

Iron overload without the C282Y mutation in patients with epilepsy

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Abstract

To test the hypothesis that iron overload predisposes to epilepsy, transferrin saturation in 130 patients with epilepsy and sex and age matched 128 control subjects without epilepsy were studied. Mean transferrin saturation was significantly higher in the epilepsy group (39.9 (SD 19.6)%) than in the control group (29.1 (SD 14.9)%). Abnormally high transferrin saturations (men>60%, women>48%) were found in 10 patients with epilepsy but in only one subject without epilepsy. Anti-epileptic drugs did not affect the transferrin saturation. Of the 11 with abnormally high transferrin saturation, two with epilepsy were heterozygotic for H63D in the haemochromatosis gene but no patient had the C282Y mutation. These results indicate that iron overload other than the C282Y mutation underlies epilepsy.

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Iron accumulation results in the formation of free radicals and subsequent brain injury.¹ Neurological diseases associated with iron overload vary and include asymptomatic deposition of iron in the basal ganglia,² psychiatric diseases,³ mental retardation,⁴ parkinsonism,^{5,6} dementia, ataxia, and myoclonic jerks.⁷ Siderosis in the brain is associated with epilepsy.^{8,9} Animal studies suggest that iron accumulation may underlie the pathophysiology of epilepsy.^{10,11}

The aim of the study was to test the iron metabolism of epileptic patients. Transferrin saturation was measured as an index of iron overload in patients with epilepsy and age and sex matched control subjects. Mutations in the haemochromatosis gene (*HFE*) were also examined in patients with high transferrin saturations.

Patients and methods**PATIENTS**

A total of 258 subjects were studied, 130 patients with epilepsy (63 men, 67 women, mean age 38.7 (SD 10.3) years) and 128 sex and age matched control subjects without epilepsy (63 men, 65 women, mean age 40.8

(10.3) years). Subjects with pica, and those receiving drugs containing iron, blood transfusions, or alcohol were excluded. None of the subjects studied had haematological diseases or active liver diseases. All subjects, whether epileptic or not, were mentally retarded and cared for by the nursing staff of the Ranzan Institute in Saitama, Japan. All of them could eat and did not receive forced nutrition. Although a quantitative comparison was not made, no obvious difference between the two groups in daily activities was found.

METHODS*Measurement of serum iron, transferrin, and ferritin*

Serum samples after an overnight fast were obtained from each subject. Serum iron was measured by standard spectrophotometry. Serum transferrin concentrations were determined by rate immunoturbidimetry on an automated analyser (model TBA-20FR, Toshiba Medical, Tokyo, Japan). Serum ferritin concentrations were measured by chemiluminescence immunoassay (Eiken Chemical Co, Ltd, Tokyo) in patients with high transferrin saturation (men>60%, women>48%).

Identification of the C282Y and H63D mutations in HFE

HFE mutations were also examined in 11 patients with abnormally high transferrin saturation. The mutation study was approved by the ethics committee at Ranzan institute. As the subjects could not understand the explanation of the study due to mental retardation, after detailed explanations of the study written informed consent was obtained from their parents or legal guardians.

HFE contains two common missense mutations.¹² One mutation (guanine to adenine at nucleotide 845) in *HFE* results in the substitution of tyrosine for cysteine at amino acid 282 and is termed the C282Y mutation. The other mutation (cytosine to guanine at nucleotide 187) in *HFE* results in the substitution of aspartate for histidine at amino acid 63 and is termed the H63D mutation.

Polymerase chain reaction amplification of the regions containing the missense mutations was performed with the primer sequences of Feder *et al.*¹² The C282Y and the H63D muta-

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Table 1 Effects of antiepileptic drugs on transferrin saturation in the patients with epilepsy

Drug	Phenytoin		Phenobarbital		Carbamazepine		Valproate	
	-	+	-	+	-	+	-	+
Men	42 (17) [19]	44 (19) [44]	45 (19) [50]	42 (17) [13]	43 (18) [51]	52 (21) [12]	44 (19) [39]	46 (19) [24]
Women	33 (16) [26]	36 (21) [41]	35 (20) [59]	38 (14) [8]	34 (20) [50]	38 (18) [17]	32 (14) [19]	36 (21) [48]
Total	37 (17) [46]	41 (21) [84]	40 (20) [110]	41 (16) [20]	39 (20) [99]	44 (20) [31]	39 (19) [73]	41 (21) [57]

Values are mean (SD) [%] with the sample size in parentheses. +=Those who were taking one of the four antiepileptic drugs; -=indicates those who were not.

Table 2 Biochemical characteristics of the subjects with high transferrin saturations

Patient No	Age	Sex	Epilepsy	Fe ($\mu\text{g/dl}$)	UIBC ($\mu\text{g/dl}$)	TrfSat (%)	Ferritin (ng/ml)	HFEcDNA845	HFEcDNA187
1	53	W	Y	104	106	50	110	845G/845G	187C/187G
2	27	M	Y	140	62	69	34	845G/845G	187C/187C
3	46	W	Y	104	79	57	30	845G/845G	187C/187C
4	52	M	Y	138	86	62	37	845G/845G	187C/187C
5	53	M	Y	128	45	74	41	845G/845G	187C/187C
6	32	M	Y	184	44	81	24	845G/845G	187C/187G
7	42	M	Y	148	82	64	57	845G/845G	187C/187C
8	51	W	Y	102	108	51	ND	845G/845G	187C/187C
9	61	W	Y	114	113	50	53	845G/845G	187C/187C
10	60	W	Y	140	102	58	41	845G/845G	187C/187C
11	22	W	N	107	110	49	8.9	845G/845G	187C/187C

UIBC=Unsaturated iron binding capacity; TrfSat=transferrin saturation; ND=not done.

tions were identified with allele specific oligonucleotide hybridisation.¹³

STATISTICAL ANALYSIS

All values are presented as means (SD). Differences between means were analysed by a two tailed Student's *t* test or Mann-Whitney *U* test.

Results

Serum iron was significantly higher ($p < 0.01$) in the epilepsy group (106 (8) $\mu\text{g/dl}$) than in the control group (88 (8) $\mu\text{g/dl}$) whereas the unsaturated iron binding capacity was significantly lower ($p < 0.01$) in the epilepsy group (173 (78) $\mu\text{g/dl}$) than in the control group (228 (76) $\mu\text{g/dl}$). Thus, the transferrin saturation was significantly higher ($p < 0.01$) in the epilepsy group (39.9 (19.6)%) than in the control group (29.1 (14.9)%). On the assumption that some antiepileptic drugs may affect iron metabolism, the degrees of transferrin saturation in the subgroups within the epilepsy group were compared according to the prescribed drugs (table 1). There was no significant difference in transferrin saturation between the subjects who were taking one of the four antiepileptic drugs and those who were not.

Table 2 shows that an abnormal increase in transferrin saturation (men >60%, women >48%) was found in 11 patients (five men and six women) consisting of 10 patients with epilepsy and only one in the control group. No cause of secondary iron overload in these patients was found. Serum ferritin was not increased in any of them. Among the 11 patients, two were heterozygous for H63D. Both of them were epileptic. No C282Y mutation was found in any of the 11 patients with abnormally high transferrin saturations.

Discussion

Transferrin saturation was significantly higher in patients with epilepsy than in those without epilepsy. Moreover, abnormally high transfer-

rin saturations were found in 10 patients in the epilepsy group but only in one in the control group. These data indicate iron overload in the patients with epilepsy. Factors which cause secondary iron overload, including diet, blood transfusions, alcohol, liver injury, and haematological diseases, were ruled out. Antiepileptic drugs could not explain the iron overload in epileptic patients, either. As phenytoin is an iron chelator,¹⁴ it would reduce iron load rather than increase it. Previous studies on rats and mice have shown that administration of phenytoin, phenobarbital, or primidone does not change the iron concentration in the serum or brain.^{15,16} My data, showing that none of the antiepileptic drugs affected the transferrin saturation, also provide evidence that the higher transferrin saturation in the epilepsy group is not due to antiepileptic drugs.

I then studied mutations in *HFE* because haemochromatosis is the most common disease of primary iron overload. Two patients were heterozygous for the H63D mutation, but no patient with the C282Y mutation. Haemochromatosis is thought to be uncommon in Japanese people,¹⁷ but the frequency is unknown. Merryweather-Clarke *et al*¹⁸ reported that the C282Y mutation was most frequent in northern European populations and absent from 484 Asian chromosomes. The positive predictive value of the transferrin saturation test—that is, the possibility that a patient with a positive result actually has haemochromatosis—is unknown in Japanese people.

I do not assume that heterozygosity for H63D affects iron metabolism in epileptic patients, because its high frequency in control populations, ranging from 16 to 23%,¹⁹ makes the heterozygosity for H63D unlikely to be pathogenic. H63D is probably deleterious only in compound heterozygotes (heterozygous for both C282Y and H63D).²⁰ To determine the cause of iron overload in patients with epilepsy, I am continuing studies of other genes regulating iron metabolism in these patients.

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