Prevalence of HFE genotypes, C282Y and H63D in patients with hematologic disorders

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Background and Objectives. Iron status has implications for normal erythrocyte and leukocyte function and for platelet count, size and activation. Increased storage of iron is considered a potential risk factor participating in the pathogenesis of malignant diseases. Since *HFE* gene mutations have recently been implicated in unbalanced iron homeostasis, we set out to examine the prevalence of these mutations in patients with hematologic disorders.

Design and Methods. C282Y and H63D mutations were determined in 232 patients with various hematologic disorders treated at Oulu University Hospital between 1987 and 2000. DNA samples extracted from either the peripheral blood or bone marrow of these patients were amplified by a polymerase chain reaction (PCR) method using sequence-specific primers, and the products were analyzed on agarose gels.

Results. There was a slight tendency towards lower frequencies of the C282Y allele in patients with acute myeloid leukemia (AML) (3.8%, n=53) and higher frequencies in those with essential thrombocythemia (ET) (16.2%, n=37). Contrary to some expectations, however, the frequency of the C282Y allele in acute lymphoblastic leukemia turned out to be normal (7.0%, n=43). Our data showed no significant deviations in H63D mutation frequency in any of the categories of patients.

Interpretation and Conclusions. Our results do not show any significant association between *HFE* gene mutations and hematologic malignancies. The divergent frequencies observed for the C282Y mutation in patients with AML and ET highlight the need for larger population studies of *HFE* mutations in patients with hematologic diseases.

Key words: hematologic disorder, hereditary hemochromatosis, *HFE*, iron.

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HFE gene mutations were first reported in patients with hereditary hemochromatosis (HH), 80-90% of whom were found to be homozygous for the C282Y mutation in this gene,[1,2] while the importance of another mutation causing HH, H63D, remains controversial. What is clear, however, is that C282Y-mutant HFE protein has an unfavorable effect on iron homeostasis that is manifested as excessive iron storage in HH patients. As a result of HFE protein dysfunction, transferrin receptor (TfR)-mediated cellular iron uptake is decreased in the duodenal crypt cells which sense the iron concentration in the body,[3,4] leading to increased intestinal iron absorption in the mature villus cells. So far, it has been demonstrated that HFE protein is expressed not only in gastrointestinal epithelial cells but also in tissue macrophages, circulating granulocytes and monocytes.[5] Moreover, Chitambar and Wereley[6] have shown that HFE protein is produced in B-lymphoid cell lines.

It is known for a fact that iron status has implications for normal erythrocyte and leukocyte function, and interestingly, it also influences platelet count, the size of platelets and their activation.[7-14] Iron may also play an important role in the pathogenesis of malignant diseases by inducing free radicals and promoting the maintenance and multiplication of malignant cells.[15,16] Furthermore, both iron overload and iron deficiency are associated with considerable aberrations in immune function.[17] Nelson[18] reported that heterozygosity for HH is associated with an increased risk of hematologic malignancy, an argument that has been supported by an investigation into childhood acute lymphoblastic leukemia (ALL)[19] which showed that the C282Y mutation enhances the risk of hematopoietic malignancies in males. It has also been found that a high iron burden in the circulation can affect the survival of ALL patients.[20] By contrast, no meaningful relationship has been reported between the prevalence of acute myeloid

leukemia (AML) and the *HFE* gene mutations C282Y and H63D.[21] Similarly, the C282Y mutation alone showed no association with the incidence of multiple myeloma (MM), even though patients with the HFE Tyr 282 allele together with TfR Ser 142 homozygosity appeared to have an increased risk of MM.[22,23]

The present study was designed to analyze *HFE* genotypes in patients with various hematologic disorders. The divergent frequencies observed for the C282Y mutation in the group of patients with AML and those with essential thrombocythemia (ET) provide a foundation for larger population studies of *HFE* mutations in patients with hematologic diseases.

Design and Methods

Patients

Peripheral blood or bone marrow samples were collected from 232 patients (106 men and 126 women) with various hematologic disorders; ALL (n=43), AML (n=53), chronic myeloid leukemia (CML, n=16), ET (n=37), myelodysplastic syndromes (MDS, n=35), MM (n=33), and polycythemia vera (PV, n=15). The samples were taken from patients during routine diagnostic and treatment evaluation at Oulu University Hospital between 1987 and 2000. ALL and AML were classified on the basis of their lymphoid or myeloid morphologic appearance, respectively, according to the French American British (FAB) criteria.[24,25] In addition, diagnoses of acute leukemia were based on the immunophenotype as assessed by flow cytometry using standard lineage immunophenotype markers. The diagnosis of MDS was also based on the FAB criteria,[26] while that of PV was based on the criteria of the PV Study Group,[27] and that of CML on the presence of the Philadelphia chromosome t(9;22).[28] The diagnosis of ET was based on the criteria described by Hoffman,[29] and those of MM on the criteria described by Kyle.[30] The investigation was approved by the Ethics Committee of Oulu University Hospital and was performed in accordance with the guidelines of the Declaration of Helsinki.

HFE genotyping

DNA was extracted either from peripheral blood or from bone marrow using a blood kit (Nucleospin, Macherey-Nagel, Düren, Germany). A polymerase chain reaction (PCR) to amplify the extracted DNA was run using the sequence specific primers (SSP) described by Smillie.[31,32] Both the C282Y and the H63D mutations were tested. The control primers designed by Steffensen *et al.*[33] were used to ensure that the PCR method was working properly. The PCR products were analyzed by electophoresis on a 1.5% agarose gel containing ethidium bromide and visualized by ultraviolet light stimulation. Positive and negative controls were included in every group of samples analyzed.

All the statistical differences between the prevalences of *HFE* genotypes in the various hematologic disorders and in the control group were assessed using Fisher´s exact probability test. *p* values of < 0.05 were considered statistically significant.

Results

The frequencies of the C282Y mutations in the various hematologic diseases and in the control group are summarized in Table 1. The frequencies of the H63D mutations are given in Table 2.

Discussion

One of the most interesting observations of our study was that the frequency of the C282Y mutation was markedly low in patients with AML (3.8%, n=53), as previously published data for a control group from the same hospital district (n=128) had indicated frequencies of 10.2% for the C282Y mutation and 20.3% for the H63D mutation.[34] The difference between the frequencies in the AML group and that in the present control group did not, however, reach statistical significance (p=0.237). The low frequency of the C282Y mutation in the AML patients was in contradiction to the previous assumption that the gene mutation responsible for HH would increase the risk of hematologic malignancies.[18] It should not be forgotten, either, that our results did not provide any evidence to support the recently published data showing that the C282Y mutation is a male-specific risk factor for childhood ALL.[19] Dorak *et al.*[19] analyzed C282Y genotypes in two independent groups of children with ALL from South Wales and the West of Scotland. They reported C282Y mutation frequencies of 23.4% and 37.4% in the two groups of male childhood ALL patients, respectively. In our study, both the ALL and childhood ALL patients (43 ALL patients, of whom 32 were aged 16 years or less at the time of diagnosis) had normal C282Y mutation frequencies and none of the male childhood ALL patients (n=14) carried the C282Y mutation. Whether the divergent mutation frequencies observed in the present and previous[19] studies reflect a genetic heterogeneity in two distant populations remains to be elucidated.

Iron overload is relatively common in patients

Table 1. Presence of the C282Y mutation in patients with hematologic disorders.

	HFE-CY				
	HET		*WT*		*Total*
	Count	*%*	*Count*	*%*	*count*
Diagnosis					
ALL	3	7.0	40	93.0	43
AML	2	3.8	51	96.2	53
CML	1	6.3	15	93.8	16
ET	6	16.2	31	83.8	37
MDS	4	11.4	31	88.6	35
MM	2	6.1	31	93.9	33
PV	1	6.7	14	93.3	15
Total	19	8.2	213	91.8	232

The frequency of the C282Y mutation for a control group (n=128) from the same hospital district was 10.2%.[34]

Table 2. Presence of the H63D mutation in patients with hematologic disorders.

	HFE-HD						
	HET		*HOMOZ*		*WT*		*Total*
	Count	*%*	*Count*	*%*	*Count*	*%*	*count*
Diagnosis							
ALL	6	14.0			37	86.0	43
AML	8	15.1	1	1.9	44	83.0	53
CML	4	25.0			12	75.0	16
ET	6	16.2			31	83.8	37
MDS	9	25.7	1	2.9	25	71.4	35
MM	4	12.1			29	87.9	33
PV	3	20.0			12	80.0	15
Total	40	17.2	2	0.9	190	81.9	232

The frequency of the H63D mutation for a control group (n=128) from the same hospital district was 20.3 %.[34]

with AML, due to multiple RBC transfusions.[35,36] Hence, AML patients need a functional machinery to handle excessive iron. It is known that macrophages normally play a central role in these processes and cellular iron uptake by macrophages is defective in homozygous C282Y patients because of HFE protein dysfunction.[37] Based on these facts, we propose that the C282Y mutation in conjunction with multiple RBC transfusions could cause severe iron overload in AML patients, which could in turn lead to serious complications. Higher mortality among C282Y mutant AML patients due to iron toxicity could theoretically cause a low C282Y mutation frequency. However, this still does not apparently explain the low mutation frequency observed in our AML patients.

Another interesting finding was that the patients with ET appeared to have a slightly higher tendency for having the C282Y mutation (16.2%, n=37). Although this allele may represent a passenger mutation frequently associated with ET, we cannot exclude the possibility that it may be more directly involved in the pathogenic mechanisms of the disease. The fact that iron has effects on platelet count, the size of platelets and their activation points to a possibility that this mutation, even in heterozygous form, could alter the platelet count and/or platelet function in some individuals. Our unpublished data have shown that HFE protein is expressed in platelets, which makes the present finding even more attractive.

We also determined the prevalence of another typical *HFE* mutation, H63D. Since the relevance of this mutation has remained questionable, even in HH patients, the significance of its slightly elevated frequency in our MDS patients naturally remains open. The percentage of H63D mutations in any of the patient categories did not show any statistically significant difference from that recorded for a control population from the same hospital district.

In summary, we found slightly divergent frequencies of the C282Y mutation in patients with AML (3.8%, n=53) and ET (16.2%, n=37). Even though these frequencies did not differ significantly from those observed in the control population (10.2%), the present results suggest that *HFE* mutations may play a role in the pathogenesis of certain hematologic disorders. However, a larger study population would be required to show or exclude a significant association between *HFE* gene mutations and the prevalence of hematologic disorders.

Contributions and Acknowledgments

JH wrote the paper, processed the data and assessed the statistical analyses. SP, ERS and PK assisted with writing and interpretations of the data with JH. ERS and PK were responsible for patient referral to our laboratory. All authors were equally responsible for conception and design of the study and approved the final version of the paper. The order of names takes into account the time, work and scientific contribution given by all authors. SP as a senior author, is cited last. We thank Kirsi Kvist-Mäkelä for the help with HFE genotyping.

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Disclosures

Conflict of interest: none.

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References

1. Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet 1996; 13:399-408.
2. Bacon BR, Olynyk JK, Brunt EM, Britton RS, Wolff RK. HFE genotype in patients with hemochromatosis and other liver diseases. Ann Intern Med 1999; 130:953-62.
3. Waheed A, Parkkila S, Saarnio J, Fleming RE, Zhou XY, Tomatsu S, et al. Association of HFE protein with transferrin receptor in crypt enterocytes of human duodenum. Proc Natl Acad Sci USA 1999; 96:1579-84.
4. Parkkila S, Niemelä O, Britton RS, Fleming RE, Waheed A, Bacon BR, et al. Molecular aspects of iron absorption and HFE expression. Gastroenterology 2001; 121:1489-96.
5. Parkkila S, Parkkila AK, Waheed A, Britton RS, Zhou XY, Fleming RE, et al. Cell surface expression of HFE protein in epithelial cells, macrophages, and monocytes. Haematologica 2000; 85:340-5.
6. Chitambar CR, Wereley JP. Iron transport in a lymphoid cell line with the hemochromatosis C282Y mutation. Blood 2001; 97:2734-40.
7. Berger M, Brass LF. Severe thrombocytopenia in iron deficiency anemia. Am J Hematol 1987; 24:425-8.
8. Soff GA, Levin J. Thrombocytopenia associated with repletion of iron in iron-deficiency anemia. Am J Med Sci 1988; 295:35-9.
9. Kellar KL, Bridges NB, Monroe MC, Bell BA, Callaway CS, Evatt BL. Maintenance of normal platelet mass in anemic Belgrade rats and their response to iron. Exp Hematol 1990; 18:979-84.
10. Yang M, Collis CS, Kelly M, Diplock AT, Rice-Evans C. Do iron and vitamin C co-supplementation influence platelet function or LDL oxidizability in healthy volunteers? Eur J Clin Nutr 1999; 53:367-74.
11. Pratico D, Pasin M, Barry OP, Ghiselli A, Sabatino G, Iuliano L, et al. Iron-dependent human platelet activation and hydroxyl radical formation: involvement of protein kinase C. Circulation 1999; 99:3118-24.
12. Loo M, Beguin Y. The effect of recombinant human erythropoietin on platelet counts is strongly modulated by the adequacy of iron supply. Blood 1999; 93:3286-93.
13. Caliskan U, Oner AF, Kabakus N, Koc H. Diminished platelet aggregation in patients with iron deficiency anemia. Clin Appl Thromb Hemost 1999; 5:161-3.
14. Kurekci AE, Atay AA, Sarici SU, Zeybek C, Koseoglu V, Ozcan O. Effect of iron therapy on the whole blood platelet aggregation in infants with iron deficiency anemia. Thromb Res 2000; 97:281-5.
15. Reizenstein P. Iron, free radicals and cancer. Med Oncol Tumor Pharmacother 1991; 8:229-33.
16. Weinberg ED. The role of iron in cancer. Eur J Cancer Prev 1996; 5:19-36.
17. Good MF, Powell LW, Halliday JW. Iron status and cellular immune competence. Blood Rev 1988; 2:43-9.
18. Nelson RL, Davis FG, Persky V, Becker E. Risk of neoplastic and other diseases among people with heterozygosity for hereditary hemochromatosis. Cancer 1995; 76: 875-9.
19. 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; 94:3957-8.
20. Potaznik D, Groshen S, Miller D, Bagin R, Bhalla R, Schwartz M, et al. Association of serum iron, serum transferrin saturation, and serum ferritin with survival in acute lymphocytic leukemia. Am J Pediatr Hematol Oncol 1987; 9:350-5.
21. 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; 23:597-8.
22. Van Landeghem GF, Beckman LE, Wahlin A, Markevärn B, Beckman L. Interaction between haemochromatosis and transferrin receptor genes in multiple myeloma. Lancet 1998; 352:1285-6.
23. Beckman LE, Van Landeghem GF, Sikstrom C, Wahlin A, Markevärn B, Hallmans G, et al. Interaction between haemochromatosis and transferrin receptor genes in different neoplastic disorders. Carcinogenesis 1999; 20: 1231-3.
24. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, et al. Proposals for the classification of acute leukaemias. French-American-British (FAB) cooperative group. Br J Haematol 1976; 33:451-8.
25. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, et al. Proposed revised criteria for the classification of acute myeloid leukemia. A report of the French-American-British Cooperative Group. Ann Intern Med 1985; 103:620-5.
26. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, et al. Proposals for the classification of the myelodysplastic syndromes. Br J Haematol 1982; 51:189-99.
27. Wasserman LR. The management of polycythaemia vera. Br J Haematol 1971; 21:371-6.
28. Rowley JD. A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. Nature 1973; 243:290-3.
29. Hoffman R. Primary thrombocythemia. In: Hoffman R, Bentz EJ Jr, Shattil SJ, Furie B, Cohen HJ, Silberstein LE, McGlave P, editors. Hematology, Basic Principles and Practice. New York: Churchill Livingstone; 2000. p. 1188.
30. Kyle RA. Diagnostic criteria of multiple myeloma. Hematol Oncol Clin North Am 1992; 6:347-58.
31. Smillie D. A PCR-SSP method for detecting the Cys282Tyr mutation in the HFE gene associated with hereditary haemochromatosis. Mol Pathol 1997; 50:275-6.
32. Smillie D. A PCR-SSP method for detecting the His63Asp mutation in the HFE gene associated with hereditary haemochromatosis. Mol Pathol 1998; 51:232-3.
33. Steffensen R, Varming K, Jersild C. Determination of gene frequencies for two common haemochromatosis muta-

tions in the Danish population by a novel polymerase chain reaction with sequence-specific primers. Tissue Antigens 1998; 52:230-5.
34. Parkkila S, Niemela O, Savolainen ER, Koistinen P. HFE mutations do not account for transfusional iron overload in patients with acute myeloid leukemia. Transfusion 2001; 41:828-31.
35. Lichtman SM, Attivissimo L, Goldman IS, Schuster MW, Buchbinder A. Secondary hemochromatosis as a long-term complication of the treatment of hematologic malignancies. Am J Hematol 1999; 61:262-4.
36. Barton JC, Bertoli LF. Transfusion iron overload in adults with acute leukemia: manifestations and therapy. Am J Med Sci 2000; 319:73-8.
37. Montosi G, Paglia P, Garuti C, Guzman CA, Bastin JM, Colombo MP, et al. Wild-type HFE protein normalizes transferrin iron accumulation in macrophages from subjects with hereditary hemochromatosis. Blood 2000; 96: 1125-9.

Peer Review Outcomes

What is already known on this topic

Iron overload is typically found in patients with HFE-related genetic hemochromatosis, and individuals who are heterozygous for the HFE C282Y mutation may have marginally elevated iron stores. Some reports have associated excess iron with cancer risk, particularly of the colorectum and of the liver. Little is known about any association between increased iron stores or HFE mutations and risk of hematologic malignancies.

What this study adds

This study excludes any significant association between HFE gene mutations and hematologic malignancies. Although this was expected, the present study provides a formal demonstration.

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Mario Cazzola, Editor-in-Chief. The final decision to accept this paper for publication was taken jointly by Prof. Cazzola and the Editors. Manuscript received September 12, 2001; accepted November 21, 2001.

Potential implications for clinical practice

This study demonstrates that gene mutations responsible for HH do not increase the risk of hematologic malignancies. The results imply that HFE genotyping is not necessary in patients with hematologic malignancies treated with multiple RBC transfusions.

Mario Cazzola, Editor-in-Chief