High prevalence of the Cys282Tyr HFE mutation facilitates an improved diagnostic service for hereditary haemochromatosis in South Africa

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Objective. The aim of the study was to investigate the molecular basis of hereditary haemochromatosis (HH) in South Africa in order to establish a reliable, cost-effective molecular diagnostic service for this potentially lethal disorder.

Design. DNA samples of patient and control groups were screened for two common haemochromatosis (HFE) gene mutations. The local frequencies of mutations C282Y and H63D were determined and the DNA results correlated with biochemical parameters.

Setting. Patients were referred from private practitioners, health workers and pathologists for a molecular diagnosis of HH at the University of Stellenbosch Medical School. Twenty-two of the 244 referrals were clinically diagnosed with HH, while the remaining patients were family members of the probands or unrelated subjects referred solely on the basis of an abnormal iron profile.

Results. Seventeen of the 22 patient referrals (77%) diagnosed with HH were homozygous for the C282Y mutation, 3 (14%) were compound heterozygotes for mutations C282Y and H63D, and 2 patients (9%) did not exhibit either mutation. Screening of 458 control individuals from the general South African population demonstrated a carrier frequency of approximately 17% for the C282Y mutation among whites, implying that up to 1 out of every 115 South Africans of

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European descent may be homozygous for this founder-type mutation. Among 64 healthy blood donors of mixed ancestry, we detected 2 individuals heterozygous and 1 homozygous for the C282Y mutation.

Conclusions. The detection of mutations C282Y and H63D at a high frequency in the majority of affected South African patients facilitates accurate pre-clinical and confirmatory diagnosis of HH in South Africa. Early detection by DNA screening and subsequent treatment by repeated phlebotomy can prevent disease onset in affected individuals. DNA diagnosis is particularly applicable to a common genetic disease such as HH, which is underdiagnosed and potentially lethal, but treatable.

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Hereditary haemochromatosis (HH) is a common autosomal recessive iron-overloading disorder characterised by a progressive increase in total body iron, leading to fibrosis and organ failure.[1] In mid-life this can result in a range of clinical complications including cirrhosis, cardiomyopathy, arthritis, diabetes, endocrine dysfunction, arthropathy and susceptibility to hepatic carcinoma. The incidence of HH is approximately 1/300 in individuals of Northern European descent, with a carrier frequency of 1/8 - 1/10.[2] Within the South African Afrikaner population, the HH gene frequency was reported to be approximately 1/100, with a carrier frequency of 1/5.[3] The high prevalence of HH among Afrikaners may be the consequence of a founder gene in this relatively homogeneous population.

Identification of a candidate gene for HH (HFE)[4] now allows mutation screening to determine the molecular basis of the disease in different population groups. A missense disease-causing mutation C282Y, comprising a G to A base change at nucleotide position 845 in exon 4 of the HFE gene, was identified in approximately 70 - 100% of clinically diagnosed HH patients.[4-6] The phenotypic effect of a C to G sequence change in exon 2, designated H63D,[4] has been uncertain, even though it has been demonstrated that the compound heterozygous state (C282Y +–/H63D+–) may be associated with iron overload.[4] Studies on the affinity of the transferrin receptor (Tfr) for transferrin have recently provided the first direct evidence for a functional consequence of the H63D mutation and clearly demonstrated the role of HFE in regulation of iron uptake.[7,8]

Despite the fact that HH is considered to be the most common genetic disease among Caucasians, the condition is largely underdiagnosed. This may in part be due to the nonspecific nature of the presenting features and the wide variety of disorders associated with iron overload. The most reliable biochemical parameters used to determine high iron storage are measurement of serum ferritin levels, serum transferrin saturation and iron binding capacity, but these are subject to various environmental influences. In order to establish a molecular diagnostic service for this treatable genetic disease, we attempted to determine the molecular basis of HH in South Africa by initially screening for two common HFE gene mutations.

SUBJECTS AND METHODS

Blood samples were obtained with informed consent from 22 unrelated South African Caucasians (14 men, 8 women), aged 28 - 59 years, who had been clinically diagnosed with HH (Table I).

Table I. Genotype distribution and allele frequencies of the C282Y and H63D mutations in patients and controls

	Patients		Controls		
	Clinically diagnosed (*N* = 22)	General referrals (*N* = 222)	Black (*N* = 200)	Mixed ancestry (*N* = 156)	Caucasian (*N* = 102)
Genotype					
C282Y++/H63D– –	17 (77%)	40 (18%)	0	1*	2
C282Y+–/H63D+–	3 (14%)	20 (9%)	0	0	1
C282Y+–/H63D– –	0	52 (23%)	1	4*	14
C282Y– –/H63D+–	0	9 (4%)	0	19	24
C282Y– –/H63D++	0	2 (0.9%)	0	2	0
C282Y– –/H63D– –	2 (9%)	99 (45%)	199	130	61
Allele					
282–G (normal)	0.16		0.998	0.98	0.91
282–A (mutant)	0.84		0.002	0.02	0.09
Carrier frequency			0.5%	3.9%	16.9%
63–C (normal)	0.93		0	0.93	0.88
63–G (mutant)	0.07		0	0.07	0.12
Carrier frequency			0	13.9%	21%

*Three of 5 subjects (1 homozygote, 2 heterozygotes) were detected among 64 healthy blood donors of mixed ancestry; percentages of mutations detected in a specific group are shown in parentheses.

noses were based on serum iron and ferritin levels, liver Diag[illegible]sies and response to phlebotomy.[9] The study was biops[illegible]squently extended to include 244 subjects (from 215 subs[illegible]rently unrelated families) who were referred for DNA appa[illegible]sis by medical practitioners, pathologists and health analy[illegible]ers. Most referrals were made solely on the basis of work[illegible]rmal iron profiles in order to confirm or largely exclude abno[illegible]at the molecular level. Relatives of affected cases were HH [illegible]red for pre-clinical diagnosis of HH based on family refer[illegible]ry or DNA results in the proband. Serum iron, transferrin histo[illegible]serum ferritin concentrations were determined for 89 of the and [illegible]nt referrals. Non-fasting iron levels were also determined patie[illegible] controls (medical staff), consisting of 38 Caucasians and 8 in 46[illegible]viduals of mixed ancestry (coloured population). These indiv[illegible]viduals, 10 men (aged 21 - 45 years) and 36 women (aged 21 - [illegible]58 years), were asked to complete a questionnaire in order to ob[illegible]ptain informed consent and to identify blood donors. DNA samp[illegible]ples of an additional 148 individuals of mixed ancestry, 64 Cauc[illegible]casians and 200 black individuals (Table I) were included for r[illegible]nutation frequency determination. The study protocol was appr[illegible]roved by the Ethics Committee of the University of Stell[illegible]enbosch.

Se[illegible]rum iron, transferrin and ferritin concentrations were dete[illegible]rmined by standard methods. Genomic DNA was extracted from 5 ml whole blood preserved in ethylenediaminetetra-acetic acid (EDTA), using a standard lysis method.[10] Exons 2 and 4 of the HFE gene were amplified by the polymerase chain reaction (PCR)[11] and screened for mutations H63D (*Mbo*I) and C282Y (*Rsa*I), respectively, using restriction enzyme analysis.[6] The digested products were resolved on a 10% polyacrylamide gel, stained with ethidium bromide and visualised by ultraviolet light. Dr E Beutler from the Scripps Research Institute in the USA kindly supplied appropriate control DNA samples.

RESULTS AND DISCUSSION

Sin[illegible]e initiation of a molecular diagnostic service for HH in 199[illegible]7, 244 subjects from 215 apparently unrelated families have bee[illegible]n referred for DNA analysis. Of the 22 unrelated patients clin[illegible]ically diagnosed with HH, 17 (77%) were homozygous for the C282Y mutation (C282Y++), 3 (14%) were compound het[illegible]erozygotes for C282Y and H63D (C282Y+–/H63D+–) and 2 (9%[illegible]) did not exhibit either mutation. Mutation C282Y creates a *Rsa*[illegible]I endonuclease recognition site while mutation H63D abo[illegible]olishes a *Mbo*I site, which enables accurate mutation det[illegible]ection by restriction enzyme analysis of PCR-amplified DN[illegible]A (Fig. 1). The genotype distribution of the C282Y and H6[illegible]3D mutations is summarised in Table I, together with the res[illegible]ults obtained after screening 222 general referrals. We also cor[illegible]mpared allele frequencies of the C282Y and H63D mutations in t[illegible]he patient group with that found in the general South Afr[illegible]ican population (Table I). The carrier frequency of C282Y

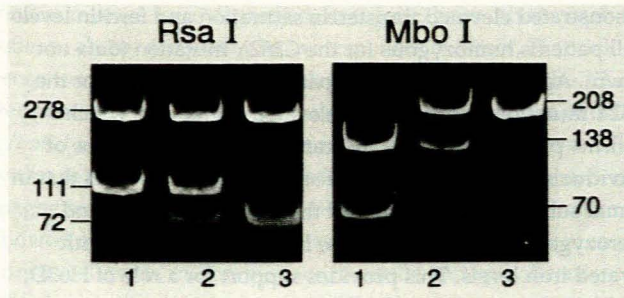

Fig. 1. Restriction enzyme analysis of HFE gene mutations. PCR-amplified DNA from a normal control (lane 1), heterozygote (lane 2) and homozygote (lane 3) for the C282Y and H63D mutations are shown following digestion with RsaI *(left) and* MboI *(right), respectively. Fragment sizes are indicated in base parts.*

heterozygotes was 16.9% in Caucasians, 3.9% in individuals of mixed ancestry and less than 1% in black controls. Detection of the C282Y mutation in a single Xhosa individual can probably be ascribed to recent Caucasoid admixture. Paucity of the known disease-causing HFE gene mutations in the South African black population may explain the virtual absence of HH in this ethnic group, as opposed to a relatively high prevalence of African iron overload, which may involve gene-environmental interaction.[12]

Mutation screening in the general population identified three C282Y homozygotes (Table I). The subject of mixed ancestry with this disease-related genotype was detected among 64 healthy blood donors and was advised to continue with this practice on a regular basis. Determination of serum iron, transferrin and ferritin concentrations in a 45-year-old man homozygous for the C282Y mutation demonstrated severely elevated tranferrin saturation and ferritin levels of 100% and 2 224 ng/ml, respectively. Interestingly, this individual reported that he had been diagnosed with hepatitis A and B when hospitalised less than a year ago due to sudden illness. A diagnosis of HH was not considered, despite the fact that the patient reported that he had had hepatitis in childhood and had been immunised in the past. Regular phlebotomies on a weekly basis over the past 4 months have now lowered his ferritin levels to 1 043 ng/ml and transferrin saturation to 57%. Considering the 25% risk of inheritance of an autosomal recessive condition, and to avoid unnecessary blood sampling from the couple's four young children, the spouse was also genotyped. She screened negative for both the C282Y and H63D mutations. This finding infers that all their children will be carriers of the C282Y mutation.

The control individual shown to be a compound heterozygote for mutations C282Y and H63D presented with a normal iron profile (ferritin 36.2 ng/ml; transferrin saturation 22%). This can probably be explained by low penetrance and possible mild effect of the H63D mutation[4,7] and/or the fact that she is a blood donor. Genotype-phenotype correlations in 89 patient referrals for whom iron levels were determined have

demonstrated elevated transferrin saturation and ferritin levels in all patients homozygous for the C282Y mutation (data not shown). Approximately 50% of patients heterozygous for the C282Y mutation also exhibited elevated iron levels, which confirms previous findings indicating that the phenotype of individuals carrying a single defective gene differs from that in normal subjects.[13] We also found that 6 out of 8 compound heterozygous patients (C282Y+–/H63D+–) presented with elevated iron levels. This provides support for a role of H63D in the phenotypic expression of HH, which appears to depend on coexistence of this mutation with C282Y. Detection of a lower frequency of the mutant G-allele of the H63D mutation in the HH population (0.07) than in Caucasian controls (0.12) is probably a consequence of the predominance of the C282Y mutation in HH patients.[14]

Increased frequencies of the C282Y and H63D mutations have recently been reported in patients with sporadic porphyria cutanea tarda (PCT).[15,16] This finding suggests the involvement of the HFE gene in the pathogenesis of PCT. South African patients with PCT and their first-degree relatives should also be screened for mutations in the HFE gene.[17] We have identified several novel missense mutations and polymorphisms in the HFE gene; these are currently being investigated further in the context of both iron overload and porphyria in South Africa (J N P de Villiers, C L Scholtz, C F Hoogendijk, E J Cawood, M J Kotze — unpublished data).

The results obtained in the South African population provided a valuable framework for the implementation of a diagnostic service for HH mutation screening. The high frequencies of the C282Y and H63D mutations detected in the study population confirmed the high prevalence of HH in South Africans of European descent.[3] The major advantage of DNA analysis over biochemical testing is that the genotype is invariant, not being influenced by environmental factors that may result in misdiagnosis. Tracing of the disease-causing mutation(s) in a family can be carried out rapidly on a small quantity of DNA, facilitating more accurate detection of those at high risk of developing iron overload. DNA testing may change the unfortunate present situation, wherein most HH-affected patients are only identified after some form of organ damage, to one of implementation of preventive treatment early in life.

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The authors dedicate this article to the memory of Gert Coetzee, a founder member of the South African Haemochromatosis Society, who dedicated his life to assisting others with HH. He was the first South African patient diagnosed with HH at the molecular level, and died in 1998.