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The *HFE* Gene Is Associated to an Earlier Age of Onset and to the Presence of Diabetic Nephropathy in Diabetes Mellitus Type 2

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We initiated the present work to determine whether the presence of the *HFE C282Y* or *H63D* mutations could be related to the clinical expression of diabetes mellitus type 2. Two hundred and twenty five type 2 consecutive diabetic patients were included and the HFE genotypes were determined. Younger ages of onset of diabetes as well as a longer duration of the disease were detected in patients carrying at least one *C282Y* allele ($p = 0.007$). An increased prevalence of retinopathy ($p = 0.014$) and of nephropathy ($p = 0.04$) were also detected in individuals carrying at least one *C282Y* allele in comparison with patients carrying the other alleles. The increased prevalence of retinopathy in *C282Y* carriers is related to the increased duration of the disease, but we not have detected that the prevalence of nephropathy is associated with the duration of the disease. However, multivariate logistic regression confirms that the prevalence of nephropathy is higher in the group of patients carrying at least one *C282Y* allele or the *H63D/H63D* genotype as compared to the group of patients with the wild-type (N/N) or the *N/H63D* genotype. To our knowledge our study is the first one to report an earlier age of onset in type 2 diabetic patients carrying *HFE* mutations.

Key Words: Diabetes; HFE; C282Y; H63D; nephropathy; retinopathy.

Introduction

It has been known for a long time that between 50% and 80% of patients with hemochromatosis have type 2 diabetes *(1–3)*. More recently, it was discovered that the main cause of hemochromatosis in individuals of European ancestry is the presence of mutations in the *HFE* gene *(4)*. Therefore, it could be expected that the frequency of *HFE* gene mutations could be increased among type 2 diabetes patients. However, while some studies did report an increased frequency of *HFE* mutation in type 2 diabetic patients *(5–11)*, other studies failed to detect such association *(9,12–20)*. Thus, the overall interpretation of these studies is that the presence of *HFE* mutations is not a major cause of type 2 diabetes.

A quite different issue is whether the presence of *HFE* alleles could affect the clinical expression of type 2 diabetes. Type 2 diabetic patients with *HFE* mutations have been described to have increased iron parameters *(21)*, increased frequency of diabetic nephropathy *(8)*, and lower systolic blood pressure *(22)*. These correlations could be either due to minimal dysfunction of iron metabolism in carriers of *HFE* alleles or to an association of the detected alleles with genes closely linked to *HFE*. Individuals with a low penetrance *HFE* genotype (*C282Y/H63D*) or heterozygous *C282Y* carriers have been reported at increased risk for different disorders *(23,24)*.

We have previously shown that HFE mutations are also present in most of hemochromatosis patients from our population *(25–28)*. Subsequently, we have also detected in 5370 blood donors that the C282Y *HFE* allelic frequency is $3.16 \pm 0.46\%$, and that the allelic frequency of the H63D mutation is $20.8 \pm 1.09\%$, which is among the highest in the world *(28)*. Biochemical expression of iron overload was detected in 80% of C282Y homozygous males in Spain *(28)*. The Hardy–Weinberg equilibrium predicts that, in our population, 1 out of every 16 individuals is a heterozygous carrier of the C282Y mutation, 1/1004 is a C282Y homozygote, 1/3 is a heterozygous carrier of the H63D mutation, 1/23 is a H63D homozygote, and 1/72 is a compound heterozygote *(28)*. However, the prevalence of type 2 diabetes in Spain ranges between 3.1% and 7.5% *(29–31)*. This value is much larger than the predicted expression of hemochromatosis derived from the prevalence of C282Y homozygotes in

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Table 1
HFE Genotypes Detected in Type 2 Diabetic Patients and Basic Clinical Data

	Genotype				
	N/N	*H63D/N*	*H63D/H63D*	*C282Y/C282Y* or *C282Y/H63D* or *C282Y/N*	*p*
Number	129 (57.3)[a]	71 (31.6)	15 (6.7)	10 (4.4)	
Gender (Male)	82 63.6%	35 49.3%	7 46.7%	5 50.0%	$\chi^2 = 4.81^{\dagger}$ $p = 0.184$
Age[b]	64.51 ± 0.90	62.52 ± 1.21	70.06 ± 2.64	63.80 ± 3.24	$F = 2.32^{\ddagger}$ $p = 0.076$
Age at Diagnosis[b]	51.50 ± 1.03	50.22 ± 1.39	59.20 ± 3.02	§[B]43.10 ± 3.71*	F = 4.10 $p = 0.007$
Years after diagnosis[b]	13.17 ± 0.84	2.25 ± 1.23	10.53 ± 2.44	20.80 ± 2.99	F = 2.76 $p = 0.043$
Blood hypertension	67 54.5%	33 47.1%	10 71.4%	7 70.0%	$\chi^2 = 4$ $p = 0.253$
Diabetic retinopathy	30 26.1%	15 24.2%	2 15.4%	7 70.0%	$\chi^2 = 10.5$ $p = 0.014$
Diabetic nephropathy	37 34.6%	12 20.7%	6 60.0%	5 62.5%*	$\chi^2 = 10.5$ $p = 0.014$
Fasting C-Peptide levels[b]	2.67 ± 0.26	2.93 ± 0.31	2.12 ± 0.86	1.16 ± 1.05	F =1.04 $p = 0.381$
Plasma creatinin levels[b]	1.38 ± 0.12	1.29 ± 0.16	1.09 ± 0.34	1.63 ± 0.43	F = 0.387 $p = 0.762$
Microalbuminuria[c]	15.30 4.02–131.5	10.30 4.10–25.00	54.60 24.50–145.90	101.90 55.42–419.00	χ^2(KW) = 6.8[¶] $p = 0.077$

[a]Numbers in parenthesis indicate percentage of total.
[b]Expressed as mean ± standard error.
[c]Expressed as median and 25–75 percentile interval.
*p value < 0.05.
[†]χ^2 chi square or Fisher exact test.
[‡]F ANOVA test by genotype.
§[B] Bonferroni correction.
[¶]χ^2(KW) Kruskal–Wallis test.

our general population (1/1004) or from the prevalence of other *HFE* genotypes *(28)*. Thus, it is clear that the presence of *HFE* mutations is an infrequent cause of type 2 diabetes in Spain *(16)*. However, we were intrigued by our particularly high population prevalence of *H63D* alleles that, together with the *C282Y* allele, predicted a substantial proportion of low penetrance genotypes. Thus, we undertook the present work in order to determine whether the presence of the *HFE C282Y* or *H63D* alleles in our population could be related to some aspects of the clinical expression of diabetes mellitus type 2.

Results

The clinical data of the type 2 diabetic patients as a function of the *HFE* genotypes is shown in Table 1. The univariate analysis (Table 1) shows a significantly earlier age at diagnosis and a longer duration of the disease in patients harboring the *C282Y* allele. These two variables are interdependent so that a younger age at diagnosis correlates with a longer evolution time ($r = -0.55, p < 0.01$). The multivariate analysis confirms that the age at diagnosis is significantly different and younger for genotypes with the *C282Y* allele, and these differences are present equally in both sexes and are not influenced by any other variable.

Univariate analysis also shows differences among genotypes in the clinical expression of retinopathy and nephropathy, these being more frequent in individuals with at least one *C282Y* allele (Table 1). However the multivariate logistic regression models only confirm a significant difference for nephropathy (Fig. 1). To reach this conclusion it has been necessary to collapse all subjects into two groups: N/N plus *H63D/N* in one group, and *H63D/H63D*, *H63D/C282Y*, *N/C282Y*, and *C282Y/C282Y* in the other group. Patients with genotypes other than N/N or *H63D/N* have an increased risk of expressing nephropathy (odds ratio = 3.72; CI 95%: 1.36–10.19, $p = 0.01$). Retinopathy is associated with a longer duration of the diabetes.

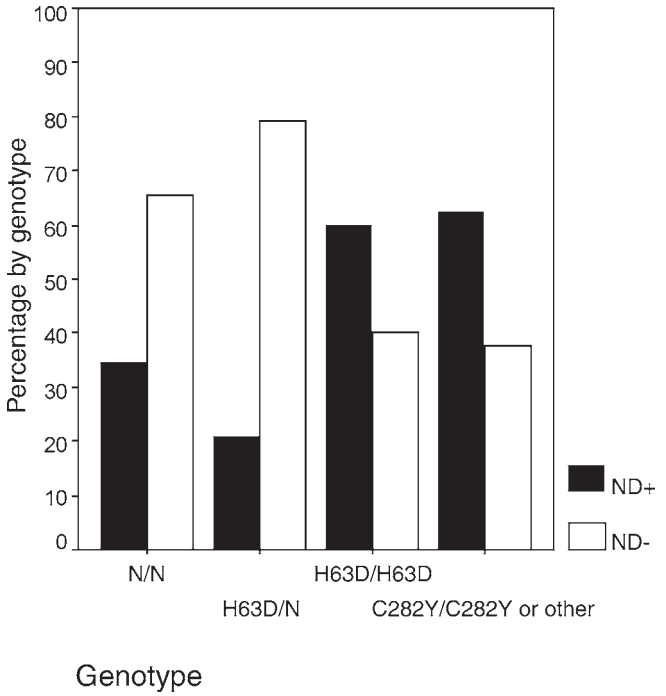

Fig. 1. Distribution of type II diabetic patients stratified by HFE genotypes according to the presence (ND+) or absence (ND–) of nephropathy.

Discussion

In this article we report an earlier age of onset and an increased incidence of nephropathy in type 2 diabetic patients who are carriers of at least one *C282Y HFE* allele or have the *H63D/H63D* genotype. Logistic regression demonstrates that the increased incidence of nephropathy is not only related to the increased duration of diabetes but it is associated with the *HFE* genotype. In contrast, an increased incidence of retinopathy is also detected in diabetic patients who are carriers of at least one *C282Y HFE* allele, but in this case the association is entirely due to the longer duration of the diabetes in patients with the *C282Y* allele.

Our observation of increased nephropathy associated with the *HFE H63D/H63D* and *C282Y* genotypes (Fig. 1) is consistent with the observation in the Polish population of a nonsignificant tendency for increased *C282Y* patients with nephropathy and a significant association with the *H63D* allele *(8)*. Type 2 diabetic patients with *HFE* mutations have been described to have increased iron parameters *(21)* indicating dysfunction of iron metabolism. Increased tubular lysosomal iron concentration has been found in patients with diabetic nephropathy *(32)*. Thus, it is plausible that minimal dysfunction in iron metabolism caused by the presence of *HFE H63D/H63D* and *C282Y* genotypes could lead to the increased diabetic nephropathy detected in the present study.

A lower systolic blood pressure has been described in individuals who are carriers of *HFE* mutations *(22)*, but we have not found any association with the presence of hypertension (Table 1).

To our knowledge our study is the first to report an earlier age of onset and an increased evolution time in type 2 diabetic patients carrying *HFE* mutations. This observation could be consistent with the increased risk for developing type 2 diabetes observed in individuals carrying *HFE* mutations *(5–11)*. An earlier age at onset is commonly observed associated with the presence of genetic risk factors in many different diseases *(33,34)*. The findings reported in this article should be extended in other studies addressing the potential association between the presence of *HFE* alleles and type 2 diabetes.

Materials and Methods

Patients

The total study population included 225 unrelated Spanish (or Caucasian) type 2 diabetic patients that were consecutively selected from our outpatient clinic between 1999 and 2002 and fulfilled the World Health Organization criteria for type 2 diabetes *(35)*. Informed consent was obtained from each individual and approved by the Ethical Committee of the Hospital Clinic of Barcelona.

After inclusion, a total of 41 clinical and laboratory data were collected for each patient from the clinical charts. However, only the following variables were considered and analyzed in the present study: gender, age, age at diagnosis, duration of the disease, blood hypertension, retinopathy, nephropathy, fasting C-peptide levels in plasma, plasma creatinin, and microalbuminuria.

Hypertension was defined as the presence of hypertension treatment or blood pressure levels at control visits over 130 mmHg (systolic) and/or 85 mmHg (diastolic). Diabetic retinopathy was assessed by a yearly ophthalmologic evaluation. The diagnosis of diabetic nephropathy required at least the presence of microalbuminuria (annual determination of microalbuminuria in 24 h urine collection). Microalbuminuria was considered positive when two different determinations above 20 μg/24 h were detected. Plasma creatinine levels (mg/dL) are the mean of creatinine levels measured during the last year of the follow-up. Fasting C-Peptide levels in plasma were expressed in ng/mL. Plasma C-peptide levels were assayed by radioimmunoassay (Byk-Sangtec Diagnostica, Dietzenbach, Germany) with an intra- and interassay CV of 2.6% and 4.4%, respectively. Creatinine was determined with an ADVIA 1650 BAYER instrument using the Jaffè method without deproteinization. Microalbuminuria was detected with the ADVIA 1650 BAYER instrument using the Olympus Corporation specific antibody by turbidimetry.

Genetic Analysis

DNA was isolated from blood and the *HFE* genotype was determined by PCR amplification and RFLP analysis with *RsaI* for the *C282Y* mutation and with *Dpn*II for the *H63D* mutation *(25–28)*.

Statistical Analysis

In order to increase the statistical power, the genotype variable has been recoded by grouping together the genotypes carrying at least one *C282Y* allele (*C282Y/N, C282Y/H36D*, and *C282Y/C282Y)*. The data for the genotype groups are presented as mean ± standard error if the variables are quantitative and symmetrical, with median and 25 and 75 percentiles if the variables are quantitative and asymmetrical, and with percentages if the variables are qualitative. Mean and median values were analyzed using ANOVA models and the Kruskal–Wallis test. A chi-square test was used to compare the frequency of clinical expression of disease based on the evolution time and gender.

Multivariate logistic regression was performed taking into account the effect of gender, age, and years of disease evolution. ANCOVA models were used to evaluate differences in the evolution time between taking into account the effect of gender and age. To evaluate differences in the biochemical variables between genotypes, we used ANCOVA models that take into account the effect of gender, age, and evolution time. Multiple comparisons were carried out with a Bonferroni correction. Statistical analyses were undertaken using the statistical package SPSS 11 with a type I error with an alpha = 0.05.

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