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## Point Mutations in Ferroportin Disease: Genotype/Phenotype Correlation

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### 1. Introduction

The identification of gene mutations involved in the pathogenesis of hereditary hemochromatosis (HH) provided a better understanding of primary iron overload syndrome (Feder, 1996). HH is a genetic disorder characterized by increased dietary iron absorption despite an excess in iron stores. The result is a progressive increase in total body iron with abnormal iron deposition in the liver and the endocrine glands. Transferrin saturation and serum ferritin level are the most reliable tests for the detection of individuals with HH. Mutations in HLA-linked HFE gene result in the classical type 1 HH inherited in an autosomal recessive pattern. One major mutation, the C282Y is responsible for 85 to 90% of the cases with an estimated prevalence of 1 in 200 in Northern Europe. However, the bioclinical penetrance is incomplete and only a few suffer from overt disease. The second most common mutation is H63D and accounts for 3 to 5% of cases. Its relationship with HH is less obvious. Compound heterozygous H63D/C282Y genotype represents less than 1% and simple heterozygous genotype is not associated with biochemical abnormalities. Less common mutations in the transferrin receptor 2 (TfR2), hemojuvelin (HJV) and Hephcidin (HAMP) genes, also in the homozygous state, result in the type 2 and type 3 HH (Pietrangelo, 1999, 2007, 2010).

The non-HFE-related form caused by Ferroportin 1 (SLC40A1) gene mutation is associated with an autosomal dominant pattern of inheritance. This type 4 HH also known as ferroportin disease is emerging as the second most common inherited iron metabolic disorder. Serum ferritin levels in these patients are elevated early in the course of the disease, whereas the transferrin saturation is not elevated until later in life. The accumulation of excess iron is seen predominantly in Kupffer cells rather than in hepatocytes and there is a marginal anemia with low tolerance to phlebotomy (Pietrangelo, 1999).

Most SLC40A1 mutations occur in exon 5 and affect valine in position 162 and asparagine in position 144. Recently, two different phenotypes of ferroportin disease have been described. The M phenotype or “type 4A” characterized by iron accumulation in macrophages and due to a loss-of-function mutation leading to non-functional iron transport mutant (e.g. V162del) and the H phenotype, also called “type 4B” characterized by iron accumulation in hepatocytes and due to gain-of-function mutation leading to

hepcidin-induced degradation resistance (e.g. N144H). Some mutations (e.g. Q248H) results only in genetic polymorphism without clinical manifestations (Mayr, 2010; Barton, 2003; De Domenico, 2006).

Controversial data reported before the era of molecular testing, suggested that patients with beta-thalassemia trait may develop iron overload (Edwards, 1981). However, most of these data, did not exclude the possible role of other endogenous factors including ferroportin disease (FD). Beta-thalassemia trait, when coinherited with a single H63D allele has rarely been associated with iron overload however; its co-inheritance with SLC40A1 mutation has not yet been studied.

Here we report a classical phenotype of ferroportin disease due to V162del in a Lebanese family with heterozygosity for H63D and beta-thalassemia trait. We also discuss the clinical expression of FD in the presence of genetic cofactors.

## 2. Family description and search strategy

The proband was a 54 years old male who presented in 1998 with fatigue and anemia. Clinical examination revealed skin pallor, splenomegaly and painful hepatomegaly. Relevant medical history included a blood transfusion 18 years earlier after a car accident and type 2 diabetes mellitus. There was no alcohol abuse or viral hepatitis. The serum ferritin concentration was 1011 ng/ml (Normal range < 400 ng/ml) and the transferrin saturation was 45%. The hemoglobin level was 10.1 g/dl (Normal: 11.5 - 17 g/dl), and the peripheral blood film showed hypochromia and aniso-poikilocytosis with target cells. The hemoglobin electrophoresis revealed a beta-thalassemia minor. The ceruloplasmin level was 474 mg/l (Normal: 155-592 mg/l) and the alpha-fetoprotein concentration was 21.37 IU/ml (Normal <5.80 IU/ml). The MRI study showed a splenomegaly, a portal vein thrombosis and a dysmorphic liver with hypertrophy of segment I. The liver biopsy revealed a fatty infiltration of the liver with iron deposits in hepatocytes and Kupffer cells and moderate portal fibrosis. Sections of liver tissue were stained with periodic acid-Schiff, Masson trichrome, Sweet's reticulin, hematoxiline/eosin and Perls Prussian blue (Figures 2 -6).

The proband's mother and one of his brothers died before the age of 50 from chronic liver disease. Their serum ferritin levels were higher than 1000 ng/ml (Figure 1). His oldest brother died from beta-thalassemia major. His 26 and 22 years old sons were healthy and presented elevated serum ferritin levels - 643 ng/ml and 744 ng/ml - with normal transferrin saturations. No liver biopsy was performed on them. His third son, 19 years old, had normal hemoglobin pattern and serum ferritin level.

The DNA from peripheral blood samples was tested with a reverse transcriptase-based assay, the Haemochromatosis StripAssay A® (Vienna Lab, Labordiagnostika, GmbH, Vienna, Austria), according to the manufacturer's instructions. This assay can detect twelve mutations in the HFE gene: V53M, V59M, H63D, H63H, S65C, Q127H, P160delC, E168Q, E168X, W169X, C282Y and Q283P, four mutations in the Tfr2 gene: E60X, M172K, Y250X and AVAQ594-597del, and two mutations in the SLC40A1 gene: N144H and V162del.

A systematic review of the literature was undertaken using search in Medline from 1981 for beta-thalassemia and hemochromatosis and from 2001 to 2011 for SLC40A1 novel mutations and new phenotypes.

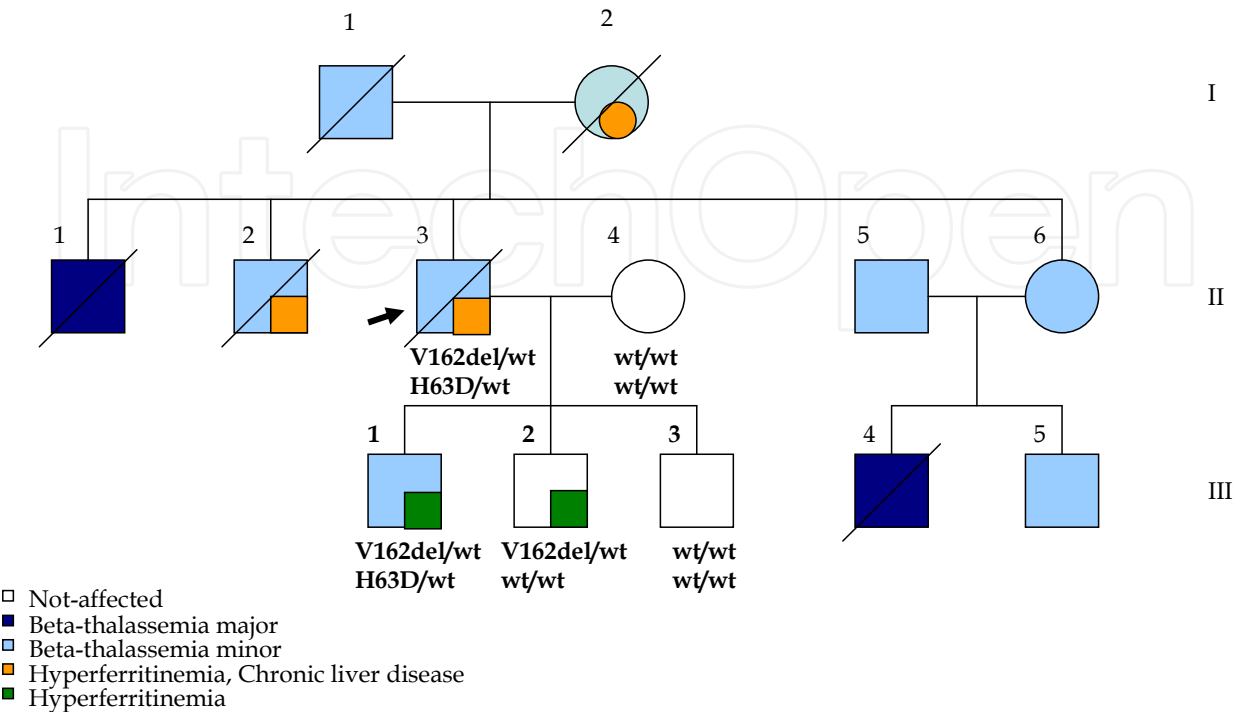


Fig. 1. Pedigree of a Lebanese family with beta-thalassemia and primary iron overload. Individuals II 3, II 4, III 1, III 2, III 3 were tested for SLC40A1 and HFE gene mutations.

2.1 Discussion and systemic review

2.1.1 Analysis of the pedigree

As shown in figure 1, the proband, his mother and one of his older brothers affected by beta-thalassemia minor died from chronic liver disease related to primary iron overload syndrome. The genetic testing showed that he was carrier of one copy of V162del mutation in SLC40A1 gene and one copy of H63D mutation in HFE gene. The two sons who carry the V162del mutation presented high serum ferritin levels with low transferrin saturation. One of them carries a single copy of H63D and beta-thalassemia trait but still remains asymptomatic. Most probably the primary iron overload syndrome transmitted in an autosomal dominant manner within this family is caused by the ferroportin disease due to V162del mutation. This mutation has a high penetrance and segregates with the clinical phenotype. The phenotypic expression is characterized by a high serum ferritin level, normal transferrin saturation; age related massive hepatocellular and macrophage iron accumulation leading to liver fibrosis. Coincidental association with H63D in two affected members and with beta-thalassemia trait in four affected members was noticed.

The role of these genetic co-factors in the aggressiveness of ferroportin disease remains to be elucidated.



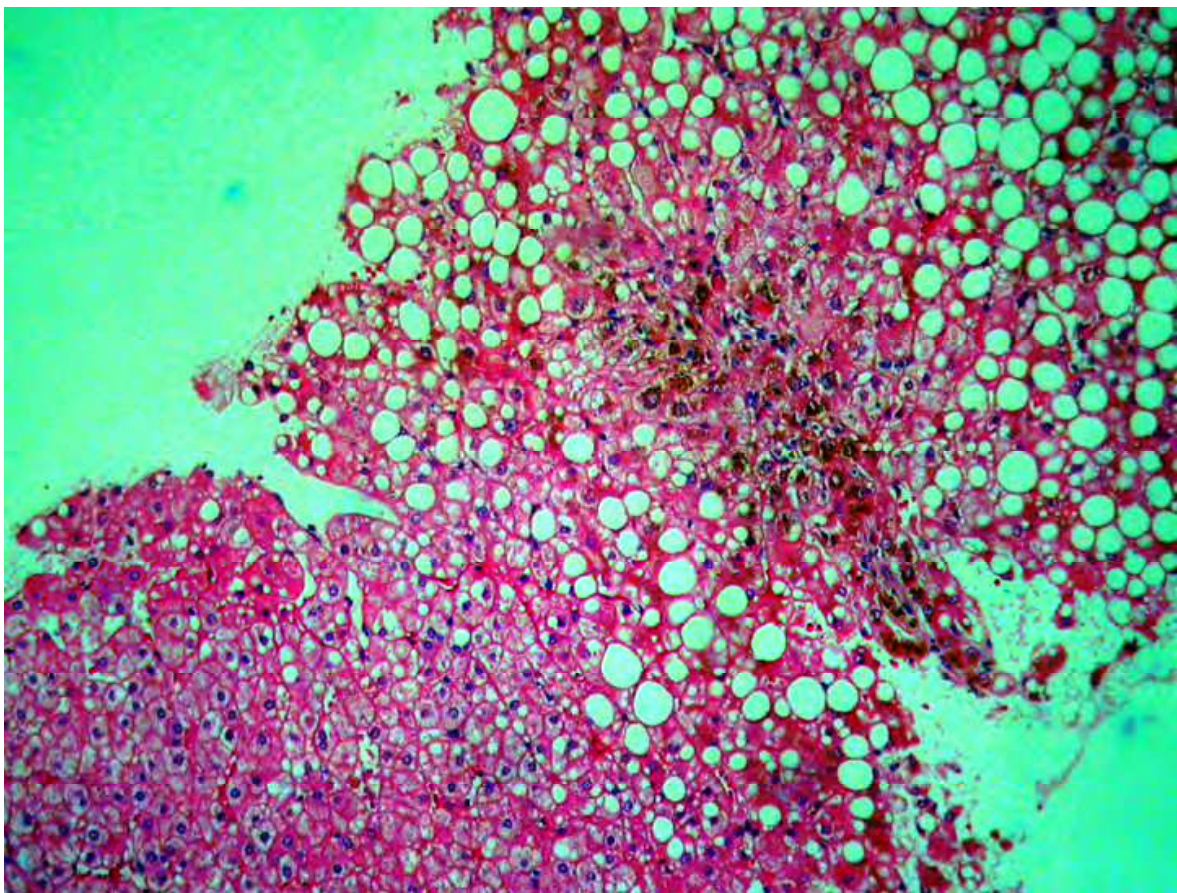
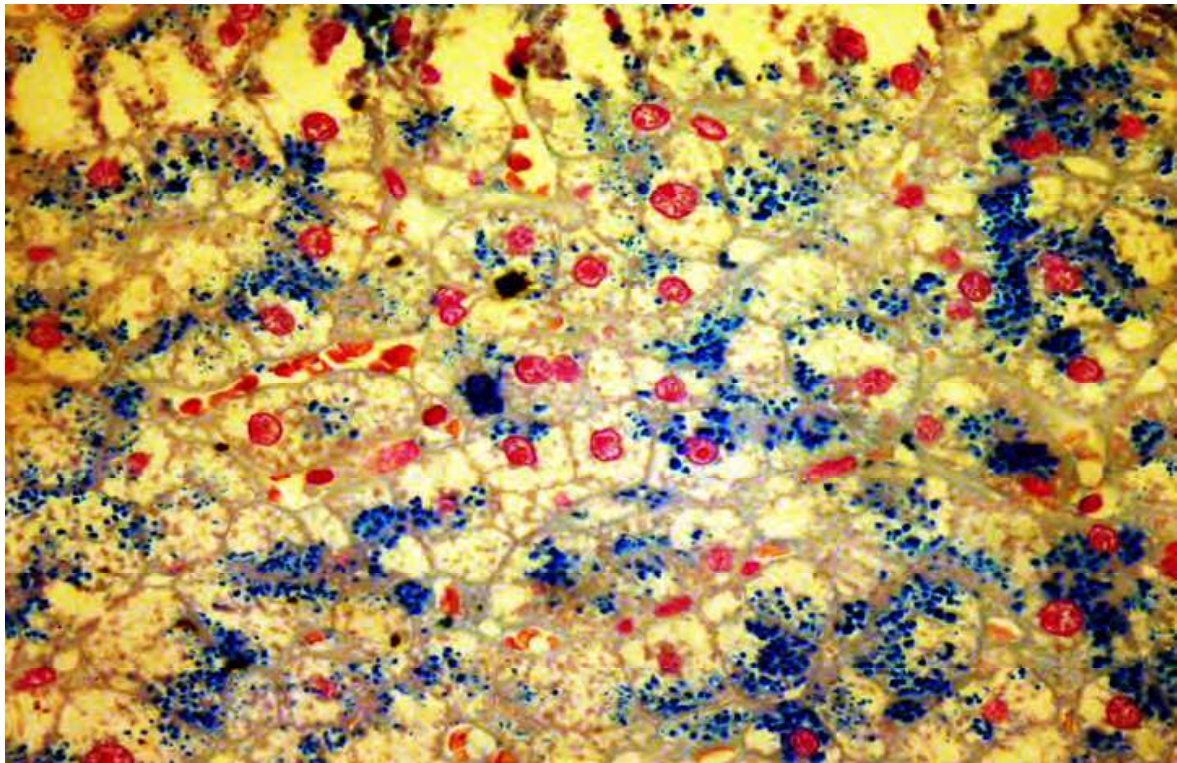
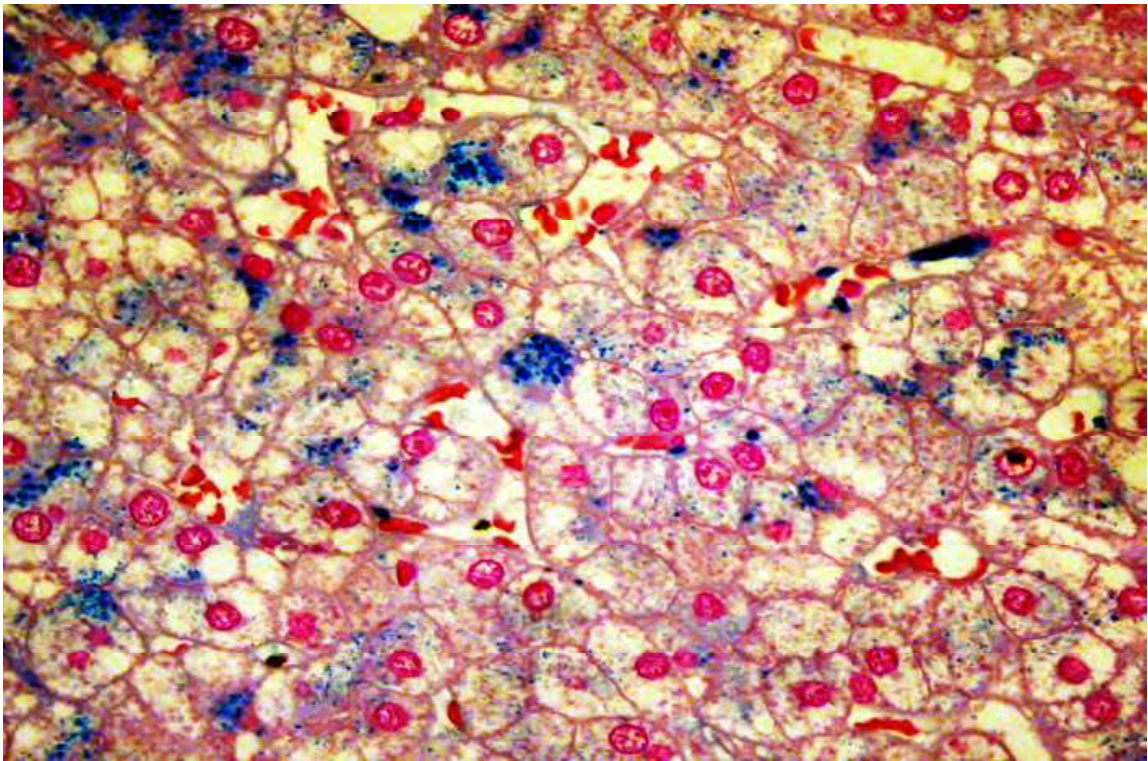


Fig. 2. Hematoxiline and Eosin stain x40. Liver siderosis and steatosis.



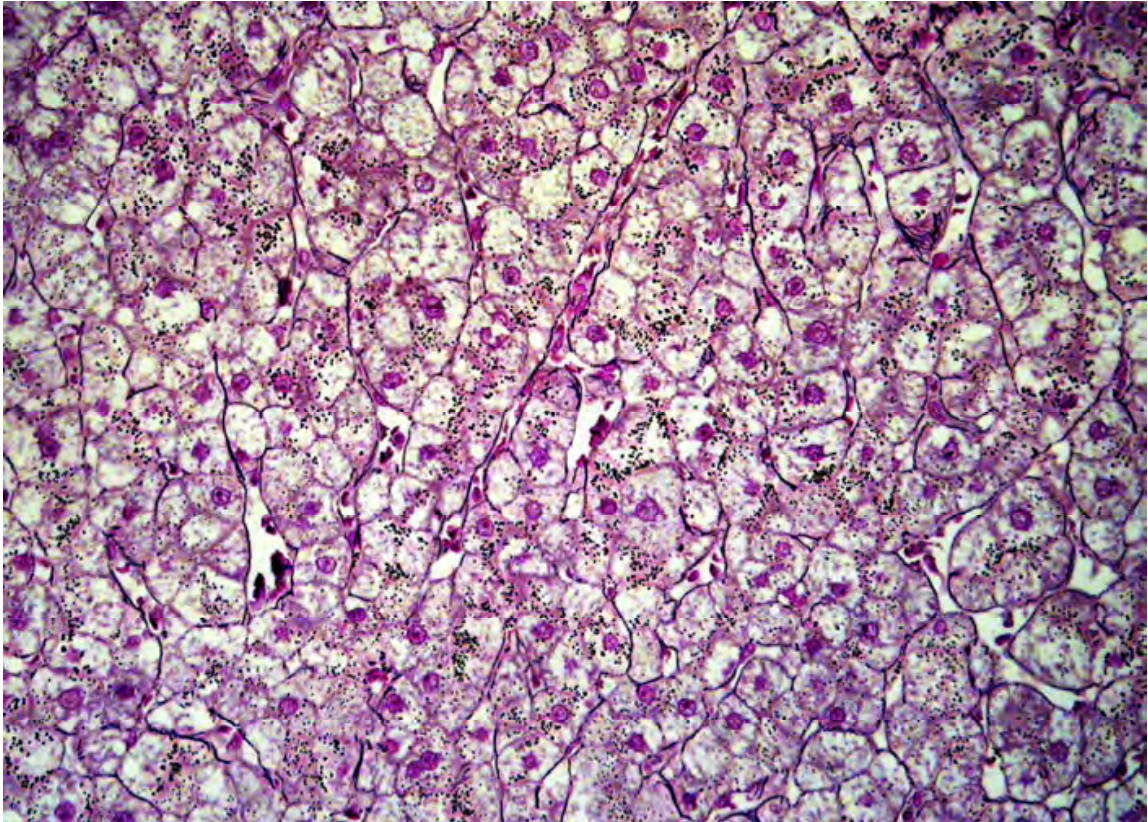
(A)





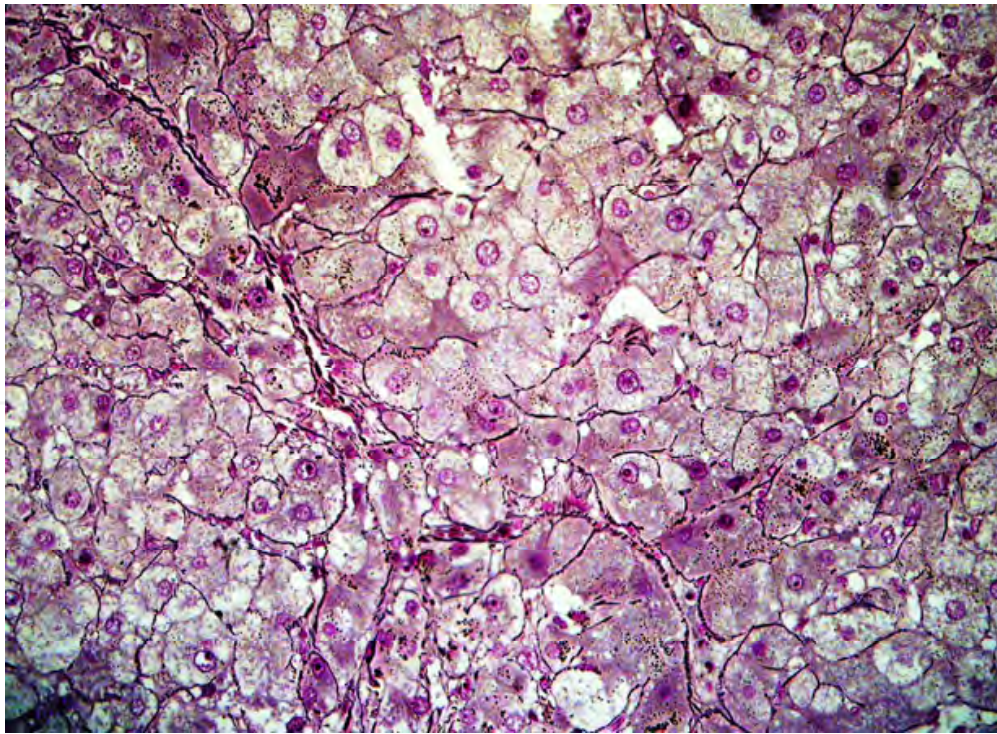
(B)

Fig. 3. Perls Prussian blue stain x400 (A and B). Grade 3 siderosis. Iron accumulation in Kupffer cells and hepatocytes



(A)





(B)

Fig. 4. Gordon Sweet's reticulin stain x100: Thick trabecula (A), and x200: Disoriented reticulin strings around the thickened trabecula (B)

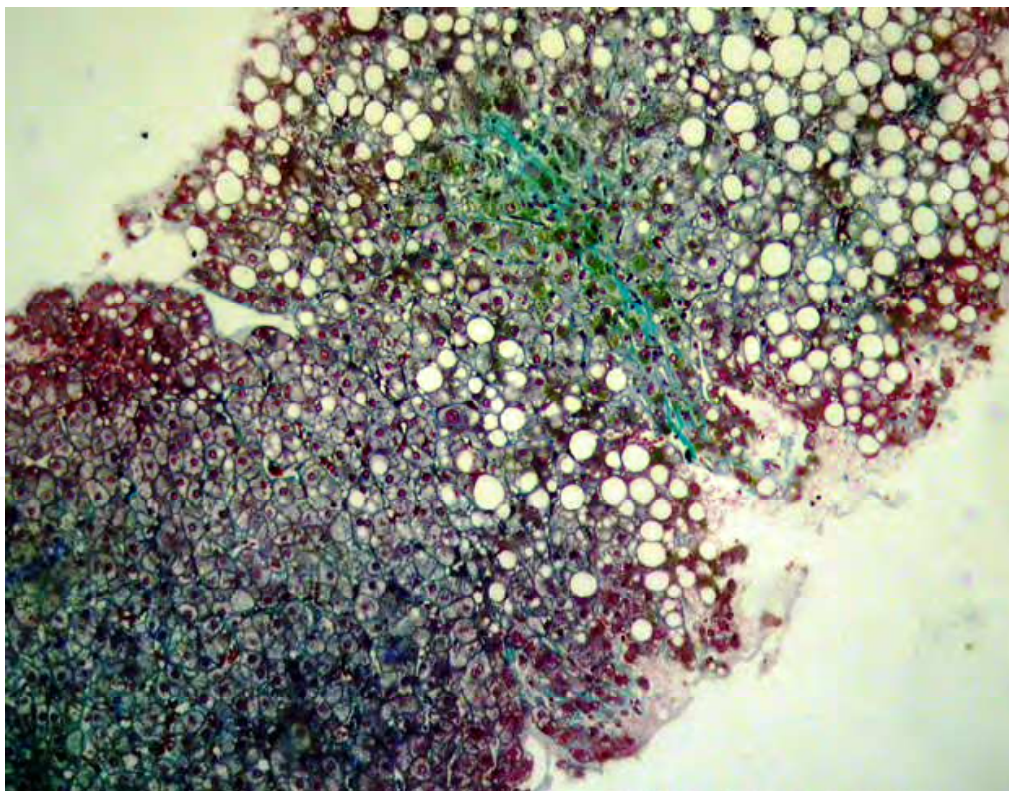


Fig. 5. Masson trichrome stain x40: Steatosis with minimal collagenous fibrosis within the stroma.



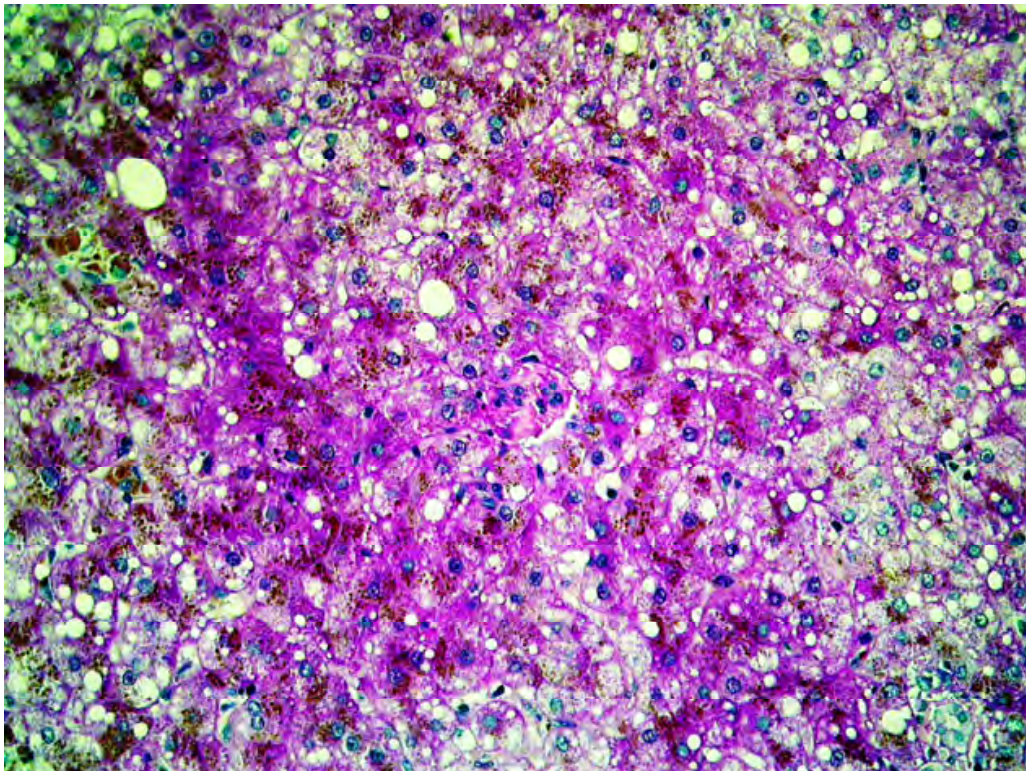


Fig. 6. Periodic-Acid Schiff stain x100. Common glycogen storage foci.

### 2.1.2 Genetic background and iron homeostasis

It is well known that different mutations in the same gene can result in different phenotypes and mutations in different genes can be expressed as the same phenotype. As for penetrance, carriers inherit their genotype in an autosomal dominant or recessive manner, yet they may not develop the diseased-phenotype because the altered inherited genes are incompletely penetrant. Modifier genes may affect the expression of some alleles which may increase or decrease the penetrance of a germline mutation. Furthermore, sporadic cases or phenocopies within the same pedigree have phenotype similar to affected mutation carriers. Reliable genetic testing may determine the hereditary nature of the disease and careful pedigree analysis, especially when multiple genetic conditions are co-inherited in members within the same family may help to sort out what genotype is causing what phenotype..

Iron homeostasis is balanced by the iron store. Hemoglobin level reflects the functional iron status, transferrin saturation percentage reflects the iron in transport status and the serum ferritin level parallels the concentration of iron storage within the body regardless of the cell type in which it is stored. Two proteins regulate the outflow of iron through cells, Hepcidin and Ferroportin. Ferroportin is the only known mammalian iron exporter, expressed in macrophages and the basolateral membrane of enterocytes and hepatocytes. It serves as a channel through which iron is transported across the cell membrane into plasma. Up-regulation of ferroportin function enables increased iron absorption from the intestine and increased iron export to plasma from macrophages. Hepcidin mediates down-regulation of ferroportin action. Imbalances cause primary iron overload states and anemias. There is currently no available routine test to detect these two proteins in clinical practice.



Transferrin iron represents the normal form of circulating iron. Non-transferrin bound iron (NTBI) has been identified in the plasma of patients in whom transferrin saturation is significantly elevated. NTBI represents a potentially toxic form and is avidly taken-up by parenchymal cells especially hepatocytes (Brissot,2011).

### 2.1.3 Genetic hemochromatosis

Hereditary hemochromatosis (HH) is a primary iron overload (PIO) syndrome characterized by increased dietary iron absorption despite an excess in iron stores resulting in high transferrin saturation, high serum ferritin level and iron accumulation in liver parenchyma. It is one of the most common inherited diseases worldwide.

#### 2.1.3.1 Hereditary hemochromatosis type 1, HFE-related

Hemochromatosis or High Fe gene (HFE) encodes for a membrane protein that is similar to MHC class I-type proteins. Following the identification of HFE gene mutations in 1996, three major mutations have been recognized to be responsible for HH in homozygous state, C282Y, H63D and S65C. The C282Y mutation has been found in more than 90% of northern European patients and in more than 80% of American patients of European origin. However, only 50-65% of Southern European patients are homozygous for this mutation (Pietrangelo, 2007, 2010). No conclusive evidence of abnormal gene expression in the heterozygous state has been found. Penetrance of HH is incomplete, usually age related and may be affected by other conditions such as hemoglobinopathies and congenital dyserythropoietic anemia. Little information about epigenetic factors influencing the penetrance is known. The precise function of the HFE protein is not fully clear.

#### 2.1.3.2 Hereditary hemochromatosis type 4, Ferroportin disease

The availability of molecular tools has allowed investigators to identify novel mutations, non-HFE related, causing different clinical forms of hemochromatosis. The autosomal dominant form of HH was first reported in 1990, in a large family from the Solomon Islands (Eason, 1990). Serum iron indices from the family members resemble a classical HH but liver biopsies showed a pattern of iron staining in both hepatocytes and Kupffer cells with a certain degree of fibrosis and cirrhosis. In 1999, a typical pedigree was described and linked two years later to a point mutation (A77D) in ferroportin 1 gene also called FPN1, IREG1, MTP1 and SLC40A1 (Pietrangelo,1999; Montosi, 2001). At the same time a large Dutch family was identified in the Netherlands (Njajou, 2001), presenting another point mutation in the same gene (N144H). Mutations in SLC40A1 gene have then been reported from many countries throughout the world (Table 1), some of them are genetic polymorphism with slightly elevated serum ferritin levels. The most frequently reported mutations involve asparagine and valine residues in position 144 and 162. Various controversial data have been reported on the structure-function relationships of the various SLC40A1 mutants. However, phenotypic manifestations of this autosomal dominant HH named ferroportin disease (FD) are heterogeneous even between family members sharing the same SLC40A1 mutation.

Unlike HFE-related HH, FD is a genetically heterogeneous iron overload syndrome with more than 40 SLC40A1 mutations reported. Most mutations have been associated with the classical form of FD characterized by hyperferritinemia, normal to low transferrin saturation and Kupffer cell iron storage. Other mutations have been associated with the non-classical

form of the FD with increased transferrin saturation and hepatocellular iron storage in addition to high ferritin level and Kupffer cell iron storage. Few mutations have been reported as ferroportin gene polymorphism.

A loss-of-function mutation leads to hepcidin-independent down-regulation of ferroportin resulting in intracellular retention of the iron export pump. A gain-of-function mutation renders ferroportin resistant to inactivation by hepcidin. Functional studies have associated the classical phenotype of FD, also called M phenotype or type 4 A with the cellular iron export deficiency due to non-functional mutant and non-classical phenotype, also called H phenotype or type 4 B with the increased absorption of dietary iron due to a hyperactive mutant (De Domenico, 2006; Mayr, 2010).

#### 2.1.4 Clinical expression of ferroportin disease and genetic cofactors

Ferroportin disease has a mild clinical expression in the absence of cofactors. Le Lan et al (Le Lan, 2011) in studying 70 affected subjects from 33 families with 19 different mutation in SLC40A1 gene showed that non-genetic co-factors such as obesity and excessive alcohol consumption are responsible for cirrhosis, fibrosis and malignant transformation cases which rather are sporadic cases in families with FD. The identification of a genetic variant in SLC40A1 is not sufficient to confirm the diagnosis of FD in patients with hyperferritinemia. Other genetically co-inherited conditions in the same individual may contribute to the development of PIO. In presenting this pedigree we mainly aimed to discuss the coexistence of multiple genetic factors that contribute to the primary iron overload condition particularly in Mediterranean areas where the  $\beta$ -thalassemia trait is prevalent in almost all populations and where FD seems to be present and underestimated. Although, when molecularly diagnosed FD could not by itself explain an aggressive phenotype, a comprehensive analysis of all co-inherited conditions and acquired factors seems to be relevant.

$\beta$ -thalassemia minor is an asymptomatic common condition in Lebanon and the Mediterranean coast characterized by a mildly ineffective erythropoiesis that induces compensatory excess in iron absorption usually without iron overload. Moreover,  $\beta$ -thalassemia major and intermedia result in iron overload only through repetitive blood transfusions. Heterozygotes for HFE hemochromatosis are also asymptomatic but have been associated with mildly increased iron absorption (Zimmerman, 2008).

Whereas C282Y shows a distribution similar to HH type 1, H63D mutation is common in areas where the disease is not prevalent and its allelic frequency has great variability worldwide. It has been reported that  $\beta$ -thalassemia trait might increase the severity of hemochromatosis in subjects with C282Y and H63D homozygous but not heterozygous state (Piperno, 2000; Melis, 2002; Estevao, 2011).

Melis et al (Melis, 2002) demonstrated in 2002 that the H63D mutation in the homozygous state resulted in higher levels of serum ferritin and presumably iron overload in patients with  $\beta$ -thalassemia trait. There was no effect on ferritin levels in those with wild type/H63D or wild type/wild type genotypes. In contrast, Martins et al (Martins, 2004) showed in 2004 that the  $\beta$ -thalassemia trait, already related with the potential development of iron overload, tended to be aggravated with the coinheritance of H63D mutation, even when present in the heterozygous state. In a recent Brazilian analysis of 138 beta-thalassemic patients, Estevao et



al found that the high levels of serum ferritin observed in beta-thalassemia heterozygotes do not depend on the inherited mutation in the beta-globin gene, and the association of beta-thalassemia heterozygous with the H63D/wild type state does not modify the iron profile in these individuals (Estevao, 2011; Oliveira, 2006). Garewal et al showed that H63D mutation which is prevalent in north Indians did not affect the iron indices in beta-thalassemia trait carriers (Garewal, 2003).

The role of SLC40A1 mutation on iron overload in beta-thalassemia carriers and inversely the role of beta-thalassemia minor on the phenotypic expression of ferroportin gene mutation have not yet been studied. Barton et al evaluated genotype and phenotype characteristics of unselected African American index patients with primary iron overload who reside in central Alabama and concluded that primary iron overload is not the result of the mutation of a single gene HFE, C282Y or ferroportin 744 G-->T (Q248H), but that common forms of heritable anemia appear to account for increased iron absorption or retention in some patients (Barton, 2003).

Arada et al in studying polymorphisms of the HFE gene and iron indices in 815 healthy Spanish subjects not affected by beta-thalassemia showed that the C282Y heterozygote, the H63D heterozygote and homozygote and the H63D/S65C compound heterozygote genotypes were associated with increased transferrin saturation relative to the wild type genotype. The latter compound genotype had the higher phenotypic expression (Arada, 2010). The SLC40A1/H63D compound heterozygosity has not yet been studied however; it may contribute to the aggravation of the iron overload picture. Table 1 shows the reported cases with this condition and the associated phenotypes. Four out of five reported cases with H63D mutation represented the mild classical form of FD while only one case report described an aggressive non-classical form with 100% transferrin saturation rate.

FD is a mild form of iron overload with heterogenous clinical presentation. It has been reported in a large cohort of patients that sex; environmental and/or acquired cofactors have prominent roles in determining the variability of the phenotypic expression. The role of co-inheritance of conditions that may interfere with the iron metabolism remains to be elucidated. In the present Lebanese pedigree, the V162del mutation is most probably the cause of the iron overload syndrome. The  $\beta$ -thalassemia and the H63D heterozygous state may have contributed to the aggressive phenotype in the proband.

In a systemic review of the literature and a meta-analysis of clinical, biochemical, pathological and molecular findings of FD, Mayr et al found that the biochemical penetrance of FD was 86%. Eighty probands out of 176 reported families were classified as having the classical phenotype and 53 probands the non-classical phenotype. The mean age at presentation for the non-classical form was higher. Cirrhosis was reported in only 4 patients of whom two had N144H, one C326S and one I180T mutation. Liver fibrosis was related to age but neither to hepatic iron concentration nor serum ferritin. Of the 31 different SLC40A1 mutations, six were unequivocally associated with the classical form and five with the non-classical form. Variable phenotypes were reported for nine mutations. The remaining probands were incompletely assessed. The authors concluded that because not all mutations were unambiguously correlated with the classical or non-classical phenotype in all reported patients with a particular mutation, the genotype to phenotype correlation suggests that FD has a multifactorial cause (Mayr, 2010). To determine whether bio-informatic tools SIFT (Sorting Intolerant From Tolerant) (Ng, 2003) and PolyPhen (Polymorphism phenotyping)

Author/year	Age sex	Family origin	Mutation (Protein)p.	Mutation (DNA) c.	Main symptoms	Ferritin (ng/ml)	TS	Coexisting conditions	Liver changes	Fe in liver cells
Montosi/2001	59 m	Italy	A77D	230C>A	Asymptomatic	5750	75%	None	Cirrhosis	REC
Njajou/2001	80 f	Netherlands	N144H	430A>C	Arthralgia, diabetes	>2000	88%	-	Fibrosis	REC ± HC
Wallace/2001	56 m	Australia	V162del	484-486del3	Hepatomegaly, pancytopenia	>12000	-	None	Portal fibrosis	Both
Devalia/2002	38 f	UK	V162del	484-486del3	Fatigue	>2000	40%	None	No fibrosis	Both
Cazzola/2002	-	Italy	V162del	484-486del3	-	High	<50%	None	No fibrosis	REC
Roetto/2002	26 f	Italy	V162del	484-486del3	Anemia	>1000	<20%	None	No fibrosis	REC ± HC
	58 f	Italy	V162del	484-486del3	Asymptomatic	>5000	<40%	None	No fibrosis	REC ±HC
	30 m	UK	V162del	484-486del3	Asymptomatic	>1000	<30%	None	No fibrosis	REC ±HC
Hetet/2003	61 m	France	D157G	774A>G	Asymptomatic	4069	44%	H63D/WT	-	-
	63 m	France	E182H	850G>T	Cataract	3018	16%	-	-	-
	49 f	France	G323V	1272G>T	Cataract	>1700	N	-	-	-
Jouanelle/2003		France (Asian)	G490D	1468G>A	-	>2000	<40%	None	-	REC ± HC
Gordeuk/2003	-	USA (African)	Q248H	744G>T	Mild anemia	>500	N	None	-	REC
Beutler/2003	-	USA (African)	Q248H	744G>T	-	High	N	-	-	REC
Rivard/2003	24 m	Canada (French)	Y64N	190T>C	Fatigue ,tremor,diarrhea	647	77%	None	Steatosis	Both
Arden/2003	48 m	Solomon Islands	N144T	431A>C	Cardiac murmur	2937	80%	None	Fibrosis	Both
Wallace/2004	32 f	Australia	N144D	430A>G	hepatomegaly	High	N	Sarcoidosis	Cirrhosis	Both
Zoller/2005	68 f	Austria	V162del	484-486del3	Cirrhosis	High	N	Sarcoidosis	Cirrhosis	Both
Sham/2005	young	USA	C326S	977G>C	Cholecystectomy (iron in lymph node)	5265	37%	None	-	REC
Morris/2005	young	Canada	N185D	553A>G	Hepatomegaly	High	>50%	-	-	HC
Koyama/2005	43 m	Japan	R489S	1467A>C	-	High	High	-	-	-
Subramaniam/2005	45 m	Australia	A77D	230C>A	Asymptomatic	822	25%	None	No fibrosis	REC ± HC
					Lethargy	3500	29%	None	No fibrosis	REC
Wallace/2005	36 f	Sri Lanka	V162del	484-486del3	Asymptomatic	3145	29%	None	No fibrosis	Both
Cremonesi/2006	34 m	Italy (Italian)	D181V	846A>T	Fatigue	1400	40%	None	No fibrosis	Both
	Young	Italy (Italian)	G80V	239G>T	Asymptomatic	1311	33%	None	No fibrosis	-
	Young	Italy (Chinese)	G267D	1104G>A	Asymptomatic	1153	32%	None	No fibrosis	-
Liu/2005 & Hayashi/2006	43 f	Japan	A117G	-	Chronic hepatitis	9660	92%	Chr. Hep	No fibrosis	Both
	43 m	Japan	R489S	1467A>C	Asymptomatic	822	25%	None	No fibrosis	REC
	79 m	Japan	R489S	1467A>C	Asymptomatic	2283	62%	None	No fibrosis	REC
Bach/2006	-	Spain	R88T	236G>C	-	High	N	-	-	-
	-	Spain	I180T	539T>C	-	High	N	-	-	-
Lee/2007	m	USA (Scottish)	G468S	1402G>A	-	>1000	-	H63D/WT	-	REC
Wallace/2007	72 m	New Zealand	S338R	1014T>G	-	1990	90%	None	Steatosis	Both
					Liver function changes					
Girelli/2008	59 f	Italy	I152F	758A>T	Liver funct. Changes	1771	23%	H63D/WT, VHC	Fibrosis	HC
	64 m	Italy	L233P	1012T>C	Diabetes	9000	75%	VHC	Fibrosis	REC +HC
Sussman/2008	47 m	USA (African)	R561G	1681A>G	Cirrhosis	2960	100%	VHC, XLSA	Fibrosis	HC
Speletas/2008	25 f	Greece	V162del	484-486del3	Mild anemia	>1000	<40%	None	-	REC
	58 f	Greece	R178G	532C>A	-	>1000	<40%	None	-	REC
Rosmorduc/2008	48 m	France	N144H	431A>G	Asymptomatic	2195	100%	None	Mild Fibrosis	HC
Pelucchi/2008	-	Italy	D157N	469G>A	-	High	N	-	-	-
	-	Italy	V72F	214G>T	-	High	N	-	-	-
Mougiou/2008	34 m	Greece	G80S	239G>?	Asymptomatic	1800	32%	None	-	REC
Lok/2009	38 m	Thailand	C326Y	977G>A	Abnormal liver function tests	4326	99%	None	-	-
Letocart/2009	35 m	Italy	Y501C	1502A>G	Asthenia, arthralgia	642	94%	None	-	-
Saja/2010	58 m	UK	D157G	470A>C	Asymptomatic	4123	32	None	Steatosis	REC
Griffith/2010	57 f	UK	R489K	1466G>A	Asymptomatic	685	39	H63D/WT	No fibrosis	REC
Mayr/2011	54 m	UK	W158C	474G>T	None	3015	21%	Ulc. Colitis	No fibrosis	REC
	41 m	UK	H507R	1520A>G	Lethargy	3097	100%	H63D/WT	Steatosis	HC
Del-Castillo-Rueda/2011	45 m	Spain	K240E	718A>G	Asymptomatic	422	71%	Chondro-sarcoma	-	-

Table 1. Reported phenotypes for various SLC40A1 gene mutations (Proband’s characteristics and iron indices) HC: Hepatocytes, REC: Reticulo endothelial cells (Kupffer cells), TS: Transferin saturation, Fe in liver cells: Liver cells where iron was predominantly accumulated, XLSA: X-linked sideroblastic anemia, VHC: Viral hepatitis C, -: Data not available.

can discriminate between disease causing SLC40A1 mutation and polymorphism Mayr et al have assessed them as alternatives to predict the effect of SLC40A1 mutation on protein function and found that PolyPhen has 99% sensitivity and 67% specificity in identifying disease-causing gene variants by scoring newly-identified mutations in patients with primary iron overload as “possibly” or “probably” damaging (Mayr, 2010).



### 3. Conclusion

Point mutations in ferroportin gene SLC40A1 result in highly penetrant ferroportin disease genetically and phenotypically heterogeneous with mild clinical expression in the absence of cofactors. Functional analysis have identified two phenotypes, classical with normal transferrin saturation and non-classical with high transferrin saturation but locus heterogeneity still exist for each form. Variability in phenotypic expression may be related to co-inherited genetic modifiers or environmental factors.

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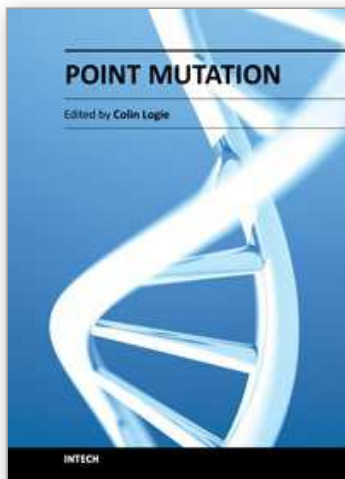
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This book concerns the signatures left behind in chromosomes by the forces that drive DNA code evolution in the form of DNA nucleotide substitutions. Since the genetic code predetermines the molecular basis of life, it could have been about any aspect of biology. As it happens, it is largely about recent adaptation of pathogens and their human host. Nine chapters are medically oriented, two are bioinformatics-oriented and one is technological, describing the state of the art in synthetic point mutagenesis. What stands out in this book is the increasing rate at which DNA data has been amassed in the course of the past decade and how knowledge in this vibrant research field is currently being translated in the medical world.

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