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Testing families with HFE-related hereditary haemochromatosis

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ABSTRACT

HFE-related hereditary haemochromatosis is the most common autosomal recessive disorder in the Caucasian population. In 1996 the responsible gene (called HFE) was identified. Two mutations (C282Y and H63D) are considered most important and occur frequently in the Caucasian population.

We describe a family of an affected proband in which first- and second-degree relatives were tested phenotypically and genotypically. In second-degree relatives both C282Y homozygosity as well as compound heterozygosity were found. Family testing can be useful to detect persons who will possibly develop iron overload. We must be aware that testing first-degree relatives only carries a 2.5% chance that persons at risk of developing iron loading will not be detected. Cascade screening of second-degree relatives might be cost-effective.

INTRODUCTION

HFE-related hereditary haemochromatosis (HFErHH) is the most common autosomal recessive disorder in the Caucasian population,[1] the prevalence ranging from 0.25 to 0.5%.[2-4] A common trait of the disease is iron loading in tissues and organs such as liver, heart, joints, pancreas and pituitary gland. Ultimately this can lead to liver cirrhosis, cardiomyopathy, arthropathy, diabetes mellitus and hypogonadism.

In 1996 the gene responsible for HFE-related hereditary haemochromatosis (called HFE gene, coding for the HFE protein) was identified.[5] The HFE protein is involved in the regulation of iron absorption.[6] Multiple mutations in the HFE gene have been described.[7] The most important one is the C282Y mutation, which means that at amino-acid 282 cysteine is substituted by tyrosine. A second (H63D, aspartate-to-histidine substitution at amino-acid 63) is also considered of importance.

The C282Y and H63D mutations occur frequently in the Caucasian population (see *table 1*).[8] Among all Caucasian HFErHH patients 80 to 90% are homozygous for the C282Y mutation, 1% is homozygous for the H63D mutation, 5% are compound heterozygous (which means heterozygous for both the C282Y as well as the H63D mutation), and 3 to 10% are heterozygous for either C282Y or H63D mutation, possibly with other mutations.[9]

Since the discovery of the HFE gene, it is possible to detect persons at risk of developing haemochromatosis in a presymptomatic stage. The Dutch Haemochromatosis Association advises testing of first-degree relatives of affected probands.[10] In this original article, we will describe a family in which C282Y homozygosity and compound heterozygosity were found in second-degree relatives. The role and extent of genetic testing of families of affected probands will be discussed.

CASE REPORT

Patient A, 60 years old and diagnosed with diabetes mellitus nine years ago, presented with complaints of chronic fatigue. Physical examination did not reveal any abnormalities. Blood examination had the following results (reference range between brackets): haemoglobin 10.3 mmol/l (8.5-10.9), mean corpuscular volume 94 fl (80-100), aspartate aminotransferase 76 U/l (<40), alanine

Table 1
Distribution of genotype in the Caucasian population and in HH patients

	CAUCASIAN POPULATION	HFE-RELATED HEREDITARY HAEMOCHROMATOSIS PATIENTS
C282Y/C282Y (homozygosity)	0.5%	80-90%
H63D/H63D (homozygosity)	2%	1%
C282Y/H63D (compound heterozygosity)	2%	5%
C282Y/WT (heterozygosity)	10%	Rest
H63D/WT (heterozygosity)	20%	Rest

WT = wildtype = unmutated gene.

aminotransferase 79 U/l (<45), lactic dehydrogenase 976 U/l (<450), γ-glutamyl transpeptidase 216 U/l (<50), alkaline phosphatase 154 U/l (40-120), ferritin 1318 µg/l (30-300), iron 39 µg/l (14-28), iron-binding capacity 55 µg/l (45-77) and transferrin saturation (iron divided by iron-binding capacity) 71% (<45%). Ultrasound examination of the liver showed no abnormalities. Serological testing for infection with hepatitis A, B and C, Epstein-Barr virus and cytomegaly virus was negative. Liver biopsy showed iron loading with mild periportal fibrosis. The patient turned out to be C282Y homozygous.

Testing of family members
All first- and second-degree relatives of the patient were tested both phenotypically (ferritin and transferrin saturation) and genotypically. The spouses of some of the first-degree relatives were tested as well. The results of this screening are shown in the family tree (*figure 1*) and *table 2*. Among the ten first-degree relatives one C282Y homozygote was found. Among ten second-degree relatives two compound heterozygotes and one C282Y homozygote were found.

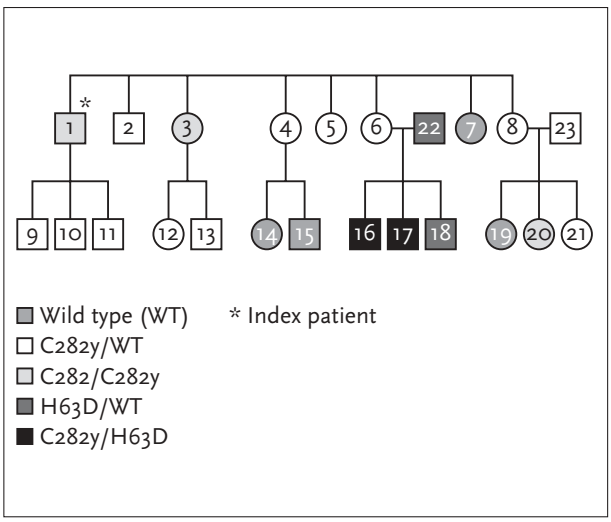

Figure 1

DISCUSSION

HFE-related hereditary haemochromatosis seems to be a favourable disorder for screening. The disease has a long presymptomatic phase, an economical and simple treatment is available and – if treated in time – patients have the same life-expectancy as healthy persons.[11]
Two modalities exist for screening haemochromatosis: phenotypic testing (ferritin level and transferrin saturation) and genotypic testing (DNA examination).
Phenotypic testing detects persons with elevated iron stores. However, phenotypic testing is a one-time measurement, so a normal test result does not exclude future iron loading. Genotypic testing detects persons who are at risk of developing iron overload, but not all detected persons will develop iron loading, indicating that HFE-related hereditary haemochromatosis is not a monogenetic disorder. Therefore, genotypic population screening for HFE-related hereditary haemochromatosis is still a matter of debate, because the clinical penetrance of the disorder (that means the percentage of persons with an 'at risk' genotype who will develop symptomatic iron loading) seems to be much lower than previously thought. Although C282Y homozygosity has a phenotypic expression (elevated ferritin and transferrin saturation) of 50 to 90%,[12,13] the importance with respect to morbidity and mortality seems to be low. Beutler *et al.* found 152 C282Y homozygotes in a group of 41,038 healthy volunteers.[13] Complaints and symptoms that could be ascribed to haemochromatosis were not more prevalent in these C282Y homozygotes than in a control group, so that the clinical penetrance in this study was only 1%.
In contrast to population screening, family testing is an accepted screening strategy to detect asymptomatic persons with (an elevated risk for) iron loading. Detecting a C282Y homozygote with elevated iron stores implies phlebotomy therapy, whereas C282Y homozygotes without phenotypic expression should be re-tested phenotypically within several years. In contrast to C282Y homozygosity, the relevance of compound heterozygosity appears to be much lower

Table 2
Phenotype of the tested family members

Family member	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Iron	39	5	*	17	18	15	8	19	5	28	15	23	37	14	22	26	24	63	4	11	16	12	22
TIBC	55	52	*	63	75	72	77	89	74	67	70	98	68	87	67	84	76	88	115	57	83	76	74
Tf-saturation	71	10	*	27	24	20	11	21	7	41	21	23	54	16	33	31	31	71	4	19	19	16	30
Ferritin	1318	355	580	40	42	37	159	77	10	109	397	44	182	37	91	148	54	42	122	213	41	145	242
Age	60	57	55	54	53	49	46	44	36	34	31	29	26	30	27	24	22	18	21	18	13	52	49

*TIBC = total iron-binding capacity, Tf = transferritin, * not available.*

since only 1 to 2% with this genotype develop a mild iron loading.[14] As can be seen from *figure 1* and *table 2*, genotype and phenotype often correlate poorly. However, the C282Y homozygote in the second-degree relatives (no. 20) was still young and it is possible that she will develop iron loading later in life. Furthermore, elevated iron parameters can be caused by conditions other than HFE-related hereditary haemochromatosis, as was recently discussed by Jacobs *et al.*[15]

The Dutch Haemochromatosis Association advises testing of first-degree relatives (siblings, children, parents) of C282Y homozygous patients both phenotypically and genotypically. In our patient second-degree relatives were tested as well. Among these second-degree relatives both C282Y homozygosity and compound heterozygosity were found, which means that spouses of first-degree relatives had introduced a C282Y and a H63D mutation into the family.

As is the case in the general population, relatives of patients with HFE-related hereditary haemochromatosis with an 'at risk' genotype do not inevitably develop iron loading. However, in families the clinical penetrance of C282Y, homozygosity appears to be higher than in the general population and is reported to be 40 to 67%.[16] Bulaj *et al.* examined the penetrance in 214 C282Y homozygotes, including second-degree relatives, who were detected by family testing.[17] From all men, 85% had an increased iron supply, 38% had signs and/or symptoms that could be ascribed to iron loading, including liver cirrhosis in 12%. However, a control group was not included in this study. Because of this higher clinical penetrance it can be questioned whether family testing for HFE-related hereditary haemochromatosis should include second-degree relatives. Extended family testing (first to third degree) has been advocated as an alternative to population screening, leading to detection of 40% of C282Y homozygotes.[18] It is thought that the higher penetrance in families is caused by additional genetic and environmental factors that promote penetrance, although a search for such genetic factors was unrewarding.[19] In second-degree relatives a dilution of these penetrance-promoting factors is expected. However, the magnitude and effect of this expected dilution is unknown so the penetrance in second-degree relatives is difficult to predict. A current two-year multicentre study of the clinical penetrance in first-degree relatives of Dutch HFE-related hereditary haemochromatosis patients will hopefully shed some light on this issue (www.zonmw.nl). If penetrance in these first-degree relatives turns out to be low, screening second-degree relatives will probably not be cost-effective. However, if the penetrance is in the reported range of 50%, a study of penetrance in second-degree relatives is needed. The chance of C282Y homozygosity in second-degree relatives of a C282Y homozygous index patient can be calculated as follows:

0.5 (= the chance of having a heterozygous sibling) x 0.1 (= the chance of a heterozygous spouse) x 0.25 (= the chance that both parents pass the mutation to their offspring) = 1.25%
+
0.25 (= the chance of having a homozygous sibling) x 0.1 (= the chance of a heterozygous spouse) x 0.5 (= the chance that the spouse passes the mutation) = 1.25%
Total = 2.5%*

*In this calculation C282Y heterozygosity of the parents of the index patient is assumed and the chance of a homozygous spouse is neglected.

At first sight this chance seems rather small. However, the yield of genetic testing in second-degree relatives can be enhanced by first testing the spouse of the first-degree relative (so-called cascade screening). If the sibling is C282Y heterozygous or homozygous, the spouse should be tested. The initial increase in costs and number of persons that need to be tested will be 75%. If the spouse carries no mutation, testing the children can be omitted. However, if the spouse is C282Y heterozygous (10% chance) there is at least a 25% chance of C282Y homozygosity in their offspring, which is the same as for siblings of the proband. The above-described approach proved to be cost-effective in screening children of affected probands.[20] The difference in chance of C282Y homozygosity between children (5%)

and second-degree relatives (2.5%) is rather small. However, in the aforementioned study the clinical penetrance in children was assumed to be 40%, whereas the penetrance in second-degree relatives is unknown. The findings of this study cannot thus be automatically extrapolated to screening second-degree relatives.

It has been argued that detecting asymptomatic C282Y homozygotes would impose a psychological and economical burden upon these persons. Insurance denial has indeed been reported. However, a recent study showed that the quality of life and psychological well-being in asymptomatic C282Y homozygotes detected by screening is not different from unaffected persons.

In conclusion, testing second-degree relatives of patients with HFE-related haemochromatosis can be helpful in detecting persons who will possibly develop iron loading in the future. Testing first-degree relatives only implies a 2.5% chance that persons who are at risk for developing iron loading will not be detected. Testing second-degree relatives by means of cascade screening might be cost-effective. However, before recommendations on screening second-degree relatives can be made, studies to cost-effectiveness and penetrance are needed.

REFERENCES

1. Worwood M. Haemochromatosis. Clin Lab Haem 1998;20:65-75.
2. Edwards CQ, Griffen LM, Goldgar D, Drummond C, Skolnick MH, Kushner JP. Prevalence of hemochromatosis among 11065 presumably healthy blood donors. N Engl J Med 1988;318:1355-62.
3. Baer DM, Simons JC, Staples RL, Rumore GJ, Morton CJ. Hemoachromatosis screening in asymptomatic ambulatory men of 30 years of age and older. Am J Med 1995;98:464-8.
4. Leggett BA, Halliday JW, Brown NN, Bryant S, Powell LW. Prevalence of hemochromatosis amongst asymptomatic Australians. Br J Haem 1990;74:525-30.
5. Feder JN, Gnirke A, Thomas W, et al. A novel MHC class-I like gene is mutated in patients with hereditary hemochromatosis. Nat Gen 1996;13:399-408.
6. Fleming RE, Migas MC, Zhou X, et al. Mechanism of increased iron absorption in murine model of hereditary hemochromatosis: increased duodenal expression of the iron transportes DMT1. Proc Natl Acad Sci USA 1999;96:3143-8.
7. Camaschella C, Roetto A, DeGobbi M. Genetic haemochromatosis: genes and mutations associated with iron loading. Best Prac Res Clin Haematol 2002;15(2):261-76.
8. Steinberg KK, Cogswell ME, Chang JC, Caudill SP, McQuillan GM, Bowman BA. Prevalence of C282Y and H63D mutations in the hemochromatosis (HFE) Gene in the United States. JAMA 2001;285(17):2216-22.
9. Mura C, Raguenes O, Ferec C. HFE mutations analysis in 711 hemochromatosis probands: evidence for S65C implication in mild form of hemochromatosis. Blood 1999;93:2502-5.
10. Richtlijnen Hemochromatose vereniging Nederland. www.hemochromatose.nl.
11. Niederau C, Fischer R, Purschel A, Stremmel W, Haussinger D, Strohmeyer G. Long-term survival in patients with hereditary hemochromatosis. Gatroenterology 1996;110:1107-19.
12. Olynyk JK, Cullen DJ, Aquilia S, Rossi E, Summerville L, Powell LW. A population based study of the clinical expression of the hemochromatosis gene. New Engl J Med 1999;341:718-24.
13. Beutler E, Felitti VJ, Koziol JA, Ho NJ, Gelbart T. Penetrance of 845G-A (C282Y) HFE hereditary haemochromatosis mutation in the USA. Lancet 2002;359:211-8.
14. Bacon BR, Powell LW, Adams PC, et al. Molecular medicine and hemochromatosis: at the crossroads. Gastroenterology 1999;116:193-207.
15. Jacobs EMG, Vries RA de, Elving LD, Stalenhoef AFH, Swinkels DW. Diagnostiek bij 5 patiënten met mogelijk primaire hemochromatose. Ned Tijdschr Geneeskd 2003;14:666-70.
16. Bradley LA, Haddow JE, Palomaki GE. Population screening for hemochromatosis: expectations based on a study of relatives of symptomatic patients. J Med Screen 1996;3:171-7.
17. Bulaj ZJ, Ajioka RS, Phillips JD, et al. Disease-related conditions in relatives of patients with hemochromatosis. N Engl J Med 2000;343:1529-35.
18. Krawcak M, Cooper DN, Schmidtke J. Estimating the efficacy and efficiency of cascade genetic screening. Am J Hum Genet 2001;69:361-70.
19. Lee P, Glebart T, Wets C, Halloran C, Beutler E. Seeking candidate mutations that affect iron homeostasis. Blood Cells Mol Dis 2002;29;471-87.
20. El Serag HB, Inadomi JM, Kowdley KV. Screening for hereditary hemochromatosis in siblings and children of affected patients. Ann Int Med 2000;132:261-9.