Short Report

Distribution of C282Y and H63D mutations in the *HFE* gene in healthy Asian Indians and patients with thalassaemia major

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ABSTRACT

Background. Mutations in the *HFE* gene have been shown to be strongly associated with hereditary haemochromatosis, an autosomal recessive disease of iron overloading. The majority of patients with hereditary haemochromatosis possess a homozygous mutation C282Y that disrupts the binding of the *HFE* gene with b2 microglobulin and prevents its surface expression. Another *HFE* mutation H63D is known to increase the relative risk of developing hereditary haemochromatosis. This disease is rare in India although secondary haemochromatosis is commonly seen among children suffering from thalassaemia major. The status of *HFE* mutations has not been explored among Indians, particularly in patients with thalassaemia major. It is also possible that in India clinical haemochromatosis could be masked by iron deficiency.

Methods. We examined a cohort of 59 unrelated, healthy individuals from north India, 57 from south India and 75 thalassaemia major patients from north India for *HFE* mutations (C282Y and H63D) in cis/trans by the polymerase chain reaction sequence-specific primer method.

Results. The C282Y and H63D mutations in the *HFE* gene were rare among Indians. Although the *HFE* mutations were increased among patients of thalassaemia their effect on iron burden or disease pathogenesis remains unclear.

Conclusions. Hereditary haemochromatosis is rarely observed among Indians and so are the C282Y and H63D mutations in the *HFE* gene. Long term follow up studies would be required to determine whether the relatively higher frequency of these mutations among patients of thalassaemia has any influence on iron accumulation.

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INTRODUCTION

Hereditary haemochromatosis is an autosomal recessive disorder of iron overload affecting approximately 5 per 1000 Caucasians while 1 in every 12 Caucasians in northwestern Europe is a carrier.[1] It is a multigenic disorder with the possible involvement of several genetic loci that include *HFE*-1 (C282Y, H63D and S65C mutations in *HFE* at 6p21),[2] *HFE*-2 (1q in juvenile haemochromatosis), *HFE*-3 (Y250X and other mutations in TFR-2 at 7q22),[3] NRAMP-2 (duodenal metal transporter, DMT-1),[4] SFT (stimulator of iron transport)[5] and other unknown factors.

The *HFE* gene, originally called human histocompatibility leucocyte-H (HLA-H), is a non-peptide binding major histocompatibility complex (MHC) class I homologue that binds with b2 microglobulin and modulates iron transport by binding the transferrin receptor (TfR)[6] in a pH-dependent manner. Two mutations in the *HFE* gene, namely (i) C282Y that results from a change in cysteine (C) at position 282 to tyrosine (Y), and (ii) H63D that results from a change in histidine (H) at position 63 to aspartate (D) disrupt cell surface expression of the mutant *HFE* gene, and alter binding with TfR, respectively. These mutations are strongly associated with predisposition to hereditary haemochromatosis and are also implicated in other disorders such as rheumatoid arthritis,[7] type 2 diabetes mellitus,[8] porphyria cutanea tarda[9] and coronary heart disease.[10]

Considerable ethnic variation is observed in the frequency distribution of *HFE* mutations. Hereditary haemochromatosis is rare in India[11] and the status of *HFE* mutations is virtually unexplored. On the other hand, iron overload is frequently observed among patients with thalassaemia major, a widely prevalent haemoglobinopathy.[12] We investigated the prevalence of *HFE* mutations in two healthy populations from north and south India and in patients with thalassaemia major from north India.

METHODS

This study was done on 116 healthy, unrelated subjects (59 from north India [from the states of Punjab, Delhi, Haryana and Uttar Pradesh] and 57 from south India [from the states of Kerala, Andhra Pradesh, Karnataka and Tamil Nadu]) and 75 patients with thalassaemia major from north India referred to the All India Institute of Medical Sciences, New Delhi. All the patients with thalassaemia major had received multiple blood transfusions and iron chelation therapy. Blood samples were collected after informed consent and processed for *HFE* genotyping using the polymerase chain reaction-sequence specific primer method and primer sequences as previously published.[13] Four possible *HFE* genotypes, namely 63H-282C, 63H-282Y, 63D-282C, 63D-282Y, were analysed and their frequencies scored.

The relative frequencies of *HFE* mutations in the healthy subjects and the thalassaemia major patients were compared and the significance of association was tested by 2×2 contingency c^2 analysis and Fischer exact text. Serum ferritin levels were also measured in 27 of the 75 thalassaemia patients using the standard biochemical procedure.[14]

RESULTS

Genotyping of C282Y and H63D mutations

The C282Y mutation was seen in only 1 of the 59 healthy subjects from north India (1.7%), but in none of those from south India. On the other hand, the allele frequency of H63D was 4 of 59 (6.8%) in subjects from north India, and 6 of 57 (10.5%) in those from

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TABLE I. *HFE* genotypes in healthy Indians and patients with thalassaemia major

Genotype	Subjects from North India (*n*=59)			Subjects from South India (*n*=57)			Thalassaemia major (*n*=75)		
	HH	HD	DD	HH	HD	DD	HH	HD	DD
CC	50	8	0	48	6	3	51	17	1
CY	1	0	0	0	0	0	6	0	0
YY	0	0	0	0	0	0	0	0	0

Genotypes HD and DD represent the presence of mutation (H to D) at amino acid residue 63 in heterozygous and homozygous conditions, respectively. The genotypes CY and YY represent presence of mutation (C to Y) at amino acid residue 282 in the heterozygous and homozygous conditions, respectively.

south India. The C282Y and H63D mutations were observed in 3 (4%) and 9 (12%) patients with thalassaemia major, respectively.

An analysis of the genotypes of these mutations revealed that the frequency of genotype CY was higher among patients with thalassaemia major (6 of 75) than healthy controls (1 of 116; p=0.03). There was no significant difference in the prevalence of HH, HD or DD genotypes between thalassaemia patients and healthy controls. We did not encounter any compound heterozygotes among the subjects tested (Table I).

Analysis of ferritin levels

High ferritin levels (2050–11 000 mg/L) were observed in all but 1 (96.3%) of these patients. However, there was no correlation between C282Y and H63D mutations and serum ferritin levels.

DISCUSSION

We describe the occurrence of *HFE* gene mutations, C282Y and H63D, in two healthy populations from north and south India as well as thalassaemia major patients from north India. Generally these mutations occur at lower frequencies in Indians as compared with healthy Europeans.[1] For example, the C282Y mutation occurs with a frequency of 6.4% in the British population,[1] 5.6% in the Hungarian,[15] 3.1% in the Polish[16] and 3.9% in the Germans, but is rare in Greeks (1.4%) and is absent in the Finnish, African, Chinese and Turkish populations. Similarly, the frequency of H63D mutation is 12.8% in the British, 10.4% in Russians, 13.6% in Europeans, but only 2.8% in Chinese, and is absent in Papua New Guineans and Australian Aboriginals.

Homozygosity of the C282Y mutation has been reported in over 90% of patients with hereditary haemochromatosis in the UK by the UK Haemochromatosis Consortium, 82.3% in the USA,[17] 92.4% in Britain[18] and in 100% of Australian patients with hereditary haemochromatosis and a positive family history.[19] Thus, the C282Y mutation is a very good marker for hereditary haemochromatosis, given the fact that *HFE* is not the only gene responsible for this condition since the role of other factors (genetic, dietary and environmental) cannot be ruled out. Hereditary haemochromatosis is rarely seen in the Asia–Pacific region[20] including India, Japan,[21] China[22] and Korea.[23] The observed low frequencies of the C282Y and H63D mutations in these populations correlates with this observation.

Our results suggest that the relatively infrequent occurrence of the C282Y and H63D mutations among Indians could explain the absence or rare occurrence of hereditary haemochromatosis. These *HFE* mutations might be involved in the process of secondary iron overload observed in some patients with thalassaemia major.

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REFERENCES

1 Merryweather-Clarke AT, Pointon JJ, Shearman JD, Robson KJ. Global prevalence of putative haemochromatosis mutations. *J Med Genet* 1997;**34:**275–8.
2 Feder JN, Gnirke A, Thomas W, Tsuchihashi, Ruddy DA, Basava A, *et al*. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nat Genet* 1996;**13:**399–408.
3 Lee PL, Halloran C, West C, Beutler E. Mutation analysis of the transferrin receptor-2 gene in patients with iron overload. *Blood Cells Mol Dis* 2001;**27:**285–9.
4 Zoller H, Pietrangelo A, Vogel W, Weiss G. Duodenal metal transporter (DMT-1, NRAMP-2) expression in patients with hereditary haemochromatosis. *Lancet* 1999;**353:**2120–3.
5 Barisani D, Parafioriti A, Armiraglio E, Meneveri R, Conte D. Duodenal expression of a putative stimulator of Fe transport and transferrin receptor in anaemia and haemochromatosis. *Gastroenterology* 2001;**120:**1404–11.
6 Davies PS, Zhang AS, Anderson EL, Roy CN, Lampson MA, McGraw TE, *et al*. Evidence for the interaction of the hereditary haemochromatosis protein, *HFE*, with the transferrin receptor in endocytic compartments. *Biochem J* 2003;**373:**145–53.
7 Li J, Zhu Y, Singal DP. *HFE* gene mutations in patients with rheumatoid arthritis. *J Rheumatol* 2000;**27:**2074–7.
8 Salonen JT, Tuomainen T-P, Kontula K. Role of C282Y mutation in haemochromatosis gene in development of type 2 diabetes in healthy men: Prospective cohort study. *BMJ* 2000;**320:**1706–7.
9 Dereure O, Aguilar-Martinez P, Bessis D, Perney P, Vallat C, Guillot B, *et al. HFE* mutations and transferrin receptor polymorphism analysis in porphyria cutanea tarda: A prospective study of 36 cases from southern France. *Br J Dermatol* 2001;**144:** 533–9.
10 Rasmussen ML, Folsom AR, Catellier DJ, Tsai MY, Garg U, Eckfeldt JH. A prospective study of coronary heart disease and the haemochromatosis gene (*HFE*) C282Y mutation: The Atherosclerosis Risk in Communities (ARIC) study. *Atherosclerosis* 2001;**154:**739–46.
11 Sood A, Jain R. Idiopathic haemochromatosis with unusual CT findings. *Trop Gastroenterol* 2000;**21:**73–4.
12 Balgir RS. Control and prevention of the genetic load of haemoglobinopathies in India. *Natl Med J India* 1999;**12:**234–8.
13 Mullighan CG, Bunce M, Fanning GC, Marshal SE, Welsh KI. A rapid method of haplotyping *HFE* mutations and linkage disequilibrium in a Caucasoid population. *Gut* 1998;**42:**566–9.
14 Workwood M. Iron deficiency anaemia. In: Dacie JV, Lewis SM (eds). *Practical haematology*. London:Churchill Livingstone; 1995; pp. 437–44.
15 Tordai A, Andrikovics H, Kalmar L, Rajczy K, Penzes M, Sarkadi B, *et al.* High frequency of the haemochromatosis C282Y mutation in Hungary could argue against a Celtic origin of the mutation. *J Med Genet* 1998;**35:**878–9.
16 Moczulski DK, Grzeszczak W, Gawlik B. Frequency of the haemochromatosis C282Y and H63D mutations in a Polish population of Slavic origin. *Med Sci Monit* 2001;**7:**441–3.
17 Beutler E, Gelbart T, West C, Lee P, Adams M, Blackstone R, *et al.* Mutation analysis in hereditary haemochromatosis. *Blood Cells Mol Dis* 1996;**22:**187–94.
18 Jouanolle AM, Gandon G, Jezequel P, Blayau M, Campion ML, Yaouanq J, *et al.* Haemochromatosis and *HLA-H*. *Nat Genet* 1996;**14:**251–2.
19 Jazwinska EC, Cullen LM, Busfield F, Pyper WR, Webb SI, Powell LW, *et al.* Haemochromatosis and *HLA-H*. *Nat Genet* 1996;**14:**249–51.
20 Mortimore M, Merryweather-Clarke AT, Robson KJ, Powell LW. The haemochromatosis gene: A global perspective and implications for the Asia-Pacific region. *J Gastroenterol Hepatol* 1999;**49:**838–43.
21 Sohda T, Yanai J, Soejima H, Tamura K. Frequencies in the Japanese population of *HFE* gene mutations. *Biochem Genet* 1999;**37:**63–8.
22 Tsui WM, Lam PW, Lee KC, Ma KF, Chan YK, Wong MW, *et al.* The C282Y mutation of the *HFE* gene is not found in Chinese haemochromatotic patients: Multicentric retrospective study. *Hong Kong Med* 2000;**6:**153–8.
23 Lee JY, Yoo KH, Hahn SH. *HFE* gene mutation, C282Y causing hereditary hemochromatosis in Caucasian is extremely rare in the Korean population. *J Korean Med Sci* 2000;**15:**179–82.