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Hemochromatosis Gene Mutation and Erythrocytosis

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Abstract

In a patient group presenting with either increased Hgb level or high RBC count *HFE* gene mutations occured in two-third of the patients. These patients had normal erythropoietin levels and had no *JAK2* mutation. Out of the 22 patients, 12 had the *H63D* mutation in heterozygous / homozygous or compound heterozygous forms, 2 had *C282Y* mutation and 7 had wild type *HFE* gene. In contrast to the classical form of type I Hemochromatosis with *C282Y* homozygous mutation, characterised by high serum iron, transferrin saturation and ferritin levels, patients with the *H63D* mutation had normal iron content. In the second part of the study authors analysed iron and red cell parameters of patients available from their Hemochromatosis registry. The results were similar: those who had the *H63D* mutation, had more red cells and less iron content. It seems like in those having this mutation more iron is consumed for the red blood cell production, but the explanation for this phenomenon is still missing. The role of *H63D* mutation in this process should still be clarified.

Keywords: HFE; H63D; Erythrocytosis

Abbreviations: RBC: Red Blood Cell; JAK2: Janus Kinase 2; EPO: Erythropoietin; PV: Polycythaemia; HH: Hemochromatosis.

Introduction

Patients with high red blood cell count (RBC) and high hemoglobin concentration with janus kinase 2 (JAK2) mutation and low erythropoietin (EPO) level- represent the group of true polycythaemia (PV) and those with wt *JAK2* and high EPO are the group of the so-called secondary polycythaemia forms [1]. Those significant number of patients, however who presenting either high haemoglobin or RBC levels and has no *JAK2* mutation, and has normal EPO levels still remain a diagnostic challenge.

It has been noticed, that regular therapeutic phlebotomy –as the main treatment option for Hereditary Hemochromatosis (HH) patients- is well tolerated by them, meaning that anemia does not develop even in the first year of diagnosis, when 400 ml blood / occasion is drained many times until the goal ferritin level achieved and maintained thereafter. In contrast to this, for a voluntary blood donor five blood donations are permitted in a year to avoid the development of anemia. In a survey of voluntary blood donors, the ratio of *H63D* heterozygotes was 31% in the group of the so-called super donors who donate 30-107 occasions [2]. Whether the high iron availability of HH patients is enough to explain this extremely good compensation of blood loss? In this study authors discuss this problem based on literature data and their recent findings.

Materials and Methods

The first study population consisted of 22 (16 M / 6 F, mean age: 61,6 yrs) non selected, consecutive and naive subjects who had not phlebotomy or other therapy before, were not blood donors and the reason of enrollment was their high Hgb levels or RBC counts found on rutin laboratory analysis. They were *JAK2* wt (confirmed also by gene sequence analysis) and had normal EPO levels.

The second study population consisted of 35 hemochromatosis patients (16 with homozygous *C282Y* ,- 11 with compound heterozygous,- and 8 with homozygous *H63D* mutation) from the Hemochromatosis patients registry of our institute. All were males. Female patient were excluded from this study because of their regular menstrual blood loss and also those males, who had known co-morbidities causing bleeding or bone marrow disorders with impaired red cell production, like myelodysplastic syndromes.

The participants signed informed consents, and the study was approved by the Institutional Ethics Committee. Whole genomic DNA was isolated from anticoagulated peripheral blood with Puregene Gentra DNA Isolation kit. *HFE* gene sequence variant *C282Y* (exon 4, c.1066G>A, substitution of Cys282 to Tyr, rs1800562); and *H63D* (exon 2, c.408C>G, substitution of His63 to Asp, rs1799945) were investigated by Light Cycler technology (Roche Diagnostics) applying melting curve analysis with the hybridization probe detection format [3]. The *JAK2* gene V617F mutation (exon 12, c.1849G>T, substitution of Val617 to Phe) was detected by allele specific multiplex PCR [4].

Results

In the first study population serum iron, transferrin saturation and ferritin concentration did not differ from normal values, but red blood cell count / or hemoglobin was higher in those, who had in any type the *HFE* gene mutation Table 1.

	EPO	RBC	Hgb	Se Fe	Tf sat	Ferritin
	mU/ml	Tera/l	g/l	umol/l	(%)	ug/l
HFE wt n=7	5,6	5,7	181	23,5	37	293
HFE mut n=15	11,9	6,0	187	17,8	30	242
Normal	2,6-18,5	F/M max	F/M max	F/M max	16-45	F/M max
		5,1/5,9	153/175	28/32,2		250/300

Mean values are shown. The upper normal values are given for Females and Males.
Table 1: Iron and red cell parameters of *JAK2* wt patients with erythrocytosis having or having not *HFE* gene mutation.

Regarding *HFE* gene status the wild type/ mutant ratio was 7 / 15. When the type of *HFE* gene mutation was analysed, 2 patients had *C282Y* and 8 were *H63D* heterozygotes whereas 4 compound heterozygotes and 1 *H63D* homozygote were also found (Table 2).

	C282Y 1/0	*H63D* 0/1	*H63D* 0/2	*C282Y* /*H63D* 01/01
HFE mutant (n:15)	2	8	1	4

Abbreviations: 1/0 or 0/1 are heterozygous, 0/2 are homozygous, 01/01: compound heterozygous.
Table 2: *HFE* gene mutation results of the 15 patients with JAK2wt erythrocytosis.

In the second study population it was clearly seen that the highest red blood cell counts occurred in those who contain the *H63D* mutation and the highest iron values in those, who had the *C282Y* in homozygous form Table 3.

	RBC Terra/l	Hgb g/l	SeFe umol/l	tfsat (%)	ferritin ug/l
compound n= 11	5,1 (4,5-6,07)	164 (140-188)	25,8 (15,1-39,3)	43 (23-69)	418 (31-1678)
H63D n= 8	5,5 (4,8-6,76)	167 (158-183)	29,9 (20,5-41,6)	40 (30-48)	336 (114-547)
C282Y n= 16	4,81 (4,3-5,75)	154 (140-168)	38,1 (27,5-53,9)	75 (42-103)	1259 (450-2580)

Mean values are shown. The upper normal values are shown in Table 1.
Table 3: Iron and red cell parameters of a Hemochromatosis patient population.

Discussion

In this preliminary study *HFE* gene mutation occurred in two-third of patients presenting either high Hgb or RBC count, but did not fulfil the criteria of PV. There was a male preponderance. Literature data confirm that HH genetic polymorphism is associated with more iron availability / and more iron absorption especially in a compound heterozygous form [5,6]. Would this be enough to explain erythrocytosis? More iron mean more red cell or Hgb?. It has been shown that both types of TfRs bind to *HFE* proteins and have role in iron sensing [7]. TfR2 is recently found to be an EPO partner receptor [8]. Based on these findings we might explain erythrocytosis seen in Tfr2 knockout mice and in Type 3 hemochromatosis patients who tolerate well repeated phlebotomies. Authors of the present study therefore assumes that co-existent TfR2 gene mutation might hide in the background in JAK2wt/norm EPO / *HFE* – related or unrelated erythrocytosis, that is to be further analysed. Multiple pathways might exist influencing erythropoiesis other than EPO signaling mechanism. Among these could be- not surprisingly- genes participating in iron metabolism regulation that still should be explored.

Conclusion

The significant ratio of *HFE* gene mutation in the group of patients presenting with high red blood cell count and hemoglobin level outlines the importance to perform the test in all cases of unexplained (JAK 2 wt, normal level EPO) erythrocytosis.

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