HFE C282Y Mutation as a Genetic Modifier Influencing Disease Susceptibility for Chronic Myeloproliferative Disease

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Abstract

Iron metabolism has been implicated in carcinogenesis and several studies assessed the potential role of genetic variants of proteins involved in iron metabolism (*HFE* C282Y, *TFR* S142G) in different malignancies. Few reports addressed this issue with relation to chronic myeloproliferative disorders (CMPD). The aims of our study were (*a*) to examine the potential associations of CMPD development with genetic modifiers of iron metabolism in a large cohort of CMPD patients; (*b*) to examine associations of genetic variants of proteins involved in iron metabolism; and acquired *JAK2* V617F mutation with clinical characteristics of CMPD. *HFE* C282Y was genotyped in 328 CMPD patients and 996 blood donors as controls, *HFE* H63D, and *TFR* S142G were tested in CMPD patients and 171 first time blood donors. *JAK2* V617F mutation was tested in CMPD patients and in 122 repeated blood donors. Decreased C282Y allele frequency (allele frequency ± 95% confidence interval) was found in the CMPD group (1.8% ± 1.0%) compared with controls (3.4% ± 0.8%; $P = 0.048$). *TFR* S142G allele frequency was reduced among V617F-negative CMPD patients (34.8% ±7.6%) compared with controls (47.8% ± 5.4%; $P = 0.02$). The frequency of *JAK2* V617F was 75.9% (249 of 328) in the CMPD group. At presentation, elevated hemoglobin levels were found in V617F-positive patients compared with V617F-negative counterparts ($P < 0.000$). Vascular complications (26.6% versus 15.2%; $P = 0.039$) as well as female gender (57.4% versus 41.8%; $P = 0.019$) were more common in V617F-positive patients. We found that *HFE* C282Y might be associated with a protective role against CMPD. Because chronic iron deficiency or latent anemia may trigger disease susceptibility for CMPD, *HFE* C282Y positivity may be a genetic factor influencing this effect. (Cancer Epidemiol Biomarkers Prev 2009;18(3):929–34)

Introduction

Iron metabolism is generally suspected to play a role in carcinogenesis by influencing disease susceptibility or disease progression because free iron may induce oxidative stress and DNA damage (1). Genetic variants of proteins involved in iron metabolism have been widely tested in different solid tumors and hematologic malignancies. *HFE* gene point mutations (C282Y, H63D, and S65C) in homozygous or compound heterozygous forms are responsible for the iron overload disorder, hemochromatosis type 1. Although *HFE*-related hemochromatosis is inherited in a recessive manner, heterozygous carriers were shown to have elevated iron parameters (serum iron and ferritin levels, transferrin saturation) without progressive iron overload (2-6). Heterozygous *HFE* carriership has been implicated as a risk factor for several clonal disorders such as hematologic malignancies and solid tumors (1, 7-12). Transferrin receptor (TFR) is also a key element of the regulation of iron homeostasis. The S142G variant of *TFR* was reported to influence cancer susceptibility (colon, breast, myeloma) in combination with distinct *HFE* genotypes (13). The effects of iron homeostasis, and *HFE* and *TFR* genotypes were extensively studied in several solid tumors even in large patient cohorts (7-12, 14-18), but only a few studies with small patient cohorts tested the potential role of *HFE* in chronic myeloproliferative disorders (CMPD), a clonal disorder of the main iron using tissue of the body (19-21).

BCR-ABL–negative CMPD is classified as polycythemia vera (PV), essential thrombocythemia (ET), and chronic idiopathic myelofibrosis (CIMF) on the basis of clinical presentation. A single, common causative genetic alteration, an acquired *Janus kinase 2* (*JAK2*) V617F mutation has recently been identified in the background of each of the above disorders (22-25). The specific etiology of CMPD is unknown and obvious risk factors (irradiation, viral infections) have not been identified (26). Regular, artificial blood loss due to multiple blood donations was also reported in a surprising excess in the previous medical history of PV patients (27, 28). It is

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conceivable that regular blood loss causing chronic iron deficiency or mild anemia may enhance the formation of CMPD as a consequence of long-term stimulated state of erythropoiesis. *HFE* C282Y heterozygosity is reported to be protective against iron deficiency (2-6). C282Y heterozygous individuals show elevated hemoglobin (Hb) levels (3, 6). As the *JAK2* V617F mutation causes different clinical phenotypes, both PV and ET, additional modifier factors such as iron homeostasis have also been suggested to play a potential role in determining whether V617F-positive CMPD manifests as PV or ET (29).

The aims of our study were the following: (*a*) to examine the potential associations of CMPD development with genetic variants of proteins involved in iron metabolism (*HFE* C282Y, H63D, S65C, and *TFR* S142G single nucleotide polymorphisms); (*b*) to examine associations of inherited *HFE* C282Y, H63D, S65C, and *TFR* S142G variants and acquired *JAK2* V617F mutation with distinct clinical characteristics of CMPD; and (*c*) to test the frequency of V617F mutation among repeated blood donors.

Subjects and Methods

Subjects. Patients (328) with BCR-ABL–negative CMPD (152 males and 176 females) were enrolled in the study between 2005 October and 2006 April. The majority (n = 270) of this group was treated at the Department of Hematology and Stem Cell Transplantation of National Medical Center, whereas 58 patients were from the Department of Hematology, Kaposi Mor Hospital. The mean age of disease onset was 58 ± 14 y (interval, 16-87 y). One hundred seventy-five patients suffered from PV, 126 patients from ET, and 27 patients from CIMF diagnosed according to the WHO criteria. The presence of Philadelphia chromosome (BCR-ABL fusion gene) was excluded in all cases by standard karyotyping, fluorescence *in situ* hybridization or reverse transcription PCR methods. Laboratory (Hb, WBC, and PLT) and clinical features (splenomegaly) at presentation or at the time of CMPD diagnosis were collected retrospectively. The median follow up was 69 ± 63 mo (interval, 0-313 mo). The occurrence of thrombotic episodes, myelofibrotic, or leukemic transformation were recorded if they were present before the diagnosis or during the follow up.

The control group consisted of 996 voluntary blood donors (0-95 previous blood donations, 694 males and 302 females) with a mean age of 36 ± 12 y (interval, 18-65 y). The majority (n = 694) of donors were recruited between 1999 April and October in Budapest (Hungary), whereas 302 donors were from Szeged (Hungary) in the framework of the Hungarian National Blood Transfusion Service. All donors were tested for *HFE* C282Y as reported previously (30). One hundred seventy-one first time voluntary blood donors out of the above 996 (0-1 previous blood donations, 90 males and 81 females) with a mean age of 24 ± 7 y (interval, 18-60 y) were additionally tested for *HFE* H63D and *TFR* S142G. One hundred twenty-two repeated blood donors (32-95 previous blood donations, 93 males and 29 females) with a mean age of 47 ± 8 y (interval, 24-65 y) were investigated for the presence of *JAK2* V617F mutation. Blood donors were interviewed by a physician employee of the blood transfusion service on each occasion to exclude major diseases in their previous history such as malignant disorders or infections.

The participants signed informed consents, and the study was approved by the Institutional Ethics Committee.

Methods. Whole genomic DNA was isolated from anticoagulated peripheral blood with Puregene Gentra DNA Isolation kit.

HFE sequence variants [C282Y (exon 4, c.1066G >A, substitution of Cys282 to Tyr, rs1800562), H63D (exon 2, c.408C >G, substitution of His63 to Asp, rs1799945), and S65C (exon 2, c.414A >T, substitution of Ser65 to Cys, rs1800730)] were investigated by LightCycler technology (Roche Diagnostics) applying melting curve analysis with the hybridization probe detection format. The *TFR* S142G polymorphism (exon 4, c.687A >G substitution of Ser142 to Gly, rs3817672) was investigated with *Bsh*NI PCR-RFLP (13).

The *JAK2* gene V617F mutation (exon 12, c.1849G >T, substitution of Val617 to Phe) was detected by allele specific multiplex PCR (22) in the CMPD patient cohort and among the repeated blood donors (n = 122). To increase the sensitivity and reproducibility of the allele specific PCR technique, the minor modifications were introduced as follows: for the PCR-reaction, 0.5 μmol/L reverse and mutation-specific oligonucleotide primers with 0.15 μmol/L control forward primer were used. The PCR consisted of 36 cycles (94°C 1 min, 58°C 1 min, 72°C 1 min). In the case of V617F-positive repeated blood donor, the V617F mutational status was confirmed by PCR-*Bsa*XI-RFLP (22).

Statistical Analyses. *HFE* and *TFR* single nucleotide polymorphism allele frequencies (AF) are presented as % ± 95% confidence interval (95% CI). The Fischer's exact test or the χ^2 test (univariate analyses) were used to compare the frequencies of *HFE* genotypes between the blood donor and patient groups. Odds ratio (OR) and 95% CI values were estimated for each *HFE* genetic variant. Laboratory parameters such as Hb levels, WBC count, and platelet count (PLT) are presented as mean ± SD. The Mann-Whitney test and multinomial logistic regression were used to compare laboratory parameters observed in *JAK2* V617F–positive and *JAK2* V617F–negative CMPD patients. The Fischer's exact test was used to compare the frequencies of different complications between the *JAK2* V617F–positive and *JAK2* V617F–negative CMPD patient groups. The analyses mentioned were carried out with the SPSS (version 13.0) software package. P value below 0.05 was defined as significant. Testing for the presence of Hardy-Weinberg equilibrium was done by the Arlequin ver 2.000[6] software.

Results

Genotyping for HFE C282Y, H63D, S65C, and TFR S142G Allelic Variants. The CMPD patient cohort (n = 328) was genotyped for *HFE* C282Y, H63D, S65C,

[6] http://anthro.unige.ch/arlequin

and *TFR* S142G. The entire blood donor group (n = 996) was tested for C282Y to increase statistical power, whereas only the first time blood donor group (n = 171) was tested for *HFE* H63D, S65C, and *TFR* S142G. Genotype distributions were examined for the fulfillment of the Hardy-Weinberg equilibrium in all groups for the four single nucleotide polymorphisms by the Arlequin software. Negative results ($P > 0.05$) were found in all groups for all four single nucleotide polymorphisms, i.e., the measured distribution of the three possible genotypes did not show significant differences from the expected values.

For comparisons of *HFE* C282Y AFs, both, the total blood donor group (n = 996) and the first time blood donor group (n = 171) were used with AF figures of 3.4% ± 0.8% and 4.7 ± 2.3%, respectively, whereas for comparisons of *HFE* H63D and S65C only the first time blood donor group was used (12.0% ± 3.5% and 1.3% ± 1.8%). AF values were in the expected ranges for C282Y, H63D, and S65C. *HFE* C282Y AF (3.7% ± 2.4%) of repeated blood donors (donors with >30 previous blood donations, n = 122) was not different from those of the total and the first time blood donors. For C282Y, significantly decreased AF values were found in the entire CMPD group [1.8% ± 1.0%, compared with the total blood donor group (P = 0.048; OR, 0.53; 95% CI, 0.28-1.00) or compared with the first time blood donor group (P = 0.021; OR, 0.39; 95% CI, 0.18-0.86)]. The results are shown in Table 1. Decreased *HFE* C282Y AFs were detected in the patient group with PV [1.7% ± 1.4%, compared with the first time blood donor group (P = 0.044; OR, 0.37; 95% CI, 0.14-0.98) but not in ET (2.4% ± 1.9%; P = 0.25; OR, 0.52; 95% CI, 0.19-1.38) and CIMF (0%; P = 0.23; OR, 0.18; 95% CI, 0.01-3.16)] and in the patient group with *JAK2* V617F–positive CMPD [1.6% ± 1.1%, compared with the total blood donor group (P = 0.042; OR, 0.47; 95% CI, 0.22-0.98) or compared with the first time blood donor group (P = 0.017; OR, 0.34; 95% CI, 0.14-0.83)]. The allele frequencies of H63D and S65C in the CMPD group did not differ from control values (H63D, 13.1% ± 2.6%; and S65C, 0.5% ± 0.5%).

We found a significantly reduced *TFR* S142G AF among *JAK2* V617F–negative CMPD patients (34.8% ± 7.6%) versus first time blood donors (47.8% ± 5.4%). Heterozygous and homozygous carriers of 142G were grouped and compared together to noncarriers (S142 homozygotes) to calculate OR and 95% CI (P = 0.02; OR, 0.47; 95% CI, 0.27-0.82). Upon stratification by diagnosis, *TFR* S142G AFs were similar in patient subgroups PV and ET, but it was significantly decreased in the patient group with CIMF (29.6% ± 12.4%; P = 0.033; OR, 0.36; 95% CI, 0.16-0.83). No significant association was found between *HFE* C282Y or H63D variants and *TFR* S142G polymorphism.

We compared the following clinical (sex; age of CMPD onset; CMPD phenotype as PV, ET, or CIMF; the rate of different complications as venous or arterial bleeding; transformation to acute leukemia or to myelofibrosis during follow-up) and laboratory parameters (Hb, WBC, and PLT at presentation) in CMPD patients carrying the mutant or wild-type *HFE* and *TFR* alleles, but we found no significant differences by univariate and multivariate analyses.

Clinical and Laboratory Characteristics According to JAK2 V617F Mutational Status. The frequency of *JAK2* V617F was 75.9% (249 of 328) in the CMPD group: 87.4% (153 of 175) in PV patients, 61.1% (77 of 126) in ET patients, and 70.4% (19 of 27) in CIMF patients. We compared the same clinical and laboratory data in different CMPD groups (PV, ET, CIMF) according to the V617F mutational status (Table 2). Female predominance was observed in V617F-positive CMPD compared with V617F-negative patients [57.4% (143 of 249) versus 41.8% (33 of 79); P = 0.019]. This predominance was also present in the PV and in the CIMF subgroups but not in ET (Table 2). The age at presentation was significantly higher in V617F-positive CMPD (60 ± 12 years versus 52 ± 16 years; $P < 0.0001$) and remained significant only in the PV subgroup. By univariate analyses, we observed higher Hb values at the time of disease onset in V617F-positive patients compared with V617F-negative counterparts (Hb, 165 ± 32 g/L versus 139 ± 30 g/L; $P < 0.000$;), this difference remained significant in PV and in ET but not in CIMF subgroups. WBC and PLT at presentation were not different in the CMPD subgroups (PV, ET, CIMF) according to the V617F mutational status. Sex, the presence of V617F mutation, and splenomegaly proved to be independent variables associated ($P < 0.0001$) with

Table 1. Genotyping for *HFE* C282Y and H63D as well as for *HFE* S142G genetic variants in the control and patient groups

Polymorphism		*HFE* C282Y			*HFE* H63D			*TFR* S142G		
Control/patient group	n	AF % ± 95% CI	HET n (%)	HOMO n (%)	AF % ± 95% CI	HET n (%)	HOMO n (%)	AF % ± 95% CI	HET n (%)	HOMO n (%)
Blood donors(total)	996	3.4 ± 0.8	65 (6.5)	1 (0.1)	—	—	—	—	—	—
First time blood donors	171	4.7 ± 2.3	14 (8.2)	1 (0.6)	12.0 ± 3.5	37 (21.6)	2 (1.2)	47.4 ± 5.4	94 (54.9)	34 (19.9)
CMPD(total)	328	1.8 ± 1.0*†	12 (3.7)	0	13.3 ± 2.6	69 (21.0)	9 (2.7)	41.5 ± 3.8	170 (51.8)	51 (15.5)
JAK2-positive CMPD	249	1.6 ± 1.1*†	8 (3.2)	0	13.5 ± 3.1	53 (21.3)	7 (2.8)	43.6 ± 4.4	133 (53.4)	42 (16.9)
JAK2-negative CMPD	79	2.5 ± 2.5	4 (5.1)	0	12.7 ± 5.3	16 (20.3)	2 (2.5)	34.8 ± 7.6†	37 (46.8)	9 (11.4)
PV	175	1.7 ± 1.4†	6 (3.4)	0	12.0 ± 3.5	34 (19.4)	4 (2.3)	44.6 ± 5.3	92 (52.6)	32 (18.3)
ET	126	2.4 ± 1.9	6 (4.8)	0	14.7 ± 4.5	27 (21.4)	5 (4.0)	39.7 ± 6.2	66 (52.4)	17 (13.5)
CIMF	27	0 ± 0	0	0	14.8 ± 9.7	8 (29.6)	0 (0)	29.6 ± 12.4†	12 (44.4)	2 (7.4)

Abbreviations: HET, number of heterozygous individuals (%); HOMO, number of homozygous individuals (%).
*P values < 0.05 when patient and total blood donor groups are compared.
†P values of <0.05 when patient and first time blood donor groups are compared by χ^2 or Fischer's Exact test. In case of *TFR* S142G, carriers of 142G (heterozygous and homozygous) were grouped and compared with noncarriers (S142 homozygotes) for statistical analyses. Allele frequency values ± 95% CI are shown.

Table 2. Clinical and laboratory characteristics of CMPD patients according to *JAK2* V617F mutational status

JAK2 V617F status	PV		ET		CIMF	
	Pos. (n = 153)	Neg. (n = 22)	Pos. (n = 77)	Neg. (n = 49)	Pos. (n = 19)	Neg. (n = 8)
Age, year ± SD*	60 ± 12 †	47 ± 16 †	59 ± 14	54 ± 16	65 ± 12	58 ± 12
Male/female	77/76 †	20/2 †	21/56	19/30	8/11 †	7/1 †
Hb (g/L ± SD)*	182 ± 20	174 ± 16	144 ± 21 †	130 ± 14 †	107 ± 33	94 ± 35
WBC (10^9/L ± SD) *	12 ± 8	20 ± 5	11 ± 5	9 ± 3	13 ± 10	12 ± 5
PLT (10^9/L ± SD) *	504 ± 241 †	270 ± 145 †	917 ± 397	1,016 ± 317	333 ± 298	408 ± 278
Splenomegaly*	68/147 (46%)	7/20 (35%)	17/73 (23%)	10/48 (21%)	17/19 (89%)	6/8 (75%)
Venous thrombosis	25/149 (17%)	0/22 (0%)	10/75 (13%)	6/49 (12%)	1/17 (6%)	0/8 (0%)
Arterial thrombosis	14/149 (9%)	1/22 (4%)	5/75 (7%)	4/49 (8%)	0/17 (0%)	0/8 (0%)
Hemorrhage	14/144 (10%)	0/22 (0%)	6/75 (8%)	2/48 (4%)	1/19 (5%)	0/8 (0%)
Transformation to myelofibrosis	13/149 (9%)	1/22 (4%)	6/75 (8%)	0/49 (0%)	19/19 (100%)	8/8 (100%)
Transformation to acute leukemia	6/145 (4%)	0/22 (0%)	1/75 (1%)	0/49 (0%)	2/18 (11%)	2/7 (28%)

NOTE: Venous thrombotic complications included deep vein thrombosis and splenic vein thrombosis; arterial thrombosis included transient ischemic attack, ischemic stroke, and acute myocardial infarction. Hemorrhagic complications were the following: gastrointestinal bleeding, hemorrhagic stroke, hematuria, severe bleeding during surgery or dentistry.
Abbreviations: Hb, Hb concentration; neg, negative; pos, positive; WBC, WBC count; PLT, PLT count.
*Clinical and laboratory data at the time of first presentation.
†$P < 0.05$ between *JAK2* V617F positive and negative CMPD patient groups.

Hb level (as the dependent variable) in multinomial logistic regression analyses. In contrast, in the same comparison, age, *HFE*, and *TFR* genotype-status had no effect on Hb levels in the CMPD cohort. Vascular complications (thrombotic and hemorrhagic combined) were more common in V617F-positive patients [P = 0.039; 26.6% (64 of 241) versus 15.2% (12 of 79)]. The frequency of venous thrombosis [14.9% (36 of 241) versus 7.6% (6 of 79); P = 0.093] and hemorrhage [8.8% (21 of 238) versus 2.6% (2 of 78); P = 0.065] alone did not differ significantly in the V617F-positive and V617F-negative patient groups, possibly due to the low case numbers. The occurrence of myelofibrosis or acute leukemia was 15% and 3%, respectively, in the whole CMPD group, and there was no difference between V617F-positive and V617F-negative patients (Table 2).

Screening for the *JAK2* V617F mutation among repeated blood donors (donors with more than 30 previous blood donations, n = 122) revealed 1 V617F-positive female donor. The V617F positivity was confirmed by two independent methods namely allele-specific PCR and PCR-*Bsa*XI-RFLP (22). Our attempt to confirm the V617F positivity by direct sequencing was not successful in this case, probably because of the low relative amounts of the mutant allele. Retrospective data analysis during 6 years of blood donation history (donations in every 3-6 months, 2-3 donations per year) revealed normal or elevated Hb values (median, 153 ± 10; range, 129-166 g/L), which exceeded the level of WHO criteria for PV only once. PLT values were not regularly measured before blood donations. Control follow up investigation (7 years after the last blood donation) confirmed the V617F positivity. Peripheral blood cell counts at this time point were as follows: 4.89×10^{12}/L (RBC), 150 g/L (Hb), 43.6% (hematocrit), and 322×10^9/L (PLT). No elevation of Hb was observed after the discontinuation of regular blood donations.

Discussion

Imbalance of iron homeostasis has been implicated in cancer susceptibility, and in most instances, iron overload was defined as a risk factor. Heterozygous *HFE* carriership is associated with moderately increased iron parameters, and therefore, it has been suggested to be a genetic modifier of several clonal disorders (solid tumors and hematologic malignancies). Previous association studies regarding common *HFE* genetic variants in relation with susceptibility for solid tumors such as colon cancer (7, 8, 14-17), or breast cancer (9-11, 18), are rather conflicting. *HFE* associations were investigated in hematologic malignancies as well. In case-control studies of Welsh and Scottish childhood acute lymphoblastic leukemia patients (n = 252), an increased C282Y (but not H63D) mutation frequency was observed in male patients compared with newborns (31). On the other hand, the H63D variant seemed to contribute to adult acute lymphoblastic leukemia (n = 27; but not acute myelogenous leukemia) susceptibility in an Italian population (32). In other published reports with small acute lymphoblastic leukemia and acute myelogenous leukemia patient cohorts, increased C282Y or H63D frequency could not be shown (19, 33, 34). Our group found elevated *HFE* mutation frequencies in Hungarian patients with myelodysplastic syndrome (35), but this finding could not be replicated in Greece (36). Beckman (13) and Van Landeghem et al. (37) found no association for *HFE* and *TFR* separately, but significant interactions were reported between *HFE* C282Y and *TFR* S142G in multiple myeloma, breast, colorectal, and liver cancers. It is unknown whether the observed associations of *HFE* or TFR genetic variants with malignant disorders are due to its effect on body iron content or because of linkage disequilibrium with (an)other gene(s).

To our knowledge, larger cohorts of CMPD patients have not yet been investigated for common *HFE* variants. Although it has to be noted that the pathogenesis of leukemias is profoundly different from that of CMPD, Hannuksela et al. (19) investigated 232 patients with various hematologic disorders, including 37 patients with ET and 15 patients with PV. C282Y mutation carrier rate was not significantly elevated in ET patients (16.2%), compared with controls (10.2%). Barton et al. (20) evaluated 100 consecutive unrelated Caucasian adults with malignancy in a community medical oncology

practice, including 7 patients with CMPD (6 of whom had PV). C282Y and H63D genotype frequency was elevated in patients (21.4% and 28.6%, respectively), compared with controls (8.9% and 14.5%). C282Y and H63D were reported to be risk factors for malignancy on the basis of OR above 2.0 (2.8 and 2.4, respectively), although *P* values were not significant due to the low case numbers. Franchini et al. (21) found similar *HFE* allele frequencies (2.2% for C282Y and 11.5% for H63D) among 52 patients with PV to that found in a previous study reporting on normal Italian population.

In contrast to previous *HFE* epidemiologic studies, where *HFE* C282Y or H63D were found to be a potential risk factor in carcinogenesis, in the present study, decreased C282Y allele frequencies were found in a large CMPD patient cohort compared with healthy blood donors. Because C282Y-heterozygous individuals show elevated Hb levels, our result, that C282Y may be a protective factor in CMPD can be explained by its protective role against chronic iron deficiency and thus anemia (3, 6). Regular blood loss due to multiple blood donations was reported in excess in the previous medical history of PV patients (20.7% of the PV cases, compared with 8% estimated in the reference population; see ref. 27). In two previous studies (28, 38), decreased cancer incidence was observed among blood donors; however, PV was a remarkable exception from this observation in one study.

We found reduced *TFR* S142G AF among *JAK2* V617F–negative CMPD patients compared with first time blood donors or *JAK2* V617F–positive CMPD patients, which raises the possibility that S142G polymorphism may influence the susceptibility of V617F-negative CMPD. This suggests a different role of iron metabolism in *JAK2*-positive and *JAK2*-negative CMPDs. No significant association was observed between *HFE* C282Y or H63D mutations and *TFR* S142G polymorphism, in contrast to Beckman et al. (13), who showed a significant association between *HFE* 282Y and *TFR* S142 among multiple myeloma, colon, and breast cancer patients.

We evaluated clinical and laboratory parameters in 328 patients with CMPD with respect to *HFE, TFR*, and *JAK2* V617F mutational status. We found no difference in clinical and laboratory parameters according to *HFE* or *TFR* mutational status, suggesting that these inherited genetic variants do not influence the clinical manifestation of CMPD (disease type as PV, ET, CIMF, age of onset, rate of complications). The presence of *HFE* C282Y (3, 6) or C282Y/H63D compound heterozygosity (39) in the healthy population and *HFE* C282Y homozygosity (40, 41) in hemochromatosis patients were reported to be associated with elevated Hb levels. In contrast, we found no effect of *HFE* genotypes on Hb levels in CMPD patients. Franchini et al. (21) also found no difference in RBC indices (hematocrit, Hb concentration, MCV, MCH, MCHC) in 52 PV patients according to *HFE* mutational status. These results suggest that *HFE* variants are minor determinants of Hb level and their effects may not be able to translate to phenotypic changes in pathologic states such as CMPD.

In contrast to the inherited *HFE* and *TFR* genetic variants, the presence of acquired *JAK2* V617F mutation influenced CMPD phenotype. The incidence of *JAK2* V617F was 75.9% (249 of 328) in the CMPD group, 87.4% (153 of 175) in PV patients, 61.1% (77 of 126) in ET patients, and 70.4% (19 of 27) in CIMF patients; the values are within the range published in the literature (22-25, 42, 43). We found a female predominance among V617F-positive CMPD compared with V617F-negative patients. Levine et al. (24) also noted female predominance among V617F-positive PV patients. In a Swedish study (44), the incidence of PV did not show significant differences between males and females ($2.69/10^5$ males versus $3.12/10^5$ females), whereas that of ET was higher among women ($0.96/10^5$ males versus $2.35/10^5$ females), in which disease the difference became significant above age 70 years. Female gender is associated with lower iron stores due primarily to regular, physiologic blood loss.

Similar to previous findings (24, 29, 45), patients carrying the *JAK2* V617F mutation were older ($P < 0.001$) and had higher Hb levels ($P < 0.001$). Vascular complications (thrombotic and hemorrhagic) were more common in V617F carrier patients [$P = 0.039$; 26.6% (64 of 241) versus 15.2% (12 of 79)]. Cheung et al. (46) also observed higher venous thrombotic rate among V617F-positive ET patients. Our data confirm earlier observations that V617F-positive ET shares clinical features (elevated Hb) with PV (29, 46).

We screened repeated blood donors with >30 previous blood donations for the presence of *JAK2* V617F mutation and identified a 53-year-old female blood donor (41 previous donations) with low level of V617F positivity. However, the PV-phenotype was hardly detected even after a long period of cessation of regular blood donations. To further substantiate the true frequency of V617F positivity, a further study with a larger sample size is needed.

In conclusion, the decreased C282Y AF found in CMPD patient group compared with control group suggests that *HFE* C282Y may exert a protective role against CMPD, especially in the *JAK2* V617F–positive CMPD subgroup. Together with previous epidemiologic observations, our results raise the possibility that iron deficiency caused by regular blood loss may provide such a stimulatory environment for the erythropoiesis, which favors the subsistence and evolution of CMPD clones. In contrast, *TFR* S142G polymorphism may alter disease susceptibility for V617F-negative CMPD in a different way. Verification of both findings in larger case-control studies with age- and sex-matched control group may be warranted. However, the present study could not exclude the possibility of the effect of other genetic factors in linkage disequilibrium with the investigated allelic variants. We did not find *HFE* C282Y allele frequency alterations between different CMPD groups (PV, ET, CIMF), suggesting that *HFE* mutational status is not associated with clinical presentation of CMPD. Furthermore, our data confirm earlier observations that V617F-positive ET shares clinical features (such as elevated Hb) with PV forming a biological continuum of V617F-positive CMPDs.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Huang X. Iron overload and its association with cancer risk in humans: evidence for iron as a carcinogenic metal. Mutat Res 2003; 533:153–71.
2. Bulaj ZJ, Griffen LM, Jorde LB, Edwards CQ, Kushner JP. Clinical and biochemical abnormalities in people heterozygous for hemochromatosis. N Engl J Med 1996;335:1799–805.
3. Beutler E, Felitti V, Gelbart T, Waalen J. Haematological effects of the C282Y HFE mutation in homozygous and heterozygous states among subjects of northern and southern European ancestry. Br J Haematol 2003;120:887–93.
4. Raddatz D, Legler T, Lynen R, Addicks N, Ramadori G. HFE genotype and parameters of iron metabolism in German first-time blood donors - evidence for an increased transferrin saturation in C282Y heterozygotes. Z Gastroenterol 2003;41:1069–76.
5. Njajou OT, Houwing-Duistermaat JJ, Osborne RH, et al. A population-based study of the effect of the HFE C282Y and H63D mutations on iron metabolism. Eur J Hum Genet 2003;11:225–31.
6. Datz C, Haas T, Rinner H, Sandhofer F, Patsch W, Paulweber B. Heterozygosity for the C282Y mutation in the hemochromatosis gene is associated with increased serum iron, transferrin saturation, and hemoglobin in young women: a protective role against iron deficiency? Clin Chem 1998;44:2429–32.
7. Robinson JP, Johnson VL, Rogers PA, et al. Evidence for an association between compound heterozygosity for germ line mutations in the hemochromatosis (HFE) gene and increased risk of colorectal cancer. Cancer Epidemiol Biomarkers Prev 2005;14:1460–3.
8. Shaheen NJ, Silverman LM, Keku T, et al. Association between hemochromatosis (HFE) gene mutation carrier status and the risk of colon cancer. J Natl Cancer Inst 2003;95:154–9.
9. Gunel-Ozcan A, Alyilmaz-Bekmez S, Guler EN, Guc D. HFE H63D mutation frequency shows an increase in Turkish women with breast cancer. BMC Cancer 2006;6:37.
10. Kallianpur AR, Hall LD, Yadav M, et al. Increased prevalence of the HFE C282Y hemochromatosis allele in women with breast cancer. Cancer Epidemiol Biomarkers Prev 2004;13:205–12.
11. Kondrashova TV, Neriishi K, Ban S, et al. Frequency of hemochromatosis gene (HFE) mutations in Russian healthy women and patients with estrogen-dependent cancers. Biochim Biophys Acta 2006;1762:59–65.
12. Dorak MT, Burnett AK, Worwood M. HFE gene mutations in susceptibility to childhood leukemia: HuGE review. Genet Med 2005; 7:159–68.
13. Beckman LE, Van Landeghem GF, Sikstrom C, et al. Interaction between haemochromatosis and transferrin receptor genes in different neoplastic disorders. Carcinogenesis 1999;20:1231–3.
14. Altes A, Gimferrer E, Capella G, Barcelo MJ, Baiget M. Colorectal cancer and HFE gene mutations. Haematologica 1999;84:479–80.
15. Macdonald GA, Tarish J, Whitehall VJ, et al. No evidence of increased risk for colorectal cancer in individuals heterozygous for the Cys282Tyr haemochromatosis mutation. J Gastroenterol Hepatol 1999;14:1188–91.
16. McGlynn KA, Sakoda LC, Hu Y, et al. Hemochromatosis gene mutations and distal adenomatous colorectal polyps. Cancer Epidemiol Biomarkers Prev 2005;14:158–63.
17. van der A DL, van der Hel O, Roest M, et al. Heterozygosity for the Cys282Tyr mutation in the HFE gene and the risk of colorectal cancer (Netherlands). Cancer Causes Control 2003;14:541–5.
18. Abraham BK, Justenhoven C, Pesch B, et al. Investigation of genetic variants of genes of the hemochromatosis pathway and their role in breast cancer. Cancer Epidemiol Biomarkers Prev 2005;14:1102–7.
19. Hannuksela J, Savolainen ER, Koistinen P, Parkkila S. Prevalence of HFE genotypes, C282Y and H63D, in patients with hematologic disorders. Haematologica 2002;87:131–5.
20. Barton JC, Bertoli LF, Acton RT. HFE C282Y and H63D in adults with malignancies in a community medical oncology practice. BMC Cancer 2004;4:6.
21. Franchini M, de Matteis G, Federici F, Solero P, Veneri D. Analysis of hemochromatosis gene mutations in 52 consecutive patients with polycythemia vera. Hematology 2004;9:413–4.
22. Baxter EJ, Scott LM, Campbell PJ, et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. Lancet 2005;365:1054–61.
23. James C, Ugo V, Le Couedic JP, et al. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. Nature 2005;434:1144–8.
24. Levine RL, Wadleigh M, Cools J, et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. Cancer Cell 2005;7: 387–97.
25. Kralovics R, Passamonti F, Buser AS, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med 2005;352:1779–90.
26. Lichtman MA, Kipps TJ, Kaushansky K, editors. Williams Hematology. 7th ed. New York: McGraw-Hill; 2006.
27. Najean Y, Rain JD, Billotey C. Epidemiological data in polycythaemia vera: a study of 842 cases. Hematol Cell Ther 1998;40:159–65.
28. Merk K, Mattsson B, Mattsson A, Holm G, Gullbring B, Bjorkholm M. The incidence of cancer among blood donors. Int J Epidemiol 1990;19: 505–9.
29. Campbell PJ, Scott LM, Buck G, et al. Definition of subtypes of essential thrombocythaemia and relation to polycythaemia vera based on JAK2 V617F mutation status: a prospective study. Lancet 2005;366:1945–53.
30. Andrikovics H, Kalmar L, Bors A, et al. Genotype screening for hereditary hemochromatosis among voluntary blood donors in Hungary. Blood Cells Mol Dis 2001;27:334–41.
31. Dorak MT, Burnett AK, Worwood M, Sproul AM, Gibson BE. The C282Y mutation of HFE is another male-specific risk factor for childhood acute lymphoblastic leukemia. Blood 1999;94:3957.
32. Viola A, Pagano L, Laudati D, et al. HFE gene mutations in patients with acute leukemia. Leuk Lymphoma 2006;47:2331–4.
33. Gimferrer E, Nomdedeu J, Gich I, Barcelo MJ, Baiget M. Prevalence of hemochromatosis related HFE gene mutations in patients with acute myeloid leukemia. Leuk Res 1999;23:597–98.
34. Ruiz-Arguelles GJ, Morales-Toquero A, Cruz-Dominguez G, et al. HFE-codon 63/282 (H63D/C282Y) gene variants in Mexican Mestizos are not risk factors for leukemia. Arch Med Res 2006;37:65–7.
35. Varkonyi J, Tarkovacs G, Karadi I, et al. High incidence of hemochromatosis gene mutations in the myelodysplastic syndrome: the Budapest Study on 50 patients. Acta Haematol 2003;109:64–7.
36. Speletas M, Kioumi A, Mandala E, et al. Prevalence of hemochromatosis gene (HFE) mutations in Greek patients with myelodysplastic syndromes. Acta Haematol 2003;110:53–4.
37. Van Landeghem GF, Beckman LE, Wahlin A, Markevarn B, Beckman L. Interaction between haemochromatosis and transferrin receptor genes in multiple myeloma. Lancet 1998;352:1285–6.
38. Edgren G, Reilly M, Hjalgrim H, et al. Donation frequency, iron loss, and risk of cancer among blood donors. J Natl Cancer Inst 2008;100: 572–9.
39. Rossi E, Bulsara MK, Olynyk JK, Cullen DJ, Summerville L, Powell LW. Effect of hemochromatosis genotype and lifestyle factors on iron and red cell indices in a community population. Clin Chem 2001;47: 202–8.
40. Barton JC, Bertoli LF, Rothenberg BE. Peripheral blood erythrocyte parameters in hemochromatosis: evidence for increased erythrocyte hemoglobin content. J Lab Clin Med 2000;135:96–104.
41. McLaren CE, Barton JC, Gordeuk VR, et al. Determinants and characteristics of mean corpuscular volume and hemoglobin concentration in white HFE C282Y homozygotes in the hemochromatosis and iron overload screening study. Am J Hematol 2007;82: 898–905.
42. Jones AV, Kreil S, Zoi K, et al. Widespread occurrence of the JAK2 V617F mutation in chronic myeloproliferative disorders. Blood 2005; 106:2162–8.
43. Jelinek J, Oki Y, Gharibyan V, et al. JAK2 mutation 1849G >T is rare in acute leukemias but can be found in CMML, Philadelphia chromosome-negative CML, and megakaryocytic leukemia. Blood 2005;106:3370–3.
44. Ridell B, Carneskog J, Wedel H, et al. Incidence of chronic myeloproliferative disorders in the city of Goteborg, Sweden 1983–1992. Eur J Haematol 2000;65:267–71.
45. Speletas M, Katodritou E, Daiou C, et al. Correlations of JAK2–617F mutation with clinical and laboratory findings in patients with myeloproliferative disorders. Leuk Res 2007;31:1053–62.
46. Cheung B, Radia D, Pantelidis P, Yadegarfar G, Harrison C. The presence of the JAK2 V617F mutation is associated with a higher haemoglobin and increased risk of thrombosis in essential thrombocythaemia. Br J Haematol 2006;132:244–5.

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