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To the editor:

Effect of the new HJV-L165X mutation on penetrance of HFE

We present a new homozygous truncating mutation, L165X, of the *hemojuvelin* gene (*HJV*), observed in one male patient with severe juvenile hemochromatosis (JH). Because the C282Y-variant in the *hemochromatosis* gene (*HFE*) was also common in this family, we investigated whether the inactivating mutation HJV-L165X influenced penetrance of HFE-C282Y homozygosity.

The proband, born in 1956 and diagnosed in 1972 by family screening (B III-54),[1] presented with increased serum iron values and heavy iron accumulation in the hepatocytes.[1,2] There was no consanguinity between his parents or grandparents,[1] and linkage to *HLA* was excluded in his familybranch, providing the first clue of genetic heterogeneity in hemochromatosis.[3] In late 2005, he provided contact information on relatives. The institutional review board approved this study and informed consent was obtained from all participants (n = 20). Non-fasting blood and urine samples from the proband and his relatives were collected between 7 and 9 PM on the same day. Information on the number of phlebotomies and the time between the last phlebotomy and sample collection was provided. Urinary hepcidin was measured by mass spectometry.[4] For the proband, we sequenced the *hepcidin* (*HAMP*) and *HJV* genes. Mutations in the *HAMP* gene were absent. The *HJV* gene was sequenced using previously reported primers.[5] For exon 3 we designed new primers: Ex3a1935F 5′-GCAAACTACACTC-CGATAGAG-3′ and Ex3a2253R 5′-GCTGGATCATCAGGTCTTCG-3′, resulting in a 319 bp product, and Ex3b2202F 5′-GACCTCGCCT-TCCATTCG-3′ and Ex3b2603R 5′-GAATCTCATGAGGTGGA-TCGG-3′, leading to a 402 bp product (GenBank NT_004 434/gi:88 943 080). We observed a novel homozygous mutation in exon 3 of the *HJV* gene. The 494T→A transversion leads to a premature stop codon at position 165 of the HJV protein: L165X. This probably leads to nonsense-mediated decay of the corresponding messenger RNA. If the aberrant message is translated, however, it would code for a protein that lacks the GPI anchor signal, such that it remains in the endoplasmic reticulum.[6] In both cases, it can be anticipated that upstream regulation of hepcidin is impaired.[7]

Relatives of the proband were investigated for the HFE-C282Y and the HJV-L165X mutation by a restriction fragment length polymorphism analysis using the Ex3b2202F and Ex3b2603R primers and the restriction enzyme HpyCH4V (New England Biolabs, Ipswich, MA).

HJV-L165X homozygosity was only present in the proband, while heterozygosity was common among relatives (allele frequency: 14/40 = 35.0%). Furthermore, the HFE-C282Y mutation was observed frequently (allele frequency: 27/40 = 67.5%) (Table 1). Phlebotomies were only reported in individuals later found to be homozygous for either HJV-L165X (n = 1; proband) or HFE-C282Y (n = 8). Iron indices (current and from the early seventies[1,2]; J. P. G., unpublished data, early 1970s) are copied into Table 1.

Current serum iron parameters are not appropriate as a measure of iron burden, as most relatives have been adequately phlebotomized. We found an alternative in the following parameters: quantity of iron removed by phlebotomies (iron removed/age),[8] a rough estimate hampered by the probability that intestinal iron uptake may increase upon phlebotomy; transferrin saturation (TS) values and desferrioxamine (DFO) test results from the early seventies, before treatment; and urinary hepcidin levels, measured with our improved MS assay.[4] The iron removed/age, urinary hepcidin levels, and iron indices from the early seventies were similar for HJV-heterozygotes (L165X) and the HJV-wildtypes, also when stratified by *HFE* genotype, indicating the absence of a clinically relevant modifying effect. Others reported HJV a modifier,[9,10] although not consistently.[11] Furthermore, our data agree with recent findings that HFE and HJV participate, at least partially, in distinct regulatory pathways.[12] Finally, against a background of multiple small variations, we cannot exclude a minor effect of the heterozygous HJV-L165X mutation on iron homeostasis.

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Table 1. Descriptive data on iron parameters of the relatives, sorted by HJV and HFE genotype

					Current			Iron removed/age[b], g/years	1970s	
Code[a]	Sex	Year of birth	HFE genotype	HJV genotype	TS, %	Ferritin, µg/L	Urinary hepcidin, M Int/mmol creat		TS, %	DFO-test[c] Mmol
B III-54[de]	M	1956	Ht	Ho	87	56	0.04[i]	1.02	93	159[j]
B III-7[e]	F	1943	Ho	Ht	53	76	2.50	0.23	53	32.3
B III-12[ef]	F	1951	Ho	Ht	71	36	0.60[i]	0.53	89	32.9
B II-12[e]	M	1924	Ho	Ht	60	96	0.83	0.37	86	107[k]
B III-85[e]	M	1950	Ho	Ht	21	30	0.10[i]	0.36	83	36.9
B III-86[g]	F	1953	Ho	Ht	58	147	1.56	0.12	85	45.0
B III-2	F	1937	Ht	Ht	30	304	2.98	NA	44	19.0
B III-5	F	1940	Ht	Ht	27	185	6.12	NA	38	28.0
B III-10	F	1947	Ht	Ht	29	107	2.91	NA	40	17.1
B III-48	M	1943	Ht	Ht	34	190	8.30	NA	43	19.1
B III-50	M	1945	Ht	Ht	23	30	0.04	NA	35	31.2
B III-52[f]	F	1949	Ht	Ht	46	62	0.75	NA	46	17.6
B III-87	M	1956	Ht	Ht	23	351	6.32	NA	55	22.0
B III-3[f]	F	1938	Ho	Wt	65	354	1.93	0.89	87	46.0
B III-88[eh]	M	1959	Ho	Wt	39	53	0.31	0.30	57	21.1
B III-89[eh]	M	1959	Ho	Wt	51	87	1.06[i]	0.28	54	23.2
B III-1	F	1936	Ht	Wt	24	275	5.35	NA	41	16.8
B III-4	M	1939	Ht	Wt	18	310	7.77	NA	45	28.8
B III-11	F	1949	Ht	Wt	24	56	2.07	NA	38	29.5
B III-9[f]	M	1946	Wt	Wt	58	821	5.13	NA	40	17.1
Reference values					< 50		0.52-7.83[l]	NA	< 50	< 30.3
Men						15-280				
Women < 50 years						6-80				
Women > 50 years						15-190				

CRP (< 10 mg/L), Hb (M: 8.1-10.7 mmol/L; W: 7.3-9.7 mmol/L), MCV (M: 84-103 fl; W: 85-104 fl), and ALAT (< 45 U/L) are within reference values for all individuals, except for male II-12, whose Hb was fairly high (11.5 mmol/L) and for male III-50, whose ALAT was slightly increased (48 U/L).

Ho indicates homozygous for the mutation; Ht, heterozygous for the mutation; Wt, wildtype for the mutation; NA, not applicable.

[a]These are the codes as used before; roman numerals indicate the generation.

[b]Grams of iron removed were calculated by multiplying the number of phlebotomies by 0.20 g (assuming 500 mL whole blood was withdrawn per phlebotomy).

[c]Urinary iron concentration in 24 hrs after administration of 1000 mg Desferrioxamine intramuscular (DFO-test).

[d]Proband; the proband in the studies in the early 1970s was his sister (Ba, III-51[2]) who was born in 1947 and died in 1998 (1970s: TS: 78%; DFO-test: 376 µmol; liver iron content: 64.2 mmol/100 g dry weight).

[e]Currently on maintenance therapy with phlebotomies.

[f]Alcohol consumption > 15 units per week.

[g]Phlebotomized in the past.

[h]III-88 and III-89 are monozygotic twins.

[i]Date of last phlebotomy less than 4 weeks in the past or unknown.

[j]Liver iron content: 32.4 mmol/100 g dry weight.

[k]Liver iron content: 17.1 mmol/100 g dry weight.

[l]Based on the range of values observed in 20 healthy individuals.[4]

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