

Hypogonadism and reduced bone mineral density in heterozygous H63D mutation in the HFE gene: an unusual presentation of hereditary hemochromatosis.

Cristiano Maria Francucci¹, Cristina Gatti¹, Andrea Camilletti¹, Paola Fiscaletti¹, Renata Caudarella²
5 and Marco Boscaro¹

¹Division of Endocrinology, *Polytechnic University of Marche, Ancona, Italy.*

²*Department of Clinical Medicine and Applied Biotechnology "D. Campanacci", Alma Mater Studiorum, University of Bologna, Bologna, Italy*

10

Abbreviated title: Hypogonadism in hereditary hemochromatosis

15

20 **Correspondence:**

Dr. Cristiano Maria Francucci

Clinica di Endocrinologia,

Ospedale Generale Regionale di Torrette

Via Conca n. 61

25 60020 Ancona, Italy

tel.: +39 071 887300

Fax: +39 071 887300;

E-mail: cm.francucci@ao-umbertoprime.marche.

ABSTRACT

30 **Objective.** Hereditary haemochromatosis (HH) results in excess iron absorption from the diet
and its deposition in body tissues, including the liver, joints, pancreas and pituitary gland,
with consequent tissue damage leading to the typical presentations of cirrhosis, arthralgia and
hypogonadism. Human iron homeostasis depends on the coordinated functions of numerous
genes, the precise roles of which are in many cases still obscure. In HH transferrin saturation
35 values $> 45\%$ are an indication for genetic testing. HH should be considered if suggestive
clinical and/or laboratory test abnormalities are found, in spite of normal transferrin
saturation, particularly if heterozygosity exists for the H63D mutation that usually has a low
frequency and penetration and, as a result, a minor phenotypic expression.

Findings. Since heterozygosity H63D mutation does not appear to have clinical effects, the
40 history of this proband, a male patient with transferrin saturation $< 45\%$, marked increase of
ferritin levels, reduced bone mass, and hypogonadism, that appears to be uncommon in
patients with lesser degree of hepatic siderosis at diagnosis, represents an atypical case in
which the transferrin saturation failed to identify the correct diagnosis.

Conclusion. Considering the presence of subjects with normal values of transferrin saturation
45 and a variable degree of clinical expressiveness in heterozygous H63D mutation, like our
proband, it is conceivable to propose genetic testing in symptomatic men with high values of
ferritin ($\geq 300 \mu\text{g/L}$), after ruling out possible secondary causes of hyperferritinemia, in order
to identify individuals with hereditary haemochromatosis and prevent or minimize iron-
related organ injury.

50 **Key words:** hereditary hemochromatosis, hypogonadism, hypopituitarism, bone mineral
density, and osteoporosis

INTRODUCTION

Hereditary Hemochromatosis (HH) is the most common inherited metabolic disease in the white population. It is a disorder of iron regulation that brings about an excess absorption
55 of dietary iron, which gradually accumulates in the parenchymal cells of liver, pancreas, heart, anterior pituitary, and gonads. Human iron homeostasis depends on the coordinated functions of numerous genes, the precise roles of which are in many cases still obscure.

Classic hereditary hemochromatosis is an autosomal recessive disorder associated with a mutation of the HFE gene, which was discovered in 1996 and is located on chromosome 6.
60 The most common form of HH is related to homozygosity for the C282Y mutation of the HFE gene, resulting in a change of tyrosine for cysteine at amino acid 282 (Feder et al, 1996) This mutation leads to a chain of events that may culminate in severe damage to multiple organs. The C282Y mutation is largely confined to whites of north European origin and the frequency of homozygosity decreases from the North to the South of Europe, being lowest in Italy and
65 the Mediterranean countries (Carella et al, 1997), where haemochromatosis is genetically heterogeneous (Piperno et al, 1998). Another mutation of the HFE gene, termed H63D mutation, results in the substitution of aspartate for histidine at amino acid 63, and does not appear to have clinical effects (Gochee et al, 2002). The clinical significance of compound heterozygosity for C282Y and H63D is still controversial (Rochette et al, 1999). It is possible
70 that several genes, other than HFE, may play a role in the disease in a few patients, presenting similar forms of iron overload which act as modifiers of the phenotype, as seen in HFE knockout mice (Bensaid et al, 2004). Therefore, it is difficult to predict whether and to what extent a such mutation will be phenotypically expressed.

The Online Mendelian Inheritance in Man (OMIM) database currently lists 5 types of
75 hereditary hemochromatosis (Bomform, 2002), each caused by mutations involving a

different gene, but cases of iron overload have been diagnosed in those genes, known to be associated with this disease, that have a normal sequence, indicating that there are still other genes to identify.

In the most extreme forms of HH, the disease manifests itself as cirrhosis, hepatocellular cancer, destructive arthritis, cardiomyopathy and endocrine problems such as diabetes mellitus and sexual dysfunction due to hypogonadotropic hypogonadism. Gonadal problems in hemochromatosis generally result from the destructive action of hemosiderin in the anterior pituitary (MacDonald and Mallory, 1959). A review by Pedersen-Bjergaard et al. on the pituitary function in haemochromatosis highlighted an insufficiency of pituitary gonadotropic secretion with clinical hypogonadism in 46% of the patients, a subclinical insufficiency of the growth hormone axis in 15%, of the lactotropic axis in 8%, of the thyroid axis in 4%, and of the adrenocortical axis in 1.5% of the patients. Moreover, the same Authors underlined that lactotropic, thyroid or adrenocortical insufficiency was usually associated with hypogonadism or growth hormone insufficiency. (Pedersen-Bjergaard et al, 1996). Hypogonadotropic hypogonadism appears to be unusual in patients with lesser degree of hepatic siderosis at diagnosis (McDermott and Walsh, 2005). Therefore, partial hypopituitarism alone, in absence of any important damage to other main parenchymal organs, notably the liver, associated with reduced bone mineral density (BMD), is an unusual presentation of the disease.

We describe a male patient with secondary hypogonadism and reduced bone mass as a presenting feature of hereditary haemochromatosis.

CASE REPORT

100 The proband, a 45-year old patient, father of one 11-year-old child, was referred to our department for the evaluation of a decreased libido associated to impotence and profound asthenia, which had begun some months back.

 He had no previous medical history. Physical examination revealed bronze skin (only a mild generalized hyperpigmentation) found also, from family history, to be present in his
105 father, nicknamed the “moor”, who died when he was 50 years-old because of cardiac problems.

 Parameters related to iron metabolism and basal hormonal evaluation, verified with analogous results on two consecutive occasions showed: the absence of anemia, normal serum iron concentration and transferrin percent saturation during fasting ($\leq 42\%$); a marked
110 increase of ferritin levels (633 ng/dl with reference range 16-220 ng/ml); normal 24-hour urinary uroporphyrin and coproporphyrin concentration; reduced levels of total and free testosterone (1,2 ng/dl and 5 pg/ml with normal range 2,7-11 ng/dl and 9-47 pg/ml, respectively), associated to low levels of luteinizing and follicle stimulating hormone (1,4 mIU/ml and 1,5 mIU/ml with normal range 1,3-9 mIU/ml and 1,4-16 mIU/ml, respectively)
115 and normal levels of prolactin. Except for mild elevation of fasting glycemia and impaired glucose tolerance at 2 hours following oral glucose-tolerance test, fasting routine laboratory tests (hemochrome, aspartate aminotransferase, alanine aminotransferase, electrolytes and HbA_{1c}) gave normal results as well as hemoglobin electrophoresis, glucose 6 phosphate dehydrogenase, pyruvate kinase, lactate dehydrogenase, haptoglobin and the reticulocyte
120 count, evaluated to rule out thalassemia or hemolytic conditions. TSH, free fractions of T₄ and T₃, IGF-I, plasma cortisol and urinary free cortisol were also normal as well as plasma

aldosterone (PA), plasma renin activity (PRA), and PA/PRA ratios in the basal state and in the upright position.

Decreased response of serum gonadotropins to GnRH stimulation (100 µg IV bolus) revealed a secondary hypogonadism. An insulin-induced hypoglycemia test, with a reduction of blood glucose < 40 mg/dL from the starting level, highlighted a basal GH deficiency and the lack of response of plasma ACTH and cortisol, which was also confirmed by a.m. CRH test (100 µg IV bolus). On the contrary, a stimulation test with thyrotropin-releasing hormone (TRH) (200 µg IV bolus) gave normal results. (see table 1)

In view of partial hypopituitarism, the brain MRI scan, carried out to exclude the presence of pituitary abnormalities or signs of hemosiderin deposition, was normal. MRI images of the liver were suggestive of iron deposition, and did not highlight liver and spleen enlargement.

According to the patient's wishes, we did not perform liver biopsy. Moreover, it has been demonstrated that the measure of liver iron concentration loses most of its diagnostic significance after cloning the HFE gene (Camaschella, 2005) and that subjects with normal biochemical liver function tests, without hepatomegaly and with serum ferritin levels less than 1,000 µg/L rarely have significant fibrosis (Qaseem et al, 2005; Schimitt et al, 2005; Yen et al, 2006). Nevertheless, liver biopsy can still be useful in some patients to provide additional information on histology (presence of fibrosis, cirrhosis) as well as on the distribution of iron (hepatocytes versus Kupffer cells) (Camaschella, 2005).

Considering the association of hemochromatosis and osteoporosis (Diamond et al, 1989) and that deleterious effects of hypogonadism on bone are well-known, we evaluated the BMD using dual x-ray absorptiometry densitometer. The patient showed reduced BMD at

145 lumbar spine (L_{2-4} : 0.956 gr/cm²; T-score: -2.59 DS) and at non dominant proximal femur
neck (0.831 gr/cm²; T-score: 1.83 DS). In light of these results, in order to study the bone
metabolism fasting blood samples were taken for the measurement of serum calcium,
phosphorus, intact parathyroid hormone, bone-specific alkaline phosphatase, C-terminal
cross-linking telopeptide of type I collagen (CTX), and 25-hydroxyvitamin D. All these
150 laboratory tests were within normal range, with the exception of CTX which was slightly
increased (data not shown).

The proband did not report any previous blood transfusion, iron-containing
medications or daily consumption of alcohol.

Although a normal value (<45%) of serum transferrin saturation normally rules out
155 hereditary hemochromatosis, we considered it reasonable, on account of raised ferritin levels
together with the familial and personal history of the patient, to perform the genetic testing
HFE mutations with the patient's consent. Heterozygosity for the H63D mutation of the HFE
gene confirmed the diagnosis for hereditary hemochromatosis.

In relation to these results the proband began an androgenic and corticosteroid
160 replacement therapy.

The response to therapeutic phlebotomy highlighted the decrease of serum ferritin in
parallel with transferrin saturation, in absence of anemia, and an increase of the free interval
of androgenic replacement therapy (im).

METHODS

165 ***Biochemical evaluation***

We measured: after overnight fasting, serum iron, using the ferrozine method (ADVIA 1650, Bayer), serum transferrin, using the nephelometric method (BN II Instrument, DADE Behring), and serum calcium and phosphorus by automated routine procedures [intra- and inter-assay coefficients of variation $\pm 2,0$ %]; by chemiluminescent immunometric assays: 170 serum ferritin, cortisol, estradiol, total testosterone, FSH, LH, prolactin, free fractions of T₄ and T₃, and TSH (ADVIA Centaur; Bayer; USA), IGF-1, 25-hydroxyvitamin D and PTH (Dia Sorin, Saluggia, Italy), GH (Medical Systems, Genova, Italy), CTX (beta-CrossLaps, Roche); ACTH, fasting insulin and dehydroepiandrosteron sulfate (Immulite; DPC; USA); by radioimmunoassays: androstenedione (DSL - USA), free-testosterone (Biosource, Belgium), 175 and bALP (Tandem-R Ostase, Beckman Coulter); by chromatographic purification using high-performance liquid chromatography (HPLC): twenty-four hour urinary free cortisol (CLU) and porphyrins. The intra- and inter-assay coefficients of variation of chemiluminescent immunometric assays and radioimmunoassays were $\pm 6,0$ %, except for bALP ($\pm 10,0$ %).

180 ***Bone densitometry***

Spinal (L₂-L₄) and femoral BMD at four femoral sites were measured by dual x-ray absorptiometry densitometer (Prodigy Lunar, Madison, Wisconsin): neck (FN), Ward's triangle (WT), great trochanter (TR) and total hip.

185

Genetic testing

DNA isolation and amplification:

Genomic DNA was prepared from blood, using the isolate 2 DNA extraction kit.
190 DNA fragments were amplified by PCR, using the primers described (5'-
ACATGGTTAAGGCCTGTTGC-3' and 5'-GCCACATCTGGCTTGAAATT-3' for the
C187G mutation at codon 63; 5'-TGGCAAGGGTAAACAGATCC-3' and 5'-
CTCAGGCACTCCTCTCAACC-3' for the G845A mutation at codon 282 of the HFE gene)
(Feder JN et al Nat Gen 1996). A "hot start" PCR at 96°C was followed by 35 Cycles of
195 denaturation at 96°C for 30 sec, annealing at 56°C for 60 sec, and extension at 72°C for 60
sec. PCR products were desalted using Ultrafree R-MC (30000 NMWL) filter units (Millipore
Corp).

Restriction fragment length analysis

Mutations were detected by restriction fragment length analysis. The C-to-G
200 transversion at nucleotide 187 abolishes the DNA sequence recognition site for endonuclease
BcI (New England Biolabs). Restriction fragments were separated on 2% NuSieve GTG
agarose and 1% agarose MP (Boehringer Mannheim) gels containing ethidium bromide and
visualized by ultraviolet transillumination.

205

210

DISCUSSION

Hereditary hemochromatosis is an iron overload disorder and the most common
215 recessive disease in Caucasians. About 80% of hemochromatosis patients are homozygous for
the C282Y mutation in the HFE gene (Njajou et al, 2004), but in Italy, typical HFE mutations
account for only 64% of cases of overt hereditary hemochromatosis (Carella et al, 1997) and
the estimated prevalence of C282Y homozygosity in the general Italian population is 1 in
3,900 (Cassanelli et al, 2001). Moreover, screening data from both northern (Barosi et al,
220 2002) and southern Italy (De Marco et al, 2004) revealed a high prevalence of HFE-
unrelated iron overload.

In hereditary hemochromatosis, transferrin saturation values are of great importance
because iron accumulates, first, in the transferrin pool, resulting in an increase of serum
transferrin saturation and, subsequently, in tissue stores, especially the hepatic parenchyma,
225 with a progressive increase in concentrations of serum ferritin. Therefore, morning fasting
transferrin saturation is generally regarded as the best single screening test for HH, even if
there is no agreed on cutoff for the optimal detection of disease (EASL, 2000; Pietrangelo,
2004; Yen et al, 2006). A threshold of 45% or more identifies individuals with potential iron
overload and is an indication for genetic testing (Yen et al, 2006).

230 Though this proband did not show the accepted biochemical criterium, a mild form of
HH could not be ruled out due to the marked increase of ferritin levels, the clinical and
laboratory signs, the MRI scan, suggestive of iron deposition on liver and spleen, and the
reduced BMD. In confirmation of this, genetic testing described a heterozygosity for the
H63D mutation, that usually has low penetration and, consequently, a minor phenotypic
235 expression.

Differently from C282Y homozygosity, clinical effects of the H63D mutation and its role in the pathogenesis of the disease are still unclear and appear to be limited (Carella et al, 1997), because the expression of a given mutation can be influenced by combined heterozygous or homozygous mutations of multiple hemochromatosis genes. Unfortunately, we do not have the possibility of performing genetic testing to evaluate the presence of mutations in ferroportin, TFR2, HAMP, and HJV, that could explain the gradual and highly variable progression of the disease.

In relation to our data, the history of our proband is not in agreement with the conclusions of several reports that had highlighted the lack of clinical manifestations of iron overload both in homo- (Gochee et al, 2002) and heterozygous (Gochee et al, 2002; Barosi et al, 2002) H63D mutations.

The presence in our proband of normal transferrin-saturation values, and persistently elevated serum ferritin could be explained by ferroportin-associated iron overload, currently classified as HH type 4 (Pietrangelo et al, 1999; Montosi et al, 2001), that results in a genetic disorder with two different clinical manifestations. In fact, the subjects with mutant ferroportin proteins with intracellular localization show typical ferroportin disease with low to normal transferrin-saturation and early K upffer cell iron loading, while the patients with mutant proteins with plasma membrane localization exhibit high transferrin-saturation and early hepatocyte iron loading, similar to classic HH (De Domenico et al, 2006). These two classes, after phlebotomy, respond differently because patients with mutations that lead to K upffer cell iron loading do not gain from treatment (Pietrangelo, 2004; De Domenico et al, 2006) However, at variance with ferroportin-related iron overload that prevalently shows showing a rapid decrease of transferrin saturation with persistently high serum ferritin (Pietrangelo, 2004). our proband exhibits. Therefore, in this case a ferroportin disease can be

260 excluded because our proband, before therapy, had normal transferrin saturation, and in
response to phlebotomy exhibited, in absence of anemia, a decrease of serum ferritin in
parallel with transferrin saturation. in response to therapeutic phlebotomy. A likely
dysmetabolic hemosiderosis has been excluded because this new condition is characterised
265 and insulin resistance, in absence of other causes of iron overload, particularly genetic
hemochromatosis and chronic alcoholism in almost all patients (Moirand et al, 1997). An
high ferritin diabetes can be excluded by the history of our proband, who shows a mild
elevation of fasting glycemia, a normal value of HbA_{1c} and impaired glucose tolerance at 2
hours following oral glucose-tolerance test. Moreover, the normal value (< 45%) of serum
270 transferrin saturation, and/or the milder symptomatic organ involvement can exclude, almost
certainly, the juvenile-onset phenotype (HAMP and HJV mutations) as well as TFR2 and
Cybrd1 mutations. The absence of anemia can exclude, with a good degree of certainty, the
rare DMT1 mutation.

Differently from the classic description of HH which, in symptomatic patients,
275 generally presents generalized cutaneous hyperpigmentation (69–75%) associated, in its most
extreme form, with hepatomegaly (70-95%), osteoarticular polyarthropathy (20-70%),
diabetes mellitus (10-60%), ECG abnormalities (up to 35%), and hypogonadism (up to 35%
of male) (Chalès and Guggenbuhl, 2003; Limdi and Crampton, 2004), these findings are only
partly present in our proband. In fact, he exhibited an unusual presentation of the disease with
280 partial hypopituitarism associated with reduced BMD.

To confirm the unusual presentation of this case, the largest detailed study of
hypogonadism, reported in HH, highlighted a low prevalence of male hypogonadotropic
hypogonadism (6,4%) that was related to the severity of iron overload (McDermott and

Walsh, 2005). In fact, in this paper patients with a lower degree of hepatic siderosis on liver
285 biopsy and without hepatic cirrhosis, diabetes mellitus, or markedly elevated serum ferritin
levels rarely manifested hypogonadism, that was associated with hepatic cirrhosis, diabetes
mellitus, and ferritin levels greater than 1500 ng/ml, at diagnosis, in 89%, 33%, and 77% of
the subjects, respectively (McDermott and Walsh, 2005).

The association of haemochromatosis and osteoporosis is well established, but it is
290 unclear whether this is due to iron overload, hypogonadism, hypoparathyroidism, liver
disease, or diabetes mellitus. In HH the prevalence of osteoporosis, as defined by bone density
measurements, is 25–33%, and the prevalence of spinal fractures ranges from 1.8% to 18%
(Chalès and Guggenbuhl, 2001).

Considering the influence of bone iron deposition in the mineralization process
295 remains an issue of debate, and in light of the history of our proband, the presence of reduced
BMD could be predominantly due to hypogonadism that can lead to increased rates of bone
resorption, and bone loss, as well as observed in the first years after menopause. In fact, our
patient referred a recent clinical deterioration of erectile dysfunction that could be related to a
parallel rapid decline in androgen production, even if it is not clear whether the bone loss seen
300 immediately after testosterone withdrawal mimics what is seen with estrogen deficiency. In
this respect several Authors (Stepan et al, 1989) observed in young men, who had undergone
bilateral orchiectomy because of sexual delinquency, a bone loss of 7% per year in the first 2
yr of lumbar bone density, evaluated by dual photon absorptiometry, as a function of time
after orchiectomy.

305 Differently from the increase observed immediately after estrogen deficiency of both
markers of bone formation and resorption, our proband highlighted a slight disassociation in

the levels of the markers of bone turnover. The reason for this is unclear and could be explained by a subtle longstanding hypogonadism.

This deleterious effect on bone is a further confirmation of the atypical presentation of
310 this case that was not associated with a severe form of HH.

The reduction of androgenic requirements, in response to therapeutic phlebotomy, is probably due to a partial normalization of the hypothalamic–pituitary–gonadal function. This result, if confirmed in following evaluations, is important because there is evidence that arthropathy, cirrhosis, and hypogonadism would not seem to be affected by treatment, unlike
315 malaise, fatigue, skin pigmentation, abdominal pain, and insulin requirements in diabetes (Tavill, 2001).

In patients with suspected HH (transferrin saturation values upper 45%), a genetic screening is generally recommended (EASL, 2000). Nonetheless, in view of the low frequency of compound heterozygotes in patients with HH (Bomform, 2002) and the minor
320 phenotypic expression in heterozygosity for the H63D mutation (Carella et al, 1997), it is conceivable to propose a genetic testing in symptomatic men with high values of ferritin ($\geq 300 \mu\text{g/L}$), not due to secondary causes of hyperferritinemia, even if normal values of transferrin saturation are present. This behaviour is suggested to identify, in time, individuals with HH to prevent or minimize iron-related organ injury (EASL 2000; Chalès and
325 Guggenbuhl, 2001), and because a timely treatment with phlebotomy, before onset of cirrhosis or insulin requiring diabetes, is associated with a normal life expectancy (Wojcik JP et al, 2002).

Moreover, since there is the risk that over reliance on HFE mutations for a diagnosis of haemochromatosis can lead to an incorrect assessment of iron stores, a careful assessment

330 of cofactors for liver damage in haemochromatosis has to be performed, because a proportion
of patients could develop additional disease processes.

The history of this proband represents an atypical case of hereditary hemochromatosis (heterozygosity for the H63D mutation of HFE) in which the best single screening test failed to identify the correct diagnosis.

335 To conclude, if clinical and/or laboratory test abnormalities suggestive for hereditary hemochromatosis are found, a genetic screening should be considered in male adults of all age groups, above all to exclude heterozygous H63D mutation, which does not appear to have clinical effects, and to prevent or to reduce iron-related organ injury

340 **Acknowledgments**

We are grateful to Professor Riccardo Sarzani and to Mr Giorgio Tesei of the Internal Medicine Department to have performed the genetic test for the HFE gene.

345

BIBLIOGRAFY

- 350 Barosi G, Silvaneschi L, Grasso M, Martinetti M, Marchetti M, Bodini U, Reggiani A, D'Agostino F, Nalli G, Degiuli A, De Silvestri A, Arbustini E. High prevalence of a screening-detected, HFE-unrelated, mild idiopathic iron overload in Northern Italy. *Haematologica*. 2002; 87(5): 472-8.
- 355 Bensaïd M, Frushon S, Mazeres C, Bahram S, Roth MP, Coppin H. Multigenic control of hepatic iron loading in a murine model of hemochromatosis. *Gastroenterology*. 2004; 126(5): 1400-8.
- Bomform A. Genetics of haemochromatosis. *Lancet*. 2002; 360:1673-81.
- 360 Camaschella C. Understanding iron homeostasis through genetic analysis of hemochromatosis and related disorders. *Blood*. 2005; 106(12):3710-7.
- Carella M, D'Ambrosio L, Totaro A, Grifa A, Valentino MA, Piperno A, Girelli D, 365 Roetto A, Franco B, Gasparini P, Camaschella C. Mutation analysis of the HLA-H gene in Italian Haemochromatosis patients. *Am J Hum Genet*. 1997; 60:828-832.
- Cassanelli S, Pignatti E, Montosi G, Garuti C, Mariano M, Campioli D, Carbonieri A, Baldini E, Pietrangelo A. Frequency and biochemical expression of C282Y/H63D 370 hemochromatosis (HFE) gene mutations in the healthy adult population in Italy. *J Hepatol*. 2001; 34 (4):523-8.

Chalès G, Guggenbuhl P. Ostéoporose de l'hémochromatose génétique. *Rev Rhum.* 2001; 68:749–51 [Ed Fr]

375 Chalès G, Guggenbuhl P. When and how should we screen for hereditary hemochromatosis? *Joint Bone Spine.* 2003; 70:263–270.

De Marco F, Liguori R, Giardina MG, D'Armiento M, Angelucci E, Lucariello A, Morante R, Cimino L, Galeota-Lanza A, Tarantino G, Ascione A, Budillon G, Vecchione
380 R, Martinelli R, Matarazzo M, De Simone V. High prevalence of non-HFE gene-associated haemochromatosis in patients from southern Italy. *Clin Chem Lab Med.* 2004; 42(1):17-24.

EASL. International Conference on Haemochromatosis. III. Jury document. *J Hepatol.*
385 2000; 33:496-504.

De Domenico I, McVey Ward D, Musci G, Kaplan J. Iron overload due to mutations in ferroportin. *Haematologica* 2006; 91:92-95.

390 Diamond T, Stiel D, Posen S. Osteoporosis in hemochromatosis: iron excess, gonadal deficiency, or other factors? *Ann Intern Med.* 1989; 110:430–436.

Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, Dormishian F, Domingo R Jr, Ellis MC, Fullan A, Hinton LM, Jones NL, Kimmel BE, Kronmal GS,
395 Moore T, Morikang E, Prass CE, Quintana L, Starnes SM, Schatzman RC, Brunke KJ,

Drayna DT, Risch NJ, Bacon BR, Wolff RK. A novel MCH class 1-like gene is mutated in patients with hereditary haemochromatosis. *Nat Genet.* 1996; 13:399-408.

400 Gochee PA, Powell LW, Cullen DJ, Du Sart D, Rossi E, Olynyk JK. A population based study of the biochemical and clinical expression of the H63D haemochromatosis mutation. *Gastroenterology.* 2002; 122:646-51.

Limdi JK et Crampton JR. Hereditary Haemochromatosis. *Q J Med.* 2004; 97:315-324.

405 MacDonald RA, Mallory GK. Hemochromatosis and hemosiderosis: study of 211 autopsied cases. *Arch Intern Med.* 1959; 105:686-700.

McDermott JH, Walsh CH. Hypogonadism in hereditary hemochromatosis. *J Clin Endocrinol Metab.* 2005; 90(4):2451-5.

410

Moirand R, Mortaji AM, Loreal O, Paillard F, Brissot P, Deugnier Y. A new syndrome of liver iron overload with normal transferrin saturation. *Lancet.* 1997; 349(9045):95-7.

415 Montosi G, Donovan A, Totano A, Garuti C, Pignatti E, Cassanelli S, Trenor CC, Gasparini P, Andrews NC, Pietrangelo A. Autosomal dominant hemochromatosis is associated with mutation in ferroportin (SLC11A3) gene *J Clin Invest.* 2001; 108: 619-62.

420 Njajou OT, Alizadeh BZ, Van Duijn CM. Is genetic screening for hemochromatosis worthwhile?. *Eur J Epidemiol* 2004; 19 (2): 101-8.

Pedersen-Bjergaard U, Thorsteinsson B, Kirkegaard BC. Pituitary function in hemochromatosis. *Ugeskr. Laeger.* 1996; 158(13):1818-22.

425 Pietrangelo A, Montosi G, Totaro A, Garut CI, Conte D, Cassanell SI, Fraquelli M, Sardin CI, Vasta F, Gasparin PI. Hereditary Hemochromatosis in adults without pathogenic mutations in haemochromatosis gene *N Engl J Med.* 1999; 341:725-32.

Pietrangelo A. Hereditary Hemochromatosis: a new look at an old disease. *N Engl J Med.* 430 2004; 350:2383-97.

Piperno A, Sampietro M, Pietrangelo A, Arosio C, Ludica L, Montosi G, Vergani A, Fraquelli M, Girelli D, Pasquero P, Roetto A, Gasparini P, Fargion S, Conte D, Camaschella C. Heterogeneity of hemochromatosis in Italy. *Gastroenterology.* 1998; 435 116:193-20.

Qaseem A, Aronson M, Fitterman N, Snow V, Weiss KB, Owens DK; Clinical Efficacy Assessment Subcommittee of the American College of Physicians. Screening for hereditary hemochromatosis: a clinical practice guideline from the American College of 440 Physicians. *Ann Intern Med.* 2005; 143(7):517-21.

Rochette J, Pomton JL, Fisher CA, Perera G, Arambepola M, Arichchi DS, De Silva S, Vandwalle JL, Monti JP, Old JM, Merryweather-Clarke, AT, Weatherall DJ, Robson KJ. Multicentric origin of haemochromatosis gene (HFE) mutations. *Am J Hum Genet.* 1999; 445 64:1056-62.

Schmitt B, Golub RM, Green R. Screening primary care patients for hereditary hemochromatosis with transferrin saturation and serum ferritin level: systematic review for the American College of Physicians. *Ann Intern Med.* 2005; 143(7):522-36.

450 Stepan JJ, Lachman M, Zverina J, Pacovsky V, Baylink DJ. Castrated men exhibit bone loss: effect of calcitonin treatment on biochemical indices of bone remodeling. *J Clin Endocrinol Metab.* 1989; 69:523–527.

455 Tavill AS. Diagnosis and management of haemochromatosis. *AASLD Practice Guidelines Hepatology.* 2001; 33:1321-8.

Wojcik JP, Speechley MR, Kertesz AE, Chakrabarti S, Adams PC. Natural history of C282Y homozygotes for hemochromatosis. *Can J Gastroenterol.* 2002; 16:297–302.

460 Yen AW, Fancher TL, Bowlus CL. Revisiting hereditary hemochromatosis: current concepts and progress. *Am J Med.* 2006; 119(5):391-9.

465

TABLE 1

Tests	Hormone	Baseline	30'	60'
<i>Hypoglicemia Insulin Test</i> <i>(0.1 U/kg IV bolus)</i>	GH (ng/ml)	0,21		0,19
	ACTH (pg/ml)	13,00		14,00
	Cortisol (mcg/dl)	20,80		21,10
<i>CRH Test</i> <i>(100 mcg IV bolus)</i>	ACTH (pg/ml)	19,00		17,00
	Cortisol (mcg/dl)	26.30		20.2
<i>GnRH test</i> <i>(100 mcg IV bolus)</i>	LH (mUI/ml)	1,50	1,80	
	FSH (mUI/ml)	1,80		1,80
<i>TRH test</i> <i>(200 mcg IV bolus)</i>	TSH (mcU/ml)	0,66	4,07	

Dynamic responses and basal hormone concentrations