The Effects of *HFE* Polymorphisms on Biochemical Parameters of Iron Status in Arab Beta-Thalassemia Patients

Suad AlFadhli¹ · Matra Salem¹ · D. K. Shome² · Najat Mahdi³ · Rasheeba Nizam¹

Received: 16 May 2015 / Accepted: 28 December 2016 / Published online: 18 January 2017
© Indian Society of Haematology & Transfusion Medicine 2017

Abstract In this study, the potential effect of three *HFE* gene polymorphisms (C282Y, H63D and S65C) and the *SLC40A1* A77D polymorphism on iron balance was investigated in 234 subjects (91 Arab beta-thalassemia major (BTM) patients, 34 beta-thalassemia trait (BTT) individuals and 109 health controls). Genotyping was done using restriction-fragment-length polymorphism and direct-sequencing. Serum-iron, total iron binding capacity, transferrin and ferritin were estimated in all BTT and BTM, and in 65 healthy controls. H63D was the only polymorphism detected in our cohort. Allele frequency was 13% in both BTM and BTT and 10% in controls with no significant difference. Serum iron, ferritin and transferrin saturation were significantly higher in normal males heterozygous for H63D as compared to homozygous wild-type males. Ferritin was significantly higher in BTT males

with or without H63D polymorphism when compared to the healthy males with H/H genotype. No such difference was observed between H/H versus H/D BTT subgroups. We conclude that H63D is the only significant hemochromatosis-associated polymorphism in the Arabian Gulf region. The heterozygous state of H63D may significantly alter iron parameters in normal males. In BTT, it appears that the beta-thalassemia allele has an overriding influence on ferritin values, and this generally manifest in males.

Keywords Beta-thalassemia · Iron · Ferritin · Transferrin · Total iron binding capacity · Arabs

Introduction

Hemochromatosis is characterized by a progressive increase in total body iron stores with abnormal iron deposition in multiple organs [1]. Primary hemochromatosis is a heterogeneous genetic disease associated with single nucleotide polymorphisms (SNPs) in five prominent genes such as hemochromatosis (*HFE*), Transferrin receptor2 (*TfR2*), Hemojuvelin (*HJV*) and solute carrier family 40, member 1 (*SLC40A1*) [2, 3].

Defects in *HFE* gene are most commonly related to primary hemochromatosis. *HFE* plays a major role in regulating iron absorption as the binding of *HFE* to *TfR* reduces its affinity for iron-loaded transferrin [4–7]. So far, more than 20 variations have been identified in the *HFE* gene that cause primary hemochromatosis [5]. The first common genetic SNP is a C to G transversion in exon 2 of the *HFE* gene that results in a histidine to aspartic acid substitution at position 63 (H63D). This occurs at the $\alpha 1$ domain and results in a reduction in the affinity of the *HFE* gene product for its ligand [8, 9]. The second is an A to T

✉ Suad AlFadhli
suadq8@yahoo.com; s.alfadhli@hsc.edu.kw

Matra Salem
matrasalem@yahoo.com

D. K. Shome
dr.dkshome@gmail.com

Najat Mahdi
NAl2@health.gov.bh

Rasheeba Nizam
rasheebanizam@gmail.com

¹ Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, Kuwait University, PO Box 31470, Sulaibekhat, Kuwait

² Department of Pathology, College of Medicine and Medical Sciences, Arabian Gulf University, Manama, Bahrain

³ Ministry of Health, Alsalmiya Medical Complex, Manama, Bahrain

transversion in exon 2 in the vicinity of H63D that results in a serine to cysteine substitution (S65C) [11]. The role of this variation on the protein function is still unclear and needs to be further examined. The third SNP is a G to A transition that results in cysteine to tyrosine substitution at position 282 (C282Y). This SNP has been reported to interfere with the formation of a disulfide bond in the $\alpha 3$ domain of *HFE* leading to the prevention of *HFE* assembly with β_2 -microglobulin, preventing its cell surface localization. The mutant protein is retained in the Golgi compartments and it is degraded before it reaches the cell surface [10, 12, 13]. The *SLC40A1* is another prominent gene suggested to be involved in iron export from the enterocytes and macrophages in mammals [14]. It majorly tends to influence the synthesis of ferroportin [15]. One of the most common polymorphisms found in the *SLC40A1* gene is a C to A change in exon 3 that results in an alanine to aspartic acid substitution at the 77 position (A77D) which in ferroportin (ferroportin disease) leads to much larger reticuloendothelial iron stores [16].

Many studies have attempted to evaluate the phenotypic expression of *HFE* genotypes in groups of beta-thalassemia patients (major, minor and intermedia) by assessing the variation of biochemical markers of iron metabolism [17–19]. The role of the *HFE* polymorphism in beta-thalassemia trait has been controversial with variable effects on iron metabolism [20–22]. In contrast, there are also evidences that indicate this genotype could induce iron overload in beta-thalassemia subjects [23–25]. Co-inheritance of the H63D SNP with other *HFE* polymorphisms may also cause severe iron overload [25]. Very limited studies have explored the effect of the *SLC40A1* gene variations on the iron status of beta-thalassemia major patients [26, 27].

In an attempt to further clarify the role of these genetic variations on iron body status, we intended to screen for polymorphisms in *HFE* (C282Y, H63D and S65C) and *SLC40A1* (A77D) along with biochemical parameters such as serum iron, total iron binding capacity (TIBC), transferrin and ferritin in Arab beta-thalassemia major (BTM) and beta-thalassemia trait (BTT) patients compared to healthy controls (HC). The present study would be the first of its kind to explore the genetic association and contribution of these polymorphisms to body iron status in Kuwaiti- Bahraini Arab population.

Materials and Methods

Study Subjects

Blood was withdrawn from 234 Arab subjects: 91 BTM (beta thalassemia major), 34 BTT (beta thalassemia trait)

patients and 109 healthy controls. The BTM group consisted of 52% females, with male to female ratio of approximately 0.9:1 and an average age of 20 ± 7 years. The BTT group consisted of 61% females, with male to female ratio of approximately of 0.6:1 and an average age of 21 ± 10 years. In addition, healthy controls included 24% females with male to female ratio of approximately 3:1 and an average age of 23 ± 5 years. The clinical and biochemical profiles were conducted in Al Amiri Hospital (Kuwait) and Salmaniya Hospital (Bahrain) as part of the patient diagnosis and follow-up. BTM patients were all diagnosed on the basis of characteristic CBC (severe microcytic hypochromic anemia) and hemoglobin fractions analyzed by HPLC (marked elevation of HbF, absence or minor amount of HbA and variable HbA₂). In many cases this was supported by molecular testing for beta-thalassemia variations. In this group 51% of patients had been splenectomised and 20% of cases had osteoporosis and endocrine failure syndromes. BTT patients showed low red cell mean corpuscular volume (MCV < 80 fl), low mean corpuscular hemoglobin (MCH < 27 pg) and increased HbA₂ (>4%). The patients in the BTM group were regularly transfused and chelated. All these patients had begun transfusions within the first 2 years of life.

Healthy volunteers (HC) were randomly selected from Kuwait by conducting a thorough survey involving demographic and clinical parameters. The inclusion criteria were general good health and no first degree relatives with history of genetic blood disorders. The exclusion criteria were any recent history of acute or chronic debilitating illness. Subjects who failed to meet the above set of criteria were excluded from the study. Iron profiling (serum ferritin, serum iron, TIBC, transferrin saturation, transferrin) was carried out for healthy controls (n = 65), and none had any abnormality. Ethnic bias within the population studied was minimized by excluding patients who were not of Arab origin. This study was reviewed and approved by the research ethics committee and written informed consent was obtained from all adult participants recruited in the study. In case of minors, the consent was obtained from their parents.

Iron Parameters

Tests for serum ferritin, iron, TIBC and transferrin were carried out using automated systems. Ferritin was measured by Elecsys 1010 analyzer by using the electrochemiluminescent method. Iron and TIBC were measured by Dimension R × L system by a chromogenic method using Ferrane. The TIBC assay involved pre-treatment of the serum with iron to saturate transferrin followed by alumina absorption of the excess and assay of the filtrate as for serum iron. Transferrin was measured by Cobas Integra analyzer by using an immunoturbidimetric assay.

Molecular Analysis

The buffy coat from a 5 ml EDTA blood sample from each subject was collected and used to extract and purify total DNA using the QIAamp Mini DNA extraction kit (QIAGEN, Germany). This DNA was used to amplify and analyse the regions of interest in the *HFE* gene exon 2 which contains H63D and S65C SNPs and exon 4 which contains the C282Y SNP. This was achieved by polymerase chain reaction (PCR) followed by restriction fragment length polymorphism analysis (PCR–RFLP) method. The C282Y creates a new *Ras1* site, whereas the H63D and S65C SNPs remove the *Mob1* and *Hinf1* sites respectively (Fig. 1). In addition, the A77D SNP in exon 3 of the ferroportin *SLC40A1* gene was amplified and analysed by sequencing by ABI 3100 automated sequencer.

Statistical Analysis

The deviation level from the Hardy–Weinberg equilibrium (HWE) in the study subjects was calculated using the Genepop program (<http://wbiomed.curtin.edu.au/gene-pop>). To compare the prevalence of gene markers tested in the study groups Pearson's χ^2 -test was employed. Any *P* value <0.05 was considered as significant. The iron homeostasis parameters were analyzed and presented as mean $\pm$ standard deviation (SD). The mean value of each variable related to the biochemical iron parameters in the mutant and wild type groups were compared by the student T-test. Data analysis was done by using SPSS (v. 19) software.

Results

Screening of *HFE* (C282Y, H63D and S65C) and *SLC40A1* (A77D) polymorphisms revealed the presence of only H63D, while the rest of the genetic variations including C282Y, S65C and A77D were not detected. No significant deviation from the Hardy–Weinberg equilibrium was found for the H63D polymorphism in the study subjects (*P* > 0.05). The marker showed a minor allele frequency (MAF) larger than 0.01 in both healthy controls subject and thalassemia patients. In the 91 patients with BTM, 16 patients were heterozygous for the H63D (H/D) and 4 patients were homozygous (D/D). Among BTT patients 9 out of 34 patients had the heterozygous genotype. No homozygous D/D genotype was detected. Among the normal controls, 21 out of 109 subjects had heterozygous H63D genotype; homozygous genotype was not detected. It was observed that the male/female ratio of subjects with H63D polymorphism in the thalassemia major group was equal whereas it was 1:2 in thalassemia minor and 20:1 in the control group. As negative iron balance is common in females in the reproductive age group comparisons of iron parameters were also done within gender groups where this was statistically feasible.

The allelic and genotypic frequencies of H63D in the tested population failed to show any significant association with BTT and BTM groups (*P* > 0.05). The frequency of the D allele was 0.13 in both BTM and BTT patients and 0.1 in normal controls. The D/D genotype was not present in the control and BTT groups. There was no significant difference between thalassemia patients (combined BTM and BTT) and healthy controls in relation to the frequencies of the H63D genotypes and allelotype.

Fig. 1 **a** PCR products after amplification of DNA segments containing H63D and S65C (295 bp), C282Y (619 bp) and A77D (306 bp) SNPs. Lane 1 contains 100 bp DNA ladder and lane five contains negative control, (*) shows position of the 600 bp fragment. **b** *Hinf1* treatment of the 295 bp amplicon containing *HFE* S65C variation. Lane 2 contains uncut amplicon (295 bp), homozygotes (AA) produce 147, 79, 69 bp bands (Lane 3, 4). **c** *Ras1* treatment of 619 bp amplicon containing *HFE* C282Y variation. Lane 2 contains

uncut amplicon (619 bp), wild type (GG) produces 234 and 385 bp bands (Lane 3 and 4). **d** *Mob1* treatment of 295 bp amplicon containing *HFE* H63D variation. Lane 2 contains uncut amplicon (295 bp). The wild type produced 3 bands corresponding to 138, 99 and 58 bp fragments (Lane 3). The homozygous mutant type produced 237 and 58 bp bands (Lane 4). CG heterozygote produced 237, 138, 99 and 58 bp bands (Lane 5)

Iron Parameters of BTM and BTT Cases Compared to Healthy Control

The biochemical iron parameters i.e., serum ferritin, serum iron, TIBC, transferrin saturation and transferrin were assayed in each of the study groups (Table 1). These variables in patient groups and healthy control subjects were compared by the t-test with significance at a P value of <0.05 . As expected, BTM showed significant differences when compared to healthy control ($P = 0.0001$) for each of the tested variables. There was no significant difference between BTT and healthy control group (Table 1).

Association of H63D Polymorphism with Biochemical Parameters of Iron Status

We further stratified the study subjects based on their genotype and investigated its influence on iron parameters. In the BTM group, comparison between-genotype (mutant vs. non-mutant) showed no significant difference in levels of iron parameters. Similarly, BTT cases with mutant genotype versus non-mutant showed no significant differences between these groups ($P > 0.05$), whereas the control subjects with polymorphism showed significantly higher serum iron ($P = 0.0004$), transferrin saturation and ferritin ($P = 0.002$) compared to those without polymorphism. Significantly reduced TIBC was observed in healthy with mutant genotype compared to non-mutant genotype ($P = 0.0001$, Table 2).

Analysis was also done to compare the iron parameters based on gender within each study group (mutant vs. non-mutant). Significant differences that emerged as a result of this analysis were: lower serum ferritin in BTM males with H/D versus H/H genotype ($P = 0.03$) and higher serum iron ($P = 0.005$), ferritin ($P = 0.02$) and transferrin saturation ($P = 0.03$) in normal males with H/D versus H/H genotype.

As expected, comparison between the study groups showed significant differences in the levels of all tested variables in BTM and healthy controls with respective genotype ($P = 0.0001$, Table 3) except for TIBC. However, in the BTT group only mutant cases with H/D showed significantly increased TIBC (mean 80.5 vs. 48.8 $\mu\text{mol/L}$;

$P = 0.003$, Table 3) and decreased serum iron concentration (mean 12.6 vs. 18.8 $\mu\text{mol/L}$, $P = 0.01$, Table 3) compared to control subjects with H/D. Similarly, gender-wise inter group genotype comparison revealed significant differences in the levels of all variables between BTM and healthy controls ($P = 0.001$), while in BTT only males with H/H genotype showed significantly increased serum ferritin compared to control males with H/H ($P = 0.02$).

Discussion

Beta-thalassemia is not uncommon among the Arab population of the Gulf region [28]. Among the most common complications in this disorder is the occurrence of iron loading. Present study was aimed to explore the polymorphisms of *HFE* and *SLC40A1* genes and their effects on laboratory parameters that measure iron metabolism in the study groups. The frequencies of C282Y, S65C and the A77D SNPs in the Arab population are likely to be extremely low and were not detected in the study. The total absence of C282Y in Saudi Arabia, Kuwait, Bahrain and Iran signifies its greatly diminished role in causing genetic hemochromatosis in these populations [29–31]. The only significant polymorphism in Arabian Gulf region, i.e. H63D, causes a phenotypic hemochromatosis in homozygous state [32].

The results of biochemical analysis of iron status in our study population showed highly significant difference between the BTM group and both the healthy control and BTT groups. There was no significant difference between the BTT and healthy control groups in relation to the iron parameters. However, several studies have shown that the BTT have higher levels of serum ferritin compared to the control population [33, 34]. One possible explanation could be the inclusion of a larger proportion of females in the test group (62% females in test group vs. 24% in the control). All females were in the reproductive age group with likely negative iron balance that is obvious from the Table 3. Males with BTT were found to have higher serum ferritin compared to the control males, however differences were not statistically significant. In a study by da Fonseca et al. [35] no significant differences reported between female

Table 1 Biochemical iron parameters in thalassemia patients and healthy control subjects (mean $\pm$ SD)

Biochemical parameter	BTM (major) (n = 91)	BTT (trait) (n = 34)	HC (n = 65)
Serum ferritin ($\mu\text{g/L}$)	5391 $\pm$ 3652	65.4 $\pm$ 50	58.2 $\pm$ 50.5
Serum iron ($\mu\text{mol/L}$)	37.8 $\pm$ 11.4	13.2 $\pm$ 6	13.9 $\pm$ 4.7
TIBC ($\mu\text{mol/L}$)	40.5 $\pm$ 7.8	78 $\pm$ 13.7	80 $\pm$ 16
Transferrin saturation (%)	95.6 $\pm$ 36	17.8 $\pm$ 7.8	18.1 $\pm$ 8.3
Transferrin (g/L)	1.6 $\pm$ 0.3	3.1 $\pm$ 0.53	3.12 $\pm$ 0.6

BTM beta-thalassemia major, BTT beta-thalassemia trait, HC healthy control

Table 2 Iron- related biochemical profile of study groups according to H63D genotypes

Subjects groups	H63D genotype	No. of cases	Ferritin ($\mu\text{g/L}$)	Iron ($\mu\text{mol/L}$)	TIBC ($\mu\text{mol/L}$)	Transferrin saturation (%)	Transferrin (g/L)
BTM ^a	H/H	71	5533.7 $\pm$ 3840	38 $\pm$ 12.2	40.2 $\pm$ 8.3	97.4 $\pm$ 38.7	1.6 $\pm$ 0.33
	H/D	16	4809.7 $\pm$ 2405.8	36 $\pm$ 5.8	43.2 $\pm$ 3.9	84 $\pm$ 17	1.7 $\pm$ 0.2
	D/D	4	4693.8 $\pm$ 3989	35 $\pm$ 1.4	38.5 $\pm$ 1.1	91 $\pm$ 1.1	1.5 $\pm$ 0.04
BTT ^b	H/H	25	67 $\pm$ 46	13.4 $\pm$ 6.7	77.6 $\pm$ 13	18.2 $\pm$ 10	3 $\pm$ 0.5
	H/D	9	60.7 $\pm$ 42	12.6 $\pm$ 4.2	80.5 $\pm$ 15.5	17 $\pm$ 8	3 $\pm$ 0.6
HC ^c	H/H	56	49.5 $\pm$ 33.4	13 $\pm$ 4.2	79 $\pm$ 15	17 $\pm$ 7.4	3.1 $\pm$ 0.6
	H/D	9	103 $\pm$ 91	18.8 $\pm$ 5	48.8 $\pm$ 22	24 $\pm$ 10.6	3 $\pm$ 0.85

BTM beta-thalassemia major, BTT beta-thalassemia trait, HC healthy control

^a Comparison of BTM (H/H) versus HC (H/H) revealed $P = 0.0001$ for all tested iron related profile and similarly, comparison of BTM (H/D) versus HC (H/D) revealed $P = 0.0001$ for all tested variables except TIBC ($P > 0.05$)

^b Comparison of BTT (H/D) versus HC (H/D) revealed significant difference only in Iron ($P = 0.01$) and TIBC ($P = 0.003$). Rest of the comparisons and BTT (H/H) versus HC (H/H) failed to show any significant difference

^c Comparison of HC (H/D) versus HC (H/H) revealed significant difference in serum iron ($P = 0.0004$), transferrin saturation ($P = 0.002$) and ferritin ($P = 0.002$) and TIBC ($P = 0.0001$)

Table 3 Biochemical variables in study groups according to H63D genotype and gender distribution

Subjects groups	Gender	H63D genotype	No. of cases	S. ferritin ($\mu\text{g/L}$)	S. iron ($\mu\text{mol/L}$)	TIBC ($\mu\text{mol/L}$)	Transferrin (g/L)	Transferrin saturation (%)
BTM	Female	H/H	38	5339 $\pm$ 3683	36 $\pm$ 10	41 $\pm$ 8	1.5 $\pm$ 0.3	92 $\pm$ 25
		H/D and D/D	10	5959 $\pm$ 2590	34 $\pm$ 4	41 $\pm$ 4.4	1.6 $\pm$ 0.2	84 $\pm$ 15
		Total	48	5468 $\pm$ 3468	36 $\pm$ 8.9	40.9 $\pm$ 7.2	1.5 $\pm$ 0.28	90.5 $\pm$ 24
	Male	H/H	33	5962 $\pm$ 4169	40 $\pm$ 14	41 $\pm$ 9	1.6 $\pm$ 0.4	101 $\pm$ 44
		H/D and D/D	10	2895 $\pm$ 1554	33 $\pm$ 1	42 $\pm$ 6	1.6 $\pm$ 0.2	80 $\pm$ 14
		Total	43	5462 $\pm$ 40.2	39.8 $\pm$ 13.5	40.8 $\pm$ 8.8	1.5 $\pm$ 0.34	99.9 $\pm$ 43
BTT	Female	H/H	15	34 $\pm$ 21	12 $\pm$ 4	78 $\pm$ 12	3 $\pm$ 0.5	16 $\pm$ 4
		H/D	6	16 $\pm$ 5	11 $\pm$ 4	89 $\pm$ 18	3 $\pm$ 0.7	14 $\pm$ 8
		Total	21	27.3 $\pm$ 18.9	11.2 $\pm$ 3.8	83 $\pm$ 15.7	3.2 $\pm$ 0.6	14.9 $\pm$ 5.9
	Male	H/H	10	92 $\pm$ 37	15 $\pm$ 9	80 $\pm$ 12	3 $\pm$ 0.5	18 $\pm$ 9
		H/D	3	136 $\pm$ 65	14 $\pm$ 5	74 $\pm$ 15	3 $\pm$ 0.6	20 $\pm$ 10
		Total	13	101 $\pm$ 45.9	14.5 $\pm$ 7.8	78.5 $\pm$ 13	3.1 $\pm$ 0.49	18.6 $\pm$ 9.1
HC	Female	H/H	26	40 $\pm$ 32	11 $\pm$ 4	81 $\pm$ 12	3 $\pm$ 0.5	14 $\pm$ 6
		H/D	1	24.3	15	115.6	4.5	13
		Total	27	39 $\pm$ 32	11.6 $\pm$ 4	82 $\pm$ 14	3 $\pm$ 0.5	14 $\pm$ 6
	Male	H/H	30	60 $\pm$ 34	14 $\pm$ 4	78 $\pm$ 17	3 $\pm$ 0.6	18 $\pm$ 8
		H/D	8	111 $\pm$ 98	19 $\pm$ 5	75 $\pm$ 14	3 $\pm$ 0.6	26 $\pm$ 11
		Total	38	71.8 $\pm$ 57.7	15 $\pm$ 4.5	77.5 $\pm$ 16	3.1 $\pm$ 0.64	20 $\pm$ 9.3

BTT versus control females whereas the male groups in their study were significantly different from each other.

Further in our study, control males with mutant H/D genotype showed significantly higher serum iron, ferritin and transferrin saturation compared to the H/H group. Another interesting fact that males with the H/H genotype associated with BTT had significantly higher ferritin compared to the H/H control male. Yamsri et al. [22]

investigated the effect of the H63D variation on serum ferritin levels in Thai thalassemia carriers. No significant difference of median ferritin levels or number of cases with elevated ferritin could be observed between males with BTT with and without the SNP, although the proportion of cases with elevated ferritin was higher in the group with mutant allele. However, as a group, males (but not females) with BTT had higher ferritin than controls in their study,

further emphasizing the importance of gender bias in assessing iron-parameters.

In summary, it appears from our study and that of the literature that heterozygous beta-thalassemia has a stronger and overriding influence on ferritin values and this manifests in males but generally not in females [22]. Although our study group did not include any case of BTT with homozygous D/D genotype, Melis et al. [24] suggested that this genotype significantly increases iron absorption because they found that males with BTT who were homozygous for this SNP had higher ferritin levels than those who lacked the H63D variation. Since gender, ineffective erythropoiesis, transfusion and iron chelation will all influence iron parameters, it follows that significant differences are expected between healthy and thalassemia (BTT and BTM) subjects even without H63D association. Therefore, comparisons between H/H and H/D genotypes *within* the respective study groups (healthy/BTT/BTM) are probably more relevant. In this connection in our study, many of the iron parameters in both BTT and BTM groups do not indicate higher iron loading in the patients with *HFE* variant genotypes—indeed many of them have lower values. This could be due to interactions with the confounding factors that were not accounted for in this study (beta-thalassemia alleles, no. of transfusions, chelation therapy) as well as low numbers of cases (with the SNP) in some groups. Analysis of such data may have provided some estimate about the rapidity of iron overload in these patients and the possible role of HH genes in its causation. A study on a relatively larger sample size that addresses the above-mentioned concerns needs to be carried out to confirm and extend the observations made in this study. Finally, significantly higher serum ferritin, iron and transferrin saturation values in the male controls with variant versus wildtype genotypes are a more valid reflection of the influence of the H63D on iron parameters, i.e., higher iron levels.

In conclusion, it appears that in the ethnic Arab population in Bahrain and Kuwait, of the four primary hemochromatosis-related SNPs investigated in this study, only H63D in the *HFE* gene is significantly prevalent whereas the other tested polymorphisms are absent or rare. The influence of the H63D SNP on biochemical parameters of iron status is unapparent in beta-thalassemia patients possibly due to interactions with the thalassemia allele and/or therapeutic measures.

Acknowledgements This work is supported by Kuwait University Grant NM02/07.

Compliance with Ethical Standards

Conflict of interest Authors declare that no conflicts of interest exist.

References

- Schranz M, Talasz H, Graziadei I et al (1999) Diagnosis of hepatic iron overload: a family study illustrating pitfalls in diagnosing hemochromatosis. *Diagn Mol Pathol* 18:53–60
- Camaschella C (2005) Understanding iron homeostasis through genetic analysis of hemochromatosis and related disorders. *Blood* 106:3710–3717
- Gasparini P, Camaschella C (2004) Hereditary hemochromatosis: is the gene race over? *Eur J Hum Genet* 12:341–342
- Parkkila S, Waheed A, Britton RS et al (1997) Association of the transferrin receptor in human placenta with HFE, the protein defective in hereditary hemochromatosis. *Proc Natl Acad Sci USA* 94:13198–13202
- Pietrangelo A (2006) Hereditary hemochromatosis. *Biochim Biophys Acta* 1763:700–710
- Pietrangelo A (2006) Hereditary hemochromatosis. *Annu Rev Nutr* 26:251–270
- Pietrangelo A, Camaschella C (1998) Molecular genetics and control of iron metabolism in hemochromatosis. *Haematologica* 83:456–461
- <http://ghr.nlm.nih.gov/gene/HFE> (2014)
- Feder JN, Gnirke A, Thomas W et al (1996) A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nat Genet* 13:399–408
- Waheed A, Parkkila S, Zhou XY et al (1997) Hereditary hemochromatosis: effects of C282Y and H63D mutations on association with b2-microglobulin, intracellular processing, and cell surface expression of the HFE protein in COS-7 cells. *Proc Natl Acad Sci USA* 94:12384–12389
- Mura C, Raguene O, Ferec C (1999) HFE mutations analysis in 711 hemochromatosis probands: evidence for S65C implication in mild form of hemochromatosis. *Blood* 93:2502–2505
- Feder JN, Tsuchihashi Z, Irrinki A, Lee VK, Mapa FA, Morikang E et al (1997) The hemochromatosis founder mutation in HLA-H disrupts b2-microglobulin interaction and cell surface expression. *J Biol Chem* 272:14025–14028
- Fleming RE, Britton RS (2006) Iron imports Vi. HFE and regulation of intestinal iron absorption. *Am J Physiol Gastrointest Liver Physiol* 290:G590–G594
- Donovan A, Brownlie A, Zhou Y et al (2000) Positional cloning of zebrafish ferroportin1 identifies a conserved vertebrate iron exporter. *Nature* 403:776–781
- <http://ghr.nlm.nih.gov/gene/SLC40A1> (2014)
- Montosi G, Donovan A, Totaro A et al (2001) Autosomal-dominant hemochromatosis is associated with a mutation in the ferroportin (SLC11A3) gene. *J Clin Invest* 108:619–623
- Aguilar-Martinez P, Schved JF, Badens C et al (2000) Iron overload in thalassaemias and genetic haemochromatosis. *Eur J Haematol* 64:279–280
- Borgna-Pignatti C, Solinas A, Bombieri C et al (1998) The haemochromatosis mutations do not modify the clinical picture of thalassaemia major in patients regularly transfused and chelated. *Br J Haematol* 103:813–816
- Cappellini MD, Fargion SR, Sampietro M, Graziadei G, Fiorelli G (1998) Nontransfusional iron overload in thalassemia intermedia: role of the hemochromatosis allele. *Blood* 92:4479–4480
- Fargion SR, Sampietro M, Cappellini MD (2000) Thalassemia and their interactions with haemochromatosis. Cambridge University Press, Cambridge, pp 435–438
- Garewal G, Das R, Ahluwalia J, Marwaha RK (2005) Prevalence of the H63D mutation of the HFE in north India: its presence does not cause iron overload in beta thalassemia trait. *Eur J Haematol* 74:333–336

22. Yamsri S, Sanchaisuriya K, Fucharoen S et al (2007) H63D mutation of the hemochromatosis gene and serum ferritin levels in Thai thalassemia carriers. *Acta Haematol* 118:99–105
23. Fairbanks VF, Thibodeau SN, Granfortuna JM, Lubin IM, Silverman LM, Rohlf EM (1997) Iron overloading in β -thalassemia trait and spherocytosis: analysis of C282Y and H63D mutations. *Blood* 190:abstr 2740
24. Melis MA, Cau M, Deidda F, Barella S, Cao A, Galanello R (2002) H63D mutation in the HFE gene increases iron overload in β -thalassemia carriers. *Haematologica* 87:242–245
25. Ruiz-Arguelles GJ, Garces-Eisele J, Reyes-Nunez V et al (2001) Heterozygosity for the H63D mutation in the hereditary hemochromatosis (HFE) gene may lead into severe iron overload in β -thalassemia minor: observations in a thalassemic kindred. *Rev Invest Clin* 53:117–120
26. Politou M, Kalotychoy V, Pissia M, Rombos Y, Sakellaropoulos N, Papanikolaou G (2004) The impact of the mutations of the HFE gene and of the SLC11A3 gene on iron overload in Greek thalassemia intermedia and β (s)/ β (thal) anemia patients. *Haematologica* 89:490–492
27. Agarwal S, Sankar VH, Tewari D, Pradhan M (2006) Ferroportin (SLC40A1) gene in thalassemic patients of indian descent. *Clin Genet* 70:86–87
28. Zahed L (2001) The spectrum of β -thalassemia mutations in the Arab populations. *J Biomed Biotechnol* 1:129–132
29. Alsmadi OA, Al-Kayal F, Al-Hamed M, Meyer BF (2006) Frequency of common HFE variants in the Saudi population: a high throughput molecular beacon-based study. *BMC Med Genet* 3:7–43
30. Alkhateeb A, Uzrail A, Bodoor K (2009) Frequency of the hemochromatosis gene (HFE) variants in a Jordanian Arab population and in diabetics from the same region. *Dis Markers* 27:17–22
31. Karimi M, Yavarian M, Delbini P, Harteveld CL, Farjadian S, Fiorelli G, Giordano PC (2004) Spectrum and haplotypes of the HFE hemochromatosis gene in Iran: H63D in β -thalassemia major and the first E277K homozygous. *Hematol J* 5:524–527
32. de Diego C, Opazo S, Murga MJ, Martinez-Castro P (2007) H63D homozygotes with hyperferritinaemia: is this genotype, the primary cause of iron overload? *Eur J Haematol* 78:66–71
33. Mehta BC, Pandya BG (1987) Iron status of β thalassemia carriers. *Am J Hematol* 24:137–141
34. Loria A, Konijn AM, Hershko C (1978) Serum ferritin in β -thalassemia trait. *Isr J Med Sci* 14:1127–1131
35. Da Fonseca SF, Kimura EY, Kerbaui J (1995) Assessment of iron status in individuals with heterozygotic β -thalassemia. *Rev Assoc Med Bras* 41:203–206