HJV mutations causing hemochromatosis: variable phenotypic expression in a pair of twins

Hemochromatosis is a genetic condition characterized by excessive intestinal iron absorption leading to systemic iron overload. Hemochromatosis is caused by a deficiency of hepcidin, or resistance to the effects of hepcidin. The mutations that cause hepcidin deficiency affect genes that regulate hepcidin transcription, or the hepcidin gene itself. Homozygous C282Y mutation in *HFE* is the most common cause of hemochromatosis, however mutations in other genes, including *HJV, HAMP*, and *TFR2*,[1] can also cause hemochromatosis. Resistance to hepcidin is caused by certain autosomal dominant mutations in *SLC40A1*, the gene encoding the hepcidin receptor and iron transporter ferroportin.[1] Homozygous or compound heterozygous *HJV* mutations are the most common cause of juvenile hemochromatosis (JH) with an autosomal recessive pattern of inheritance. JH is characterized by early onset severe iron overload before age 30 leading to liver damage, cardiomyopathy, diabetes, arthropathy, and hypogonadism.[2] *HJV* codes for the hemojuvelin protein, a bone morphogenetic protein co-receptor, which is essential for adequate hepcidin production in response to iron.[3] Among several mutations identified, *HJV*pG320V is the most common mutation worldwide. Here we report a case of fraternal twin girls with similar *HJV* mutations but highly discordant severity of iron overload after obtaining parental consent as part of the Undiagnosed Disease Network.

The dizygotic twin girls were born at 32 weeks of gestation to a primigravida mother. They were conceived with intrauterine insemination. The pregnancy was complicated by placenta previa and placenta accreta in twin B. A Cesarian section was performed as twin B had intrauterine growth restriction (IUGR). The family history includes mother with SLE and Sjogren's syndrome and father who had a benign neuroendocrine tumor.

Twin A. She weighed 1.8 kg at birth, had a single umbilical artery, and stayed in the neonatal intensive care unit (NICU) for 10 days to establish feeding. She was discharged with ptosis as the only noted abnormality. Since then, she has been meeting all her developmental milestones and growing appropriately. At age 7, the patient had axillary and pubic hair, was evaluated for precocious puberty. The patient was using lavender lotions and soaps which apparently contributed to premature adrenarche, and the rest of the workup for precocious puberty was unremarkable. Incidentally, the patient was found to have elevated liver enzymes aspartate transaminase (AST) 101 U/L (age-appropriate normal range, 13-62 U/L), alanine transaminase (ALT) 183 U/L (age-appropriate normal range, 8-70 U/L), alkaline phosphatase 356 U/L (age-appropriate normal range, 145-335 U/L). She had a positive antinucelar antibodies (ANA) 1/640 titer, homogeneous pattern. Due to persistent elevation in liver enzymes, a liver biopsy was performed, which revealed moderate iron deposition with focal bridging fibrosis (etiology of fibrosis is unclear), a hepatic iron concentration of 17,362 μg/g/dry weight of liver tissue (age-appropriate

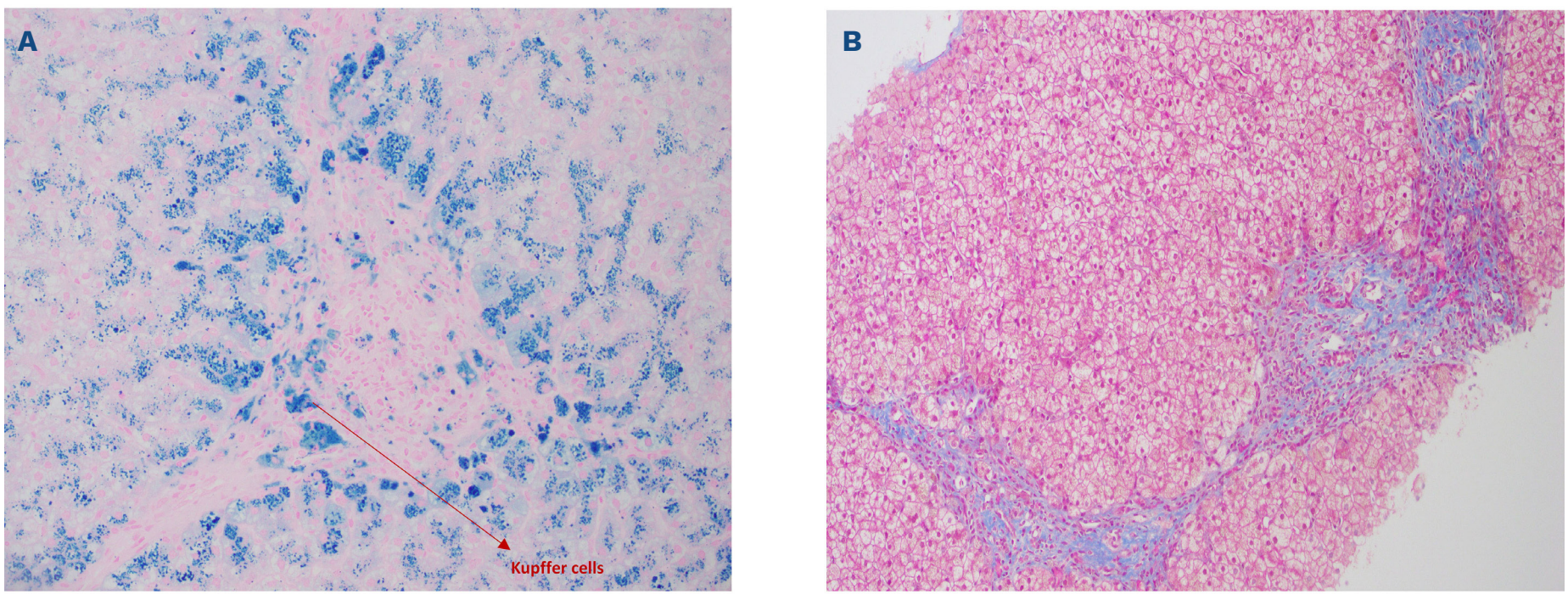

Figure 1. Liver biopsy of twin A. (A) Perl's Prussian blue iron stain, magnification x200. There is moderate hemosiderin deposition predominantly within the hepatocytes. Scattered iron-laden Kupffer cells are present. (B) Trichome stain, magnification x100. Bridging fibrosis highlighted in blue.

Table 1. Laboratory findings in the twins. (A) Genetic findings in the twins. (B) Hematological and biochemical parameters in the twins.

A						
	Gene	**Zygosity**	**Coding change**	**Frequency**	**Classification**	**Inheritance**
Twin A	*HFE*	Het.	c.187C>G p.His63Asp	14% European, non-Finnish	Risk	-
	HJV	Het.	c.302 T>C p.Leu101Pro	0.00093% European, non-Finnish	Pathogenic	-
	HJV	Het.	c.959 G>T p.Gly320 Val	0.039% European, non-Finnish	Pathogenic	-
Twin B	*BUB1B*	Het.	p.Glu215del chr15:g.40475962_40475964delAAG	-	Uncertain significance	Mother
	HFE	Het.	c.187C>G p.His63Asp	14% European, non-Finnish	Risk	-
	HJV	Het.	c.302 T>C p.Leu101Pro	0.00093% European, non-Finnish	Pathogenic	-
	HJV	Het.	c.959 G>T p.Gly320 Val	0.039% European, non-Finnish	Pathogenic	-

B						
	At the time of diagnosis		**3 months after chelation for twin A**		**11 months after chelation for twin A and 2 months after stopping PPI for twin B**	
Twin	**A**	**B**	**A**	**B**	**A**	**B**
Hemoglobin g/dL	13.0	14.4	13	13.0	12.8	13.4
Hematocrit %	38.9	41	38.9	36.4	39.2	38.2
Red blood cell count $x10^6/\mu L$	4.45	4.37	4.31	3.90	4.45	4.20
Mean corpuscular volume fL	87.4	93.8	86.3	93.3	88.1	91
Mean corpuscular hemoglobin pg	29.2	33	29.2	33.3	28.8	31.9
Mean corpuscular hemoglobin concentration g/dL	33.4	36.7	40.8	43.5	32.7	35.1
Red cell distribution width %	12.8	12.1	12.8	13	12.3	12
Reticulocyte count %	2.29	-	1.59	3.82	1.96	2.0
Absolute reticulocyte count $x10^6/\mu L$	0.10	-	0.07	0.15	0.09	0.09
Platelet count $x10^9/L$	367	260	367	231	315	204
White blood cell count $x10^9/L$	6.68	9.79	6.68	7.91	6.54	5.75
Ferritin ng/dL	1,763	196	1,480	129	416	170
Serum iron mcg/dL	308	261	305	233	327	250
Iron binding capacity mcg/dL	328	<283	603	<255	588	<272
Transferrin saturation %	94	77.42	51	82.18	56	UC
Aspartate transaminase U/L	148	25	52	27	44	27
Alanine transaminase U/L	262	17	66	13	37	17
Hepcidin levels ng/mL	-	-	-	-	7.7	12.3

Het: heterozygous. PPI: proton pump inhibitors; UC: unable to calculate.

normal range, 200-1.800 μg/g) (Figure1). Iron studies showed ferritin of 17,63 ng/mL (age-appropriate normal range, 8-180 ng/mL), serum iron 308 mcg/dL (age-appropriate normal range, 27-164 mcg/dL), TIBC 328 mcg/dL (age-appropriate normal range, 271-448 mcg/dL), serum transferrin 215 mg/dL (age-appropriate normal range, 188-341 mg/dL), and transferrin saturation of 94%. Genetic testing for hemochromatosis revealed pathogenic compound heterozygous *HJV* mutations and heterozygosity for the H63D *HFE* mutation as listed in Table 1A. Patient opted to be on iron chelation instead of phlebotomy, hence started on deferasirox 10 mg/kg/day. Magnetic resonance imaging (MRI) of the abdomen performed 3 months after starting chelation showed diffuse hepatic iron overload with estimated iron concentration of 5.9 mg iron per 1 g dry liver. Liver enzymes (AST 52 U/L, ALT 66 U/L), serum ferritin (1,480 ng/mL) and transferrin saturation (54%) showed a decreasing trend.

Twin B. She weighed 680 g at birth and spent 3 months in the NICU, course was complicated by chronic lung disease, feeding intolerance, failure to thrive, and astigmatism. Chromosomal microarray study was normal. At 8 months, she was diagnosed with infantile spasms and brain MRI revealed pachygyria. She was then tested for *IGF1R* and *PAPPA2* mutations through next generation sequencing, but no abnormality was found. She was diagnosed with gastric esophageal reflux disease and was treated with proton pump inhibitors (PPI). She also had poor weight gain of 400-900 g per year despite adequate calorie intake by mouth. Patient has received zinc, copper, and folate supplementation during infancy. Whole exome sequencing was performed. A heterozygous c.636_638delAGA, pGlu215del variant of uncertain significance in the *BUB1B* gene was identified. Variants in this gene are associated with autosomal recessive mosaic variegated aneuploidy (MVA) syndrome 1. At age 7, serum ferritin was 196 ng/dL, serum iron 261 mcg/dL, TIBC <283 mcg/dL, and serum transferrin 239 mg/dL, transferrin saturation 94 %. Genetic testing revealed the same heterozygous *HJV* mutations and heterozygosity for the H63D *HFE* mutation as in her sister. MRI of the abdomen was not performed as it was not clinically indicated. She has no clinical signs of iron overload currently. Laboratory parameters of the twins are listed in Table 1B.

Iron overload in HJV associated JH (HJV-JH) develops at an early age, with the mean age at diagnosis of 24 years. The sex distribution is equal in HJV-JH.[4] *HJV*pGy320Val and *HJV*Leu101Pro are the most common mutations in Caucasians.[5] HFE-associated hemochromatosis is inherited in an autosomal recessive pattern and p.Cys282Tyr in *HFE* is the most common pathogenic substitution in Caucasians. Both girls have a *HFE* mutation, with variant c.187C>G p. His63Asp (H63D) in addition to the *HJV* mutations. The H63D mutation is mild, and a single copy has not been known to cause any iron dysregulation. The interaction between H63D in *HFE* and *HJV* has not been established. Prior to the discovery of the HFE gene but after the linkage of the common form of hemochromatosis to human leucocyte antigen (HLA) was already established, Crawford *et al*. reported concordance in hepatic iron concentration among siblings of same sex with genetic hemochromatosis unless one of the siblings had definite reason for blood loss or discordance in HLA status.[6] Piling *et al*. report that common genetic variants that influence iron in the general population may help in estimating prognosis and treatment planning in patients with *HFE*pC282Y homozygous mutations.[7] Although twin A and B have the same known hemochromatosis (*HJV*) mutations, twin B has normal serum ferritin level and no clinical signs of iron overload. Twin B has no history of bleeding or blood donation. She has been maintaining hemoglobin >11 g/dL since discharge from the NICU. Fraternal twins share only half their genome, so it is possible that discordance in yet unknown genetic modifiers cause the phenotypic difference.

We measured serum hepcidin levels in both twins. Twin A has a low serum hepcidin of 7.7 ng/mL (normal range 4.4-47.3 ng/mL) despite elevated ferritin, and hepcidin to ferritin (H: F) ratio of 0.018 indicating classical hemochromatosis phenotype.[8] Twin B has a serum hepcidin level of 12.3 ng/mL and H: F ratio of 0.072, four-times higher than twin A. The level of hepcidin in twin B is inappropriately low considering the high serum transferrin saturation (>50%). It is unclear why the patient has high transferrin saturation but no evidence of elevated iron stores (ferritin 170 ng/mL). Iron utilization appears normal as hemoglobin (13.4 g/dL) is within the normal range. Twin B has higher mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), by ~10% consistently (Table 1B). This may indicate that she is utilizing iron for erythropoiesis at a higher rate than her sister, however their reticulocyte count is similar.

Twin B has a *BUB1B* mutation which is associated with MVA syndrome. Based on exome sequencing, she appears to be a carrier for this condition. We pursued additional testing, a sensitive genome-wide method to assess aneuploidy from blood DNA for which the whole genome sequence was generated. There was no evidence of any aneuploidy. There are no reports of any relationship between *BUB1B* mutation and iron metabolism. Twin B however has a few features of the MVA, such as IUGR, slow post-natal growth, shorter than average stature, and pachygyria with seizures. Currently, twin B weighs 15.6 kg at 8 years of age, less than the 1 percentile (compared to twin A who is 39 kg at 91st percentile). It is likely that twin B's chronic malnutrition has contributed to having normal serum iron and ferritin levels despite having high transferrin saturation levels (>50%). She may be absorbing iron at a higher rate than other nutrients because of her hemochromatosis mutations, which allows maintaining iron homeostasis and erythropoiesis at a normal level despite malnutrition. In contrast, the patient has been taking zinc supplements. Serum zinc lowest at 49 μg/dL at

age 4 years, maintains in the range of 62.6-98.6 μg/dL with zinc supplements (normal range, 60-120 μg/dL).
PPI are known to limit iron absorption,[9,10] thus it is possible that twin B absorbed less iron than what would be expected based on the hemochromatosis mutations. However, the patient stopped taking PPI for the last 2 months and the iron studies have not changed appreciably (serum iron 233 *vs*. 250 mcg/dL, TIBC <255 *vs*. <272 mcg/dL and ferritin 129 *vs*. 170 ng/mL). Longer observation is needed to make a more meaningful conclusion regarding any effect of PPI on iron parameters since she been taking them for the last 8 years.
We describe for the first time a severe case of juvenile hemochromatosis presenting with classical clinical features in contrast to her twin sister with the same mutations in the *HJV* gene with no signs of iron overload. In addition to the recognized hemochromatosis genes, other genetic and nutritional factors may modulate the severity of iron overload in hemochromatosis.

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https://doi.org/10.3324/haematol.2023.284134

Received: August 24, 2023.
Accepted: February 28, 2024.
Early view: March 7, 2024.

Disclosures

No conflicts of interest to disclose.

Contributions

AV prepared the manuscript and figures. SZ prepared pathology images. DS revised the manuscript and contributed to brainstorm discussion. GC performed whole genome sequencing. EM, EN, TG and SG revised the manuscript and contributed to brainstorm discussion.

Funding

This work was partially supported by the UCLA California Center for Rare Disease of the Institute for Precision Health and by National Institutes of Health grant U01NS134356 awarded to the UCLA Clinical Site of the Undiagnosed Diseases Network.

Data-sharing statement

Data can be accessed with the references, tables and figures submitted as part of the manuscript.

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