

HLA-H Mutations in the Ashkenazi Jewish Population

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ABSTRACT: Hereditary hemochromatosis is a common disorder in people of European origin. The HLA-H gene has been found to have two mutations that apparently cause hemochromatosis. The principal mutation, 845G→A (C282Y), is believed to have arisen relatively recently in the Celtic population. To determine the incidence of this mutation and the other hemochromatosis-associated mutation, 187C→G (H63D), among Ashkenazi Jews, a people who are believed to have arrived in Europe in about the 8th Century A.D., we have examined the DNA from 381 unrelated Jewish subjects and 206 non-Jewish white controls. The gene frequency for the 845G→A mutation among Jewish subjects was only 0.013 compared with a frequency of 0.070 among controls, a difference that is significant at the 0.00001 level. The phenotypically milder nt 187C→G mutation had a frequency of 0.155 in the non-Jewish population and 0.097 in the Jewish population, a difference that was also statistically significant at the <0.01 level.

Keywords: hemochromatosis, population genetics, gene frequency

INTRODUCTION

Hereditary hemochromatosis (iron storage disease) is arguably the most common inherited disorder among people of European ancestry. Cloning of the gene with demonstration of two hemochromatosis-related mutations, 845G→A (C282Y;845A) and 187C→G (H63D;187G) (1), has made possible precise estimation of gene frequencies in people of different ethnic origins. Surveys of persons of mixed Caucasian ancestry showed a frequency of heterozygotes of 6.4% (10/155) (1), 15% (29/193) (2), and 5.8% (8/139) (3), for a composite gene frequency of 0.048.

The gene for hereditary hemochromatosis is believed to be of Celtic origin (4). One might presume that the founder mutation arose relatively recently since it is in marked linkage disequilibrium even with the HLA-A gene which is now recognized to be approximately 4

megabases centromeric to HLA-H (1). However, the mutation does not seem to be limited to Northern Europe. Based on the screening of blood donors for transferrin saturation a Northern Italian population was estimated to have a gene frequency of 0.045 (5). We have encountered one Mexican patient with the C282Y/C282Y genotype (6). However, we recall only one Jewish patient with hemochromatosis, long before genotyping was possible, and are unaware of any cases that have been published in the literature.

Ashkenazi Jews seem to have migrated to the Rhineland by the eighth century (7). The existence of "Jewish diseases" such as Tay-Sachs disease, Gaucher disease and Niemann-Pick disease in this population, diseases that are relatively rare in the non-Jewish population, implies a considerable degree of genetic isolation. Moreover, a number of genetic markers, particularly those in the HLA system, suggest that

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the extent of genetic exchange with surrounding European populations has been meager (8).

MATERIALS AND METHODS

Materials and Methods

Three hundred eighty-one samples of DNA that had been obtained from unrelated persons of Ashkenazi Jewish ancestry were examined for the HLA-H mutations 845G→A (C282Y) and 187C→G (H63D). Eighty-nine of these samples were from patients with type I Gaucher disease. Two hundred six samples from persons of non-Jewish European origin served as controls. Most of the latter had been included in an earlier study (2) and in those cases mutation detection was performed as described previously.

Genomic DNA was isolated from leukocytes by standard methods and 0.2 µg was amplified by the polymerase chain reaction (PCR). Two fragments from the HLA-H gene, one containing the 187G mutation and the other containing the 845A mutation were simultaneously amplified in a 50 µl system. The reaction contained 34 mM Tris-HCl pH 8.8, 8.3 mM ammonium sulfate, 3.4 mM MgCl₂, 5% dimethyl sulfoxide, 85 µg/ml bovine serum albumin, 0.2 mM of each dNTP, 0.75 Units of Taq DNA polymerase (Qiagen, Inc., Santa Clarita, CA), and 200 ng of each of the four oligonucleotide primers listed in Table 1. After an initial 4 minute denaturation at 98 C, PCR was performed for 30 cycles of 92 C denaturation, 58 C annealing, and 72 C

extension, all for 30 seconds, and a final extension of 7 minutes at 72 C. The PCR was performed in a 96 well plate in a Perkin Elmer 96 well thermocycler. The amplified fragments containing nt 187 and nt 845 were 310 bp and 390 bp in length respectively. Three microliters of the amplified DNA was spotted in quadruplicate on Nytran Plus nylon membranes (Schleicher and Schuell, Keene, NH). The membranes were soaked in 1.5 M NaCl/0.5 N NaOH for 2 minutes, then in 1.5 M NaCl/0.5 M Tris-HCl pH 8.0 for 2 minutes, and finally rinsed briefly in 0.2 M Tris-HCl pH 7.5/2×SSC. The DNA on the membranes was immobilized by ultraviolet crosslinking in a Stratalinker (Stratagene, LaJolla CA). The spotted membranes were prehybridized at 42 C for 30 minutes in 6×SSC, 5× Denhardt's, 0.02 M sodium phosphate pH 7.0, 0.5 mg/ml salmon sperm DNA, and 1% SDS. Four oligonucleotide probes (Table 1) representing the normal and mutant sequences in the region of the two HLA-H mutations were labeled with γ-³²P-ATP and polynucleotide kinase (New England BioLabs Inc., Beverly, MA) according to manufacturer's instructions. After prehybridization the spotted membranes were hybridized for 2 hours at 42 C in a solution of 7×SSC, 0.02 M sodium phosphate pH 7.0, 0.5 mg/ml salmon sperm DNA, 1% SDS and 5 × 10⁶ cpm of labeled probe. The membranes were washed in 6×SSC and 0.1% SDS at the temperatures listed in Table 1. The washed membranes were exposed to XAR X-ray film for 6 hours at -70 C.

Table 1. Primers and Probes for Amplification and Detection of HLA-H Mutations

Mutations in HLA-H (nt)	PCR Primers	Oligonucleotide Probes	Wash Temperature
187C→G	5' -GCCTCAGAGCAGGACCTTGG-3'	5' -TCTATGATCATGAGAGT-3'	48C
	5' -CAGCTGTTTTCTTCAAGATGC-3'	5' -TCTATGATGATGAGAGT-3'	48C
845G→A	5' -TGGCAAGGGTAAACAGATCC-3'	5' -ATATACGTGCCAGGTGG-3'	53C
	5' -CTCAGGCACTCTCTCAACC-3'	5' -ATATACGTACCAGGTGG-3'	50C

RESULTS AND DISCUSSION

Table 2 summarizes the results of the population survey. The gene frequency of the C282Y mutation was 0.013 in the Jewish population, compared with a gene frequency of 0.070 in the non-Jewish population. This difference is highly statistically significant ($p < 0.00001$; Fisher's exact test). The gene frequency of the H63D mutation at nt187 was also less in the Jewish than the non-Jewish European population at 0.097 vs 0.155; ($p < 0.01$; Fisher's exact test).

The gene frequency of the C282Y mutation that we documented, viz. 0.07, is somewhat higher than that reported by other groups, 0.034 (1), and 0.029 (3). Based on a population consisting of 206 individuals, the standard error of our estimate is 0.018. Therefore, the difference between the gene frequencies reported by various groups could be due not only to differences in the composition of the normal population but, because of the small numbers

involved, merely statistical variation. The expected frequency of homozygotes for this mutation based on our gene frequency estimate is approximately 5/1,000 population, a frequency well within those derived by some population surveys based on serum iron saturations: 12/1,000 in New Zealand (9), 4.5/1,000 among Afrikaners (10), 4.5 and 6.6/1,000 in the U.S. (11,12), and approximately 4/1,000 among Danes (13,14). The expected homozygote frequency among Ashkenazi Jews, in contrast, would be predicted to be more than an order of magnitude lower, at 0.17/1,000. The lower frequency of the H63D mutation indicates that the proportion of compound heterozygotes among the Jewish population would be even lower.

Our studies confirm those based on other genetic markers that suggest that the admixture of the Jewish population with the surrounding European population has been relatively meager (8), and implies that hereditary hemochromatosis should be a rare disease among Ashkenazi Jews.

Table 2. HLA-H Genotypes in Ashkenazi Jewish and Control Subjects

Group	nt 187C→G(H63D)					nt 845G→A (C282Y)				
	Genotype (number of subjects)			Gene frequency		Genotype (number of subjects)			Gene frequency	
	C/C	C/G	G/G	C	G	G/G	A/G	A/A	G	A
Jewish	313	62	6	0.903	0.097	371	10	0	0.987	0.013
Non-Jewish White	149	50	7	0.845	0.155	177	29	0	0.930	0.070

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REFERENCES

1. Feder JN, Gnirke A, Thomas W, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nature Genet* 13:399-408, 1996.
2. Beutler E, Gelbart T, West C, et al. Mutation analysis in hereditary hemochromatosis. *Blood Cells Mol Dis* 22:187-194, 1996.
3. Jouanolle AM, Gandon G, Jezequel P, et al. Haemochromatosis and HLA-H. *Nature Genet* 14:251-252, 1996.
4. Jazwinska EC, Pyper WR, Burt MJ, et al. Haplotype analysis in Australian hemochromatosis patients: Evidence for a predominant ancestral haplotype exclusively associated with hemochromatosis. *Am J Hum Genet* 56:428-433, 1995.

5. Velati C, Piperno A, Fargion S, Colombo S, Fiorelli G. Prevalence of idiopathic hemochromatosis in Italy: Study of 1301 blood donors. *Haematologica (Pavia)* 75:309-312, 1990.
6. Beutler E, Gelbart T. *Unpublished* 1997.
7. Ankori Z. Origins and history of Ashkenazi Jewry (8th to 18th century). In: Goodman RM, Motulsky AG, eds. eds. *Genetic Diseases Among Ashkenazi Jews*. New York: Raven Press, p. 19, 1979.
8. Bonné-Tamir B, Ashbel S, Kenett R. Genetic markers: Benign and normal traits of Ashkenazi Jews. In: Goodman RM, Motulsky AG, eds. eds. *Genetic Diseases Among Ashkenazi Jews*. New York: Raven Press, p. 59, 1979.
9. Elliott R, Lin BP, Dent OF, Tait A, Smith CI. Prevalence of hemochromatosis in a random sample of asymptomatic men. *Aust N Z J Med* 16:491-495, 1986.
10. Ballot D, Meyer TE, Bothwell TH, et al. Idiopathic haemochromatosis. Family studies and results of a pilot prevalence survey. *S Afr Med J* 71:639-642, 1987.
11. McLaren CE, Gordeuk VR, Looker AC, et al. Prevalence of heterozygotes for hemochromatosis in the white population of the United States. *Blood* 86:2021-2027, 1995.
12. Edwards CQ, Griffen LM, Goldgar D, Drummond C, Skolnick MH, Kushner JP. Prevalence of hemochromatosis among 11,065 presumably healthy blood donors. *N Engl J Med* 318:1355-1362, 1988.
13. Wiggers P, Dalhoj J, Kiær H, et al. Screening for haemochromatosis: Prevalence among Danish blood donors. *J Intern Med* 230:265-270, 1991.
14. Milman N, Clausen JO, Jordal R. Iron status in young Danish men and women: A population survey comprising 548 individuals. *Ann Hematol* 70:215-221, 1995.