Mild Iron Overload in an African American Man with *SLC40A1* D270V

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Key Words

Ferroportin • *FLVCR1* P542S • Iron overload

Abstract

We report on a 46-year-old black man who resided in Alabama with normal transferrin saturation, mild hyperferritinemia, chronic hepatitis C, and 3+ iron in hepatocytes and Kupffer cells. Exome sequencing revealed heterozygosity for *SLC40A1* D270V (exon 7, c.809A→T), a mutation previously reported only in 1 black patient with iron overload who resided in the Republic of South Africa. The present patient was also heterozygous for: heme transporter *FLVCR1* novel allele P542S (exon 10, 1624C→T); *FLVCR1* T544M (rs3207090); hemopexin (*HPX*) R371W (rs75307540); ferritin scavenger receptor (*SCARA5*) R471H (rs61737287); and transferrin receptor (*TFR*) G420S (rs41295879). He had no *HFE*, *TFR2*, *HJV*, or *HAMP* mutations. D270V was not detected in 19 other African Americans with iron overload who resided in Alabama. The allele frequency of *SLC40A1* D270V in 258 African American adults who participated in a health appraisal clinic was 0.0019 (95% confidence interval 0–0.0057). D270V could explain 'classical' ferroportin hemochromatosis phenotypes in some African Americans.

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Introduction

The *SLC40A1* gene encodes ferroportin, an iron exporter and the hepcidin receptor [1]. Some *SLC40A1* mutations cause an uncommon, heterogeneous group of iron overload disorders characterized by an autosomal dominant pattern of inheritance [2, 3]. The *SLC40A1* polymorphism Q248H occurs in some Blacks with hyperferritinemia who reside in the US [4–7], but the odds ratio of the association of Q248H with iron overload is not significantly elevated [8]. No other *SLC40A1* missense mutations believed to cause elevated iron measures have been previously described in African Americans [2]. Herein, we report the identification of *SLC40A1* D270V in a 46-year-old African American man with mild iron overload who resided in Alabama. The relationship of D270V to his iron phenotype is discussed in the context of other mutations we identified by genome sequencing and the associations of other *SLC40A1* mutations to iron overload phenotypes in persons of sub-Saharan African native descent.

Case Report

At a routine annual examination, a 46-year-old African American man from south Alabama had hemoglobin 14.5 g/dl, mean corpuscular volume 82 fl, serum aspartate aminotransferase 51 U/l, serum iron 152 µg/dl, transferrin saturation 37%, and serum

ferritin 353 ng/ml. He was diagnosed to have hepatitis C at age 17 years and had positive serum hepatitis C antibody tests with mild intermittent elevation of serum aminotransferase levels on several occasions during the ensuing 29 years. We observed that he had hepatitis C, genotype 1. He had no symptoms or abnormal physical findings. He had a history of mild hypertension. He consumed a normal diet, did not drink alcohol, and never took iron supplements or donated blood. He had no family history of blood, iron, or liver abnormalities. His father had died of unknown cause. His mother was alive but was not available for study. His brother had died as a child in an accident.

Further testing revealed serum iron 187 µg/dl, transferrin saturation 41%, and serum ferritin 559 ng/ml. Percutaneous liver biopsy was interpreted as mononuclear infiltration and grade 2 fibrosis in the portal triads, no hepatocellular necrosis, 3+ stainable iron in hepatocytes and Kupffer cells [9], and minimal fatty change. He declined to undergo phlebotomy therapy or other treatment for iron overload or hepatitis. At age 57 years, he reported no symptoms and had a normal physical examination, and his serum levels of aminotransferases were normal. He declined to undergo repeat serum iron measurements.

Methods

Genetic Testing of the Present Patient

HFE mutation analysis to identify C282Y, H63D, and S65C alleles was performed as previously described [4]. Direct sequencing of *SLC40A1* was performed as previously described [4]. Further mutation analysis was performed by exome sequencing with Roche Nimblegen SeqCap EZ Human Exome Library version 2 according to the manufacturer's instructions. Next-generation sequencing was performed with an Illumina GAIIx. We obtained 51,236,724 pairs of reads of length 100, for a total of 10.25 billion bases of raw data. The *SLC40A1* D270V mutation (chromosome 2: 190428903) was found through computational analysis of genome-wide coding region sequences with 52× coverage and validated by Sanger sequencing [10]. Mapping, single nucleotide polymorphism (SNP) calling, and SNP annotation of exome sequencing reads were performed using Bowtie (bowtie-bio.sourceforge.net), SAMtools (samtools.sourceforge.net) and SeattleSeq (gvs.gs.washington.edu/SeattleSeqAnnotation), respectively. Details of exome sequencing are available upon request and will be described elsewhere.

Evaluation of Other African Americans for SLC40A1 D270V

We performed direct sequencing of *SLC40A1* in 19 other unrelated African American adults from Alabama who had primary iron overload phenotypes. We also estimated the allele frequency of *SLC40A1* D270V in African Americans by testing DNA specimens obtained with informed consent from the Kaiser/The Scripps Research Institute Genetics Bank from a convenience sample of 258 African American adult participants in a health appraisal clinic [11]. Genotyping was performed by real-time PCR using the Biorad CFX thermal cycler and Precision Melt software (Biorad, Hercules, Calif., USA). Amplification was performed using *SLC40A1* primers FPN ex7F (AGTACTACTAATAATTG-GCTTTT) and FPN ex7Rn (GACCCATCCATCTCGGAAGGT), and Sso Eva green Taq polymerase, with 1 cycle of 95°C at 3-min

initial denaturation, and 39 cycles of 95°C for 20 s, 57°C for 5 s, and 72°C for 5 s. The melt curve analysis was performed between 70 and 95°C at 0.1 degree increments. All ambiguous and non-wild-type genotype calls were validated by direct Sanger sequencing (Genewiz, San Diego, Calif., USA).

Results

Genetic Testing of the Present Patient

HFE mutation analysis did not detect *HFE* C282Y, H63D, or S65C alleles. Exome sequencing revealed heterozygosity for a mutation in *SLC40A1* (D270V; exon 7, c.809A→T) identified as novel by SeattleSeq but which was described previously by Zaahl et al. [12] in 1 of 22 South African black patients with iron overload. The *SLC40A1* Q248H polymorphism was not detected in the present patient. He was homozygous for *BMP2* R190S, where serine is the common allele. We identified mutations in other genes related to iron homeostasis (table 1). In particular, we found heterozygosity for: a novel allele in the heme transporter gene *FLVCR1* (P542S; exon 10, c.1624C→T); *FLVCR1* T544M (rs3207090); hemopexin (*HPX*) R371W (rs75307540); ferritin scavenger receptor gene (*SCARA5*) R471H (rs61737287); and transferrin receptor (*TFR1*) G420S (rs41295879). Exome sequencing did not detect any mutations in the hemochromatosis genes *HFE*, transferrin receptor-2 (*TFR2*), hemojuvelin (*HJV*), or hepcidin (*HAMP*).

Evaluation of Other African Americans for SLC40A1 D270V

None of the 19 other adult black patients with iron overload phenotypes had *SLC40A1* D270V. Exome sequencing of 4 of these 19 other patients confirmed that they did not have *SLC40A1* D270V. Overall, we observed D270V in 1 of 20 unrelated African Americans with iron overload phenotypes [allele frequency approx. 0.025 (1/40)]. The allele frequency of *SLC40A1* D270V mutation in 258 African American adults who participated in a health appraisal clinic was 0.0019 (1/516 alleles). Clinical observations on the one adult positive for D270V were not available.

Discussion

We excluded *FLVCR1* P542S as a candidate mutation for iron overload in the present case because the proline residue at amino acid position 542 is instead a serine at

Table 1. Selected mutations identified by exome sequencing in an African American man with mild iron overload

Gene ID	Chromosome_ position	Ref- base	Geno- type	Known/ novel	rsID	Amino acid change	cDNA Position	PolyPhen	Afrofq	Eurofq	SNPQual	ReadCnt
<i>ACVR1B</i>	12_52380898	G	A/G	known	34050429	NA	NA:1433	U/benign	17	0	231	196
<i>BMP2</i>	20_6759115	A	T/T	known	235768	R190S	570	benign/possibly damaging	0	29	228	78
<i>CD163</i>	12_7637769	G	A/G	known	61729512	T901M	2702	benign	NA	NA	210	31
<i>FLVCR1</i>	1_213068588	C	C/T	novel	NA	P542S	1624	U	NA	NA	161	185
<i>FLVCR1</i>	1_213068595	C	C/T	known	3207090	T544M	1631	benign/possibly damaging	14	45	231	176
<i>GLRX3</i>	10_131959150	C	C/T	known	2274217	P123S	367	benign	8	28	160	90
<i>HNF4G</i>	8_76468282	G	A/G	known	1805098	M227I	681	benign	1.8	42	228	96
<i>HPX</i>	11_6452889	G	A/G	known	75307540	R371W	1111	U	NA	NA	32	7
<i>IL6</i>	7_22767137	C	T/T	known	2069830	P32S	94	benign	8	0	49	4
<i>NFKB1</i>	4_103528817	T	G/T	known	4648099	H712Q	2136	benign/possibly damaging	6	0	76	18
<i>RAG1</i>	11_36597313	A	A/G	known	2227973	K820R	2459	benign	9	11	105	80
<i>SCARA5</i>	8_27729527	C	C/T	known	61737287	R471H	1412	benign	NA	NA	112	9
<i>SLC40A1</i>	2_190428903	T	A/T	novel	NA	D270V	809	U	NA	NA	222	52
<i>STAT5A</i>	17_40461436	C	C/T	novel	NA	T719M	2156	U	NA	NA	32	4
<i>TFRC</i>	3_195791240	C	C/T	known	41295879	G420S	1258	benign	NA	NA	169	86

Exome sequencing identified several genes related to iron homeostasis with known or novel mutations annotated by SeattleSeq. PolyPhen predictions, from lowest to highest degree of potential consequence, are 'benign', 'unknown' (U), 'possibly damaging', and 'probably damaging'. Minor allele frequencies are given for African (Afrofq) and European (Eurofq) populations

when known and NA is given when unknown. The minor allele is not always the same as the reference base (Refbase), as in *BMP2*, where the Refbase is the minor allele and the mutation identified by SeattleSeq is the major allele. SNPQual = SNP Single nucleotide polymorphism quality score; ReadCnt = read count; NA = not available.

the homologous position in rhesus monkey, dog, and opossum. *FLVCR1* T544M is a polymorphism that occurs at a high allele frequency in the general population. *BMP2* was eliminated as a candidate gene for the iron overload phenotype of the present patient because he was homozygous for the common allele that encodes serine at amino acid position 190. Thus, *SLC40A1* was the only gene in which we identified a mutation by exome sequencing that is known to be associated with an iron overload phenotype when present in a heterozygous configuration.

We report the occurrence of *SLC40A1* D270V heterozygosity in a black man with a mild iron overload phenotype who resided in Alabama. He was the only one of 20 unrelated black Americans tested with primary iron overload phenotypes from Alabama who had D270V (mean allele frequency 0.025; 95% CI 0–0.076). In the initial description of *SLC40A1* D270V by Zaahl and colleagues [12], only 1 of 22 black iron overload patients who resided in the Republic of South Africa was heterozygous for *SLC40A1* D270V (mean allele frequency 0.023; 95% CI 0–0.069) [12]. D270V was not detected in 20 South African black control subjects [12]. The allele frequency of

SLC40A1 D270V mutation in 258 African American adults from a health appraisal clinic sample was 0.0019 (95% CI 0–0.0057). On 9 January 2011, *SLC40A1* D270V had not been reported in the GenBank SNP database.

The aspartic acid at position 270 of ferroportin (or orthologous locus) is conserved in most species except in frog in which there is a threonine and a glycine instead of an aspartic acid residue, and in zebrafish, in which there is a methionine residue [13]. SIFT (sorting intolerant from tolerant) predicts the D270V mutation to be 'tolerated' and PolyPhen (polymorphism phenotyping) predicts the consequence of the mutation to be 'unknown' [this study and 3]. We propose that the previous identification of D270V in a South African black patient with iron overload [12] and in the present African American with a mild iron overload phenotype indicates that D270V is probably a pathogenic mutation and not a benign sequence variant, and that this allele may occur predominantly in Blacks of sub-Saharan African descent.

SLC40A1 D270V affects the third intracellular domain of ferroportin [1]. *SLC40A1* G267D (exon 7; c.1104G→A), adjacent to D270V, alters the same domain

and was associated with hyperferritinemia transmitted as an autosomal dominant trait in 5 members of a family of Chinese descent [14]. In that report, there was no mention of liver biopsy or phlebotomy therapy in family members who had hyperferritinemia and *SLC40A1* G267D. Thus, it is not possible to exclude *SLC40A1* G267D as a mutation that causes iron overload or, if so, in which hepatic cell excess iron is deposited. In contrast, *SLC40A1* L233P (exon 7; c.698T→C) affects a more proximal aspect of the third intracellular domain [1, 15]. To date, only 1 subject has been reported to have *SLC40A1* L233P: an Italian patient with a 'non-classical' ferroportin hemochromatosis phenotype and severe iron overload [15].

SLC40A1 Q248H (rs11568350), although not detected in the present case, occurs as a polymorphism in South African Blacks (allele frequency approximately 0.09) and in African Americans (allele frequency approx. 0.05) [8]. This frequency estimate of the Q248H polymorphism is consistent with the one we reported previously in African Americans [controls 0.08 (16/200); serum ferritin >350 ng/ml in females, >500 ng/ml in males, 0.17 (12/92)] [4]. Q248H is sometimes associated with hyperferritinemia, especially in men and in Q248H homozygotes [4–6]. Regardless, Q248H is not associated with a significantly increased odds ratio for iron overload in African or African American subjects [8] and the allele frequency of Q248H was not higher in African Americans with high serum iron measures than in control subjects [6]. An African American man with *ALAS2* R452S and *SLC40A1* R561G had severe multi-organ iron overload, although *ALAS2*

R452S was the predominant cause of his iron overload [16, 17]. *SLC40A1* G339D, L384M, and L384B are also common in African Americans, but have no defined association with abnormal iron phenotypes [4].

In persons with 'classical' ferroportin hemochromatosis, serum iron measures and complications of iron overload typical of 'non-classical' ferroportin and other types of hemochromatosis are relatively uncommon [3]. Observations in the present patient are consistent with 'classical' ferroportin hemochromatosis, including age of presentation, normal transferrin saturation, mild hyperferritinemia, relatively low mean corpuscular volume, and mild iron deposition in hepatocytes and Kupffer cells. Iron overload and liver phenotypes similar to those of the present patient are also relatively common in African Americans with iron overload [4, 9, 18, 19]. Alteration of the iron phenotype in the present patient, either by mutations in genes other than *SLC40A1* that we identified or by hepatitis C, cannot be excluded. Taken together, we conclude that *SLC40A1* D270V is an uncommon allele that could explain iron overload and liver phenotypes consistent with 'classical' ferroportin hemochromatosis in some African Americans.

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