The Southern French registry of genetic hemochromatosis: a tool for determining clinical prevalence of the disorder and genotype penetrance

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ABSTRACT

Background

Despite great progress in understanding the mechanisms underlying genetic hemochromatosis, data on the prevalence and the penetrance of the disorder are conflicting.

Design and Methods

A registry of patients with genetic hemochromatosis was established in the South of France and a regional health network was developed to allow the inclusion of all the diagnosed patients. C282Y homozygous patients classified in stages 2 (biological iron overload), 3 and 4 (clinical manifestations of iron overload, stage 4 being the more severe) according to the classification of the French National Authority for Health were included in the registry over a 6-year period.

Results

A total of 352 symptomatic C282Y homozygotes were identified, resulting in a total prevalence of 1.83 per 10,000 (95% CI: 1.63 to 2.02) in subjects over 20 years and 2.40 per 10,000 (95% CI, 2.15 to 2.65) among subjects of European descent. Among Europeans, the total calculated penetrance was 15.8% in stage 2 or higher, 12.1% in stage 3 or 4 and 2.9% in stage 4. The penetrance was slightly higher in males (18.7%) than in females (13.2%). It was 19.9% for individuals over 40 years of age (24.1% and 16.3% in males and females, respectively) with a maximum of 31% in subjects between 50 and 54 years old. Among 249 patients with complete records, 24% were in stage 2, the majority (58%) were in stage 3, and 18% in stage 4. There was a higher proportion of males, and excessive alcohol intake was more prevalent in stage 4 than in stages 2 and 3 combined.

Conclusions

A French Mediterranean regional hemochromatosis registry with strict inclusion criteria is a useful tool for characterizing the history of this disease, particularly for the most severely affected patients, as defined by the disease severity classification. The total prevalence of symptomatic C282Y homozygotes in the region was found to be low. However, clinical penetrance (stages 3 and 4) was not negligible.

Key words: registry, HFE hemochromatosis, prevalence, penetrance.

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Introduction

Genetic hemochromatosis is an autosomal recessive disorder, characterized by parenchymal iron overload due to increased iron absorption from the diet.[1] This definition explains many of the disease's characteristics, including the earliest known biological disorder: elevated transferrin saturation (TS). Clinical expression is polymorphic and includes non-specific signs such as general fatigue and arthropathies. Complications include hepatic disorders, diabetes, and cardiac and pituitary insufficiency. Most patients with hemochromatosis carry two copies of the C282Y mutation on the *HFE* gene.[2] Consequently, *HFE* hemochromatosis is the only type of genetic hemochromatosis with an epidemiological and public health impact. All other gene defects are rare or exceptional.

However, one of the main conclusions of epidemiological studies conducted in various countries in which the prevalence of *HFE* hemochromatosis is high is that not all individuals bearing the C282Y mutation develop a severe form of the disease as was initially thought. Indeed, many individuals remain asymptomatic throughout their entire life.[3,4] A few large epidemiological studies have indicated that the overall penetrance of the disease could be as low as 1% among subjects homozygous for C282Y.[5] The methodology used in these studies raised major criticisms, but it now obvious that the clinical penetrance of the C282Y homozygous (YY) genotype is incomplete. Thus, questions concerning the prevalence of various clinical stages and the clinical penetrance of severe forms of the disorder are still controversial.

In 2005, the French National Health Authority (*Haute Autorité de Santé* HAS) published guidelines for the management of *HFE* hemochromatosis, in which a classification of five clinical stages was proposed[6] (*http://www.has-sante.fr*). This classification is intended to describe and to follow the natural history of the disease. It also provides a prognostic approach towards the patients at diagnosis.[7] This report also highlighted a number of still unsolved questions in the field.

In order to try and answer these epidemiological questions, we chose to establish a registry of genetic hemochromatosis in the Languedoc-Roussillon (LR), a Mediterranean administrative region of South-East France. We had previously estimated the allele frequency of C282Y in this region.[8] The prospective and systematic inclusion of hemochromatosis patients in the registry over a 6-year period allowed us to estimate the prevalence of genetic hemochromatosis, both overall and according to gender and age. Clinical stage according to the HAS classification was also determined. The second objective of the study was to calculate the penetrance of *HFE* hemochromatosis in the same population.

Design and Methods

Population

We studied patients recorded in the registry of genetic hemochromatosis of the LR region, from March 1st, 2002 to June 30th, 2008. LR is an administrative region in the South of France, with a population of 1,928,682 million inhabitants over 20 years of age (data provided by INSEE, 2006, *http://www.insee.fr*).

Case definition and classification

We used the strict definition of epidemiological registries of patients proposed by the French National Registry Committee (1995) "*Continuous and exhaustive collection of personal data concerning one or more health event* [here, genetic hemochromatosis], *in a geographically defined population* [here, the LR region], *with aims of research and public health*". Criteria for inclusion in the registry were defined in 2001, according to the then recent consensus conference on hemochromatosis.[9] *HFE* genotype information was not mandatory, especially for older patients. When the *HFE* genotype was not available, a patient could be included if he/she had: (i) either typical clinical signs of genetic hemochromatosis and a liver biopsy with a liver iron distribution characteristic of genetic hemochromatosis; or (ii) typical clinical signs of hemochromatosis and more than 5 g (men) or 4 g (women) of iron removed by therapeutic phlebotomies. Among the last two groups of patients, a small number were shown to have other rare forms of *HFE* or non-*HFE* related genetic hemochromatosis, some of whom have been previously reported elsewhere.[10] However, only patients over 20 years of age, with clinical and/or biological signs of hemochromatosis and a C282Y homozygous *HFE* genotype were analyzed in the present study (Figure 1).

Patients were classified in stages from 0 to 4 depending on the clinical severity of their disease, according to Brissot *et al.* (2004).[6,7] The stages are defined in Table 1. Nine asymptomatic individuals (classified as stage 0 or 1) were excluded from analyses. Most of them had been recruited through family screening. Thirteen individuals whose stage could not be determined (due to lack of certain data) were also excluded (Figure 1).

Registry approval was obtained from the national ethical committee of the CNIL (*Commission Informatique et Liberté*). All the patients gave informed written consent to the inclusion of their data in the registry. Patients were removed from the registry in the event of loss to follow-up, death during the survey, withdrawal of consent or emigration from the LR region.

In order to obtain an accurate estimate of prevalence, data from hemochromatosis patients reported for inclusion in the registry for whom consent was not available were recorded anonymously in a separate "pre-inclusion" database.

Patient identification and inclusion

Multiple sources were regularly screened to identify all established or suspected cases of genetic hemochromatosis in the area. The identification of patients was enabled through the establishment of a regional network formed between Montpellier University Hospital (project coordinator) and all the regional health institutions or professionals involved in the follow-up of hemochromatosis patients. These included other hospitals, community clinics and blood banks (where phlebotomies are often performed), as well as specialists and general practitioners. The main sources of case identification were the two hematology laboratories of the university hospitals of Montpellier and Nîmes which performed *HFE* genetic tests. In addition, out-patient records and hospitalization reports over the previous 10 years were reviewed in all hospitals participating in the study, and patients with a diagnosis of hemochromatosis were listed. The Hospital Information System (HIS) enabled us to identify patients with a diagnosis of hemochromatosis hospitalized over the previous 3 years. Private practitioners from this area (gastroenterologists, internists, pathologists, endocrinologists, hematologists, private laboratories, as well as general practitioners) were contacted directly or via regional professional societies. Patients were also informed of the existence of the registry by the national patients' association (*Association Hemochromatose France*) and were encouraged to spontaneously give their consent to inclusion.

Data collection

The *enrollment questionnaire*, completed by practitioners or by a member of the coordination group, contained information on age, symptoms and circumstances at diagnosis, clinical and biological data, clinical complications, family history of hemochromatosis, morphological investigations including liver biopsy or magnetic resonance imaging, *HFE* genotype and phlebotomies (number, volume, duration).

Annual follow-up of patients included in the registry was performed from 2004 onwards by use of a standard short questionnaire sent by mail to the physicians. The status of each patient (dead or alive, still living in LR or not) was updated each year, together with the occurrence of complications and the frequency of phlebotomies.

Quality controls of the registry were set up by obtaining anonymous information on the number of patients declared with the disease, through Health Insurance Administrations of the area.

Statistical analysis

Prevalence rates were adjusted for age and sex by the direct method to the 2006 French population (INSEE). The penetrance was calculated as the proportion of individuals bearing the genotype who subsequently develop the phenotype (stage 2, 3 or 4). The 95% confidence intervals of the adjusted rates were calculated.[11] For categorical variables (stage, genotype…), the distribution was reported and comparisons were made using Pearson's χ^2 test. If the test was not valid, Fisher's exact test was used. For quantitative variables (age, age at diagnosis, duration), the mean and standard deviation are reported if the distribution was normal; if otherwise, the median and interquantiles (25-75%) are given. All analyses were two-tailed, with a *P* value of less than 0.05 considered statistically significant. The statistical analyses were carried out using SAS statistical software (Cary, NC, USA).

Results

Prevalence of symptomatic YY patients

In order to estimate the prevalence of symptomatic YY patients in the LR region, we pooled the patients included in the registry (n=249) and those who were notified but not included (no available consent/pre-inclusion database) (n=103) (Figure 1). There was no significant difference in age and gender between the two groups. Thus, the prevalence of symptomatic patients (stages 2, 3 and 4) among the regional population over 20 years of age was 1.83 per 10,000 [95% confidence interval (CI), 1.63 to 2.02]. The nationwide age- and sex-adjusted prevalence in France was 1.79 per 10,000 (95% CI, 1.61 to 1.99). When restricted to

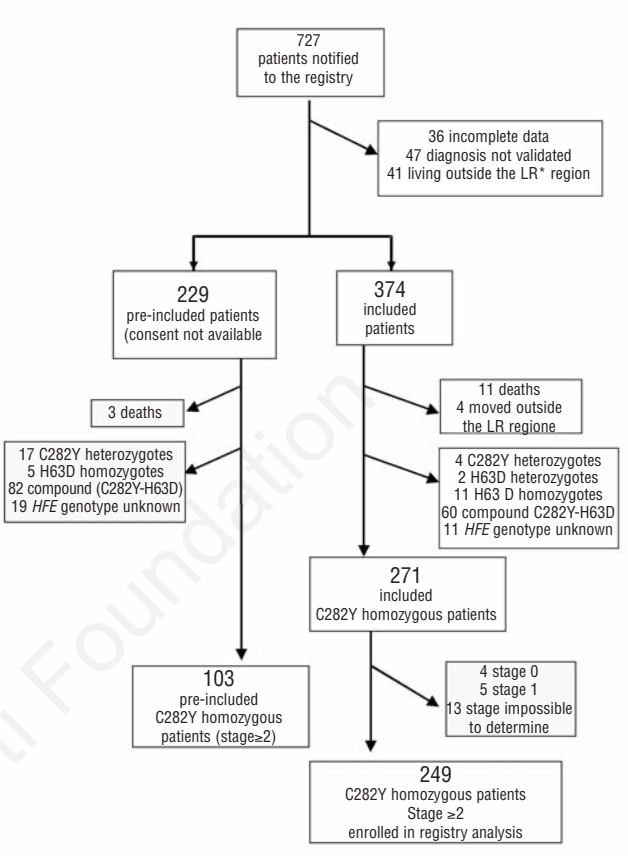

Figure 1. This flow chart starts with all the patients who were notified to the registry and describes those who fulfilled the inclusion criteria and were actually included. Due to the initial design of the registry, patients with clinical or biological signs of hemochromatosis were included regardless of *HFE* genotype. Among the included patients, the present study focuses on stage 2-4 C282Y homozygous subjects (n=249); 103 additional C282Y homozygous patients notified to and expecting inclusion in the registry, were combined in calculations in order to obtain a correct estimate of the prevalence of the disorder in the region. *LR : Languedoc-Roussillon (administrative region of Southern France) For *stage* definition see Table 1.

Table 1. Stages of severity of genetic hemochromatosis, according to the French HAS, of the YY patients studied.[6]

Stage	Definition*	All patients (n=249)	Males (n=144)	Females (n=105)	Sex ratio M/F	
2	Increased TS and SF levels (> 200 μg/L in females and > 300 μg/L in males) No clinical manifestation	59 (24%)	36 (25%)	23 (21.9%)	1.6	
3	Increased TS and SF Clinical manifestationwith no direct impact on survival (such as fatigue, arthropathies)	145 (58%)	73 (51%)	72 (68.6%)	1.01	*P*=0.004
4	Increased TS and SF Life threatening clinical manifestation with impact on survival (such as diabetes, chronic liver disease, cirrhosis, cardiomyopathy)	45 (18%)	35 (24%)	10 (9.5%)	3.5	

*TS: transferrin saturation, SF: serum ferritin. *Patients in stage 0 (YY genotype but clinically and biologically asymptomatic) (n=4) and patients in stage 1 (with only increased TS) were excluded from the study.*

individuals of European descent (76% of the population of the region),[8] the prevalence was 2.40 per 10,000 (95% CI, 2.15 to 2.65).

Penetrance estimates

We previously showed that the allele frequency of the YY genotype in the region was 0.039 among individuals of European origin (95% CI, 0.03 to 0.048).[8] Using this datum and according to the Hardy-Weinberg equilibrium, the expected number of YY subjects over 20 years of age was 2229 [95% CI, 1319 to 3377]. As 352 YY symptomatic (European) patients (in stage 2 and higher) were identified in the area, these figures anabled the evaluation of the range of total penetrance of the YY genotype in the European population of the region, which was estimated at 15.8% (10.4 to 26.7% according to the 95% CI for the number of YY subjects). Penetrance was slightly higher among males [18.7% (95% CI, 12.4-31.7%)] than among females [13.2% (95% CI, 8.7-22.2%)]. Important differences were observed according to age, with a markedly lower penetrance at 7.2% (95% CI, 4.8-12.2%) for individuals under 40 years of age. For the population over 40 years old, the total penetrance for stage 2 and higher, was 19.9% (95% CI, 13.1-33.6%); penetrance was 24.1% (95% CI, 15.9-40.8%) and 16.3 (95% CI, 10.7-27.5%) in males and females respectively. The maximum penetrance, at 31.0% (95% CI, 20.5-52.4%), was observed in patients between 50 and 54 years of age.

Description of the registry population

The general description of YY patients in stage 2 and higher included in the registry is given in Table 2. The mean age at diagnosis was 49.7 years (±13.6) and there was a slightly lower prevalence of females (sex ratio M/F: 1.4). The diagnosis of hemochromatosis was made following the onset of clinical manifestations and/or during family screening in 86.1% and 34.0% of the cases, respectively. However, a family history of hemochromatosis or iron overload was reported by 42.2% of patients. Fatigue and joint manifestations were the most frequent clinical signs, found in 64% and 46% of the patients, respectively, whereas liver disease, diabetes and skin manifestations were observed in less than 20% of cases.

Comparison between genders

Clinical and biological variables were compared in males and females, showing a significantly higher frequency of diabetes (P=0.002) and hepatic disease (P=0.002), including chronic liver disease (cirrhosis, fibrosis and hepatocellular carcinoma) (P=0.02) or cirrhosis alone (P=0.04), in males (Table 2). This resulted in a higher proportion of males in stage 4 in which the male to female sex ratio was 3.5 (P<0.0001). As a possible aggravating factor, excessive alcohol intake (>40 g/day) was found for five males and for only one female (P=0.03).

Classification into stages

Patients included in the registry were classified according to the French HAS staging system.6 Twenty-four percent were in stage 2 (preclinical stage), whereas 76% had clinical manifestations: the majority (58%) were in stage 3 and 18% were in stage 4, presenting with the most severe manifestations (Table 1).

For subjects of European origin, the penetrance of stage 4 was 2.9%, whereas the penetrance of stages 3 and 2 was 9.2% and 3.7%, respectively.

Comparison between genders within stage 4 (n=45) showed a lower age at diagnosis for males than for females (51.3±13.2 years and 62.4±13.1 years, respectively; P=0.02). There were no other differences within this group between males and females except for cardiac complications (cardiomyopathy, severe arrhythmia), which were more frequent among females (55.6% in females, 12.9% in males, P=0.01).

Discussion

In an attempt to describe the natural history of hemochromatosis, the French HAS has published a disease severity classification,[6] which follows the putative disease evolution and can be helpful to evaluate patients' risk. This classification clearly separates biological stages (stages 1 and 2) from clinical stages (stages 3 and 4) of the disease and enables evaluation of the biological and clinical penetrance of the disorder. We used this classification in order to calculate disease penetrance among patients whose data were recorded in a Southern French registry of hemochromatosis. To our knowledge, this is the first registry based on the systematic inclusion of patients with hemochromatosis in a delineated geographic area over a period of time, thus complying with the definition of "registries" according to the French "Registry committee" with rigorously defined inclusion criteria and patients' follow-up.

We found that the prevalence of symptomatic YY patients (stage 2 or higher) is 1.83 per 10,000 in the adult population (>20 years) (2.40 per 10,000 among Europeans). The population of the region studied is characterized by a high level of ethnic heterogeneity. This was shown by a study we performed in 2001 on a sample of 1276 neonates, in order to determine the incidence of *HFE* mutations in the region.8 In this study, we were able to obtain the origin of the grandfathers and grandmothers of each newborn. We found that up to 24% of the studied population had a non-European descent and originated from countries in which the *HFE* C282Y mutation is rare.[8] On the other hand, the allele frequency of the C282Y mutation was 0.039 among people of European descent, which can be considered relatively low compared to that in other regions of France, such as Brittany (YY frequency, 0.5%).[12] The prevalence of the YY genotype and prevalence estimates of symptomatic hemochromatosis (stages 2, 3 and 4) determined from the registry, allowed the total penetrance of the YY genotype in the European population of the region over 20 years old to be estimated at 15.8%. Penetrance was estimated to be 19.9% in patients over 40 years of age and was higher for men (24.1%) than for women (16.3%).

The prevalence and the penetrance of hemochromatosis have been and are still a matter of debate. Soon after the description of the *HFE* gene, evidence was published indicating that the clinical penetrance of the YY genotype was incomplete.[3] Beutler *et al.*[5] claimed that this penetrance was as low as 1% among YY subjects in a large cohort of mainly American Caucasians. Although this claim was supported by data from other epidemiological studies,[16] it led to years of controversy,[13-15] particularly since Beutler *et al.* may have excluded patients who had received a previous diagnosis of hemochromatosis. Allen *et al.*[17] recently published figures showing a penetrance of nearly 30% in men and 1.2% in women in a prospective cohort of subjects volun-

tarily enrolled to study diet and other lifestyle factors and their influence on the development of chronic diseases. One of the main sources of bias for such large epidemiological studies is the fact that they rely on surveillance of healthy populations and may exclude affected individuals. Another difficulty concerns disease description and criteria used to define penetrance.[18] Beutler *et al.*[5] argued that clinical manifestations such as fatigue or arthropathies are non-specific and are so common in the general population that they can coexist with a genetic background of hemochromatosis, without any direct link to the disease itself. Allen *et al.*[17] defined hemochromatosis-related iron overload on the basis of severe manifestations such as cirrhosis, liver fibrosis or hepatocellular carcinoma, which correspond to stage 4 conditions in our study. However, additional criteria were used to define documented iron overload, such as elevated aminotransferase levels, physician-diagnosed symptomatic hemochromatosis and arthropathy of the second and third metacarpophalangeal joints (included in stage 3 of the HAS classification). These criteria, except for the last one, are not unequivocal. Schematically, in the study by Allen *et al.*,[17] patients referred to as having documented hemochromatosis-related iron overload correspond to patients in stages 3 and 4 in our study. The total penetrance (males and females) found in this group was 29.6%, close to the previous estimates for Picardy[19] and Britany.[20] Our estimate for stages 3 and 4 combined is lower, but remains above 10% (12.1%). For stage 4 of the HAS classification, the calculated penetrance in the population of the region was lower, at 2.9%.

Most of the above cited studies, in which prevalence and penetrance of the YY genotypes were evaluated, focused on Northern European populations and voluntarily excluded other populations. The present study specifically targets a European population of mainly Mediterranean origin. In this population we found lower prevalence and penetrance than in northern Europeans. However our figures reflect minimum estimates as some factors may have contributed to underestimation of prevalence and penetrance of symptomatic hemochromatosis in this study. First, the number of symptomatic patients may be underestimated, as is the case in any attempt to perform a census of all affected patients with such a polymorphic disorder. Despite the establishment of a registry according to strict criteria and

Table 2. Characteristics at inclusion of the 249 YY patients (stage 2 or more) enrolled in the registry.

	Total	Males	Females	*P* value
Gender	(n=249)	144 (58%)	105 (42%)	NS
Sex ratio	1.37			
Age at inclusion (years)	(n=249)	(n=144)	(n=105)	NS
Mean (± SD)	54.0 (±13.2)	54.0 (±13.9)	54.0 (±12.7)	
Age at onset (years)	(n=248)	(n=144)	(n=105)	NS
Mean (± SD)	49.7 (±13.6)	48.8(±13.1)	50.9 (±14.1)	
Duration of the symptoms (years)	(n=248)	(n=144)	(n=104)	NS
Mean (± SD)	4.32 (±7.0)	5.3 (±8.4)	2.9 (±4.0)	
Transferrin saturation at diagnosis (%)	(n=207)	(n=112)	(n=95)	NS
Mean (± SD)	77.6 (±14.9)	79.5 (±13.7)	75.5 (±16.0)	
Serum ferritin at diagnosis (µg/L)	(n=227)	(n=126)	(n=101)	<0.0001
Median (IQ25-75)	1538 (525-1818)	1335 (830-2170)	538 (371-1190)	
Clinical manifestations				
- Fatigue	150 (64 %) (n=236)	70 (68.6%) (n=134)	80(59.7%) (n=102)	NS
- Joint manifestations	110 (46%) (n=240)	69 (50%) (n=138)	41 (40.2%) (n=102)	NS
- Liver disease	41 (17%) (n=237)	33 (23.6%) (n=140)	8 (8.2%) (n=97)	0.002
cirrhosis	20 (9%) (n=231)	16 (11.9%) (n=135)	4 (4.2%) (n=96)	0.04
hepatocarcinoma	5 (2.2%) (n=229)	5 (3.8%) (n=133)	0 (0%) (n=96)	NS
- Diabetes	31 (13%) (n=232)	26 (19.1%) (n=136)	5 (5.2%) (n=96)	0.002
- Skin manifestations	23 (10%) (n=233)	17 (12.6%) (n=135)	6 (6.1%) (n=98)	NS
- Heart manifestations	9 (4%) (n=226)	4 (3.0%) (n=132)	5 (5.3%) (n=94)	NS
- Pituitary insufficiency	9 (4%) (n= 216)	6 (4.8%) (n=124)	3 (3.3%) (n=92)	NS
Stage	(n=249)	(n=144)	(n=105)	
2	59(24%)	36 (25%)	23 (21.9%)	0.004
3	145 (58%)	73 (50.7%)	72 (68.6%)	
4	45 (18%)	35 (24.3%)	10 (9.5%)	
Phlebotomies (at inclusion)	(n=239)	(n=140)	(n=99)	0.004
Yes*	167 (70%)	108 (77.1%)	59 (60%)	
Calculated penetrance (%)				
Stage ≥2	15.8 (95%CI, 10.4-26.7)	18.7 (95%CI, 12.4-31.7)	13.2 (95%CI, 8.7-22.2)	
Stage 2	3.7 (95%CI, 2.5-6.3)			
Stage 3	9.2 (95%CI, 6.1-15.5)			
Stage 4	2.9 (95%CI, 1.9-4.9)			
Age >40	19.9 (95%CI, 13.1-33.6)	24.1 (95%CI, 15.9-40.8)	16.3 (95%CI, 10.7-27.5)	

the development of a wide regional network, some patients may have been missed. Indeed, patients with hemochromatosis may not necessarily enter a specific medical framework and some of them may escape recruitment. In the context of an official registry, some may refuse to give consent, which in France is mandatory for inclusion, but in our experience this was a very uncommon situation. Additionally, the diagnosis may be missed in certain patients with either mild (stage 2) or very severe symptoms (end stage-liver disease) due to the lack of specificity of clinical manifestations. It is worth noting that during our regional survey, the diagnosis of three patients was made at the terminal stage of disease, revealed by hepatocellular carcinoma.

In this study, we found only a slight difference (not statistically significant) between men and women for total penetrance for stages 2, 3 and 4 combined, (18.7% and 13.2%, respectively). In contrast, Allen *et al.*[17] found a penetrance of 28.4% for men and only 1.2% for women for hemochromatosis-related iron overload. Other studies have reported differences between genders but not to such a large extent.[19,20] Nevertheless, in our population, there was a clear majority of men (78%) in the more severe stage 4 (male to female ratio of 3.5) which corresponds to the majority of the patients reported by Allen *et al.* There was also a higher proportion of males (61%) in the biological stage 2. The proportion of male and female stage 3 patients included in the registry was similar (male to female ratio of 1.01), with no clear explanation.

This study provides a novel approach towards estimating the clinical and biological prevalence of hemochromatosis due to the YY genotype, by means of a regional registry. It indicates that the minimum prevalence of symptomatic hemochromatosis, with either biological iron overload (stage 2), or "mild" and "severe" clinical expression (stage 3 and 4, respectively), is relatively low in this Mediterranean region (2.4 per 10,000 among Europeans). However, the clinical penetrance (stages 3 and 4) was 12.1%, whereas the penetrance of the most severe manifestations (stage 4) was 2.9%, with a large predominance of men (78%).

It is currently impossible to predict whether a patient diagnosed at an early stage of the disease will develop severe manifestations if left untreated. This reinforces the need for new biological markers which can help define prognostic criteria in YY subjects and thus predict disease evolution.

Authorship and Disclosures

PA-M designed the study, conducted the research and wrote the paper; MB participated in the design of the study, examined and included patients, and wrote the paper; FB, PB, OD, CJ, AK, DL, ALQ, IR, ER, and JFS participated in the design of the study, examined and included patients, and read and approved the paper; JR participated in the design of the study, and analyzed liver biopsies; SC, MG-B, and FR managed the database, and read and approved the paper; TM, and PD participated in the epidemiological design and statistical analysis; MCP designed the study, performed the epidemiological analysis and wrote the paper.

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