

Angel F. Remacha · M. Pilar Sardà · M. Jesús Barceló ·
Vanessa Bach · Albert Altès · Montserrat Baiget ·
Carlos Guarner · Irene Blesa

Genotype–phenotype correlation in a Spanish population homozygous for the H63D mutation of the HFE gene

Received: 15 November 2005 / Accepted: 16 January 2006 / Published online: 8 March 2006
© Springer-Verlag 2006

Hereditary hemochromatosis (HH) is an autosomal recessive disorder of iron metabolism with excessive cellular iron levels resulting in tissue damage [1–3].

In 1996, Feder et al. showed that in most HH patients, the HFE gene is mutated in position 282 (replacement of cysteine by tyrosine, C282Y) [4]. In Spain, 85–90% of HH patients showed this mutation [5, 6]. However, a second mutation, the replacement of histidine in position 63 by aspartic acid (H63D), is much more frequent. In our area, the Northeast of Spain, the H63D mutation was present in 37% of the general population, and 4% were homozygous [7].

In a murine model, the contribution of the H63D homozygosity (DD genotype) was demonstrated. However, the iron overload (IO) was below that of the C282Y homozygosity (YY genotype) [8]. Moreover, data in humans support that the DD genotype could result in IO

[9–11]. Given the high prevalence of this mutation in our population, we evaluated the genotype–phenotype correlation in homozygotes for the H63D mutation.

Two studies were conducted in two Spanish populations. First, the correlation between the HFE mutations and the amount of iron in the liver was evaluated in 78 cases (7 cases were DD homozygotes) [12]. Liver biopsy was carried out because of a biochemical IO [serum transferrin saturation >50% and/or serum ferritin >350 µg/l]. Hemochromatosis was considered when the hepatic iron index (HII) exceeded 1.9 µmol/g of dry weight multiplied by the age in years, µmol/g.year [1–3]. In 30 out of 78 cases, HII exceeded 1.9 µmol/g.year, including three cases (10%) homozygous for the H63D mutation (HII=4.2, 3.9, and 3.8 µmol/g.year). Moreover, in one patient homozygous for the H63D mutation, the HII was 1.8 µmol/g.year. Therefore, four out of the seven DD homozygotes showed moderate liver IOs (57%). C282Y homozygotes showed the highest levels of HII. DD homozygotes had higher levels of HII than wild-type patients and H63D heterozygotes (Table 1).

The second study was conducted between 1998 and 2004 in 55 consecutive patients with the DD genotype. All patients came from our university hospital and were studied for all the known secondary causes of biochemical IO [13, 14]. Data were not available in one case, which was excluded from the study. Hcpidin (HAMP) gene mutations were investigated given that these mutations could modify the severity of the IO [15]. Transferrin saturation and serum ferritin were evaluated by automated methodologies (Hitachi 911 and Elecsys, Roche). Moreover, total iron removed by phlebotomy was calculated in grams. Mutations of the HFE gene (C282Y, H63D, and S65C) were analyzed with LightCycler equipment [6, 7, 14]. The HAMP gene was evaluated using single strand conformational polymorphism and direct sequencing [15].

The patients were 36 men and 18 women, with a median age was 51 years. They were chosen for the study because of a biochemical IO (41 cases) or a family study (13 cases). Forty-five patients (83%) showed a biochemical IO pattern. An underlying cause of biochemical IO was observed in 35

A. F. Remacha (✉) · M. P. Sardà · M. J. Barceló · V. Bach ·
A. Altès · M. Baiget · C. Guarner · I. Blesa
Hematology Department, Hospital de Sant Pau,
Avda Padre Claret 167,
Barcelona, 0825, Spain
e-mail: aremacha@hsp.santpau.es

A. F. Remacha · M. P. Sardà · M. J. Barceló · V. Bach ·
A. Altès · M. Baiget · C. Guarner · I. Blesa
Hematology Department, Hospital Espirit Sant,
Santa Coloma de Gramenet, Barcelona, Spain

A. F. Remacha · M. P. Sardà · M. J. Barceló · V. Bach ·
A. Altès · M. Baiget · C. Guarner · I. Blesa
Genetics Department, Hospital de Sant Pau,
Barcelona, Spain

A. F. Remacha · M. P. Sardà · M. J. Barceló · V. Bach ·
A. Altès · M. Baiget · C. Guarner · I. Blesa
Genetics Department, Hospital Espirit Sant,
Santa Coloma de Gramenet, Barcelona, Spain

A. F. Remacha · M. P. Sardà · M. J. Barceló · V. Bach ·
A. Altès · M. Baiget · C. Guarner · I. Blesa
Gastroenterology Department, Hospital Espirit Sant,
Santa Coloma de Gramenet, Barcelona, Spain

Table 1 Iron overload distributions depending on the HFE mutations

	YY	CY/HD	DD ^c	Others
HII>1.9 ^a	24	2	3	1 ^b
HII 1–1.9	2	2	1	3
HII<1		4	3	33
Total cases	26	8	7	37
HII				
Mean ± standard deviation	5.7±3.8	1.6±1.75	2.02±1.9	0.31±0.28
Maximum–minimum	1.1–15.8	0.2–5.4	0.1–4.2	0.1–1.2

^aHII hepatic iron index in $\mu\text{mol/g}\cdot\text{year}$

^bA patient with alcoholic cirrhosis and focal iron. This patient was excluded (HII=2.2 $\mu\text{mol/g}\cdot\text{year}$)

^cComparisons: YY (homozygous for the C282Y mutation) vs DD (homozygous for the H63D mutation) $p<0.0001$. Difference=2.37. 95% confident interval=1.4–3.3. DD vs CY/HD (compound heterozygotes) p =not significant. DD vs others (wild type and H63D heterozygotes) $p=0.005$. Difference=1.71. 95% confident interval=0.4–3.3

out of 54 patients (65%). However, an associated cause of biochemical IO could not be found in 35%, including three patients with clear familial patterns (two patients were brothers and the remaining patient had two sisters and a daughter with biochemical IO). The HAMP gene was normal in the 54 cases. Fourteen patients with IO were phlebotomized, but the total phlebotomized iron was always below 5 g in men and 3 g in women. Interestingly, 4 and 3.75 g of iron were removed in the three familial cases.

Our findings demonstrate that H63D homozygosity is related to a moderate IO. However, the IO was clearly lower than in the C282Y homozygotes. An underlying associated cause of biochemical IO was observed in most of the individuals (Table 2). In contrast, the patients without associated causes showed minimal iron abnormalities. A possible modifying role of the HAMP gene was discarded.

Not surprisingly, our results in humans mimicked the liver iron results observed in a murine model [8]. Interestingly, the patients with moderate IOs showed underlying causes [13, 14]. It is reasonable to assume that this association is necessary for the development of IO [13, 14] and pathological lesions in the liver [16]. Moreover, other studies in our area have demonstrated that some patients with HH criteria were homozygous for the H63D mutation [5, 17, 18].

Although a small percentage of patients with HH criteria have been observed in almost all studies, very few studies have specifically dealt with DD patients [9–11]. These studies suggested a role for the DD genotype in IO [9–11]. In a French study [11], the relationship between the DD genotype and IO has also been observed. Our DD homozygotes and the French DD homozygotes share many clinical characteristics, such as age, male predominance, and moderate IO. However, 65% of our patients showed underlying causes of IO, in contrast to 32% in the French population. This discrepancy could be due to differences in the recruitment. In our case, only patients admitted to our hospital were considered. Taking together the data of the French study and our study, a number of genetics modifiers were discarded, such as transferrin receptor (TFR) polymorphisms, the Y250X mutation of TFR2, and HAMP gene mutations.

In summary, given the high prevalence (4%) of homozygotes for the H63D mutation in Northern Spain [7, 18], the DD genotype could account for 5–10% of our HH [5, 6]. As suggested, some additional disorders such as hepatitis C virus infection, dysmetabolic syndrome, alcoholism, etc., are probably necessary for the development of moderate IO and liver pathology in some HFE genotypes [13, 14, 16]. These findings should be borne in mind when evaluating IO, especially in an area with high prevalence of the H63D mutation, such as northern Spain.

Table 2 Underlying associated disorders in individuals with the DD genotype

	Cases	Sat %	Serum ferritin $\mu\text{g/l}$	Fe (g) ^a
Associations	35 (65%)			
HCV	10 (18.5%)	72±14	600±604	0.8–1.57
DMS	20 (37%)	50±15	726±369	1–3.6
OH	10 (18.5%)	50±14	514±317	1–3.8
HEM	6 (11%)	63±28	1,086±700	–
No factors	19 (35%)			
3 IO family ^b	3 IO family ^b	44–50–67	800–825–1,500	3.75–4 ^a
16 minimal changes	16 minimal changes	38±11	163±127	

Sat transferrin saturation, HCV hepatitis C virus infection, DMS dysmetabolic syndrome, OH alcoholism, HEM hematological disorder, IO iron overload

^aPhlebotomized iron in grams

^bThree cases with a familial IO

Acknowledgements We are grateful to “Fondo Investigaciones Sanitarias” grants: FIS PI 20506, PI 020235, and PI 041120 from the Ministerio de Sanidad y Consumo (Spain), RED INERGEN C03/05 and AATRM 005/29/2004.

References

- Niederau C, Fischer R, Purschel A, Stremmel W, Haussinger D, Strohmeyer G (1996) Long-term survival in patients with hereditary hemochromatosis. *Gastroenterology* 110:1107–1119
- Wojcik JP, Speechley MR, Kertesz AE, Chakrabarti S, Adams PC (2002) Natural history of C282Y homozygotes for hemochromatosis. *Can J Gastroenterol* 16:297–302
- Pietrangolo A (2004) Hereditary hemochromatosis—a new look at an old disease. *N Engl J Med* 350:2383–2397
- 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, Lauer P, Lee VK, Loeb DB, Mapa FA, McClelland E, Meyer NC, Mintier GA, Moeller N, Moore T, Morikang E, Prass CE, Quintana L, Starnes SM, Schatzman RC, Brunke KJ, Drayna DT, Risch NJ, Bacon BR, Wolff RK (1996) A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nat Genet* 13:399–408
- Sanchez M, Bruguera M, Bosch J, Rodes J, Ballesta F, Oliva R (1998) Prevalence of the Cys282Tyr and His63Asp HFE gene mutations in Spanish patients with hereditary hemochromatosis and in controls. *J Hepatol* 29:725–728
- Baiget M, Barceló MJ, Gimferrer E (1998) Frequency of the HFE C282Y and H63D mutations in distinct ethnic groups living in Spain. *J Med Genet* 35:701
- Altes A, Ruiz A, Barceló MJ, Remacha AF, Puig T, Maya AJ, Castell C, Amate JM, Saz Z, Baiget M (2004) Prevalence of C282Y, H63D and S65C mutations of HFE gene in 1146 newborns from a region of Northern Spain. *Genet Test* 8:407–410
- Tomatsu S, Orii KO, Fleming RE, Holden CC, Waheed A, Britton RS, Gutierrez MA, Velez-Castrillon S, Bacon BR, Sly WS (2003) Contribution of the H63D mutation in HFE to murine hereditary hemochromatosis. *Proc Natl Acad Sci U S A* 100:15788–15793
- Gochee PA, Powell LW, Cullen DJ, Du Sart D, Rossi E, Olynyk JK (2002) A population-based study of the biochemical and clinical expression of the H63D hemochromatosis mutation. *Gastroenterology* 122:646–651
- Melis MA, Cau M, Deidda F, Barella S, Cao A, Galanello R (2002) H63D mutation in the HFE gene increases iron overload in beta-thalassemia carriers. *Haematologica* 87:242–245
- Aguilar-Martinez P, Bismuth M, Picot MC, Thelcide C, Pageaux GP, Blanc F, Blanc P, Schved JF, Larrey D (2001) Variable phenotypic presentation of iron overload in H63D homozygotes: are genetic modifiers the cause? *Gut* 48:836–842
- Barry M, Sherlock SA (1971) Measurement of liver-iron concentration in needle-biopsy specimens. *Lancet* i:100–103
- Schoniger-Hekele M, Muller C, Polli C, Wrba F, Penner E, Ferenci P (2002) Liver pathology in compound heterozygous patients for hemochromatosis mutations. *Liver* 22:295–301
- Altes A, Remacha AF, Sureda A, Martino R, Briones J, Brunet S, Baiget M, Sierra J (2003) Patients with biochemical iron overload: causes and characteristics of a cohort of 150 cases. *Ann Hematol* 82:127–130
- Merryweather-Clarke AT, Cadet E, Bomford A, Capron D, Viprakasit V, Miller A, McHugh PJ, Chapman RW, Pointon JJ, Wilmhurst VL, Livesey KJ, Tanphaichitr V, Rochette J, Robson KJ (2003) Digenic inheritance of mutations in HAMP and HFE results in different types of haemochromatosis. *Hum Mol Genet* 12:2241–2247
- Geier A, Reugels M, Weiskirchen R, Wasmuth HE, Dietrich CG, Siewert E, Garton C, Lorenzen J, Bosserhoff AK, Bruggmann M, Gressner AM, Matern S, Lammert F (2004) Common heterozygous hemochromatosis gene mutations are risk factors for inflammation and fibrosis in chronic hepatitis. *Liver Int* 24:285–294
- Guix P, Picornell A, Parera M, Galmes A, Obrador A, Ramon MM, Castro JA (2002) Distribution of HFE C282Y and H63D mutations in the Balearic Islands (NE Spain). *Clin Genet* 61:43–48
- Ropero Gradilla P, Villegas Martinez A, Fernandez Arquero M, Garcia-Agundez JA, Gonzalez Fernandez FA, Benitez Rodriguez J, Diaz-Rubio M, de la Concha EG, Ladero Quesada JM (2001) C282Y and H63D mutations of HFE gene in patients with advanced alcoholic liver disease. *Rev Esp Enferm Dig* 93:156–163